

Lipids Analysis

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Lipids

- **Lipids are mostly very hydrophobic**
- **Most are conjugates of fatty acids of a variety of chain lengths, which have different degrees of unsaturation, cis-trans isomers, and chiral centers**
- **The conjugating frame to which the fatty acids bind can be quite hydrophilic**
- **This results in a very wide (evergrowing) number of lipid species**

Analysis of fatty acids

- Fractional crystallization
- Thin layer chromatography (TLC)
 - Argentation TLC (to separate according to number of double bonds)
- Gas liquid chromatography
 - Packed columns
 - Capillary columns
- LC-MS
- SWATH-MS
- Differential ion mobility
- DESI-MS

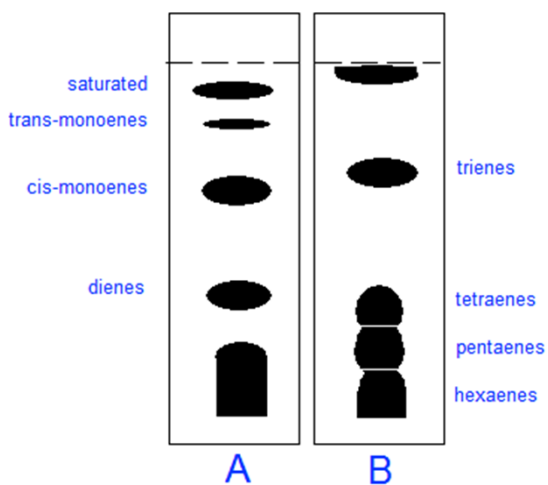
Fractional crystallization

- Still used in industry
- Crystallization is used to determine whether adulteration of butter fat by other lower quality fats has occurred
- Unsaturated fats are more soluble at lower temperatures
 - Division into “stearins” and “oleins”
 - For fatty acids, make lead salts and cool in diethyl ether or ethanol – the saturated FAs crystallize out first

Thin-layer chromatography

- Fatty acids or methylated fatty acids separated on alumina or silica gel TLC
- When AgNO_3 is incorporated into the silica slurry before making the TLC plate, the observed separation is dependent on the degree of unsaturation (π -bonding)
 - Saturated
 - Mono-unsaturated
 - Di-unsaturated, etc.

Ag^+ ion TLC of fatty acids

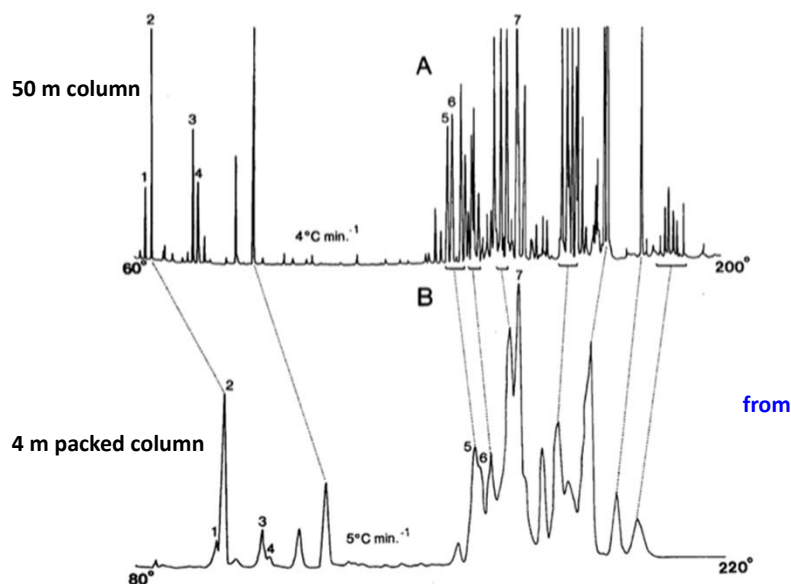


It is a class separation

Gas-liquid chromatography

- 1952 Martin and James start GC by separating volatile fatty acids (C_1 - C_6)
 - Quickly extended it to long chain FAs by methylating them
 - Used 5-6 feet x $\frac{1}{4}$ inch glass or stainless steel packed columns
- 1955 Patent for capillary, open tubular columns awarded
 - Did not enter commercial use until the mid-1970s

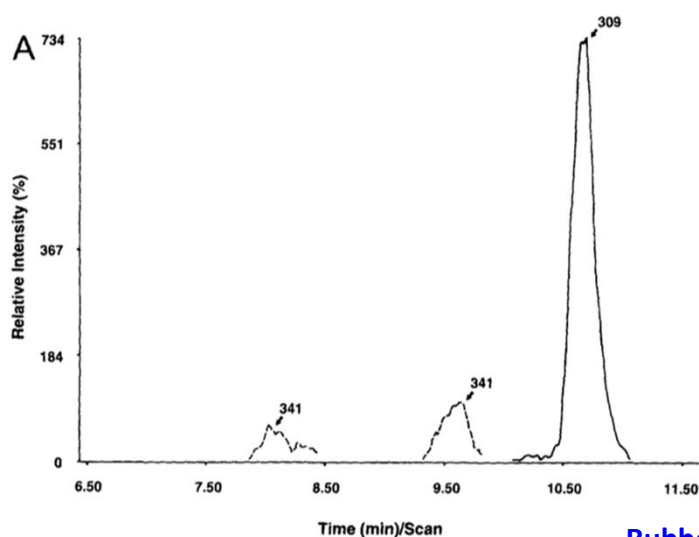
Packed vs Capillary GC of FAME



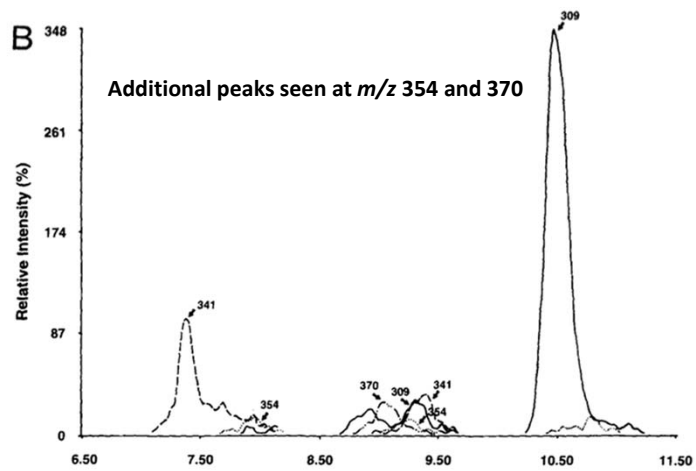
(HP)LC

- Reverse-phase LC
 - Can be used for lipid class separation based on hydrophobicity
 - Again, Ag^+ can be introduced into the medium to enhance the separation of unsaturated fatty acids
 - Very difficult to detect lipids spectroscopically
 - LC-MS is the preferred method

LC-MS of oxidized linolenate

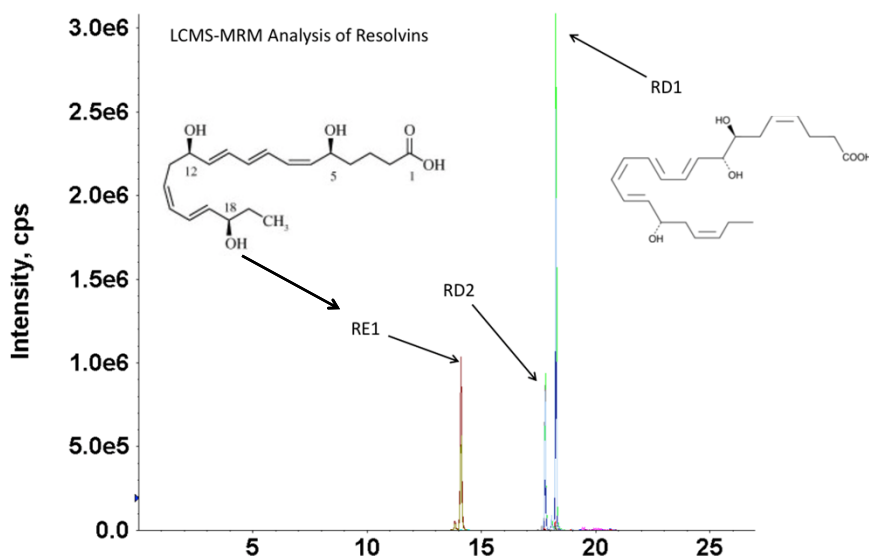


LC-MS of linolenate treated with NO

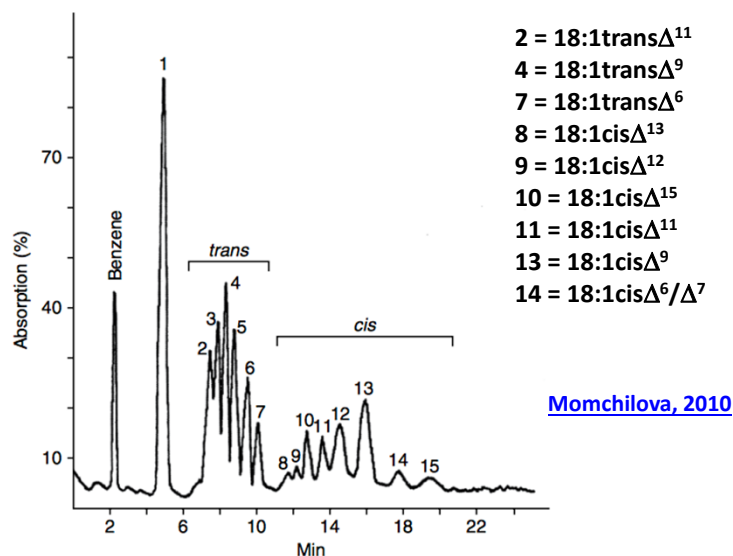


[Rubbo 1994](#)

Gradient LC-MS of resolvins

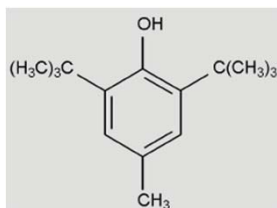


Silver ion-HPLC of unsaturated fatty acids



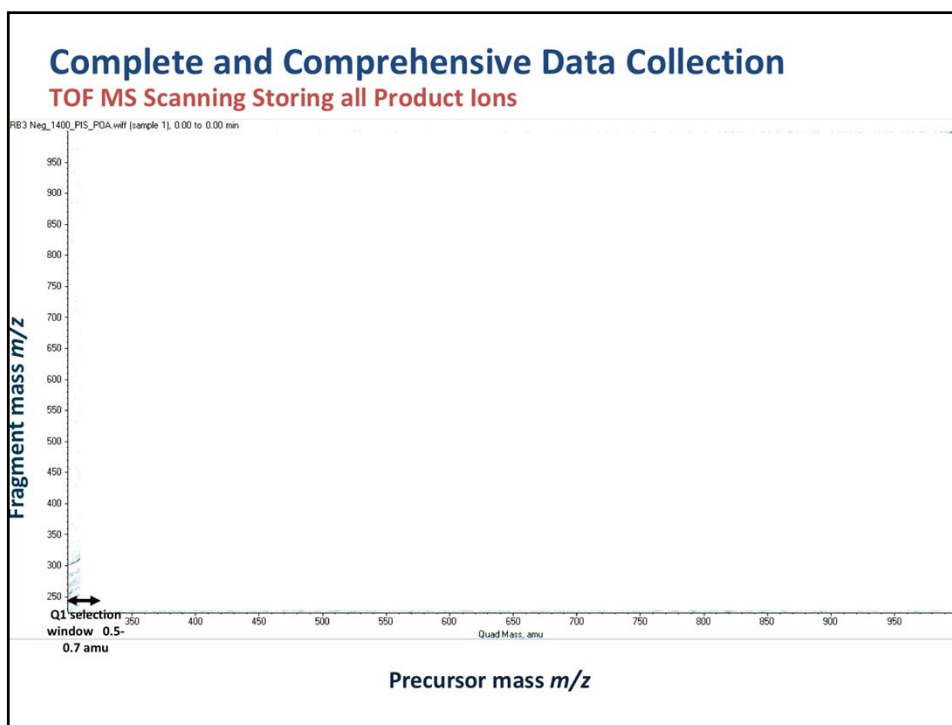
Modern lipidomics

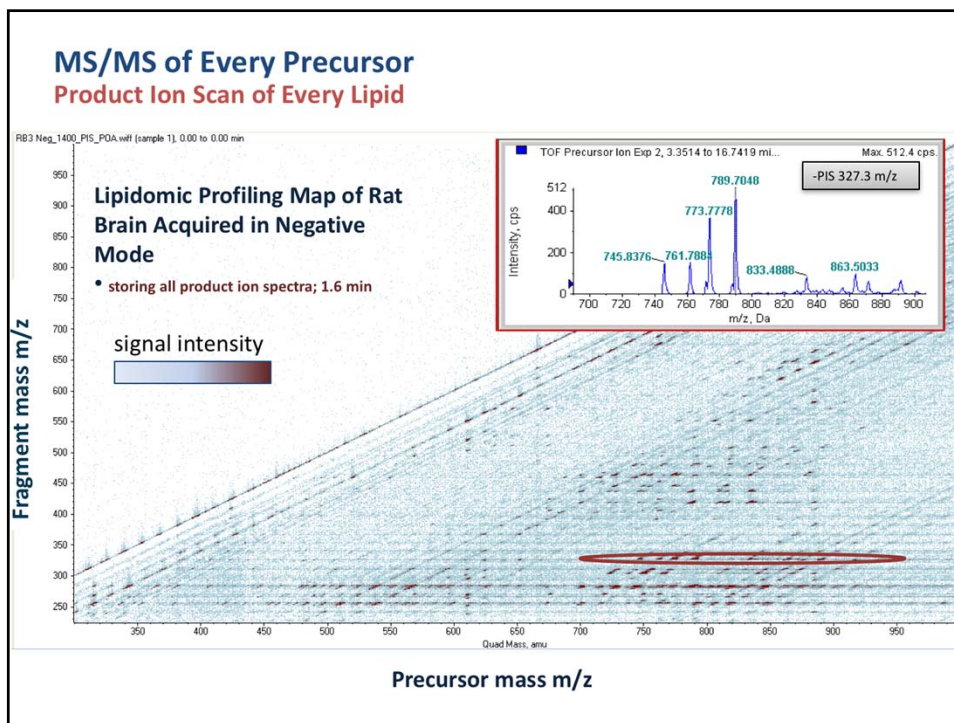
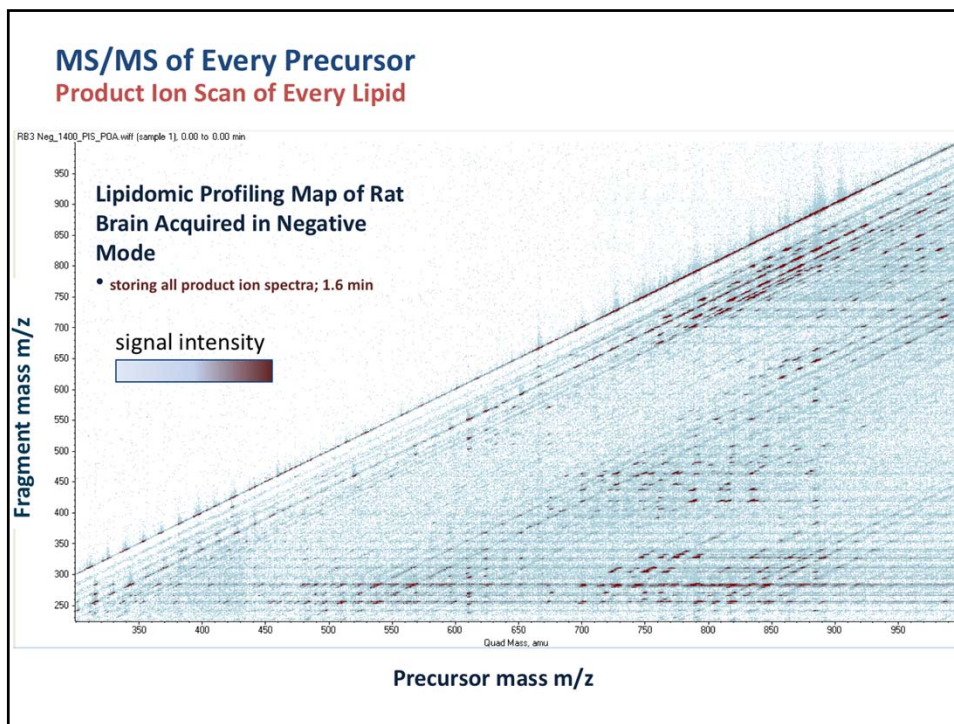
- Use of the SWATH-MS approach
- Preceded by total lipid extraction using a two-phase partition by adding $\text{CHCl}_3:\text{MeOH}$
 - Bligh-Dyer and Folch extractions
 - Crucial to do so in an atmosphere of argon and in the presence of butylated hydroxytoluene to prevent oxidation

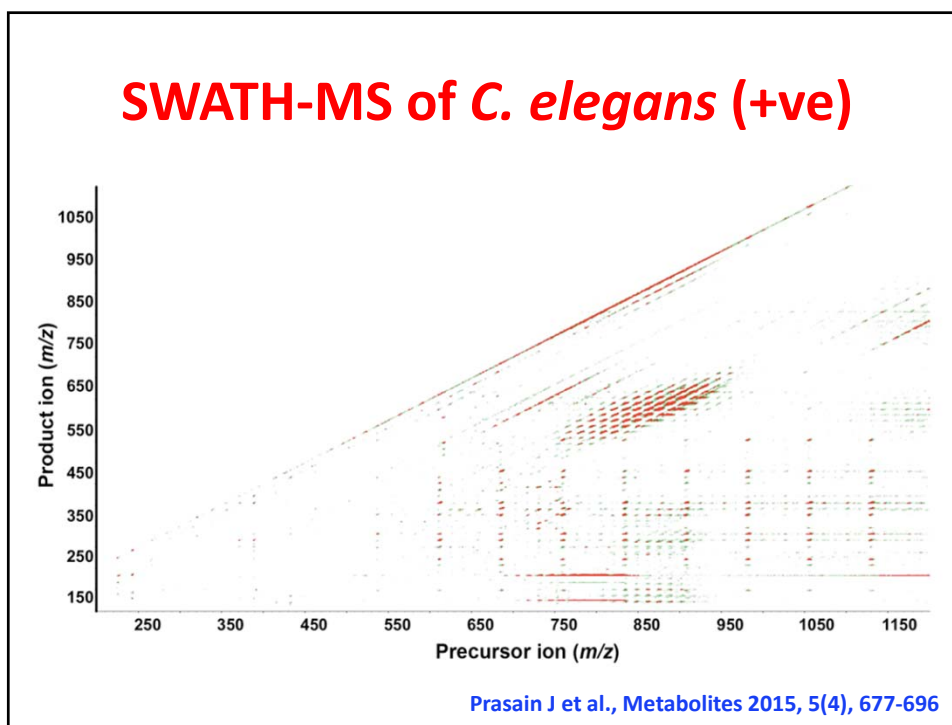
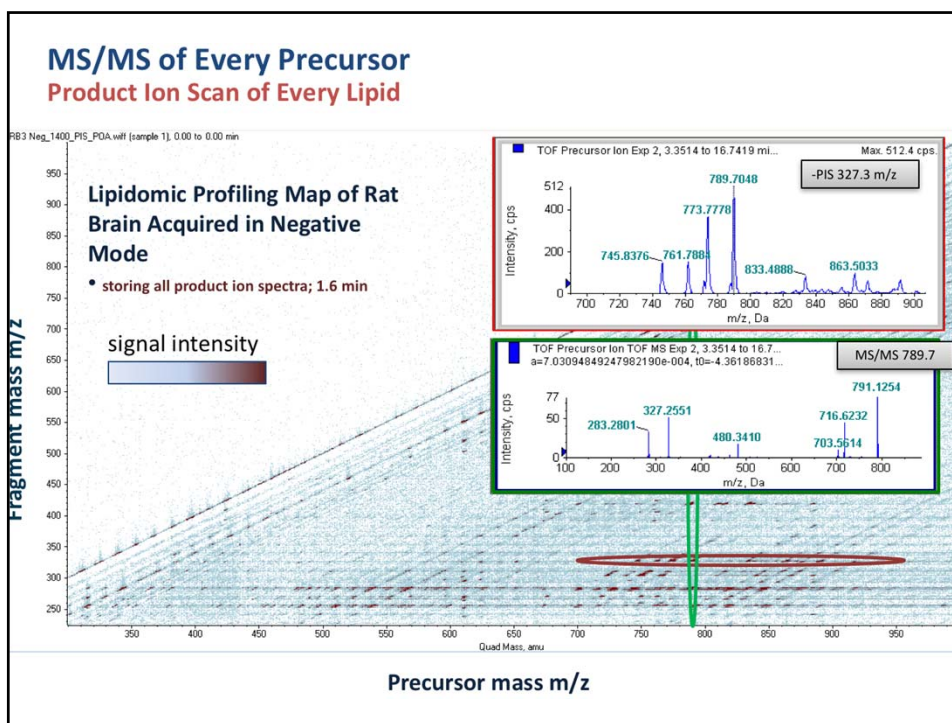


SWATH-MS

- Based on an infusion strategy on a 5600 TripleTOF
 - Ions are filtered 1.2 m/z at a time in the quadrupole over a m/z range of 200-1200
 - The filtered ions are collisionally dissociated and fragment ions analyzed by the TOF analyzer
 - MSMS spectra collected for 500 msec for each m/z , i.e., infusion for 500 sec (8.33 min)







Other MS methods for lipids

- SWATH-MS is comprehensive – no stone unturned
- However, many lipids overlap in mass and there are also isomers with the same mass
- To observe more individual lipids, it is necessary to resolve lipids before analyzing them in the mass spectrometry
- Even then, isomers can be a problem
- A form of ion mobility may be the answer to this

SelexIon™

•Differential Mobility Spectrometry (DMS) is the term used for planar geometry



Separation waveform (SV):

Radially displaces ions towards one or the other electrode, depending upon high and low field mobility characteristics

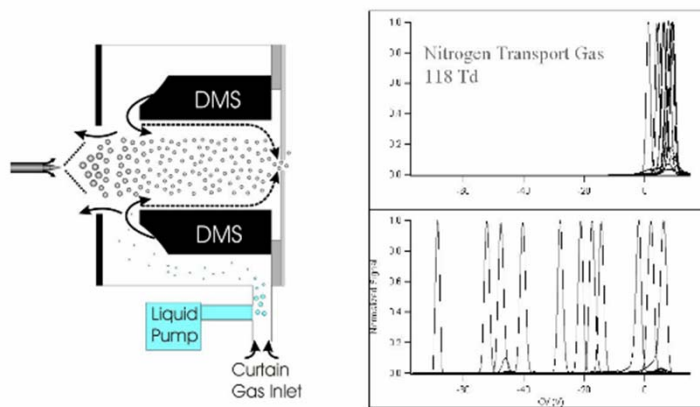
Compensation voltage (COV):

Restores the trajectory for a given ion to allow them to transmit through the DMS device and enter the mass spectrometer

AB Sciex

Liquid modifiers and SelexION™

- Liquid modifiers can be added to the curtain gas flow
- Improves separations
- More options for separation in difficult cases

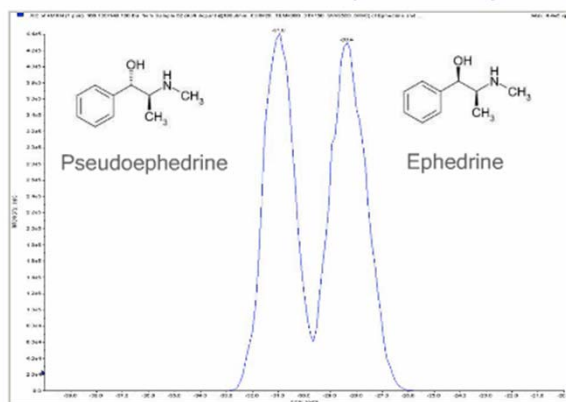


AB Sciex

11 compounds: methylthiazine, amoxicillin, ephedrine, norfentanyl, acyclovir, clobutrol, tramadol, epinephrine, pantoquin, fenofibrate, bincopria

Separating isobaric isomers

Separation of Isomers - Pseudoephedrine/Ephedrine



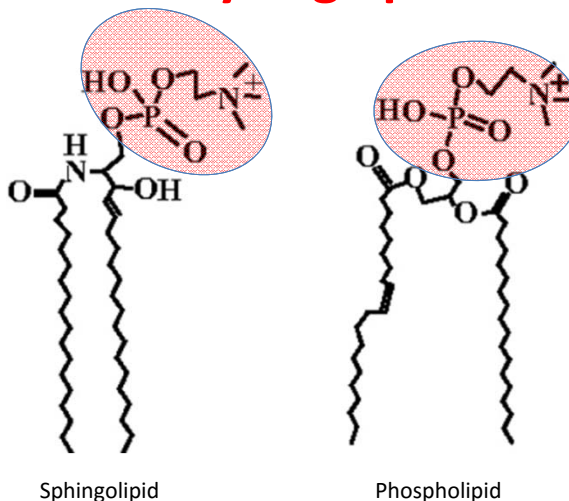
•Separation of pseudoephedrine and ephedrine with SelexION™

•Indistinguishable by MS or MS/MS

AB Sciex

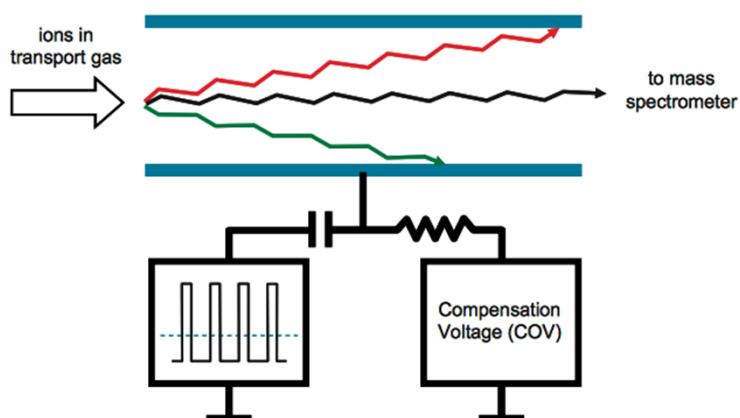
The problem of analyzing lipids

- Despite the sheer number of lipids, the *units* comprising them are closely related and therefore they have similar masses
- Sphingolipids may only be different in mass by 1 Da from their PC analog
 - ^{13}C -Isotope profiles overlap
 - Head groups are the same



Differential mobility MS is an answer

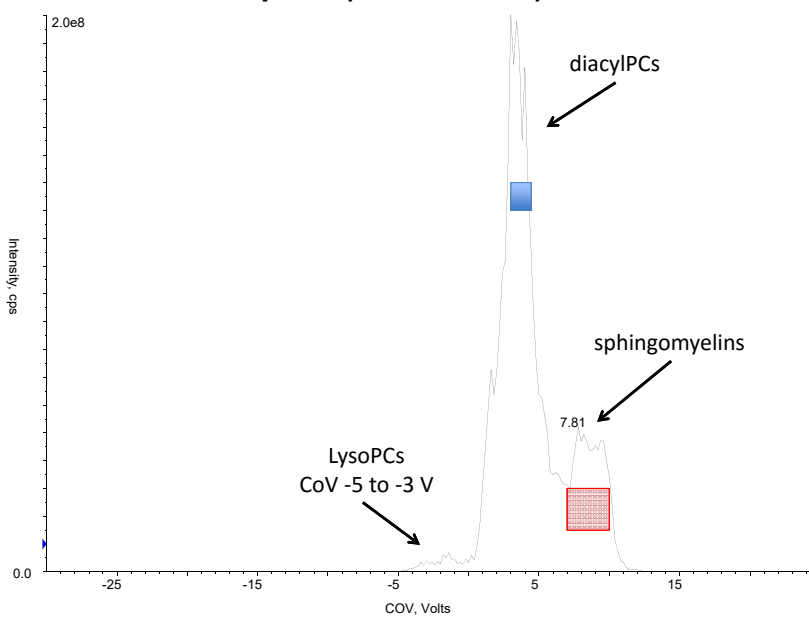
Innovative Planar Design; SelexION™ Ion Mobility Cell.

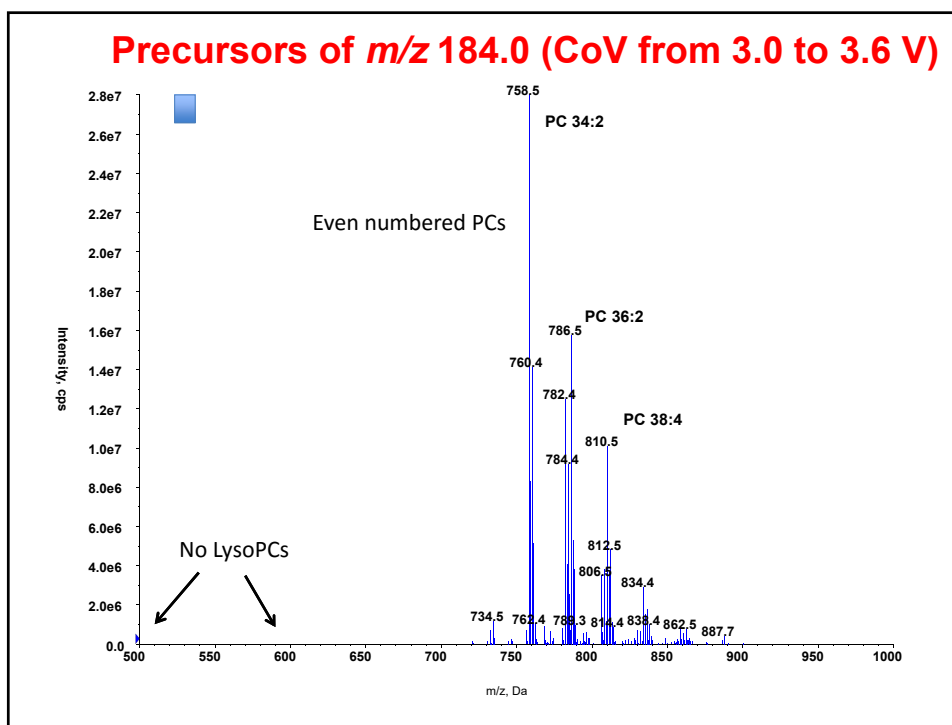
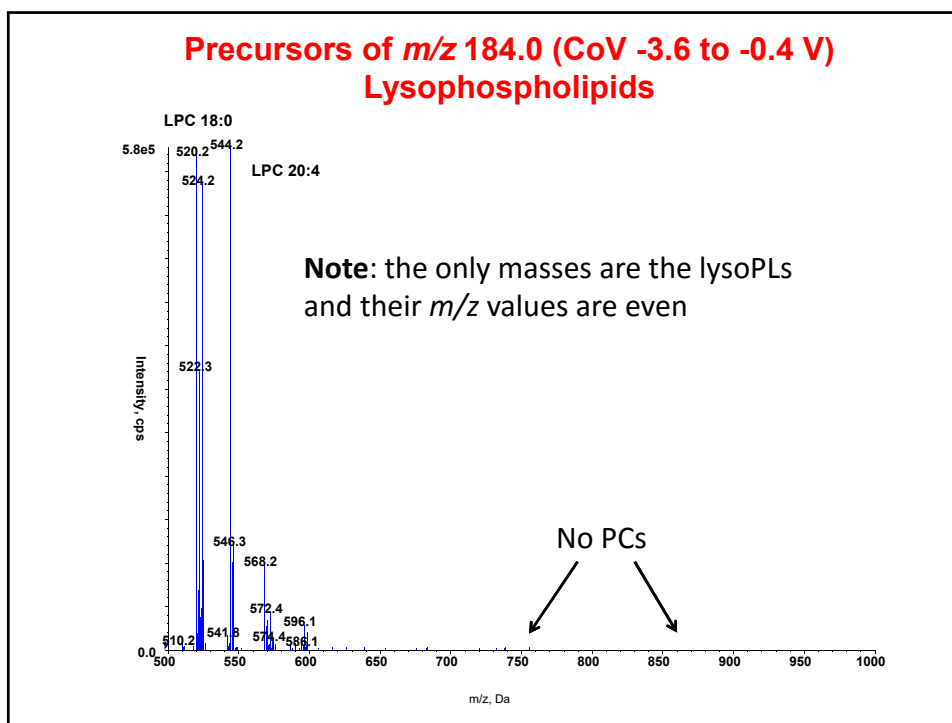


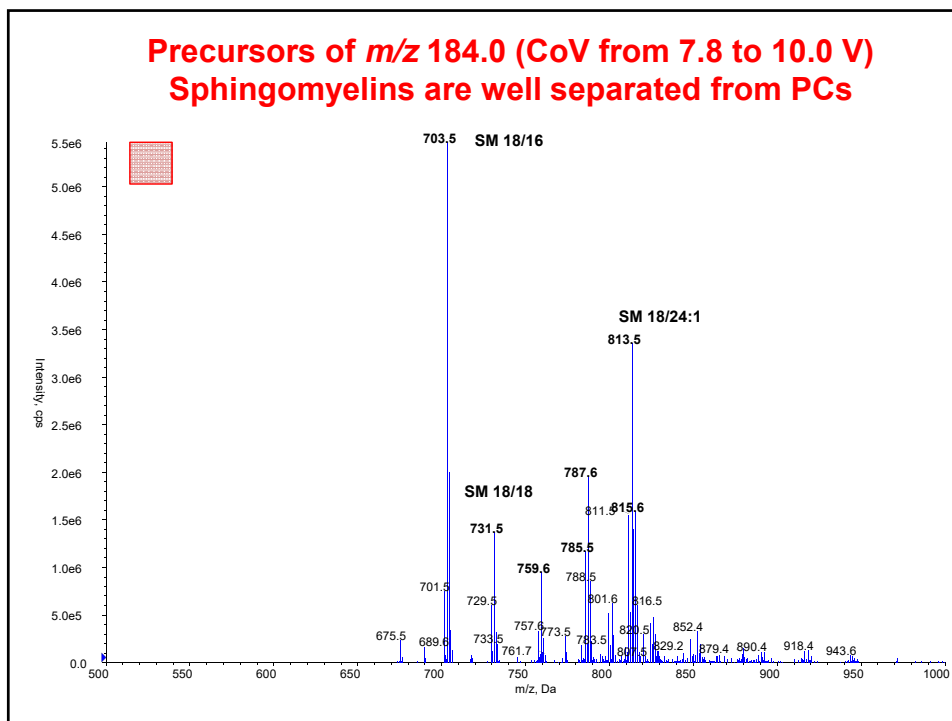
Differential mobility mass spectrometry

- A fragment ion may have multiple precursor ions
- The precursor ions may be separable by DMS before they enter the mass spectrometer
- By scanning with the compensating voltage, the precursor ions enter the mass spectrometer at different CoVs
- (Note: Further separation is possible using resolving agents, e.g., isopropanol)

Total ion current of precursors of m/z 184.0 Sample 1 (HS 07-30-15)

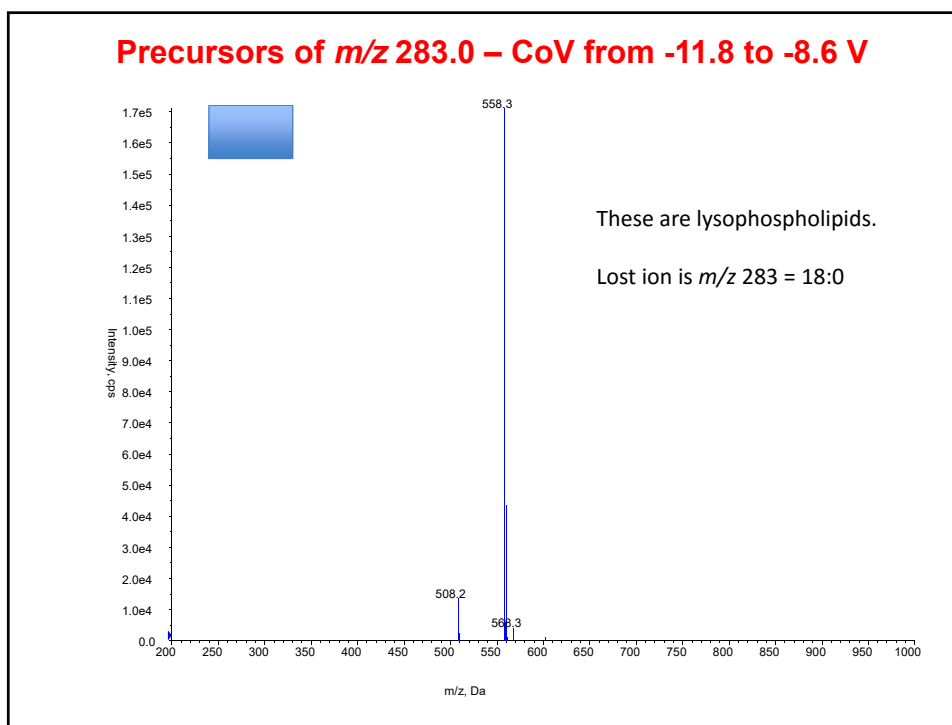
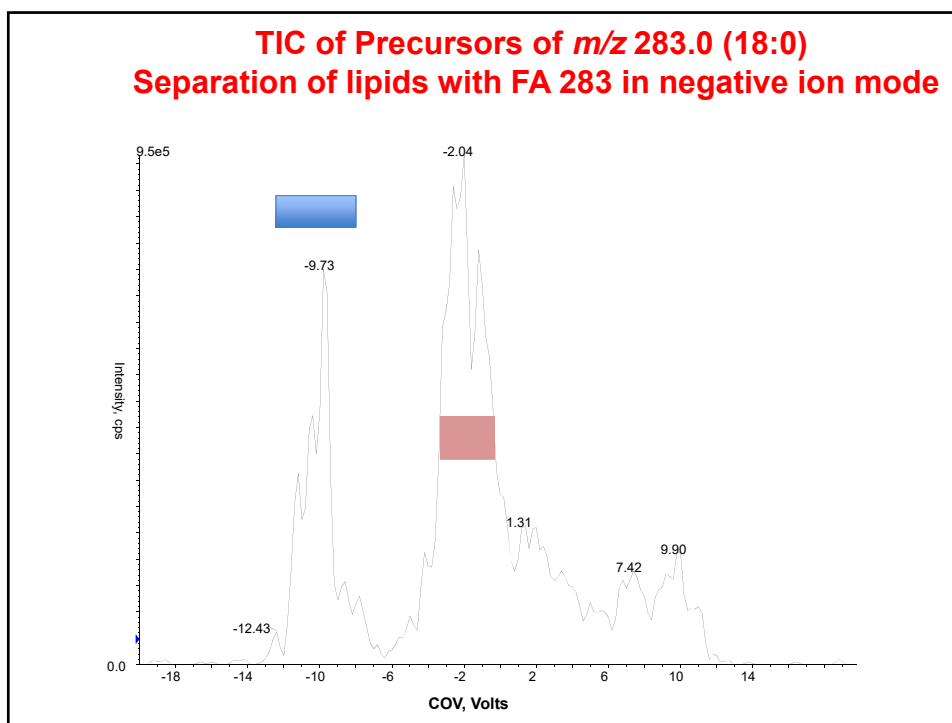


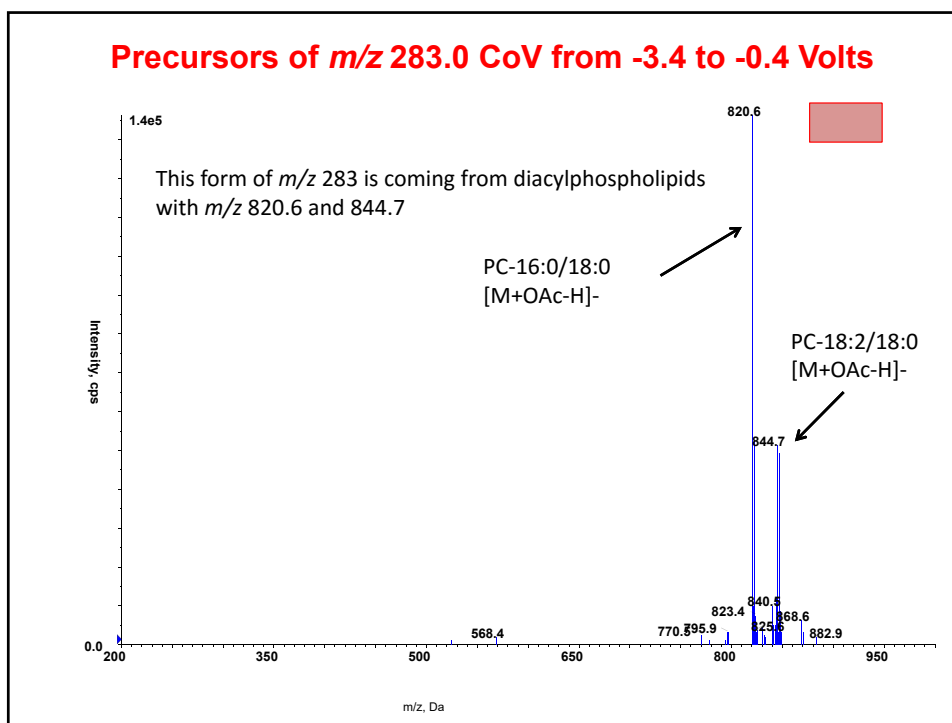




Origins of negatively charged product ions

Precursors of m/z 283 (stearate, 18:0) studied at
different compensation voltages

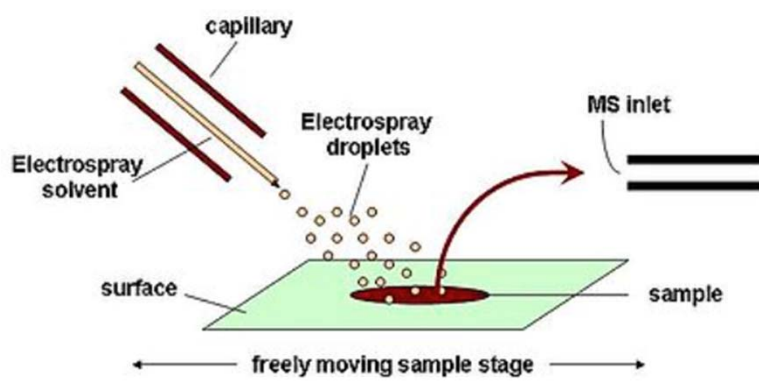




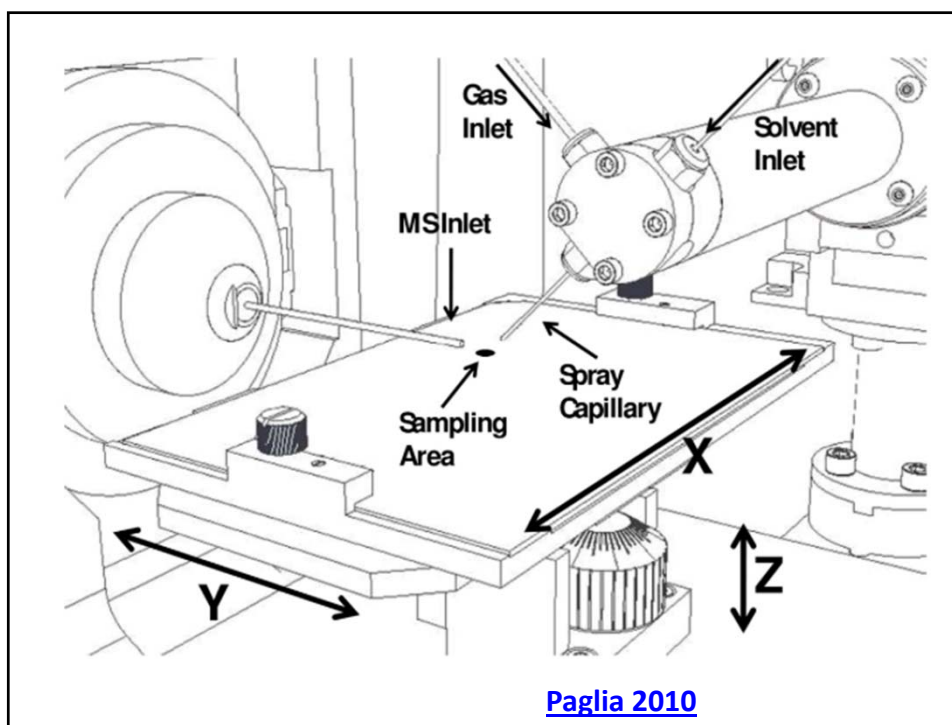
Summary

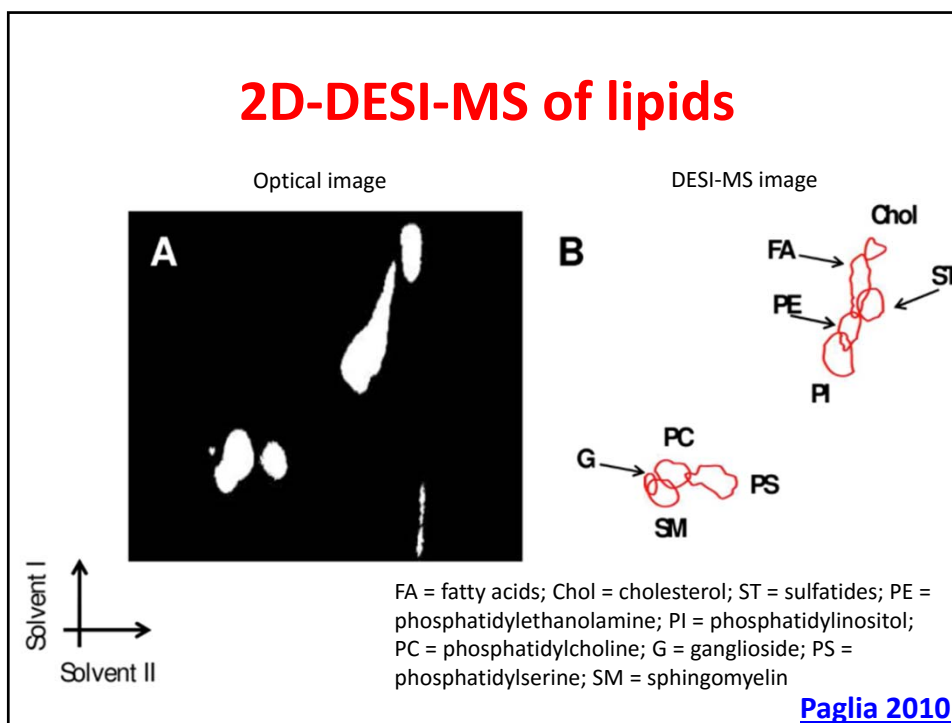
- **Differential mobility mass spectrometry (DMS) is an important new tool in the study of lipids**
 - It overcomes many of the problems that beset the analysis of lipids with overlapping masses
- **Further separation (not exploited yet) comes from differential resolution with specific solvents**
- **Metabolon has introduced a kit for the analysis of lipids where the extraction solvent contains ~1,000 deuterated lipid internal standards enabling absolute quantification of a wide range of lipids using DMS**

DESI-MS TLC of lipids



Desorption electrospray ionization mass spectrometry of the lipids on the TLC plate without extraction





References

- Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, Freeman BA. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J Biol Chem.* 1994 Oct 21;269(42) 26066-75.
- Paglia G, Ifa DR, Wu C, Corso G, Cooks RG. Desorption electrospray ionization mass spectrometry analysis of lipids after two-dimensional high-performance thin-layer chromatography partial separation. *Anal Chem.* 2010 Mar 1;82(5):1744-50.
- Momchilova SM, Nikolova-Damyanova BM. Separation of isomeric octadecenoic fatty acids in partially hydrogenated vegetable oils as p-methoxyphenacyl esters using a single-column silver ion high-performance liquid chromatography (Ag-HPLC). *Nat Protoc.* 2010 Mar;5(3):473-8.
- [AB Sciex Webinar](#) on SelexION technology