The use of mass spectrometry in lipidomics

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Outlines

- **Brief introduction to lipidomics**
- **Sample preparation/extraction**
- **Analytical methodology: MS/MS structure elucidation of phospholipids/prostaglandins**
- **Library of eicosanoid standards**

Lipidomics- A comprehensive analysis of lipid molecules in response to cellular pathophysiology

Lipids are very important!!

Nutrition

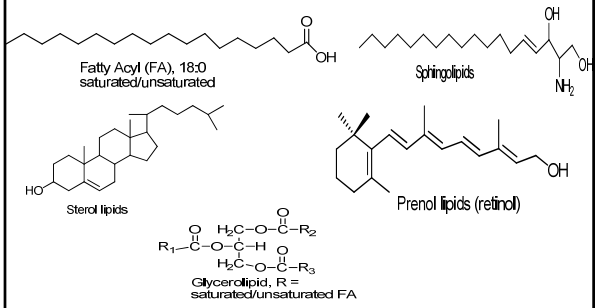
-Energy source

-Energy storage

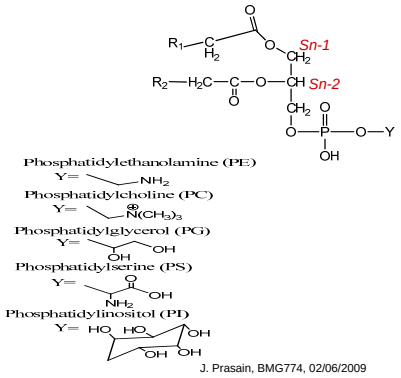
Nutrition related diseases-
Atherosclerosis, diabetes

Phospholipids are essential- membrane composition/ functional state of cells

Structures of different lipids classes



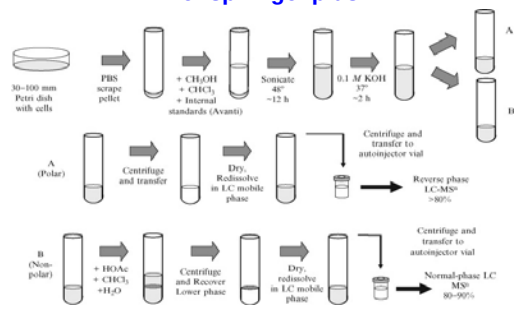
Structures of main phospholipids



Extraction of lipids from tissue by Folch method

- **Homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of the tissue sample, centrifuged**
- **Washed with 0.2 volume (4 ml for 20 ml) of water, centrifuged to separate two layers. Remove the upper phase.**
- **The lower phase is evaporated to dryness and stored at -20 ^{o}C untill analysis.**

Extraction schemes used for extraction of sphingolipids



Sullard et al. Methods in Enzymology, Vol. 432, 2007, 83-115

Strategies for Lipid Identification and Quantitative Analysis by Mass Spectrometry

A. Accurate mass value of molecular weight-related ions-
-FTICR, Q-TOF, Quadrupole-MS

A. Characteristic fragment ions
-Fragment ions of polar head groups or specific neutral loss from each class of phospholipids and glycerolipids are also very important

A. LC-MS/MS
-normal phase and reversed phase column chromatography

Mass spectrometry analysis of lipids

A. Direct infusion ESI-MS/MS
- acetonitril:methanol:water = 6:7:2 (0.1% ammonium formate)
- mass rage 400-900 m/z, injection 4-10 uL

B. LC-MS/MS
-reversed-phase LC-MS/MS, a 300 µm x 15 cm Atlantis dC18 capillary column
Gradient -10 mM ammonium acetate in MeOH/IPA/water (90:5:5)

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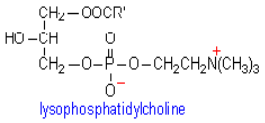
Which ionization mode for which phospholipids?

Positive ion mode	Negative ion mode
PC	PE
LPC	PA
PE	PI
LPE	PI
SM	PG
PS	PIPs



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

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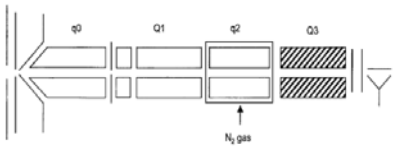
Various types of MS/MS experiments in Q-TRAP

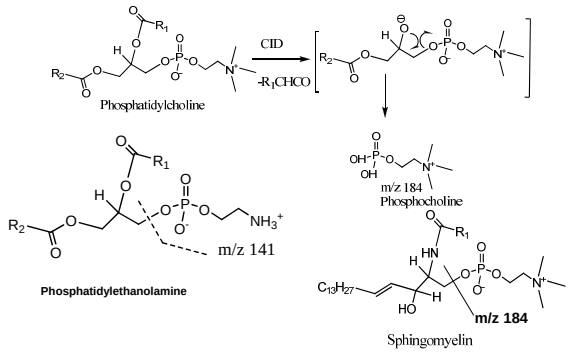


Mode of operation	Q1	q2	Q3
Q1 Scan	Resolving (Scan)	RF-only	RF-only
Q3 Scan	RF-only	RF-only	Resolving (Scan)
Product Ion Scan (PI)	Resolving (Fixed)	Fragment	Resolving (Scan)
Precursor Ion Scan (PC)	Resolving (Scan)	Fragment	Resolving (Fixed)
Neutral Loss Scan (NL)	Resolving (Scan)	Fragment	Resolving (Scan Offset)
Selected Reaction Monitoring mode (SRM)	Resolving (Fixed)	Fragment	Resolving (Fixed)

Enhanced Q3 Single MS (EMS)	RF-only	No frag	Trap/scan
Enhanced Product Ion (EPI)	Resolving (Fixed)	Fragment	Trap/scan
MS^3	Resolving (Fixed)	Fragment	Isolation/frag trap/scan
Time delayed fragmentation (TDF)	Resolving (Fixed)	Trap/No frag	Frag/trap/scan
Enhanced Resolution Q3 Single MS (ER)	RF-only	No frag	Trap/scan
Enhanced Multiply Charged (EMC)	RF-only	No frag	Trap/scan

Figure 1. Schematic of QqLIT (Q TRAP, AB/MDS, Sciex) and description of the various triple quadrupole and trap operation modes.

Fragmentation scheme of phospholipids



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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

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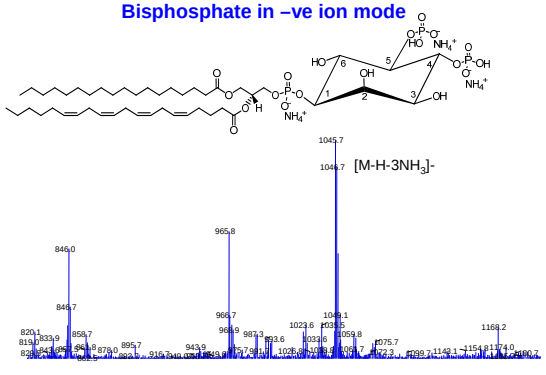
ESI-MS spectrum of L-α-Phosphatidylinositol-4,5-Bisphosphate in –ve ion mode



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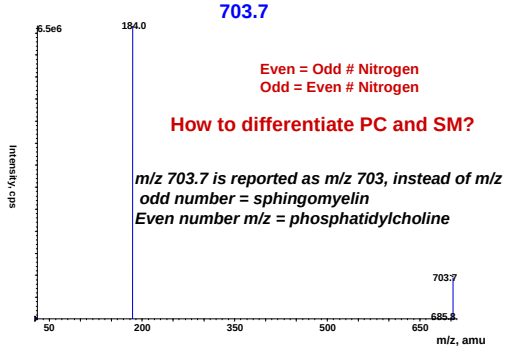
MS/MS of sphingomyelin standard (2S,3R,4E)-2-acylaminooctadec-4-ene-3-hydroxy-1-Phosphocholine m/z 703.7



Even = Odd # Nitrogen
Odd = Even # Nitrogen

How to differentiate PC and SM?

m/z 703.7 is reported as m/z 703, instead of m/z 704
odd number = sphingomyelin
Even number m/z = phosphatidylcholine

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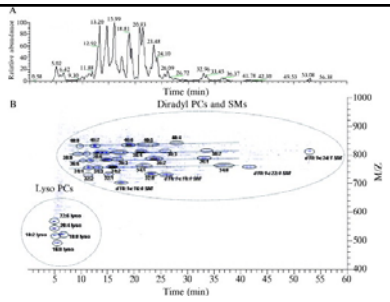

Two-dimensional map of choline-containing phospholipids detected by LC-MS/MS precursor ion m/z 184. (B) Two-dimensional map.

Source: Taguchi et al. Methods in Enzymology, 2007

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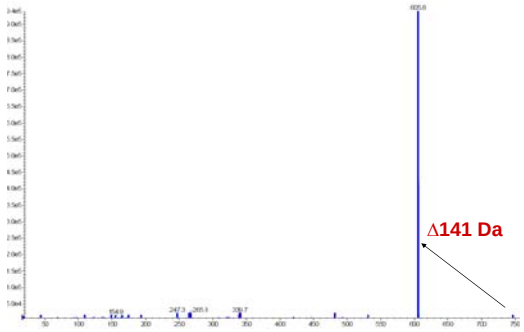
MS/MS of m/z 746.90: PE Std

Neutral loss of 141 is a characteristic for detecting PE



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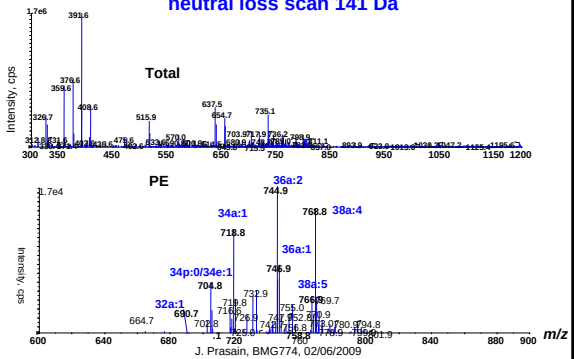
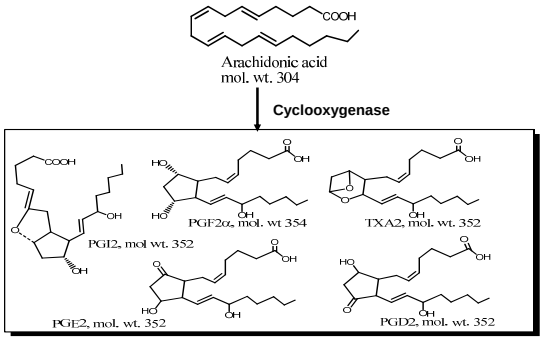
Identification of individual phosphatidylethanolamine (PE) in a lipid mixturecan be possible by neutral loss scan 141 Da

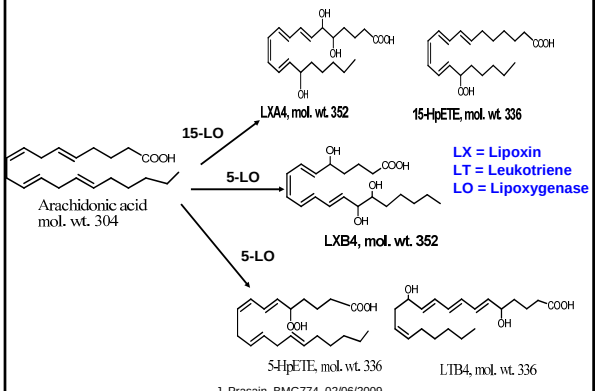


Eicosanoids, meaning 20 derived from a 20-carbon acid, arachidonic acid

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

General pathways involving oxygenase to form prostaglandins, leukotrienes and lipoxins





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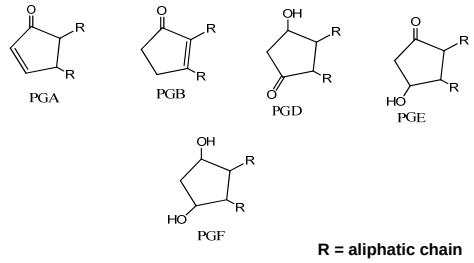
HpETE = Hydroperoxyeicosatetraenoic acid

Structural representation PG based on ring features



R = aliphatic chain

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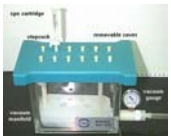
Extraction of PGs

A. Solid Phase extraction



Tissue sample (100 mg)

Homogenized in 3 mL of
15% methanol in water
Spiked with internal standard, PGE2-d4
Centrifuged, removed proteins
Supernatant acidified with 0.1 M HCl

SPE cartridges (C18)

Washed with MeOH/water
Acidified extract loaded onto the
Prewashed SPE, washed with 15%
MeOH in water, water and hexane
Finally eluted with methyl formate

Methyl formate soluble fraction

Evaporated to dryness
Stored at -20 0C

LC-MS/MS analysis

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Liquid-liquid extraction

Tissue sample (100 mg)

Homogenized in 3 mL of
acetone water (2:1)
Spiked with internal standard, PGE2-d4
Centrifuged, removed proteins

Supernatant extracted with hexane

Centrifuged and upper phase discarded
Lower phase acididfied with formic acid
to pH 3.5, extracted with CHCl3

Chloroform soluble fraction

Concentrated to dryness
Stored at -20 ºC

LC-MS/MS analysis

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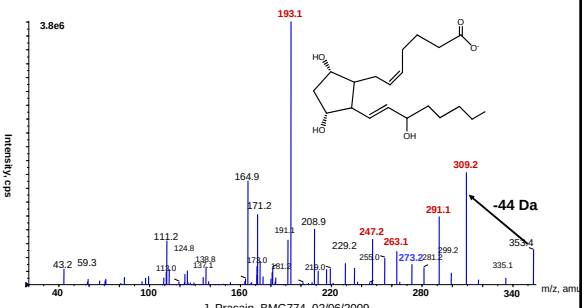
ESI-MS/MS of the [M-H]- from PGF2α m/z 353 using a quadrupole mass spectrometer



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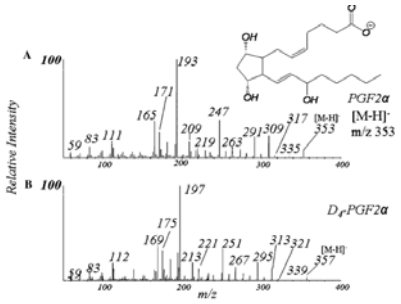
What information does deuterium labeling at C-2 and C-3 of PGF2 provide us for structure elucidation of PG?



Source: Murphy et al. Analytical Biochemistry, 2005

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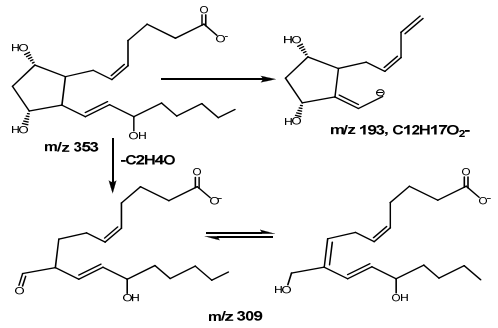
Fragmentation scheme of PGF2α [M-H]⁻ m/z 353



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

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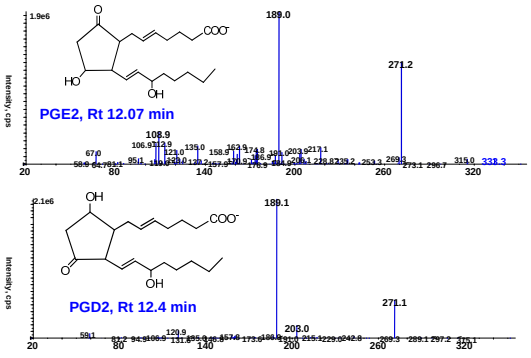
MS/MS fragmentation of PGE2 and PGD2 m/z 351.00



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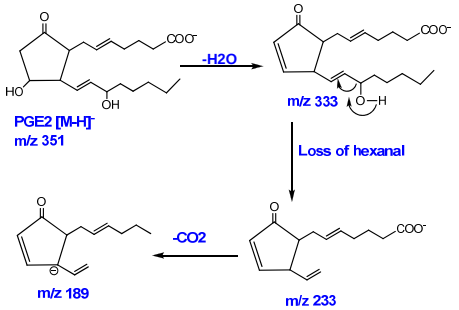
MS/MS fragmentation of PGE2 [M-H]⁻ m/z 351



The first loss of water, m/z 189 and m/z 233 are characteristics of PGE2/PGD2

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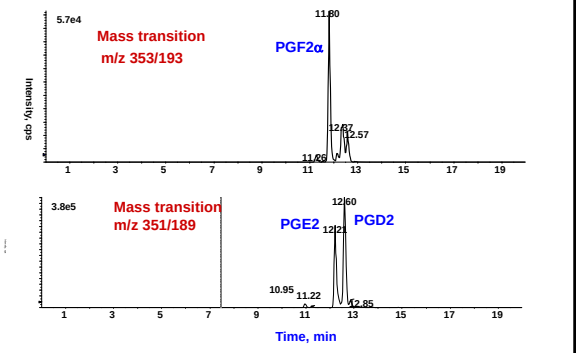
MRM chromatogram of eicosanoid standards



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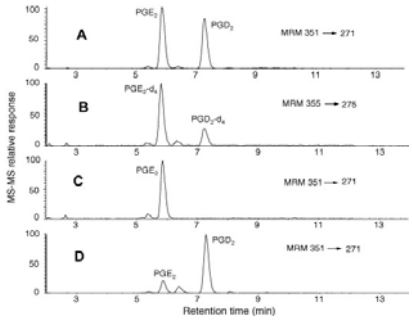
Deuterated PG standards are used for quantitative analysis of PGs in a extract



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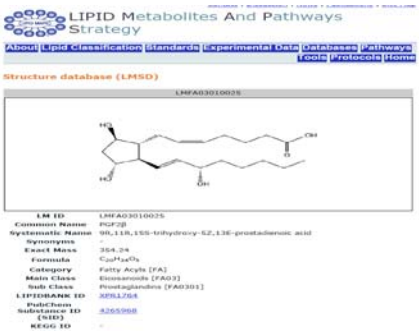
Source: Cao et al. Analytical Biochemistry, 2008

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



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