# The use of 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**
- **LC-MS/MS quantification of ceramides**

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

## Lipids are very important!!

**Nutrition**

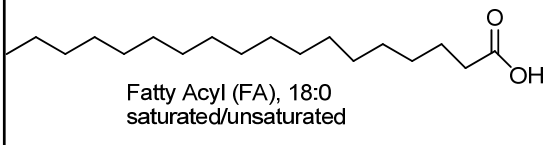
-Energy source

-Energy storage

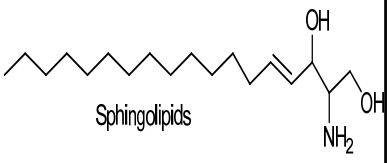
**Nutrition related diseases-**
Atherosclerosis, diabetes

**Phospholipids are essential- membrane composition/ functional state of cells**

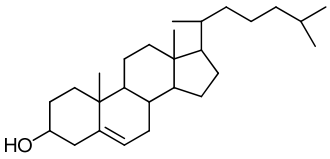
# Structures of different lipids classes



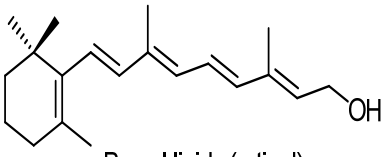

Fatty Acyl (FA), 18:0
saturated/unsaturated



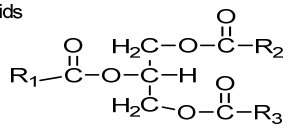

Sphingolipids



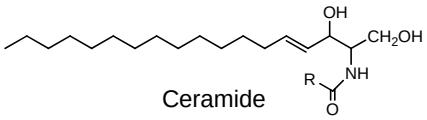

Sterol lipids




Prenol lipids (retinol)

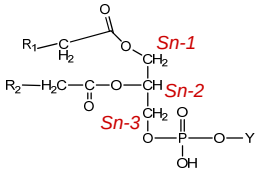



Glycerolipid, R =
saturated/unsaturated FA

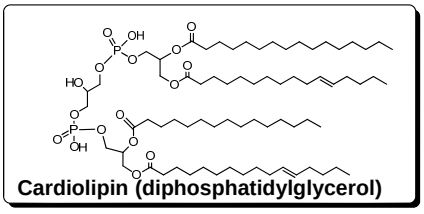



Ceramide

# Structures of main phospholipids




Phosphatidylethanolamine (PE)
Y= $NH_2$
Phosphatidylcholine (PC)
Y= $N(CH_3)_3$
Phosphatidylglycerol (PG)
Y= OH OH
Phosphatidylserine (PS)
Y= O OH $NH_2$
Phosphatidylinositol (PI)
Y= HO HO OH OH OH



**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.

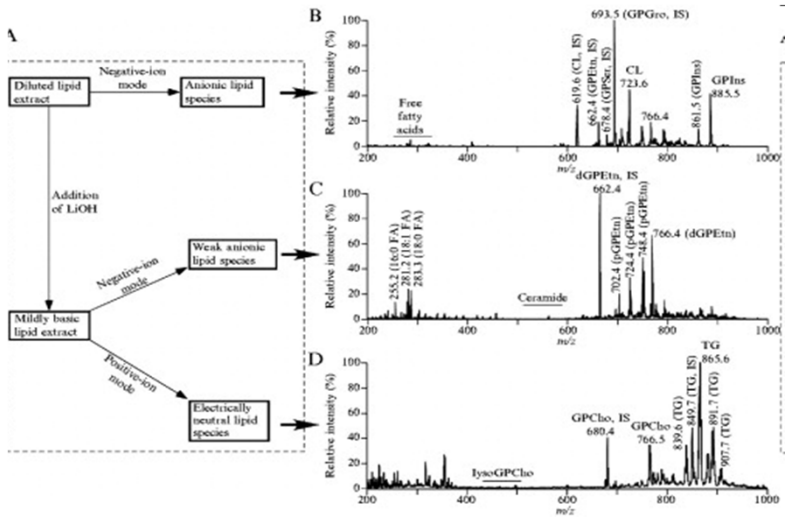
## Shotgun lipidomics: intrasource separation of lipids for quantitative lipidomics

| 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**

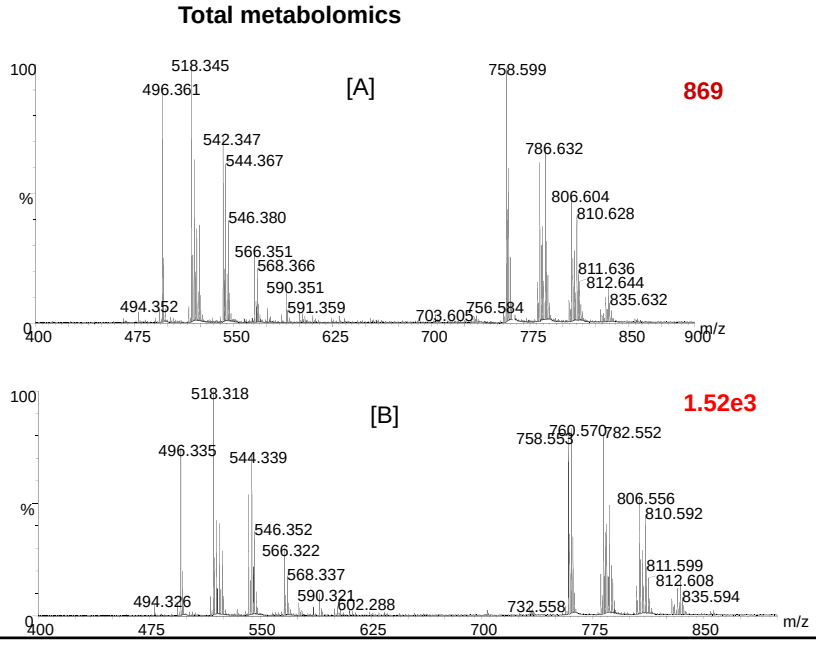
Source: Gross and Han,, 2004

## Application of shotgun lipidomics: intra-source separation of lipids

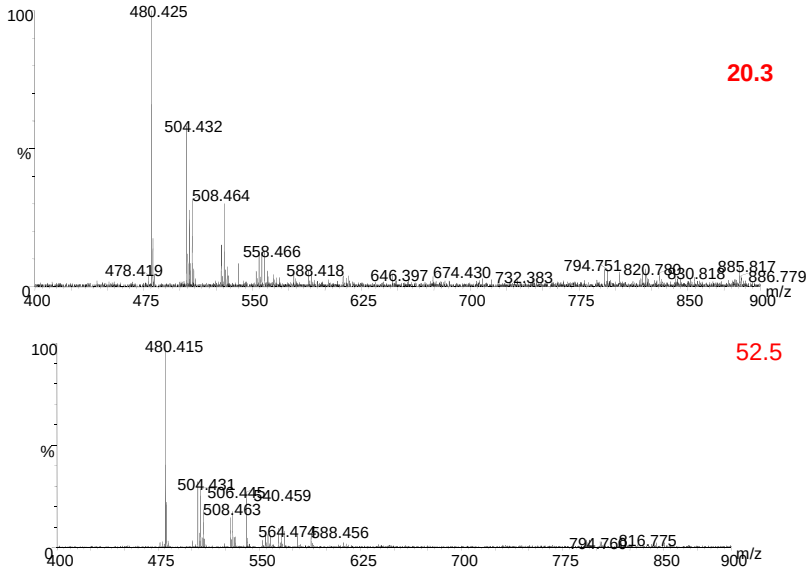



Source: Gross and Han, methods in Enzymology, 2007

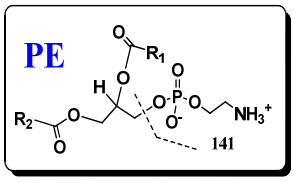
## Total scan of metabolites (Q1 SCAN + ion mode) for a plasma sample obtained from lean mouse [A]; ob/ob mouse



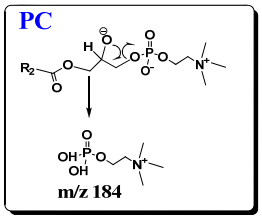
**Total scan of metabolites (Q1 SCAN -ve ion mode) for a plasma sample obtained from lean mouse [A]; ob/ob mouse**

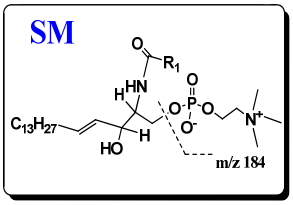



# Profiling phospholipids and sphingosines in a complex mixture using MS/MS

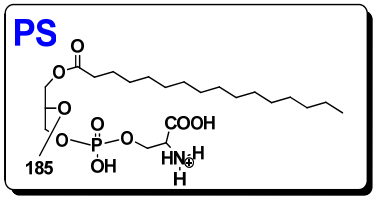



**PE**
**Neutral Loss scan 141**



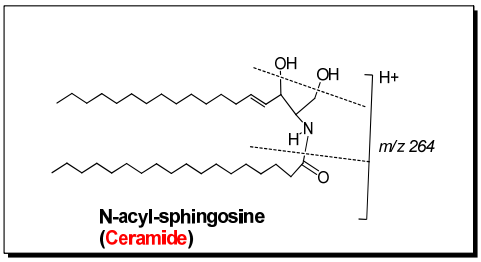



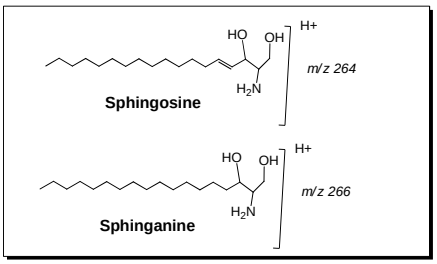

**PC & SM**
**Precursor ion scan 184**

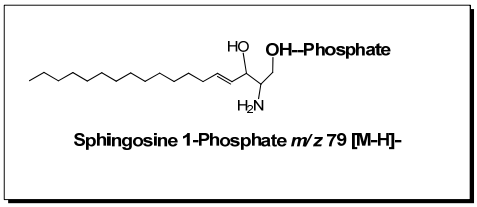



**PS**
**Neutral Loss scan 185**

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

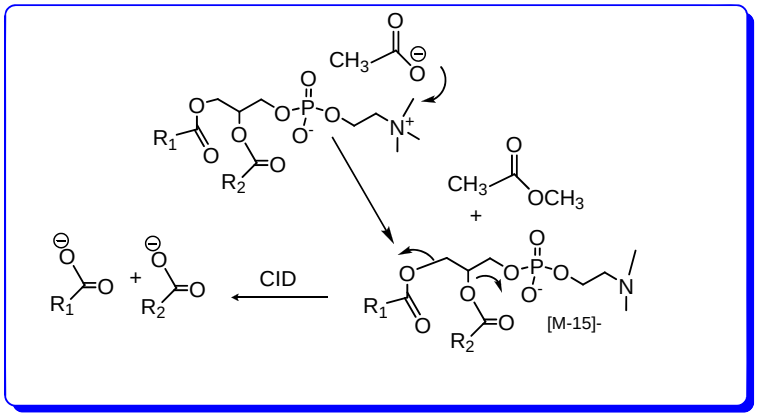



***m/z* 264 is a characteristic ion for all compounds containing a sphingosine backbone**

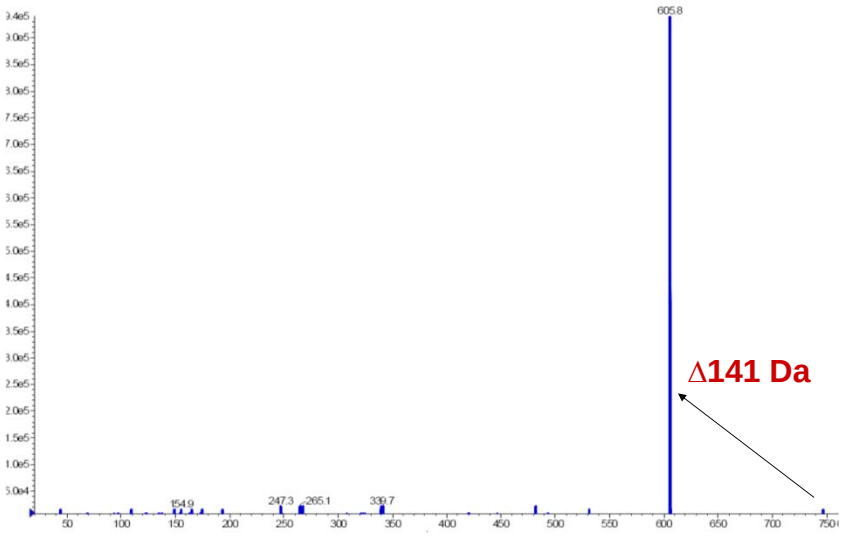
## Phosphatidylcholine loses a methyl group to form a negatively charged, pseudomolecular ion



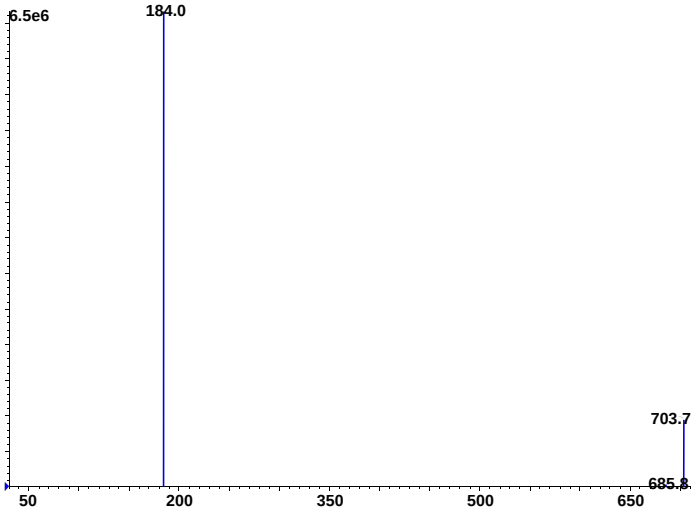

**Phospholipids may undergo demethylation and then the loss of the fatty acyl groups from glycerophosphocholine backbone.**

## MS/MS of m/z 746.90: PE Std

**Neutral loss of 141 is a characteristic for detecting PE**




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

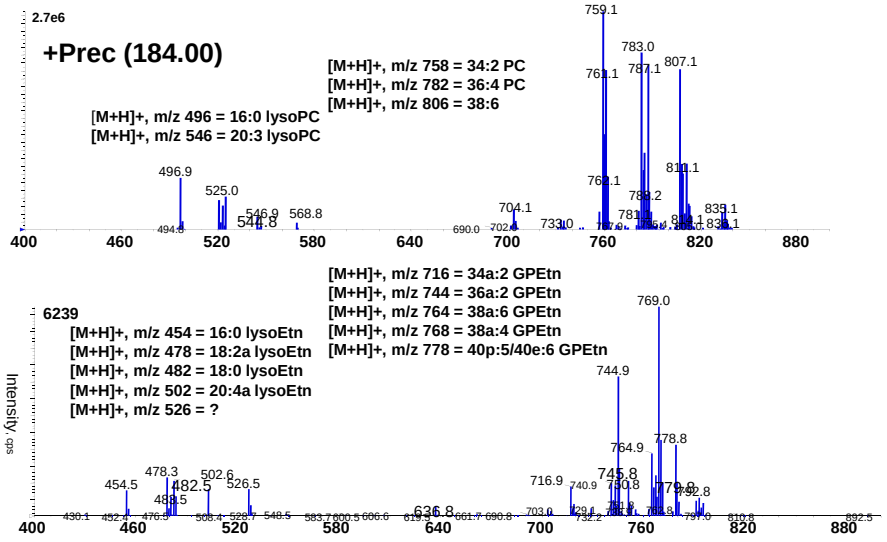


## ESI-MS/MS analyses of various lipids

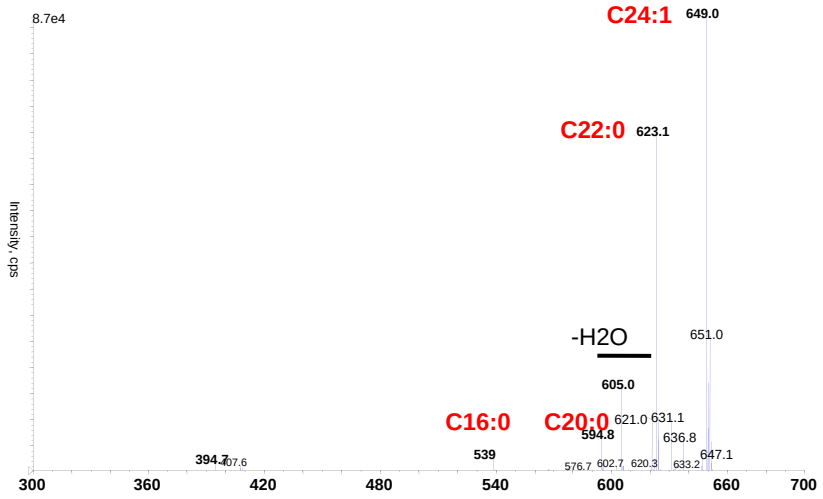
| Lipid Class(s) | Precursor Ion | MS/MS Mode & Conditions | Fragment |
|---|---|---|---|
| cardiolipin | $[M-2H]^{2-}$ | PI, *m/z* 153.0, 35 eV | glycerol phosphate derivative |
| PtdGro, PtdH | $[M-H]^-$ | PI, *m/z* 153.0, 35 eV, * | glycerol phosphate derivative |
| PtdIns | $[M-H]^-$ | PI, *m/z* 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

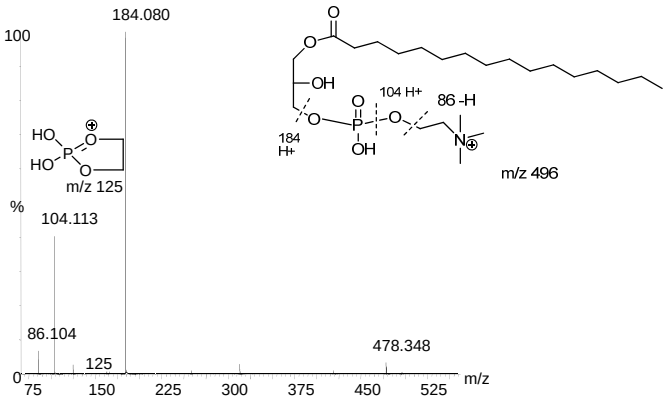
## Profiling of phospholipids using precursor ion m/z 184 and neutral loss scan 141 for PC, SM and PE



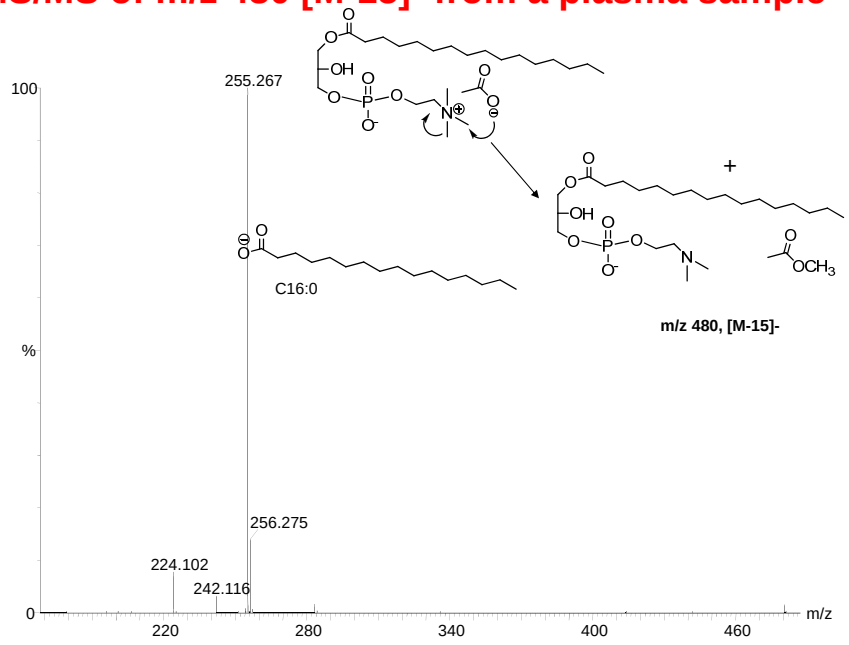
## Precursor ion scan m/z 264 in +ve ion mode is specific to identify ceramides in a sample



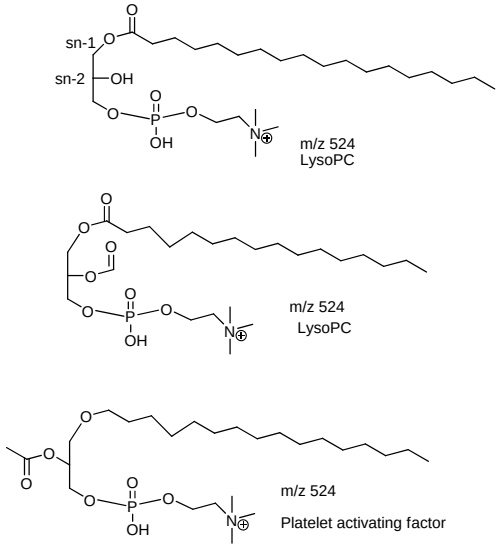

## MSMS fragmentation of *m/z* 496 obtained from a plasma sample



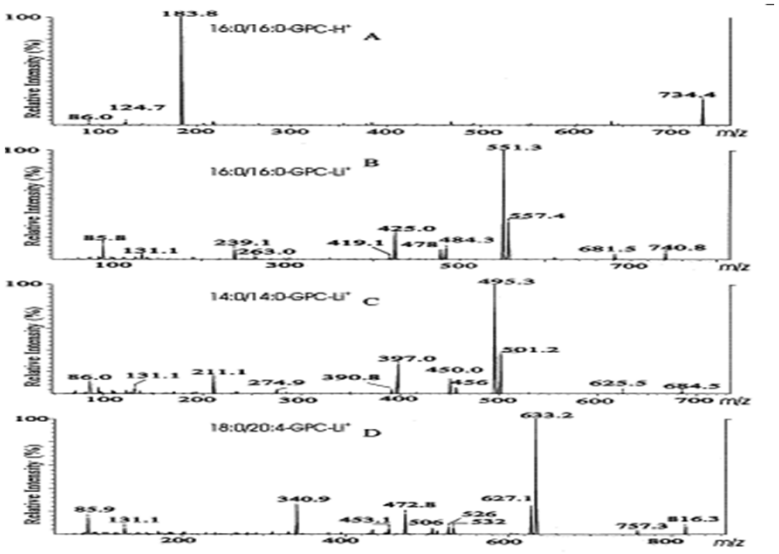
## MS/MS of m/z 480 [M-15]- from a plasma sample




## Several isomeric compounds exits and unambiguous identification is a challenge

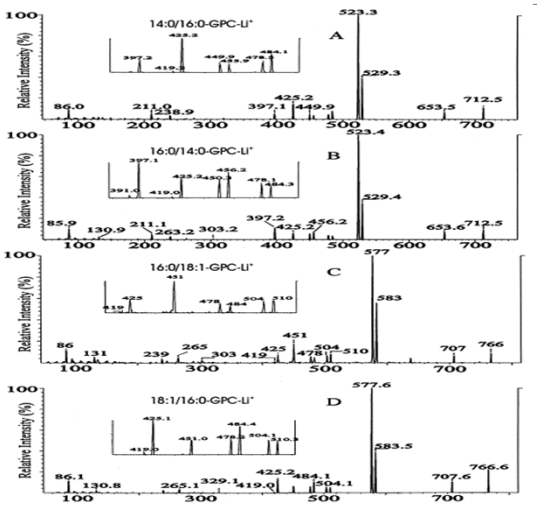


## Lithiated adducts of phosphocholine provide more structural **information** in their MS/MS spectra



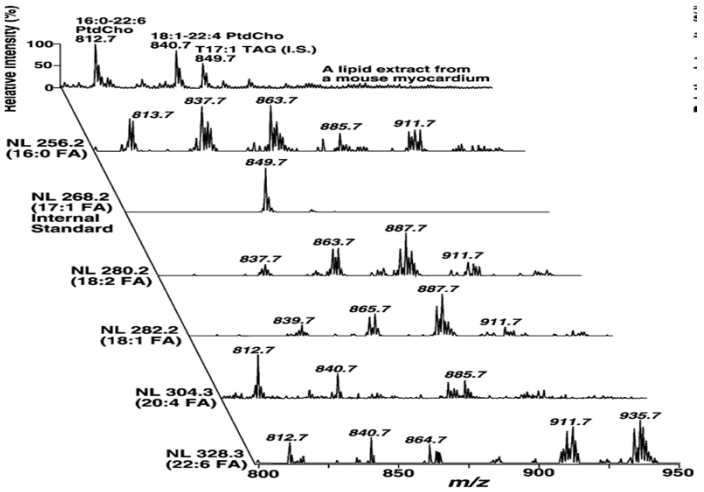

Source: Hsu et al. J. Am Soc. Mass Spectrom, 1998

## 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

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




Source: Han and Gross, 2004

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

LIPID MAPS -- LIPID Metabolites And Pathways Strategy

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LIPID Metabolites And Pathways Strategy

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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- | $C_{20}H_{32}O_6$ | 368.22 |

LIPID Metabolites And Pathways Strategy

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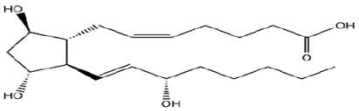
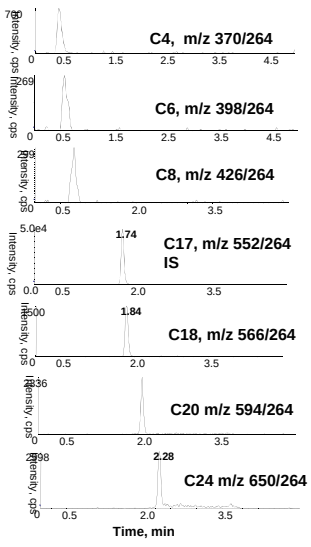
Structure database (LMSD)

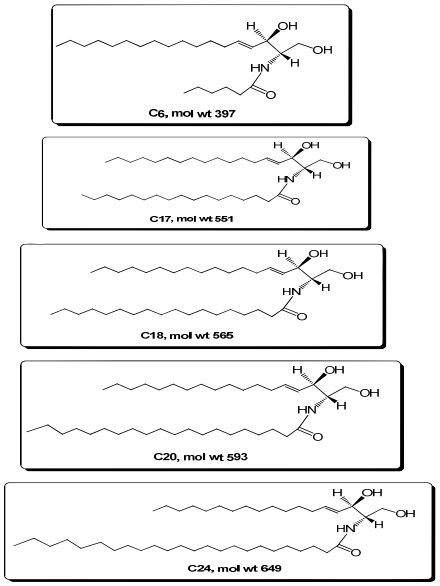
LMFA03010025



| | |
|---|---|
| LM ID | LMFA03010025 |
| Common Name | PGF2β |
| Systematic Name | 9R,11R,15S-trihydroxy-5Z,13E-prostadienoic acid |
| Synonyms | - |
| Exact Mass | 354.24 |
| Formula | $C_{20}H_{34}O_5$ |
| Category | Fatty Acyls [FA] |
| Main Class | Eicosanoids [FA03] |
| Sub Class | Prostaglandins [FA0301] |
| LIPIDBANK ID | XPR1764 |
| PubChem Substance ID (SID) | 4265968 |
| KEGG ID | - |

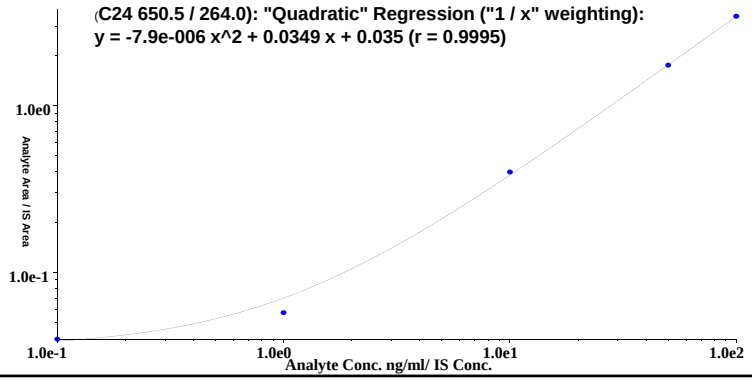
## MRM chromatograms showing simultaneous determination of ceramides (C4-C24)



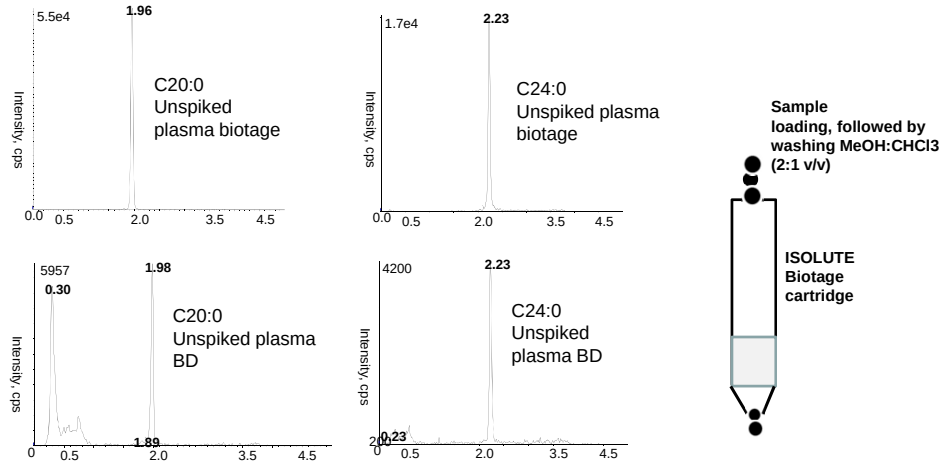



## A linear response for Cer C24:0 was observed over a range of 0.1-100 ng/ml with correlation coefficient greater than 0.99

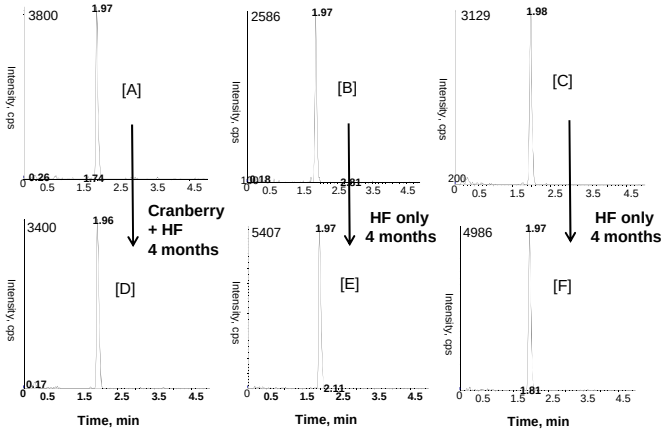
| Sample Name | Analyte Peak Name | Calculated Concentration (ng/mL) | Accuracy (%) |
|---|---|---|---|
| Ceramide Standard 100 ng/ml | C24 650.5 / 264.0 | 100 | 100 |
| Ceramide Standard 50 ng/ml | C24 650.5 / 264.0 | 49.8 | 99.6 |
| Ceramide Standard 10 ng/ml | C24 650.5 / 264.0 | 10.5 | 105 |
| Ceramide 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 |




## Sample preparation is a crucial step in quantitative analysis of ceramides; Poor recoveries of non-polar ceramides in Bleigh Dyer (BD) liquid-liquid extraction compared to Biotage (supported liquid extraction)



## 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.**