Lipidomics: analytical aspects

Metabolismo y Enfermedades Metabólicas
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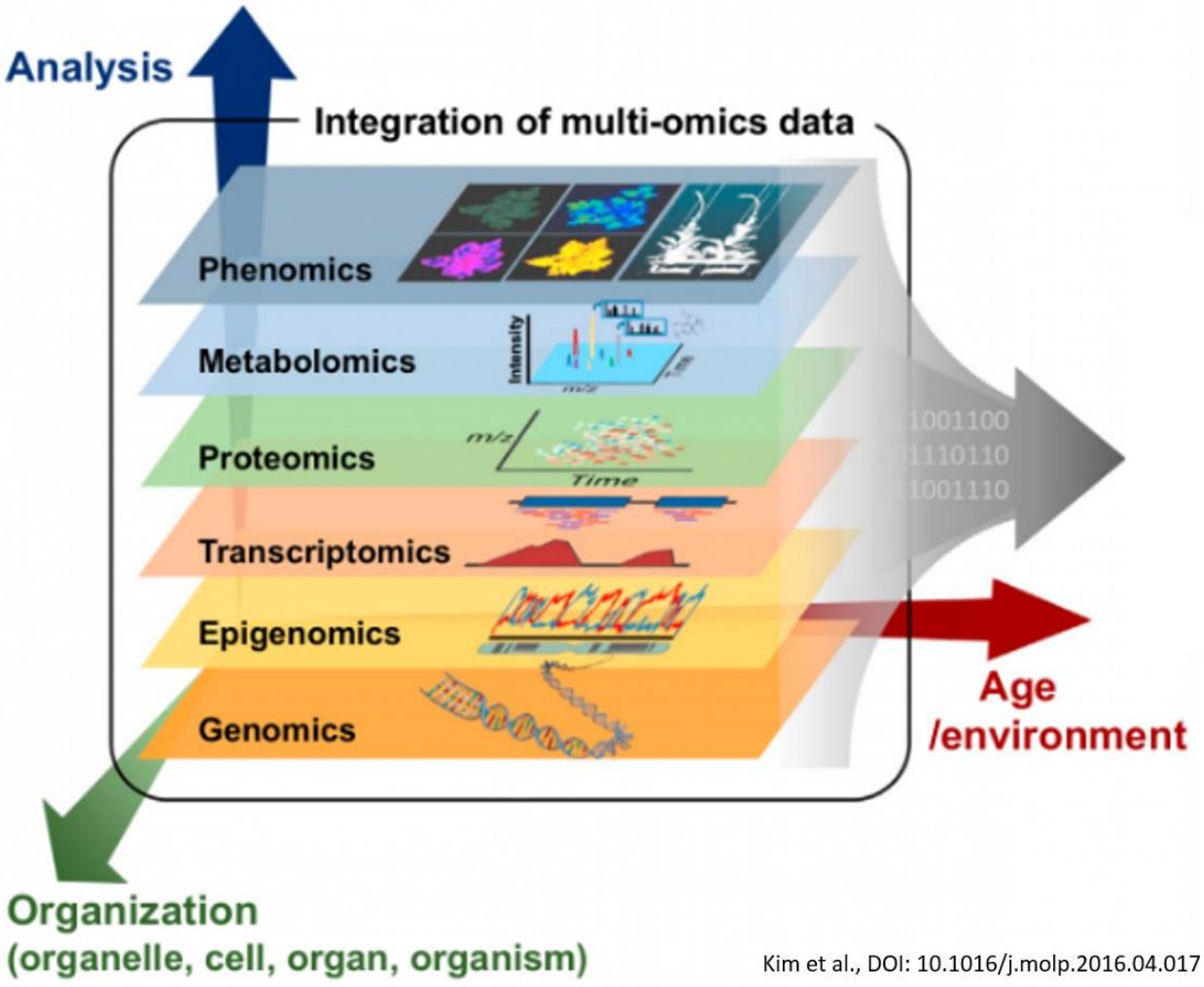
Systems biology



Kim et al., DOI: 10.1016/j.molp.2016.04.017

Omics

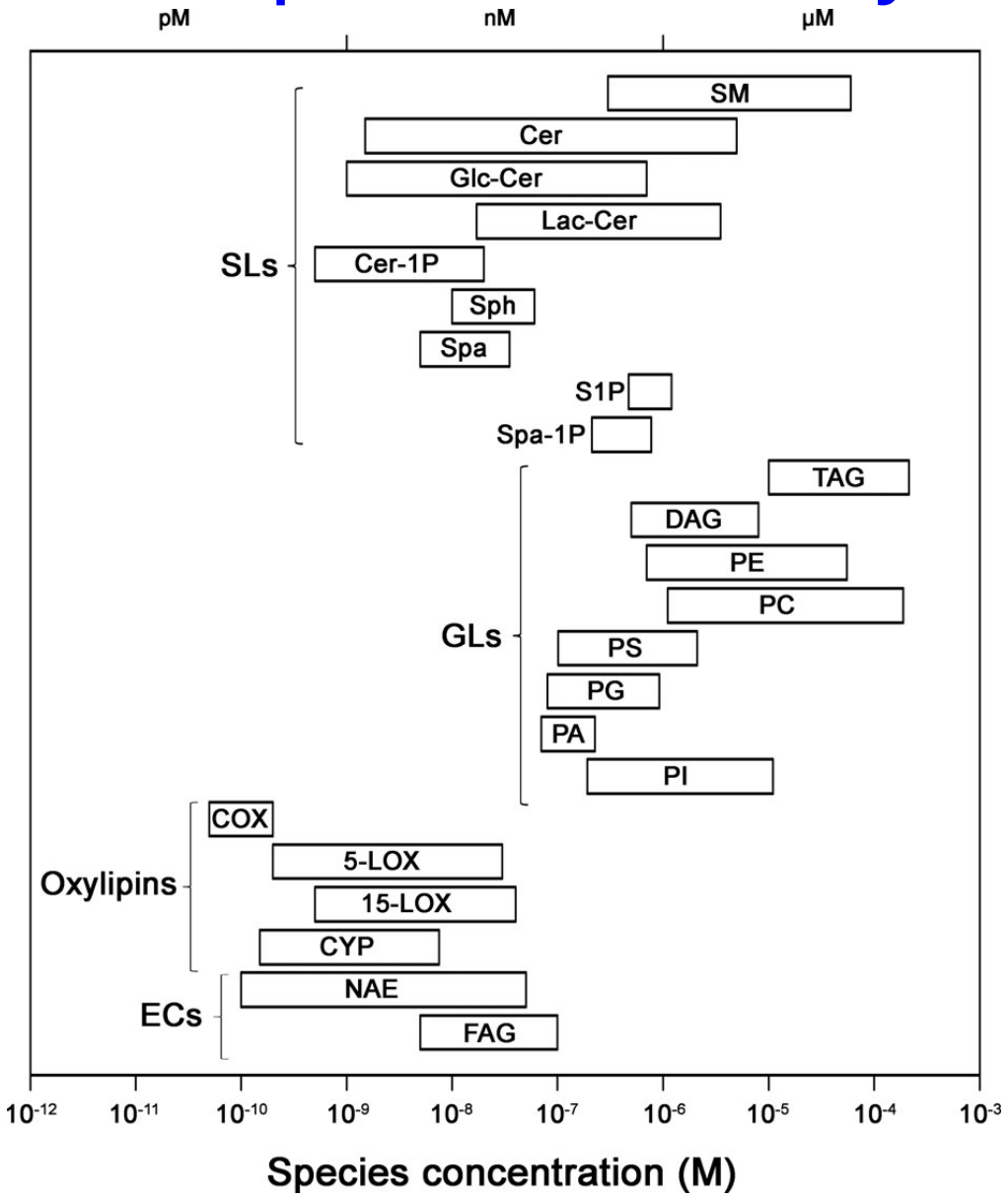
"Omics" refers to **the large-scale study** and analysis of biological molecules or systems within a specific field, such as genomics, proteomics, metabolomics, or transcriptomics. **It involves comprehensive, high-throughput approaches** to characterize and understand the entirety of a particular biological component, such as genes, proteins, metabolites, or RNA molecules, within an organism or system.

Bioinformatics plays a crucial role in omics studies by providing **computational tools and methods to manage, analyze, and interpret the vast amounts of data**, enabling researchers to derive meaningful insights from complex biological datasets

Lipidomics

Integrated in metabolomics, lipidomics studies all lipid types in a system, exploring their roles, amounts, and interactions. It uses advanced tools like mass spectrometry and bioinformatics for comprehensive lipid analysis and interpretation.

Lipidomics: analytical challenges



-Structural variability

-Big range of concentrations

Analytical technique: RMN vs MS

Aspect	Nuclear Magnetic Resonance (NMR)	Liquid Chromatography coupled to Mass Spectrometry (LC-MS)
Principle	Measures magnetic properties of atoms in molecules.	Analyzes ionized molecules based on mass-to-charge ratio.
Sensitivity	Moderate sensitivity, requires larger sample amounts.	**High sensitivity**, can detect low amounts of lipids.
Resolution	Limited resolution for complex lipid mixtures.	**High resolution**, can identify and quantify diverse lipids.
Throughput	Lower throughput, time-consuming for analysis.	**Higher throughput**, faster analysis of multiple samples.
Quantification	**Absolute quantification**	Semiquantitative or Relative quantification
Sample Prep	**Minimal sample preparation, non-destructive method**.	More extensive sample prep, potential for ionization bias.
Structural Info	Provides limited structural information.	**Offers detailed structural insight, including isomers.**
Reproducibility	**Strong among laboratories**	Low even intralaboratory

MS lipidomics: Analytical process

Aim: to obtain separated lipids ionized in gas phase to be detected

The most general elements:

-Sample preparation

-Chromatographic separation

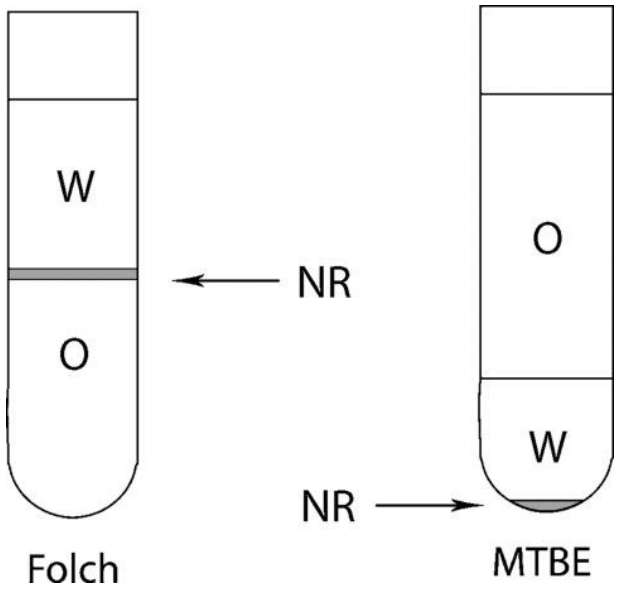
-Ionization

-m/z analyzer

-detector

MS lipidomics: Sample prep

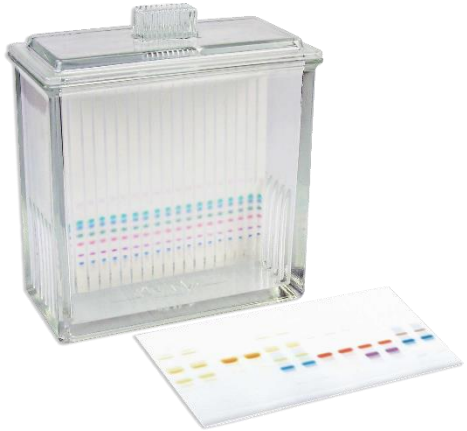
+ **Solid phase extraction (SPE): oxylipins**
+ **Protein precipitation with organic solvents**
+ **Folch and Bligh & Dyer methods: mixtures of chloroform, methanol and water**
+ **Matyash: mixtures of methyl-tert-butyl ether (MTBE)**

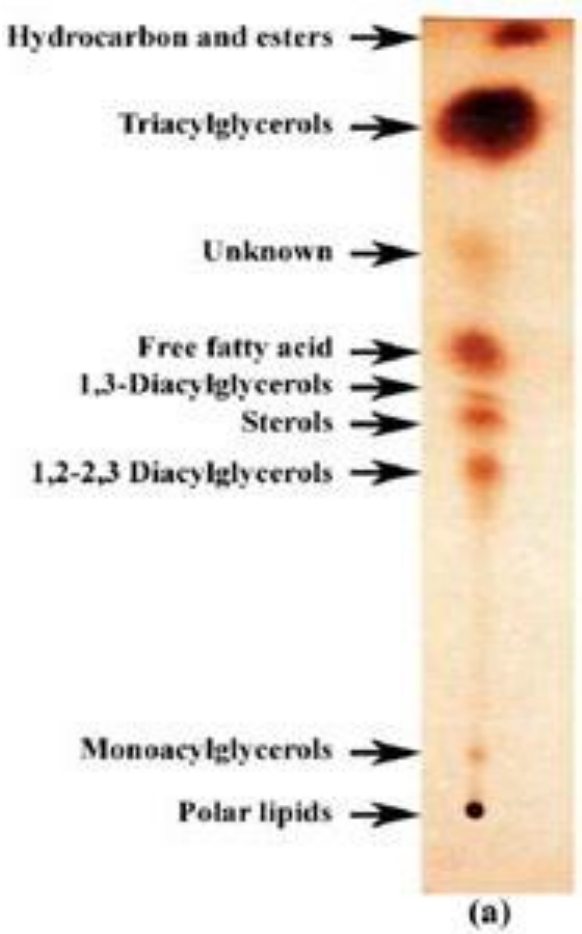


MS lipidomics: TLC

Thin layer chromatography (TLC)

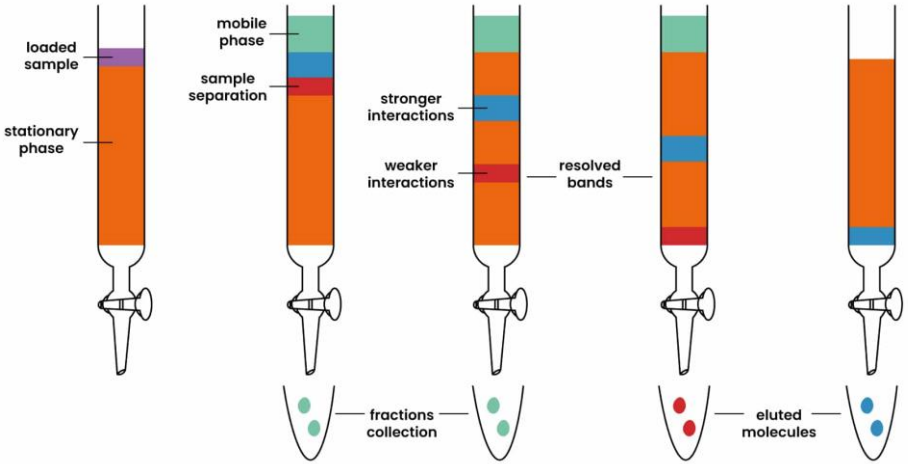
- **-Normal phase (the more polar the more retained)**
- **-Non-high-throughput**
- **-Reserved for specific analyses**





MS lipidomics: LC

Liquid chromatography (LC)



Columns in chromatograph:

-High pressure (HPLC)

-Ultra high pressure (UHPLC)

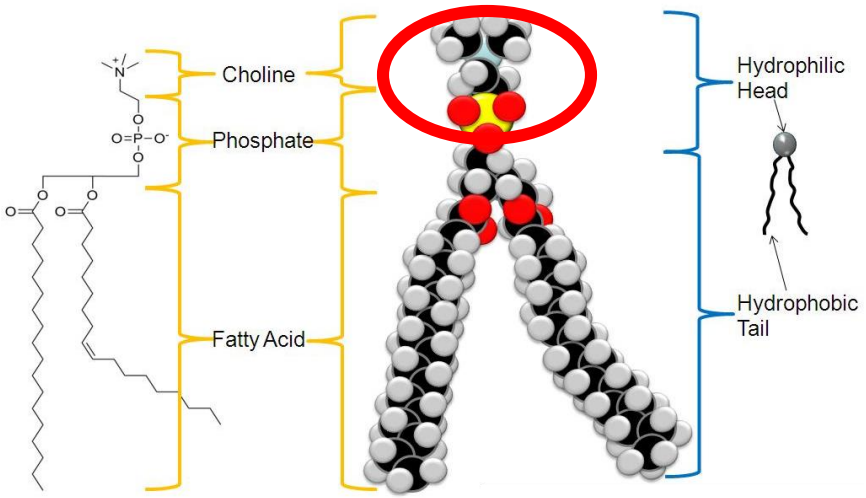
MS lipidomics: NPLC

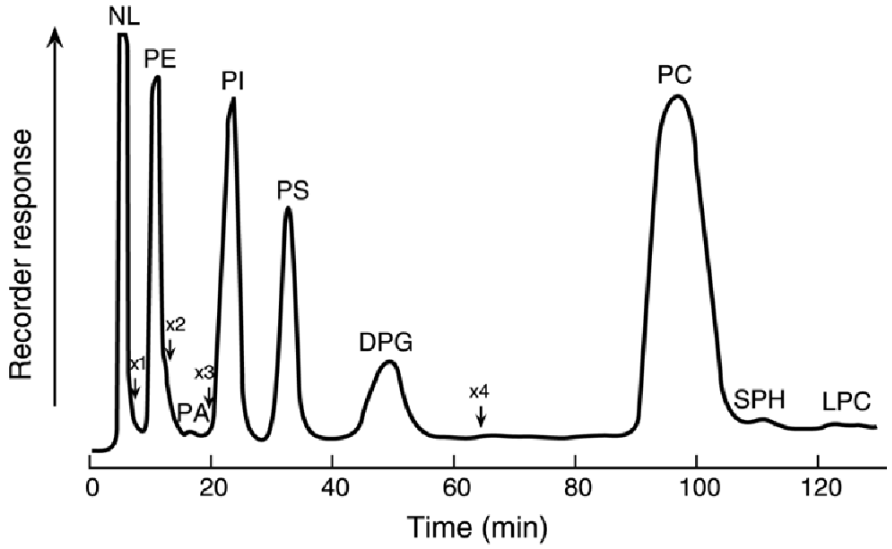
Normal phase Liquid chromatography (NPLC)

-Stationary phase more polar than the mobile phase.

-The more polar the more retained (higher retention time)

-Phospholipids mainly separated by the polar headgroup





MS lipidomics: RPLC

Reversed phase Liquid chromatography (RPLC, *fase inversa* in Spanish)

-Stationary phase more apolar than the mobile phase.

-The more polar the less retained (lower retention time)

NPLC vs RPLC:
https://www.youtube.com/watch?v=MLoitPJQH3g

MS lipidomics: RPLC

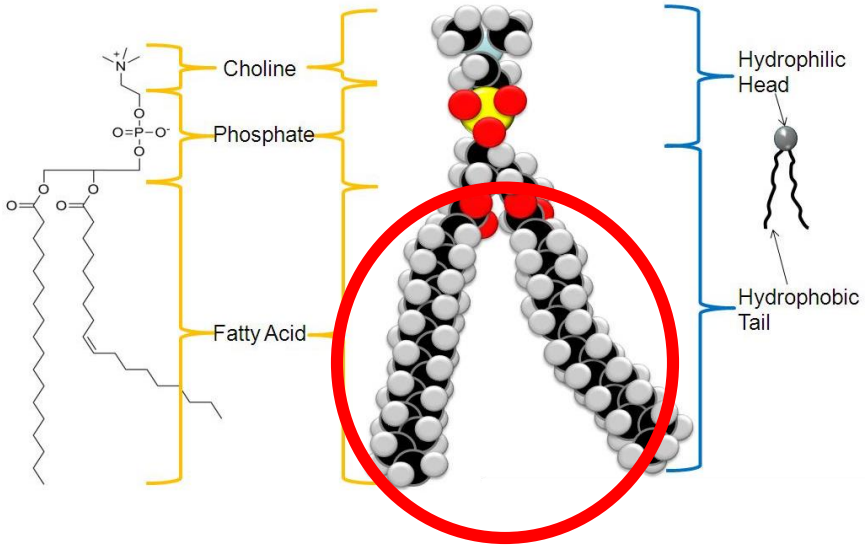
Separation of lipids by a combination of:

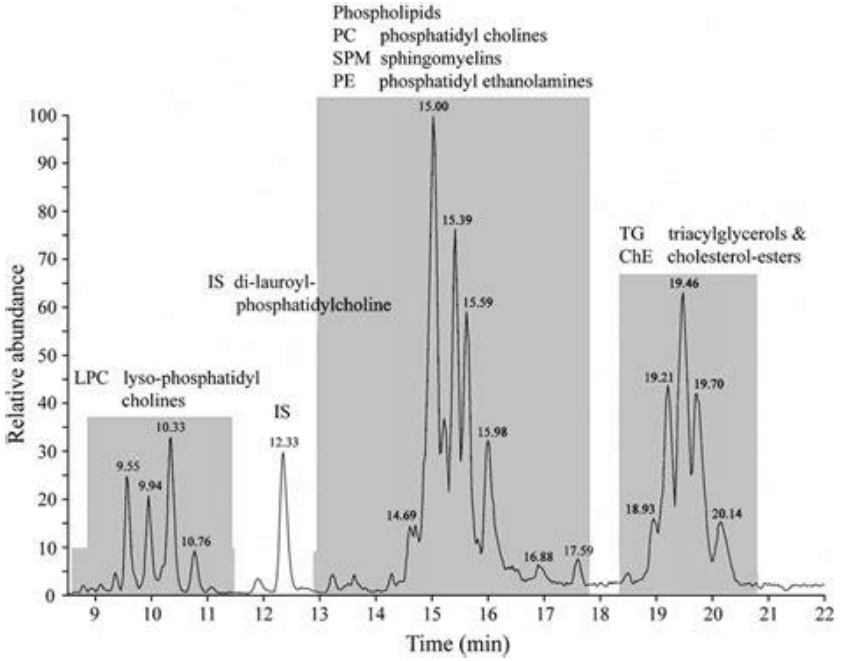
-Number of acyl chains (fatty acids)

-Polar headgroup

-Length of the fatty acids

-Number of the double bonds (unsaturations)





MS lipidomics: Quality Control

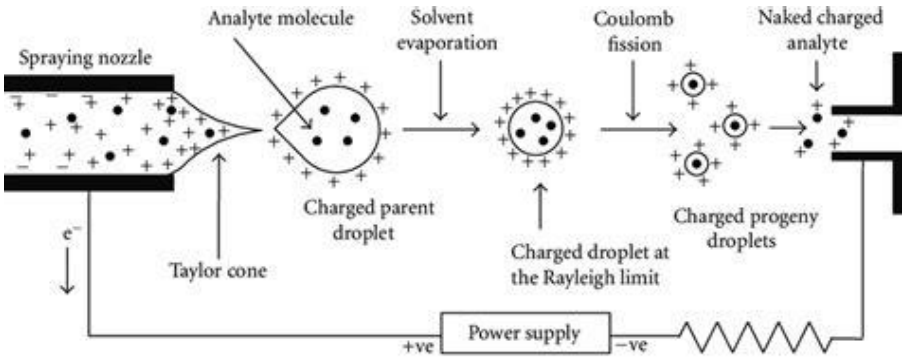
Quality control in lipidomics/metabolomics ensures data accuracy by using **reference samples** to detect and correct instrument variability.

Usually, it is a mixture of the samples that are going to be injected

Of special importance when the number of samples is high and **multiple batches** of injection are required

MS lipidomics: ionization

The most common ionization is electrospray (ESI)



Two polarities that determine how the lipid ionize:

positive | negative

We obtain ions that are transported to low pressure!

MS lipidomics: analyzers

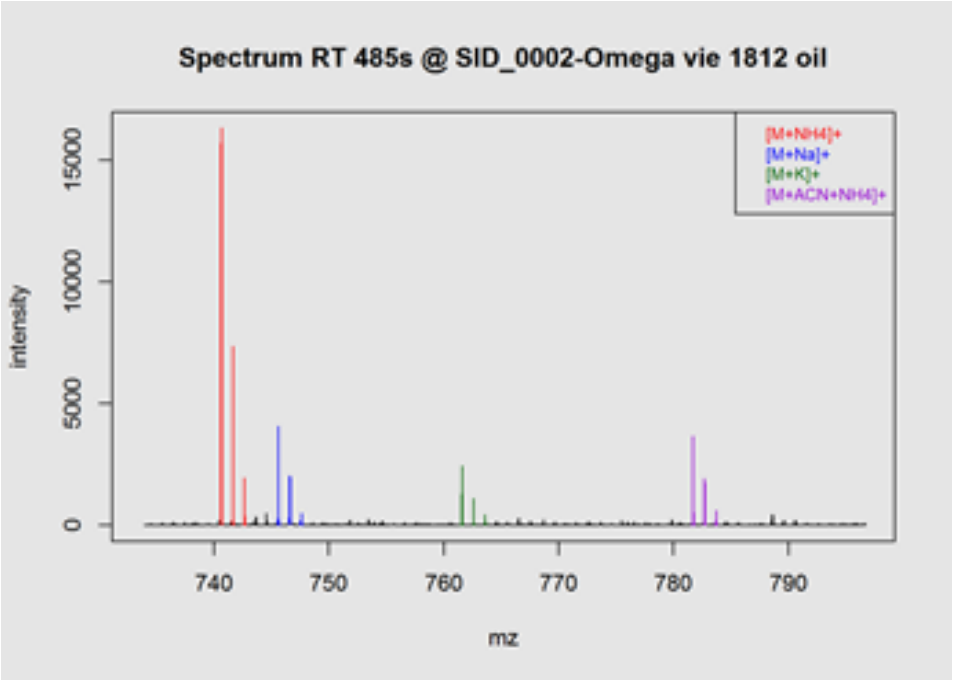
Different types

+Single quadrupole (SQ)

+Triple quadrupole (TQ)

+Time of flight (ToF)

+Orbitrap (Orbi)



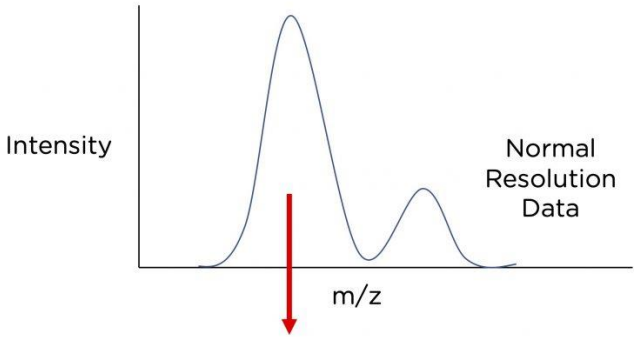
MS lipidomics: SQ analyzer

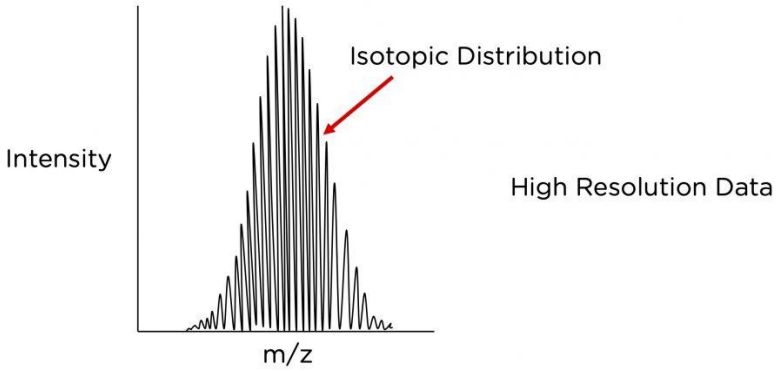
Properties

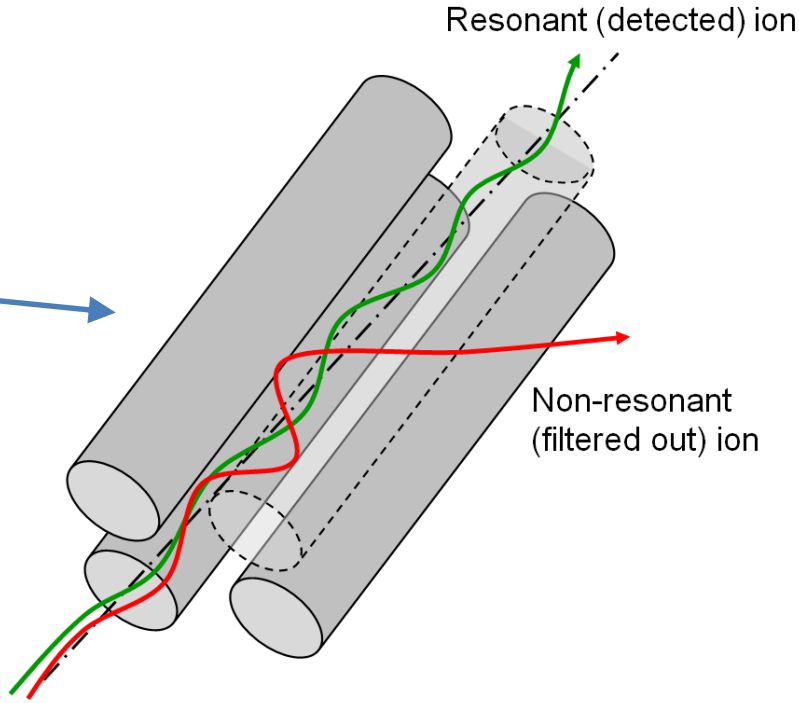
+Non efficient for scanning

+ion filter

+low resolution







Low selectivity

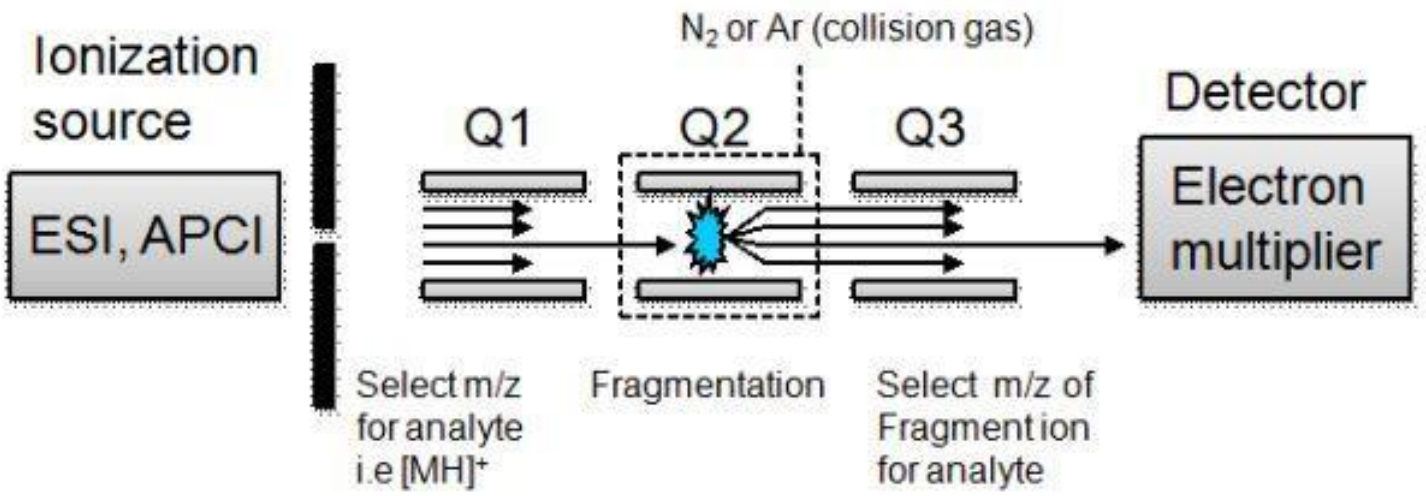
MS lipidomics: TQ analyzer

Properties

- Ion filter + collision cell + Ion filter

- Low resolution but increased selectivity

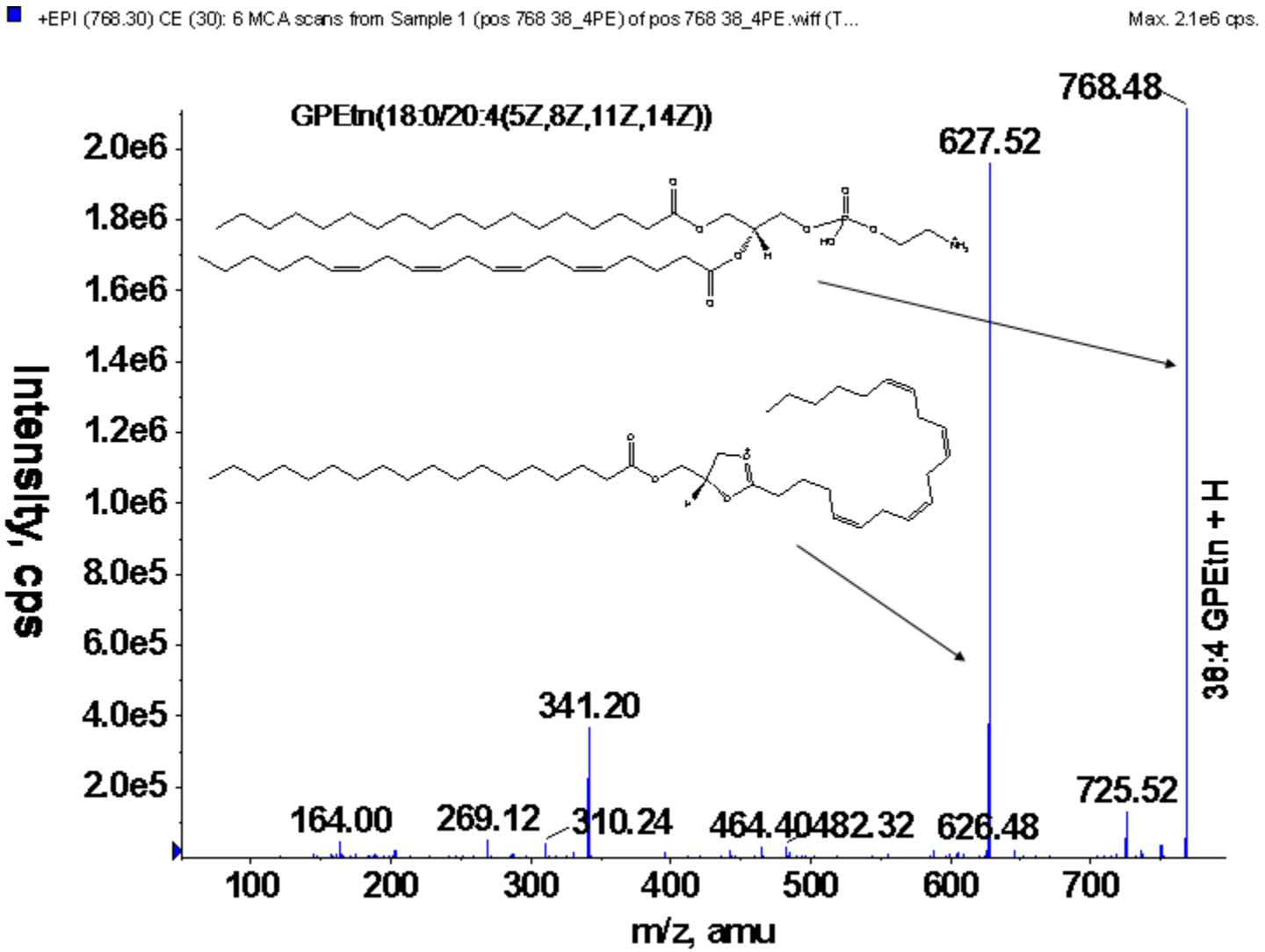
-High dynamic range (linear response)



https://www.youtube.com/watch?v=Jc1uC6EbMCs

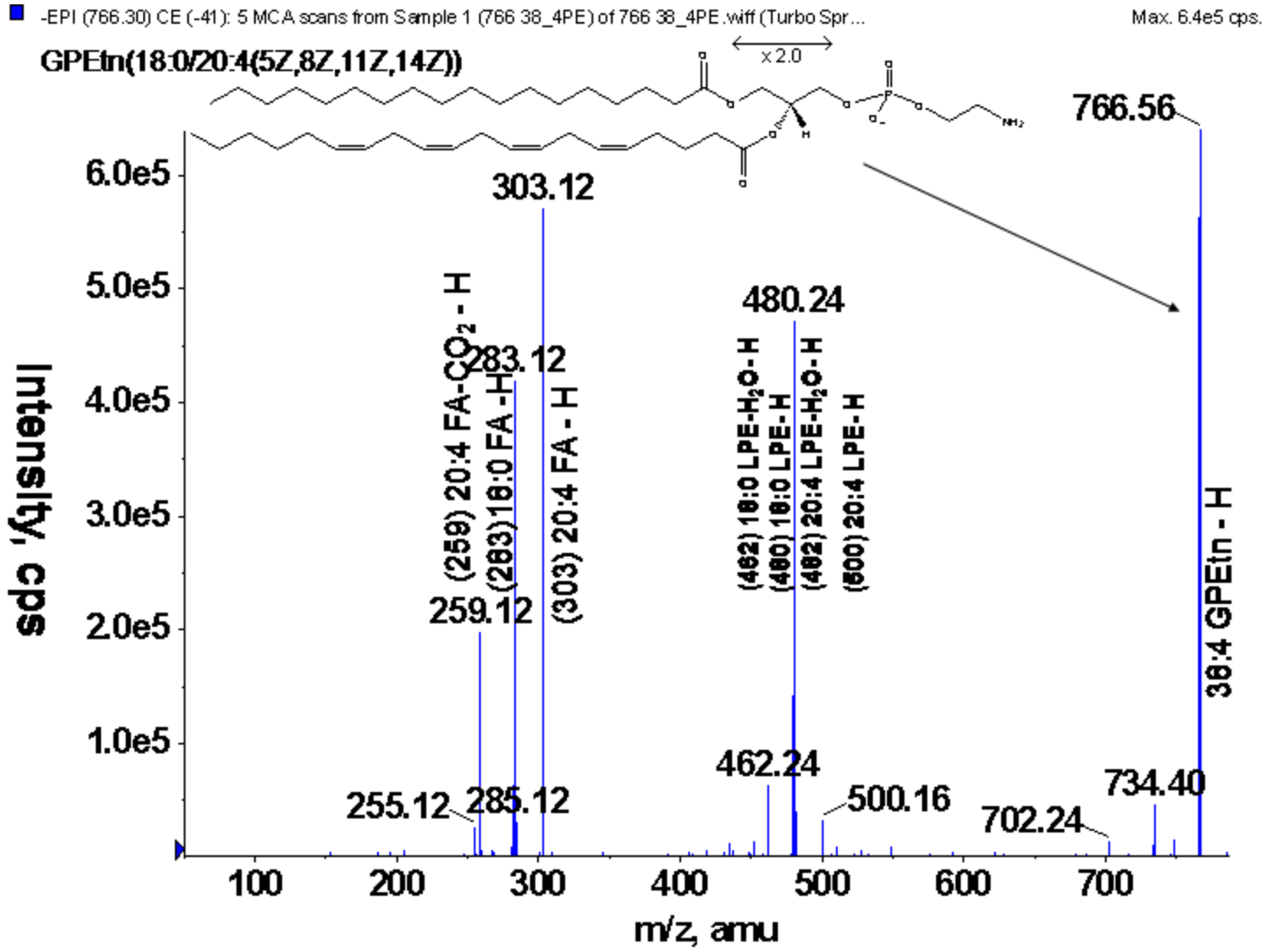
MS lipidomics: Fragmentation

PE 18:0_20:4 in positive mode



MS lipidomics: Fragmentation

PE 18:0_20:4 in negative mode

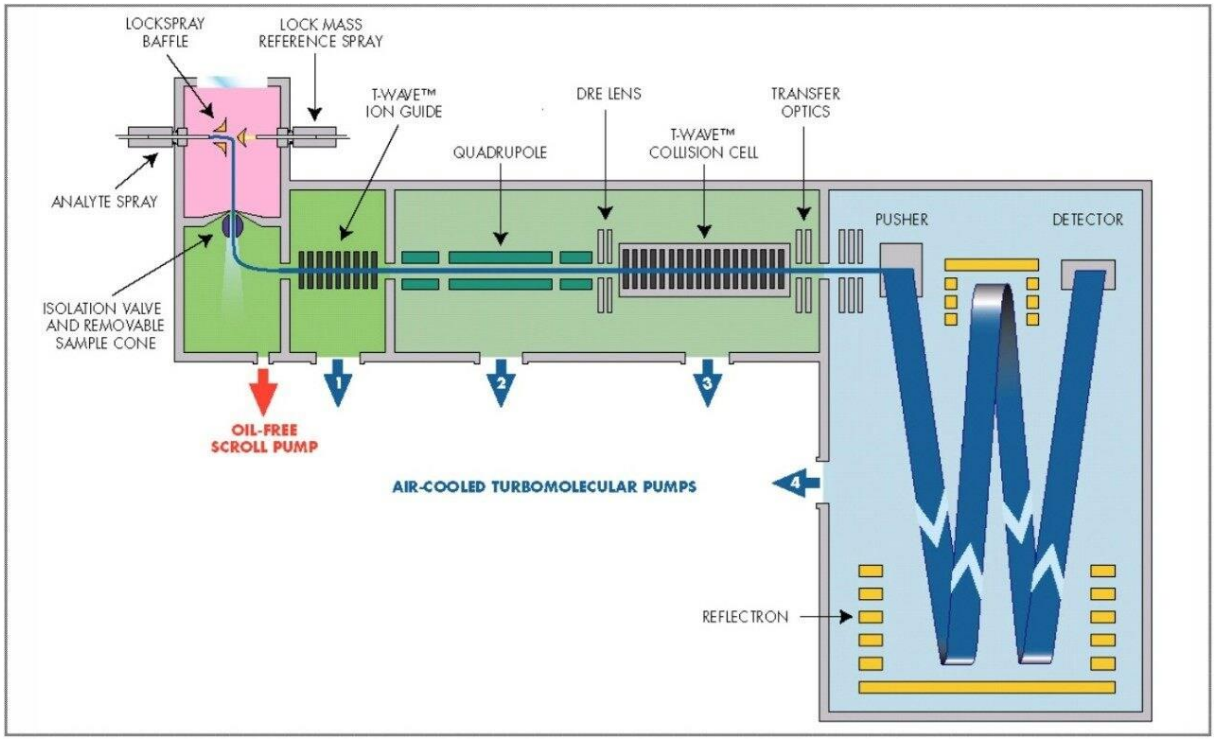


MS lipidomics: Q-ToF analyzer

Properties

- Ion filter + collision cell + Time of Flight

- ToF: medium-high resolution but lower dynamic range



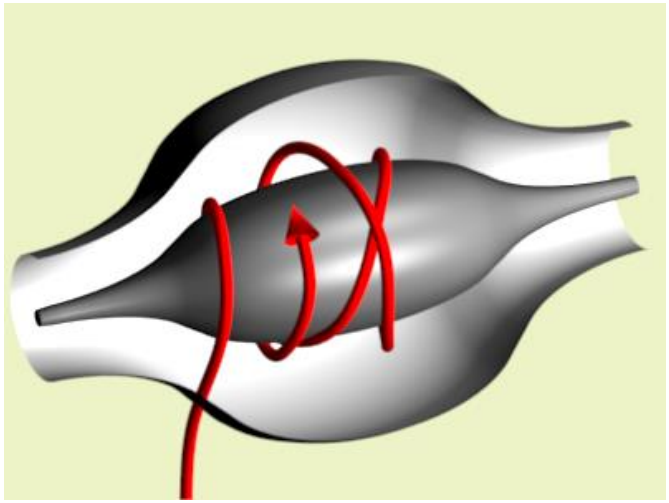
https://youtu.be/W-DRL-V2Rkg?t=29

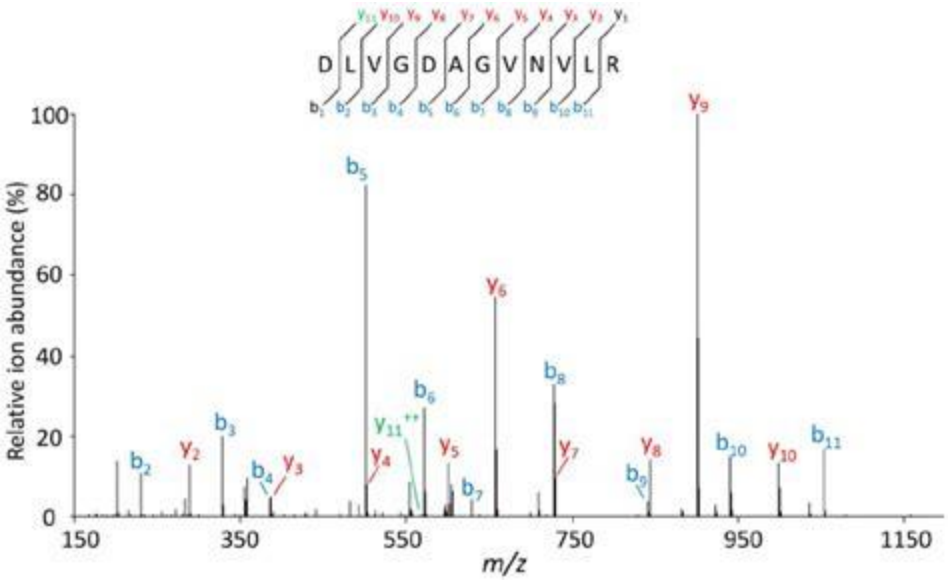
MS lipidomics: Orbitrap analyzer

Properties

- High resolution

- Very expensive, reserved for proteomics



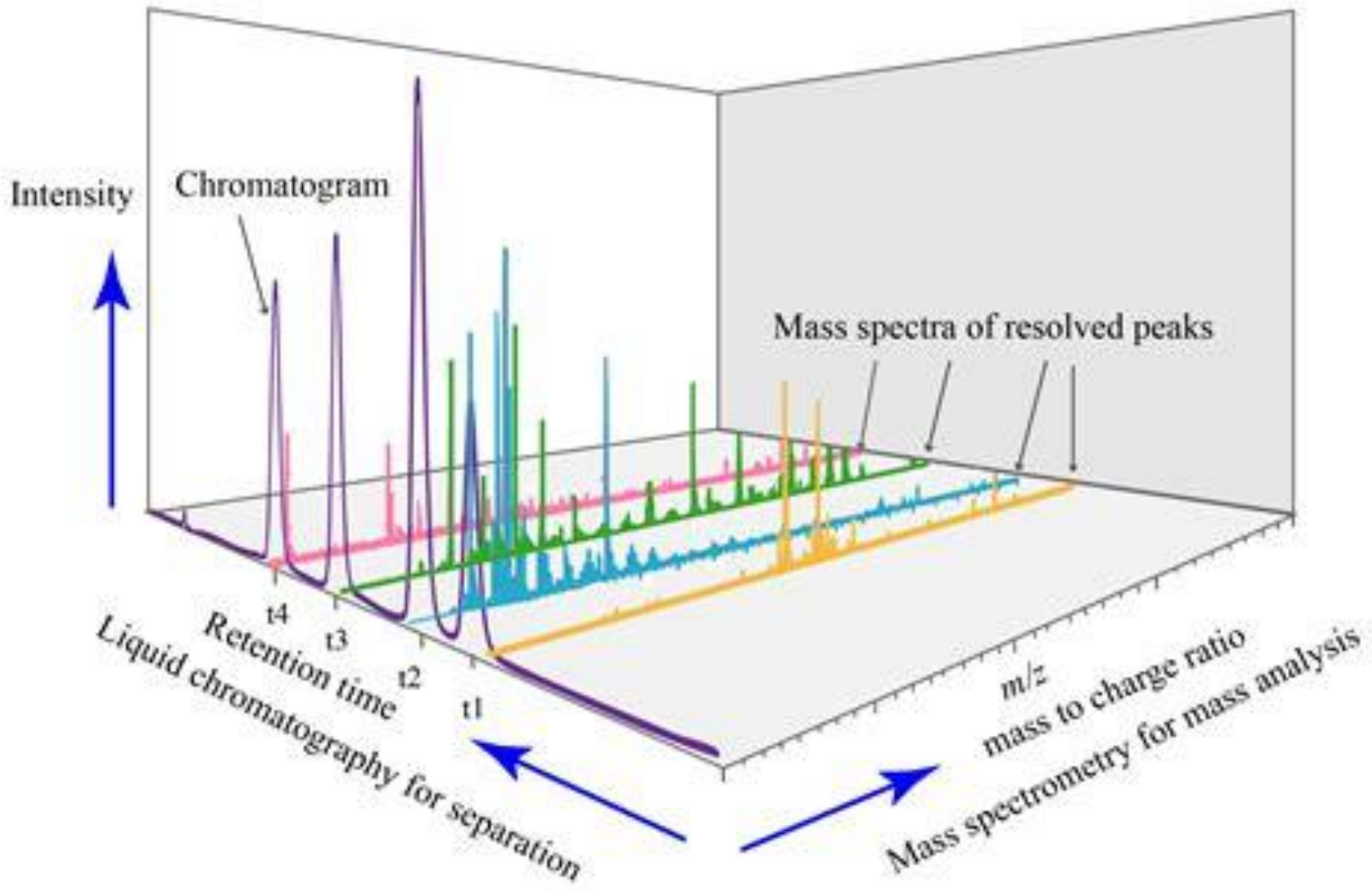


https://www.youtube.com/watch?v=K1VSYjuw6os

MS lipidomics: Analyzer Comparison

Aspect	Triple Quadrupole (Triple Quad, TQ)	Q-ToF (Quadrupole-Time of Flight)	Orbitrap
Principle	Selective mass transitions using multiple quads.	Accurate mass measurement with TOF analyzer.	**High-resolution** mass analysis with ion trapping.
Sensitivity	**High sensitivity** for targeted analysis.	Medium sensitivity for accurate quantification.	Medium sensitivity and resolving power.
Quantitation	**Excellent for targeted quantitation**.	Accurate quantitation and identification.	Accurate quantitation for complex samples.
Selectivity	**Exceptional selectivity for specific analytes**.	Offers high selectivity and comprehensive analysis.	**High selectivity for complex samples**.
Speed	**Fast analysis for high-throughput assays.**	Fast acquisition rates for high-resolution analysis.	Moderate acquisition speeds with high resolution.
Fragmentation	Limited MS/MS capabilities for targeted analysis.	**Excellent MS/MS fragmentation for structural elucidation.**	Versatile MS/MS capabilities for comprehensive analysis.
Common lipidomics applications	**Targeted: minor lipids and oxylipins**	**Glycerolipidome and sphingolipidome**	**Glycerolipidome and sphingolipidome**
Price	Low to medium range.	Medium to high range.	High range.

MS lipidomics: Multidimensional spectrum



MS lipidomics: Analytical developments

- **Coupling to Ion Mobility Spectrometry**
- **Better limits of detection**
- **Bigger dynamic range**
- **Integration of MS scans and MS/MS (fragmentation)**