INTRODUCTION TO BIOLOGICAL MASS SPECTROMETRY I.

Outline

- Definitions
- History
- Instrumentation
  - Ionization
  - Mass analyzers
- Performance
- Analyte separation
- Applications

Mass spectrometry

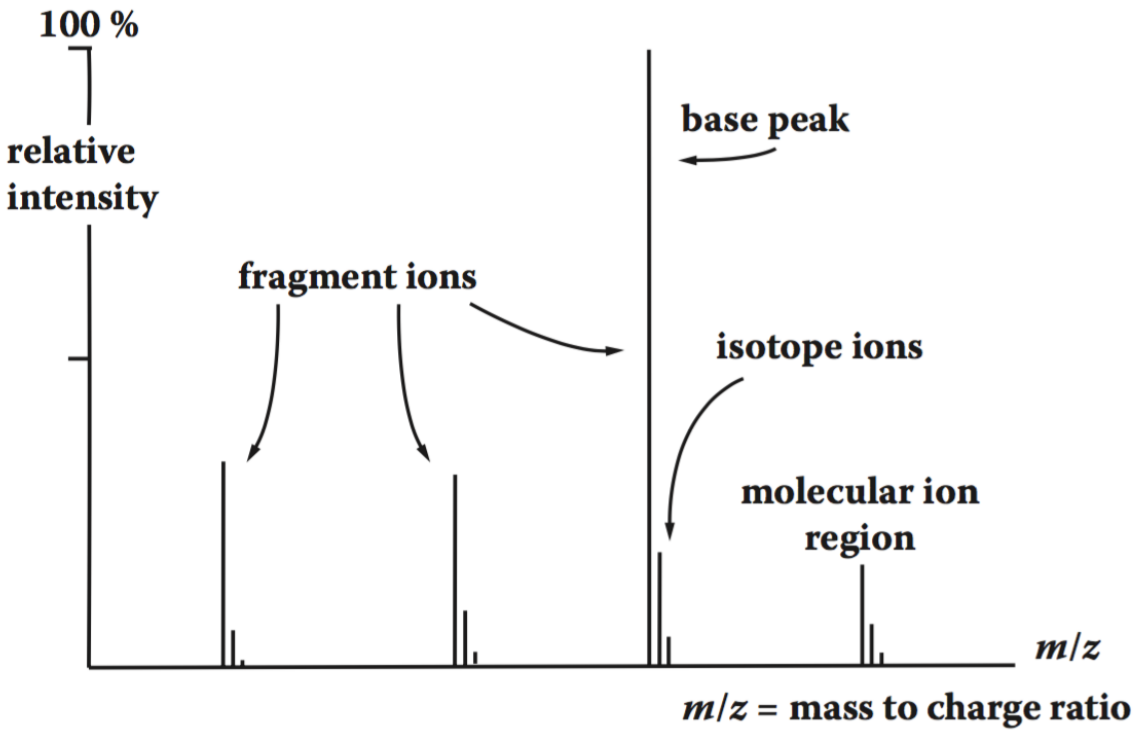
„Mass spectrometry is an essential analytical tool in chemistry, biochemistry, pharmacy, and medicine.”

The basic principle of mass spectrometry (MS) is

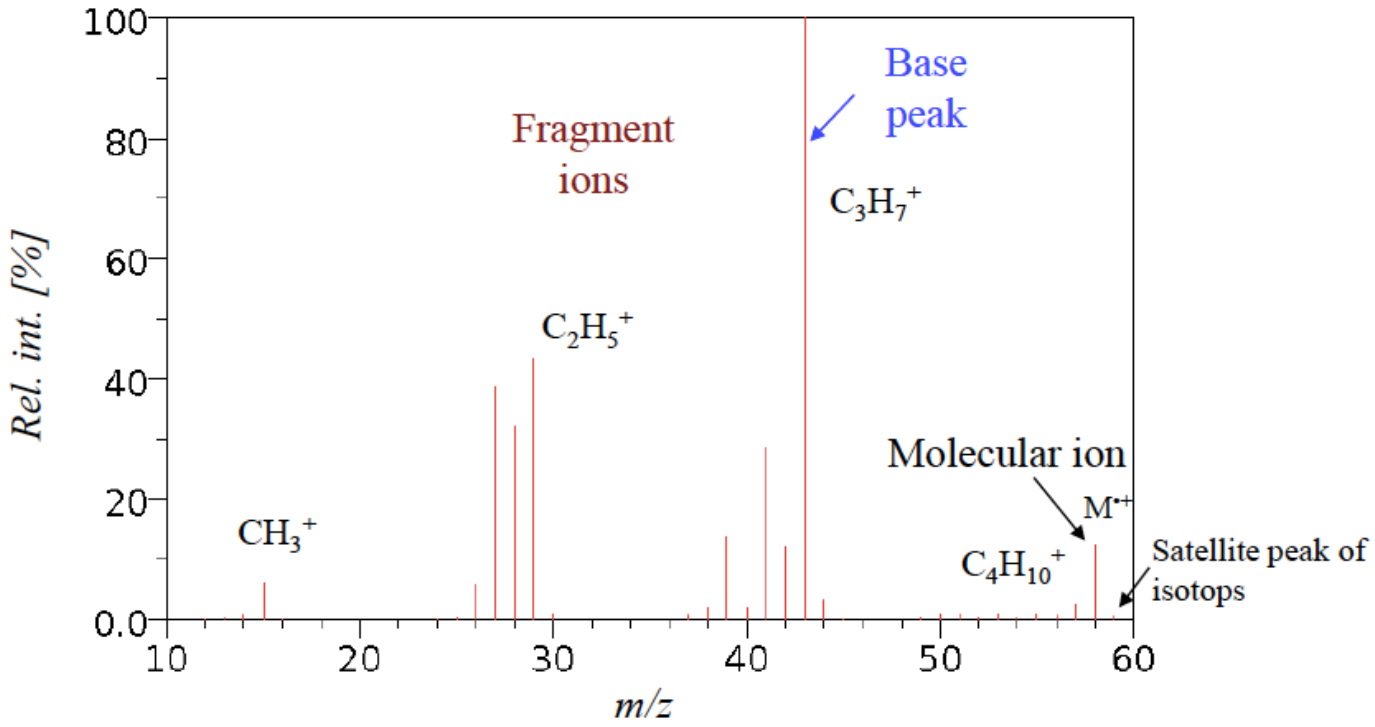
- ***to generate ions*** from either inorganic or organic compounds by any suitable method,
- ***to separate these ions*** by their *mass-to-charge ratio* (*m/z*) and
- ***to detect them*** qualitatively and quantitatively by their respective *m/z* and abundance.

Mass spectrum

The two-dimensional representation of signal intensity (ordinate) versus mass-to-charge ratio, *m/z* (abscissa).



Mass spectrum of n-butane (C_4H_{10})



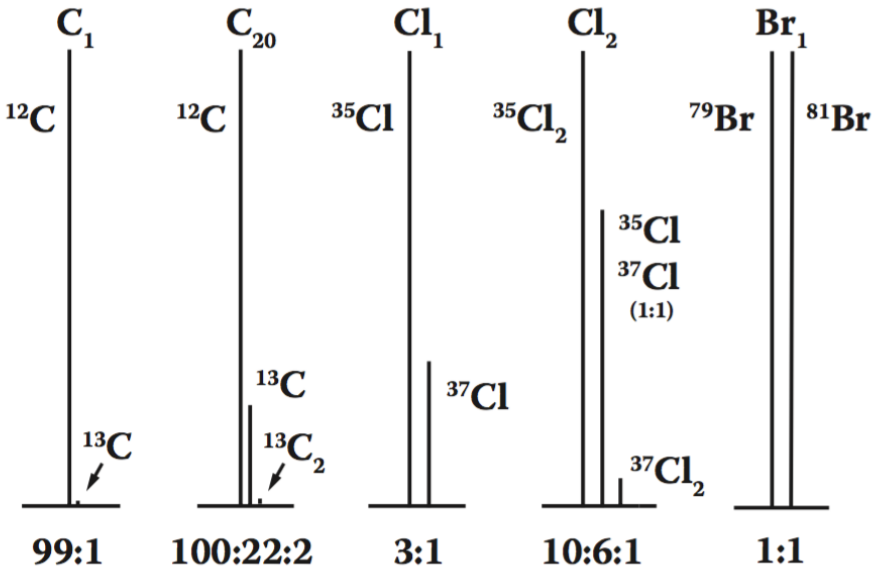
The most intensive peak is called base peak.

Usually, the intensity of the base peak is normalized to 100% relative intensity.

Information obtained from a mass spectrum

- Molecular mass
- Structure (information from fragment spectra)
- Elemental composition (molecular formula)
- Isotopic distribution

element	mass	abundance
H	1.0078 2.0141	99.985% 0.015%
C	12.0000 13.0034	98.89% 1.11%
N	14.0031 15.0001	99.64% 0.36%
O	15.9949 16.9991 17.9992	99.76% 0.04% 0.20%

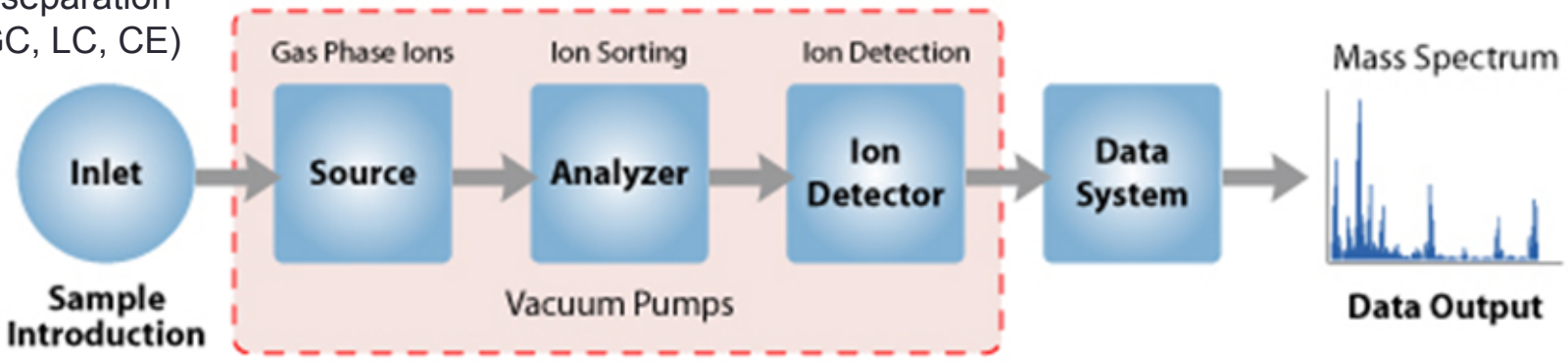


Mass spectrometer

A typical mass spectrometer comprises three parts, an ***ion source***, a ***mass analyzer***, and a ***detector system***:

1. **Ion source** – *conversion of neutral compounds to ions*
2. **Mass analyzer** – *separation of ions according to their m/z values in a gas phase at high vacuum*
3. **Detector** – *detection of ions after their separation and determination of their abundances*

Sample introduction
• Direct
(gas, liquid, solid)
• After separation
(e.g. GC, LC, CE)



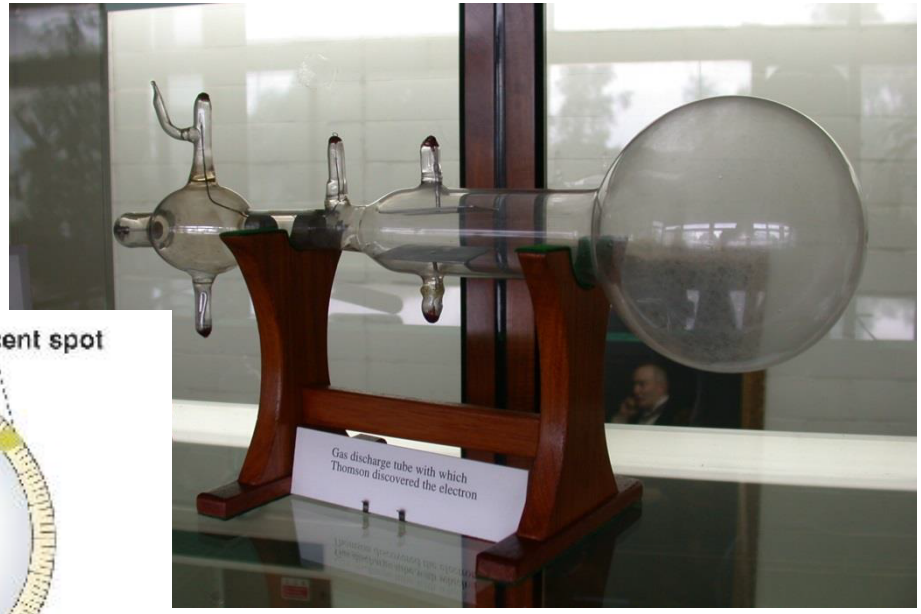
- EI, CI
- ESI
- APCI, APPI
- MALDI
- FAB

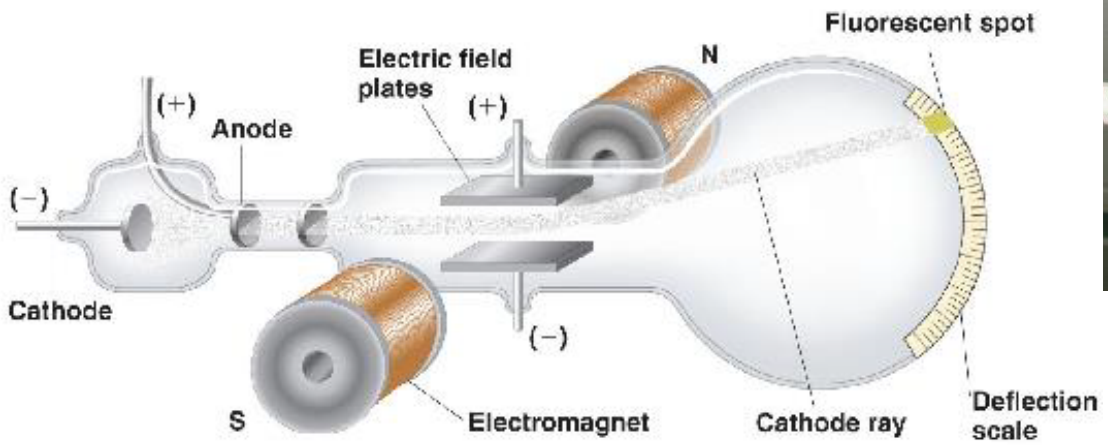
- Quadrupole (Q)
- Ion trap (IT)
- Time of flight (ToF)
- Magnetic sector
- FT-ICR, Orbitrap

History

Joseph John Thomson

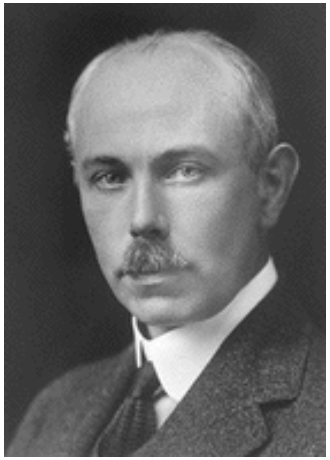
In 1897, Thomson set out to prove that the cathode rays produced from the cathode were actually a stream of negatively charged particles called electrons.





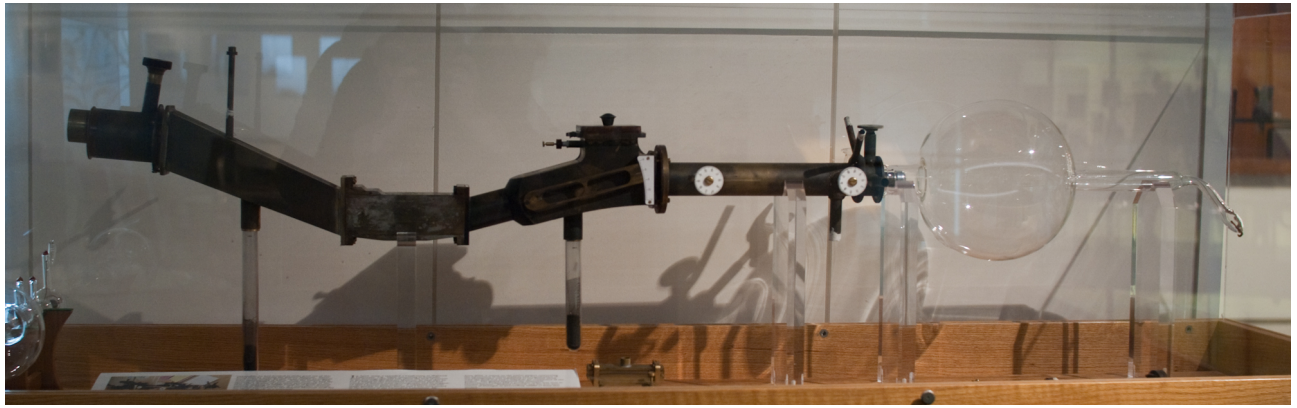
History

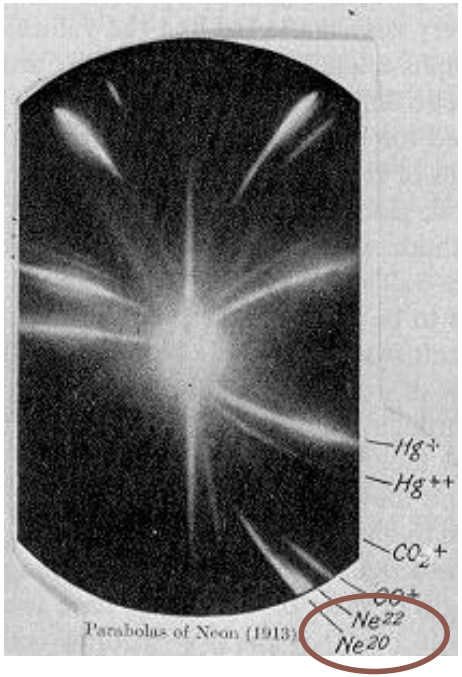
Francis William Aston



He channeled a stream of neon ions through a magnetic and an electric field and measured its deflection by placing a photographic plate in its path.
Two patches of light (two different parabolas of deflection) → two Ne atoms with different atomic masses

The first mass spectrograph

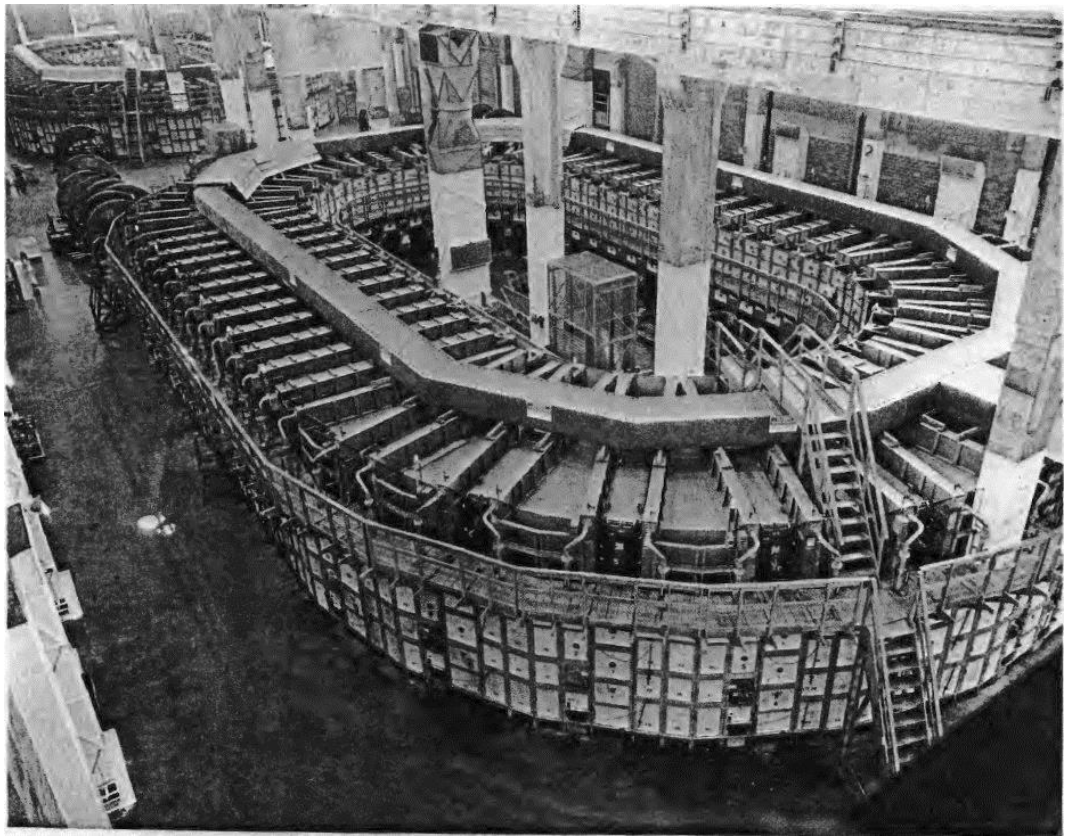




History

The Manhattan project

Sector mass spectrometers known as ***calutron***s were developed by Ernest O. Lawrence and used for separating the isotopes of uranium during the Manhattan Project.



Joseph John Thomson
1906 Nobel Prize for Physics
"in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases"

Francis William Aston
1922 Nobel Prize for Chemistry
"for his discovery, by means of his mass spectrograph, of isotopes, in a large number of non-radioactive elements, and for his enunciation of the whole-number rule"

Wolfgang Paul
1989 Nobel Prize for Physics
"for the development of the ion trap technique"

John Bennet Fenn
2002 Nobel Prize for Chemistry
"for the development of soft desorption ionisation methods (ESI) for mass spectrometric analyses of biological macromolecules"

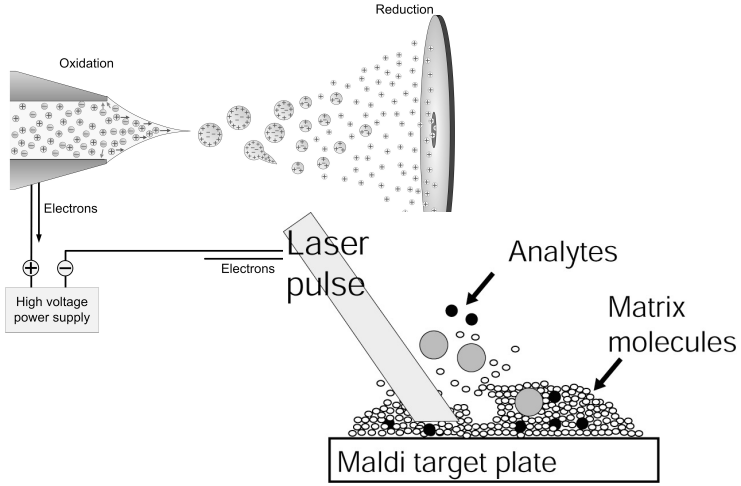
Koichi Tanaka
2002 Nobel Prize for Chemistry
"for the development of soft desorption ionisation methods (MALDI) for mass spectrometric analyses of biological macromolecules"

1890 1900 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020

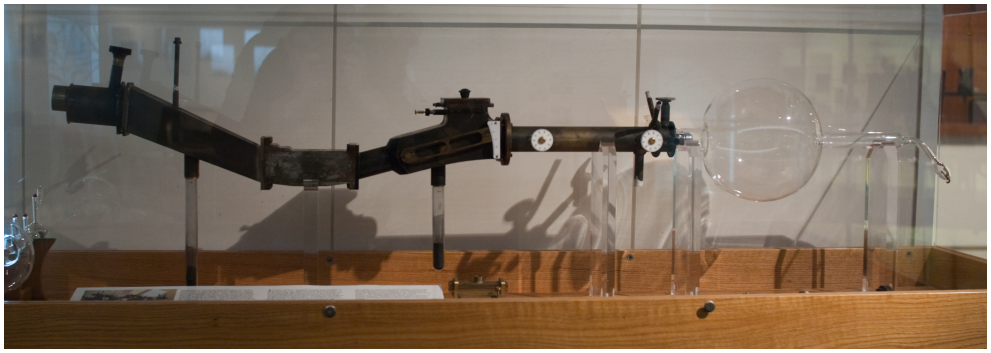
Analysis of small volatile compounds

Analysis of proteins

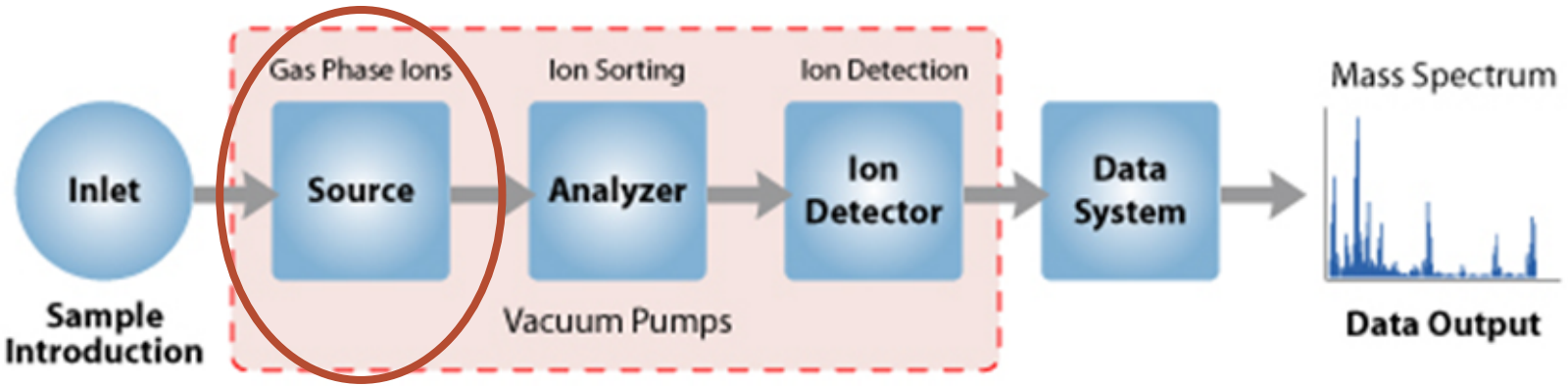
Proteins are difficult to ionize



Mass spectrometers



Mass spectrometer



Ion sources

Tasks:

- to ionize the sample
- to produce coherent ion beam
- to accelerate the ions towards the mass analyzer

Ionization types:

- electron-impact ionization (EI)
- chemical ionization (CI)

} gas-phase ionization

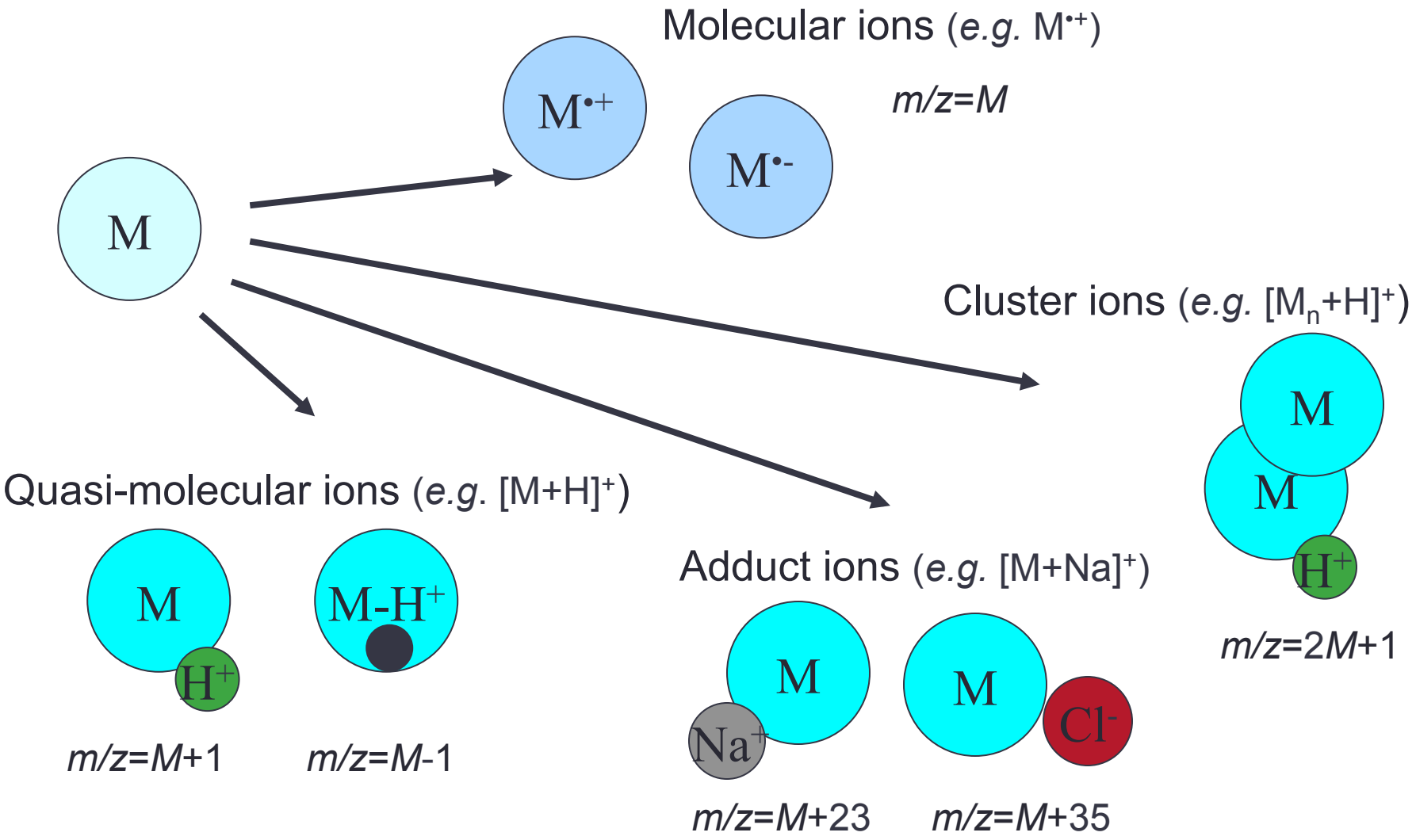
- electrospray ionization (ESI)
- atmospheric pressure chemical ionization (APCI)
- atmospheric pressure photo ionization (APPI)

} liquid-phase Ionization

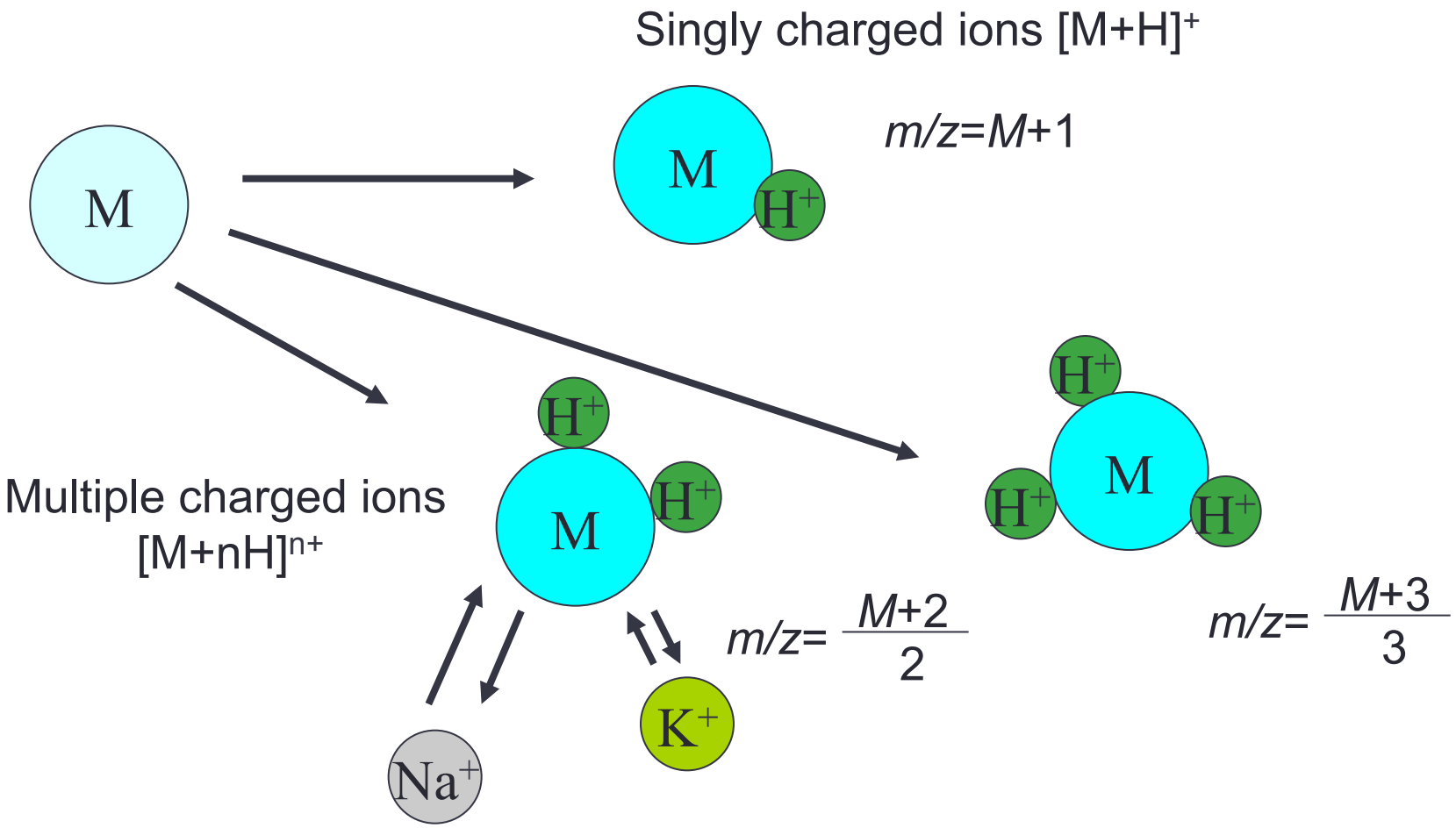
- matrix assisted laser desorption ionization (MALDI)
- secondary ion mass spectrometry (SIMS)
- plasma desorption (PD)
- fast atom bombardment (FAB)

} solid-state ionization

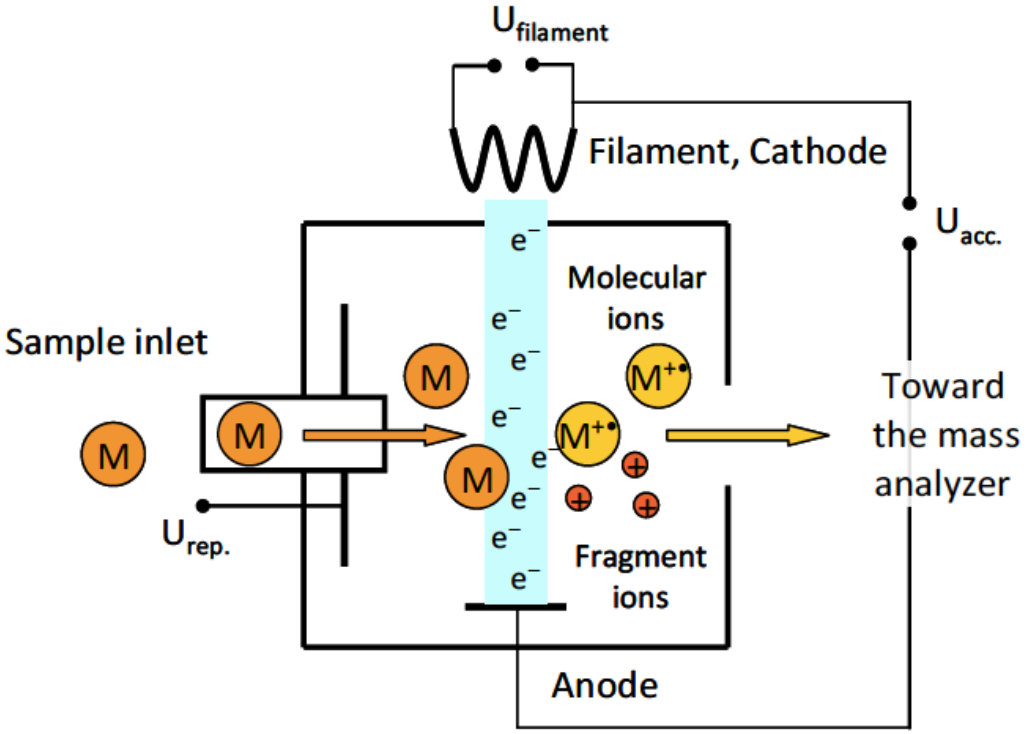
Generated ions



Generated ions



Electron-impact ionization (EI)



- Electron impact
- Heated filament (cathode)
  - W, Re
  - T_{fil} = 2000 °C
- Ionization chamber
  - p= 10^{-5} mbar
  - T= 150-250 °C
- Anode
  - U=10-70V
- Ionization
  - Molecular ion
- Fragmentation
  - Fragment ions

$$M + e^- \longrightarrow M^{+\bullet} + 2e^-$$

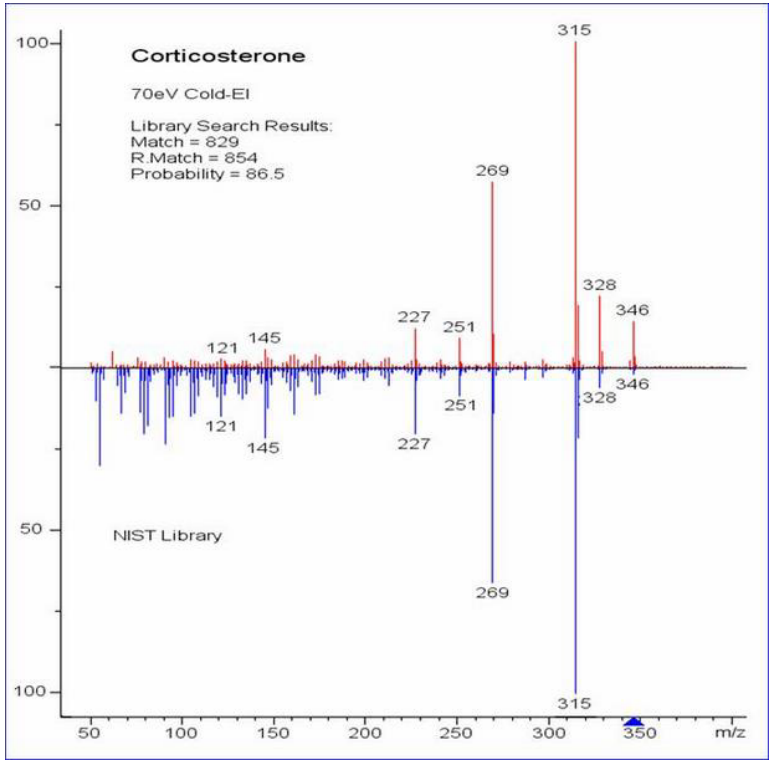
Electron-impact ionization (EI)

Applicable for

- volatile and thermally stable sample
- low- to medium-polarity, non-ionic organic compounds
- up to ~1000 Da.

Besides the molecular mass information (M+·), it also provides ***structural information*** due to the extensive and characteristic fragmentation that occurring in EI.

EI spectra measured under standard conditions, therefore
it has very ***good reproducibility*** and allows ***similarity search*** in databases.

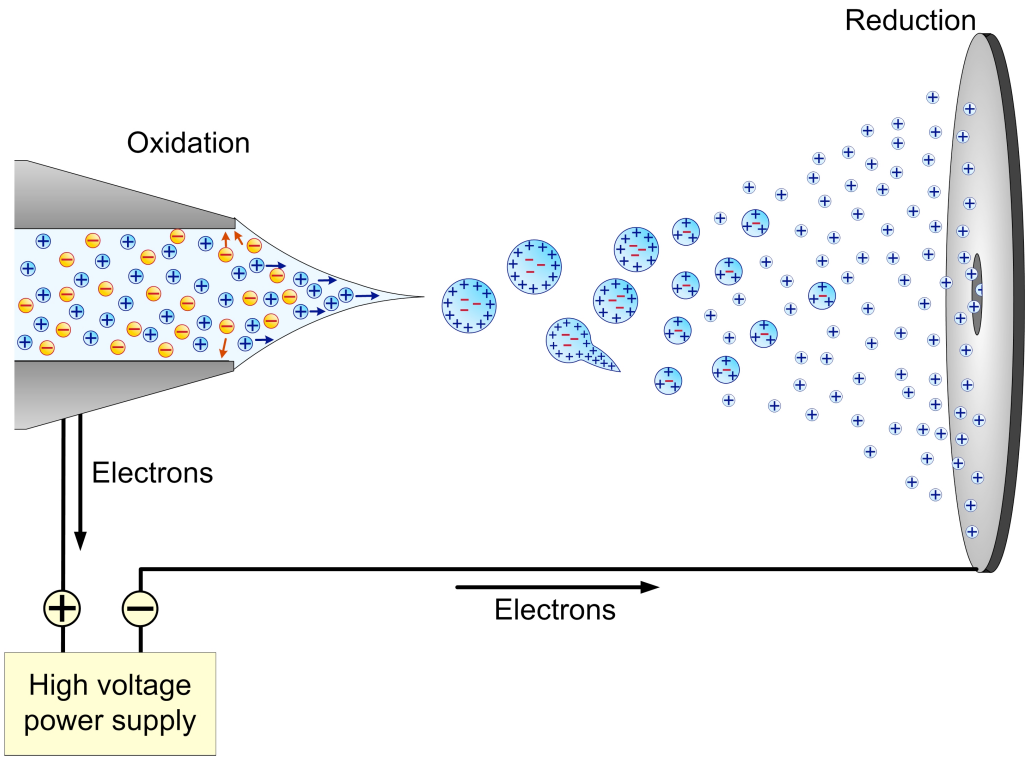


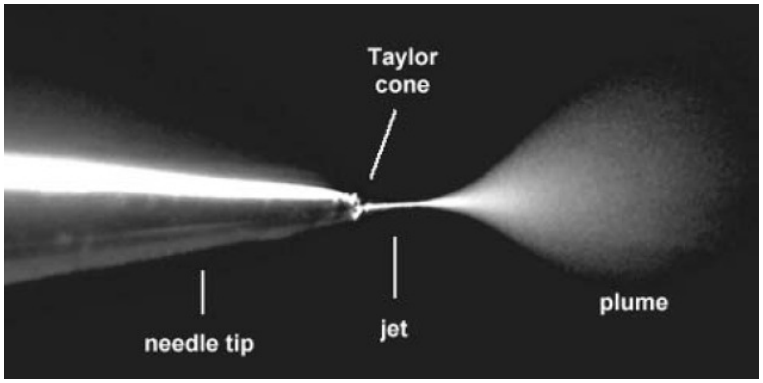
Databases:
NIST/EPA/NIH Mass Spectral Library
Wiley / NBS Mass Spectral Database

Electrospray ionization (ESI)

Applying strong electric field under atmospheric pressure to a liquid.
The high electric field generates a mist of highly charged droplets.
The droplets reduce in size by evaporation of the solvent or by "Coulomb explosion" (droplet subdivision resulting from high charge density).

Fully desolvated ions result from complete evaporation of the solvent.





Electrospray ionization (ESI)

Advantages

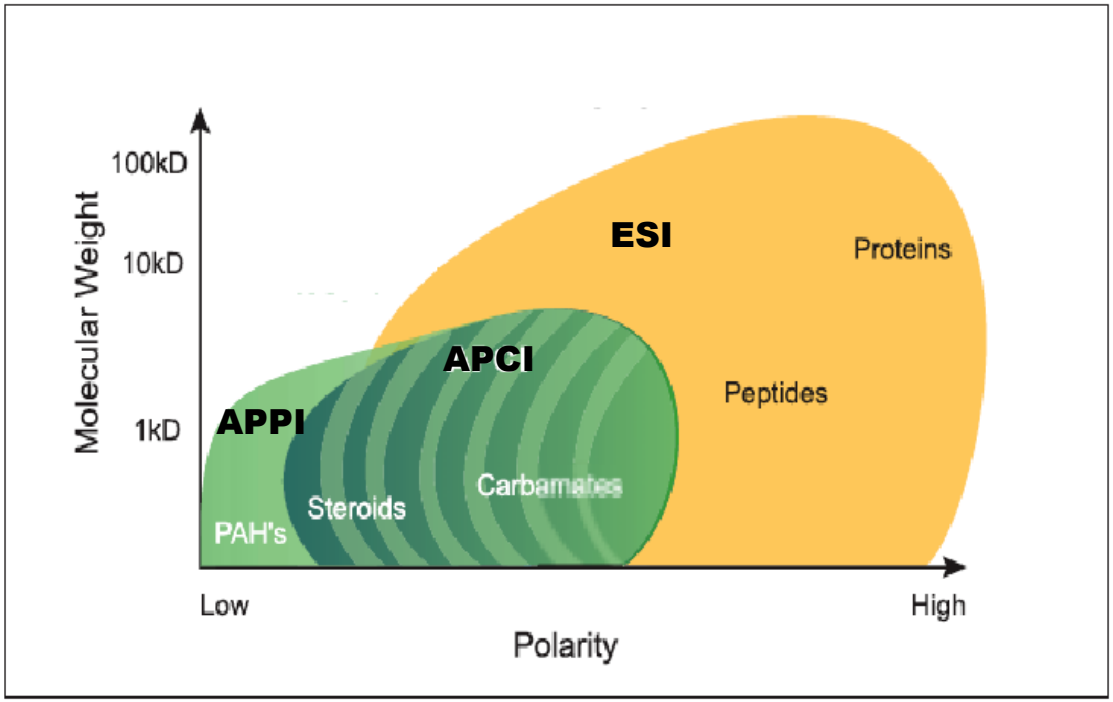
- Highly sensitive (10^{-12} – 10^{-15} mol)
- Positive and negative ions (quasi-molecular ions)
- Highly polar/ionic compounds
- Wide mass range of molecules
- Easy to couple to LC or CE
- Basic tool in proteomics for peptide and protein analysis

Disadvantages

- Salts and other non-volatile additives should be avoided
- Only volatile buffers should be used!
- Mixtures without separation can not be or hardly analyzed
- Non-polar molecules can not be analyzed with this technique

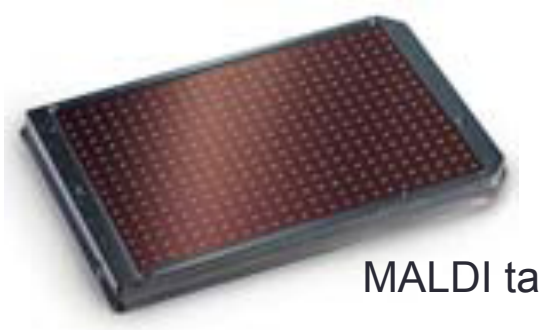
Ionization under atmospheric pressure

- ESI (electrospray ionization)
- APCI (atmospheric pressure chemical ionization)
- APPI (atmospheric pressure photoionization)

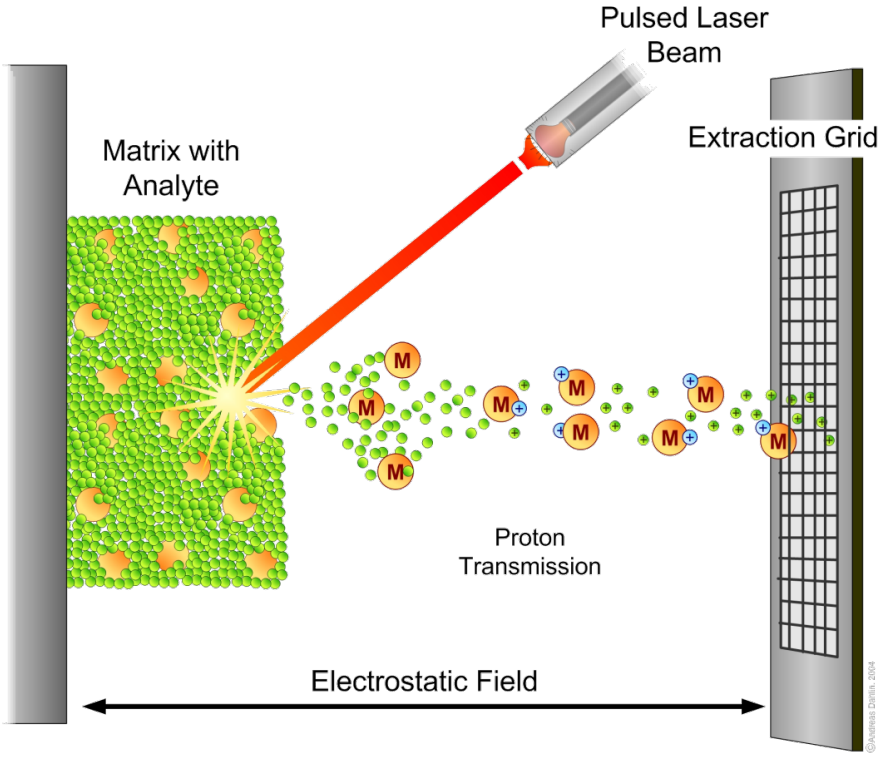


Matrix-Assisted Laser Desorption Ionization (MALDI)

Absorption of the laser light by a **solid sample layer.**
Energy uptake ➔ evaporation and ionization of the sample.

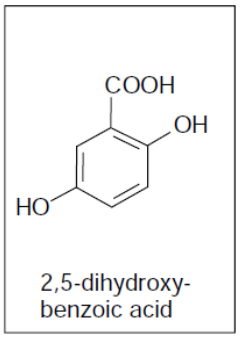


MALDI target plate

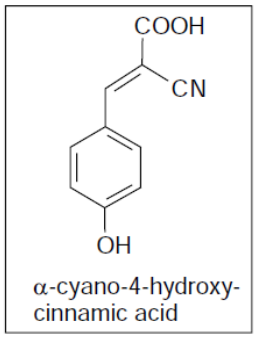


$$\text{Matrix (S)} \xrightarrow{hv} \text{Matrix* (S)} \rightarrow \text{Matrix (S*)} \rightarrow \text{Matrix } (S^+ + e^-) \xrightarrow{E} \text{Matrix} + M^+$$

Matrix-Assisted Laser Desorption Ionization (MALDI)



2,5-dihydroxy-benzoic acid



α-cyano-4-hydroxy-cinnamic acid

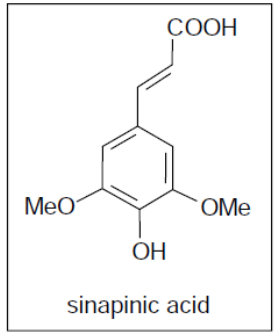
Advantages

- Highly sensitive (10^{-12} – 10^{-15} mol)
- Positive and negative ions
- Polar or easily polarizable compound
- High molecular mass (up to 300 kDa)
- Basic tool in proteomics for peptide and protein analysis

Disadvantages

- Salts should be avoided
- Low-molecular mass ions and the matrix ions can overlap
- Off-line coupling to LC

Common matrices: 2,5-dihydroxybenzoic acid (DHB)
α-cyano-4-hydroxycinnamic acid (CHCA)
sinapinic acid (SA)

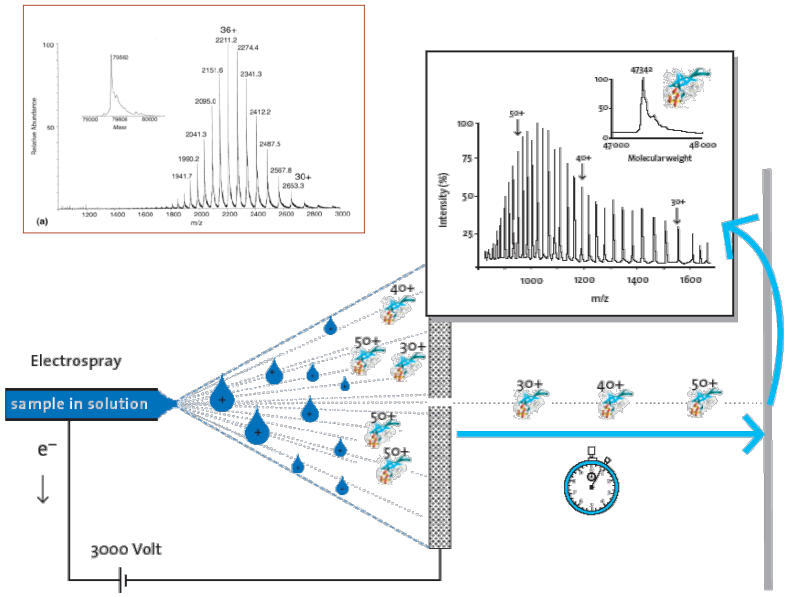


sinapinic acid

Ionization of biomolecules

ESI - Electrospray ionization

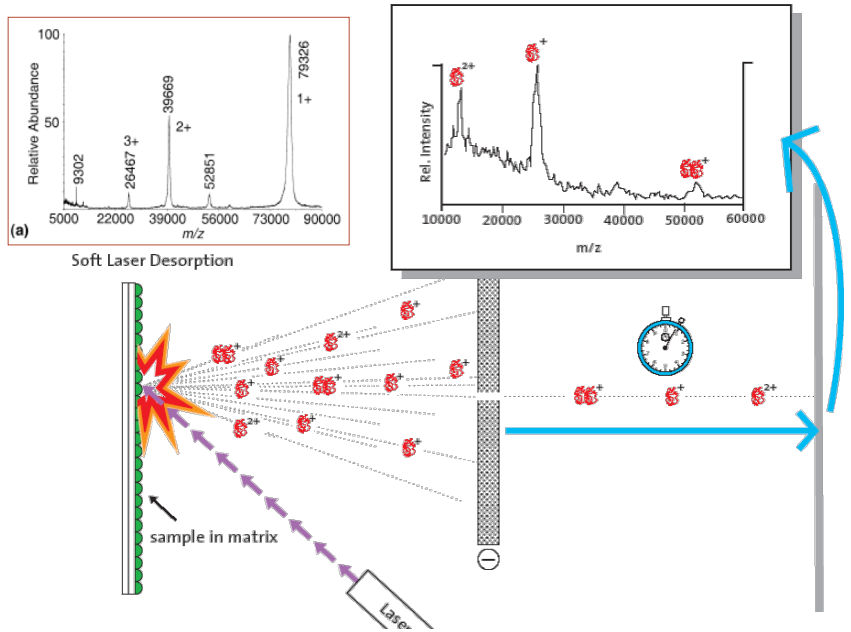
- Soft ionization technique
- Sample in liquid
- Purification is needed
- Multiple charges



Fenn et al. *Science* **1989**, *246,* 64-71

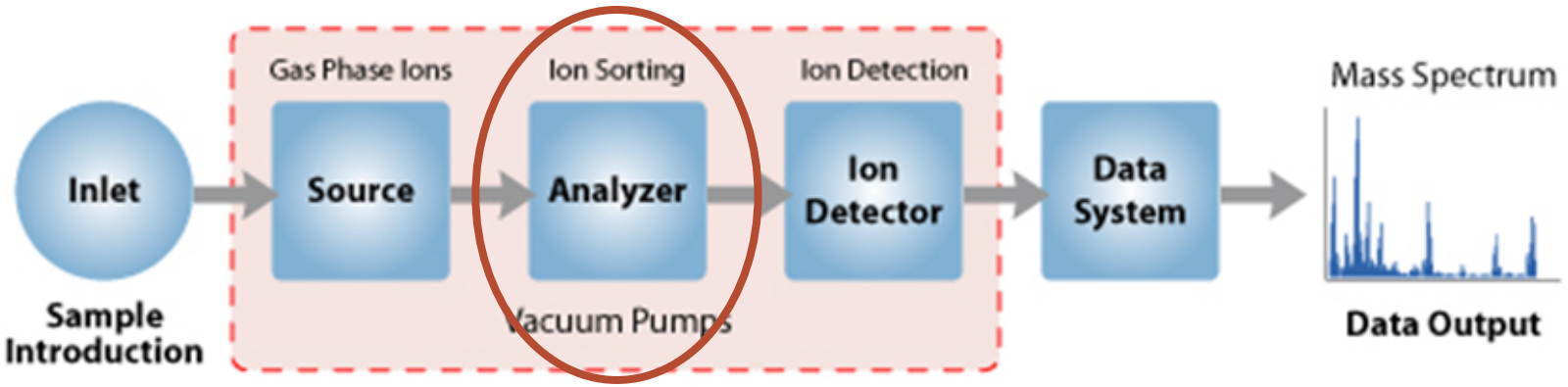
MALDI - Matrix assisted laser desorption/ionization

- Soft ionization technique
- Sample in solid state
- Purification optional
- Single charges



Karas & Hillenkamp *Anal. Chem.* **1988** *60*, 2299-2301.

Mass spectrometer



Mass analyzers

Tasks:

To ***separate ions*** by their *mass-to-charge ratio* (*m/z*) and to ***drive or*** to ***focus*** these discrete ion packages toward the detector.

Ionization types:

- Magnetic (B)
- Electrostatic (E)

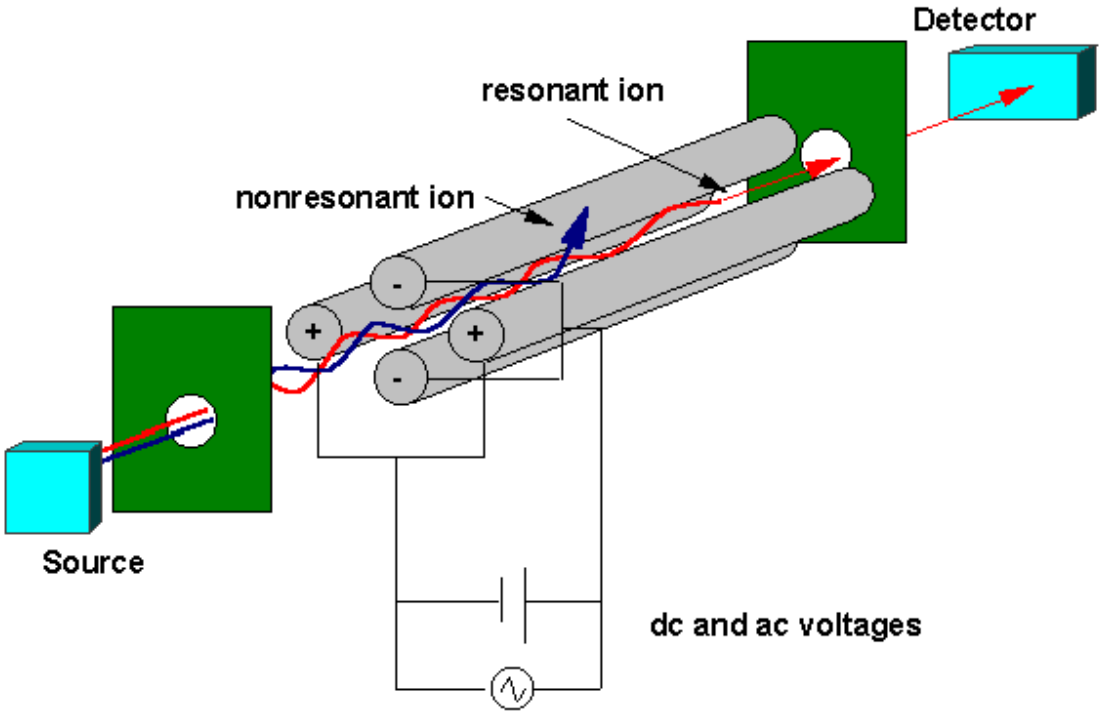
} Double focusing sector instruments (BE, EB)

- Quadrupole (Q)
- Ion trap (IT) / Linear ion trap (LIT)
- Time-of-flight analyzer (TOF)
- Orbitrap (OT)
- Fourier transform ion cyclotron (FTICR)

- Combined analyzers (QqQ, QTrap, QTOF, etc.)

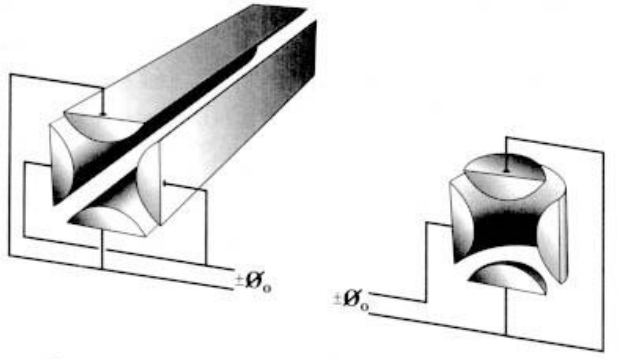
Quadrupole

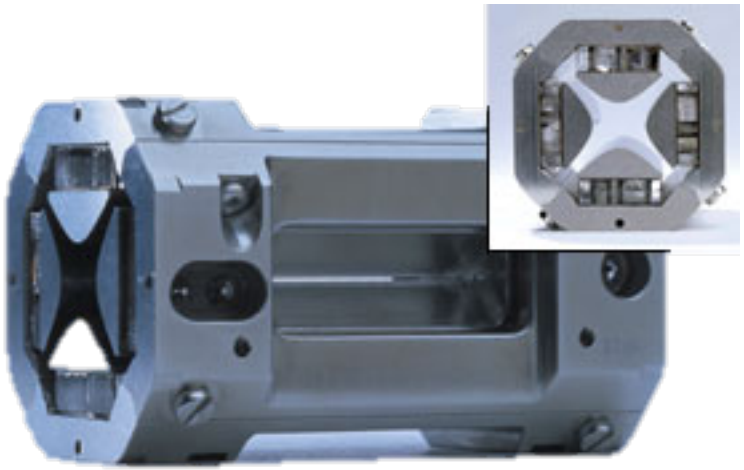
Consists of four hyperbolically or cylindrically shaped rod electrodes mounted in a square configuration.
The pairs of opposite rods are each held at the same potential which is composed of a DC voltage (U) and an AC component (V with ω frequency) → 2D quadrupole electric field

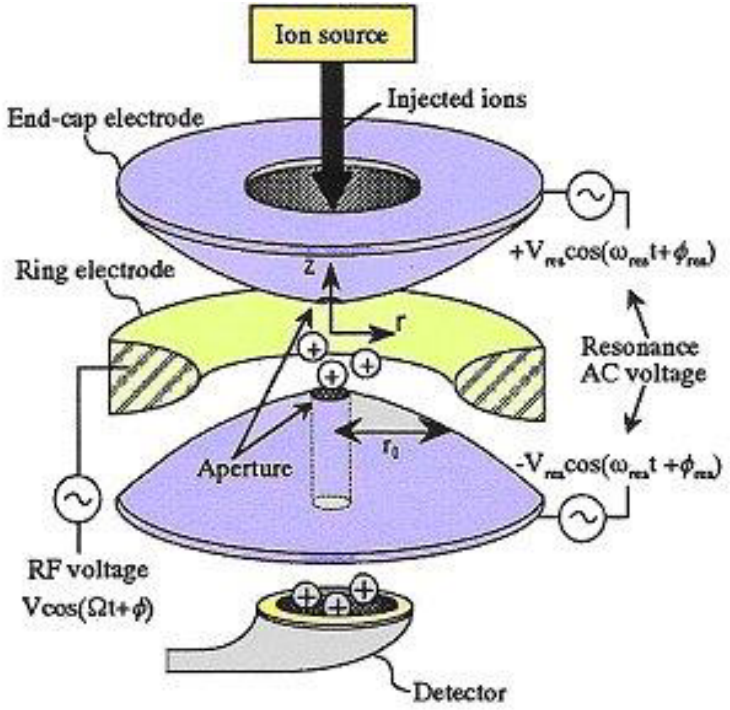


Iontrap

Based on quadrupole technology
Higher ion capacity
Limited mass resolution
Ability to manipulate ions (fragmentation)
3D ion trap (IT) or linear ion trap (LIT)

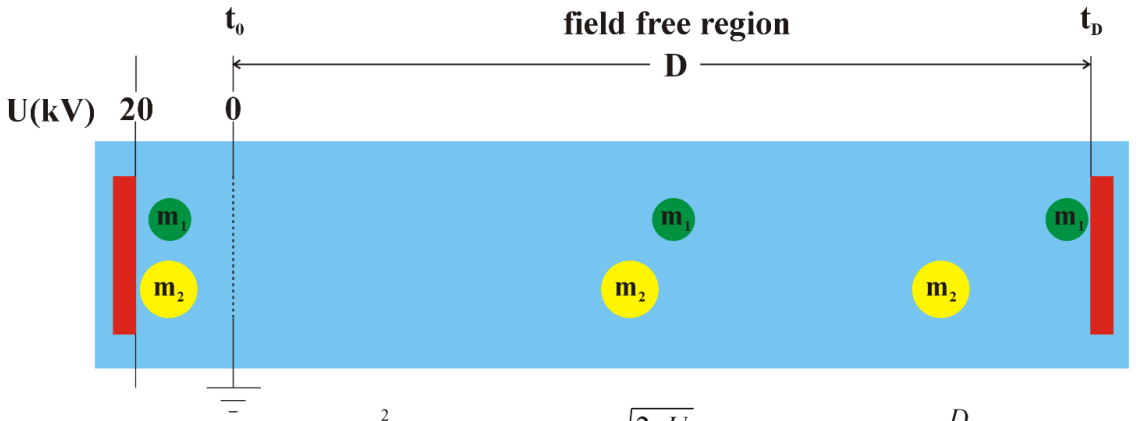






Time of flight (TOF)

Ions, produced mostly by MALDI, are accelerated down a long flight tube via a brief ‘pulse’ electric field.
The ions travel through this region with a velocity that depends on their m/z ratios.
The former potential energy of a charged particle in an electric field is converted into kinetic energy.



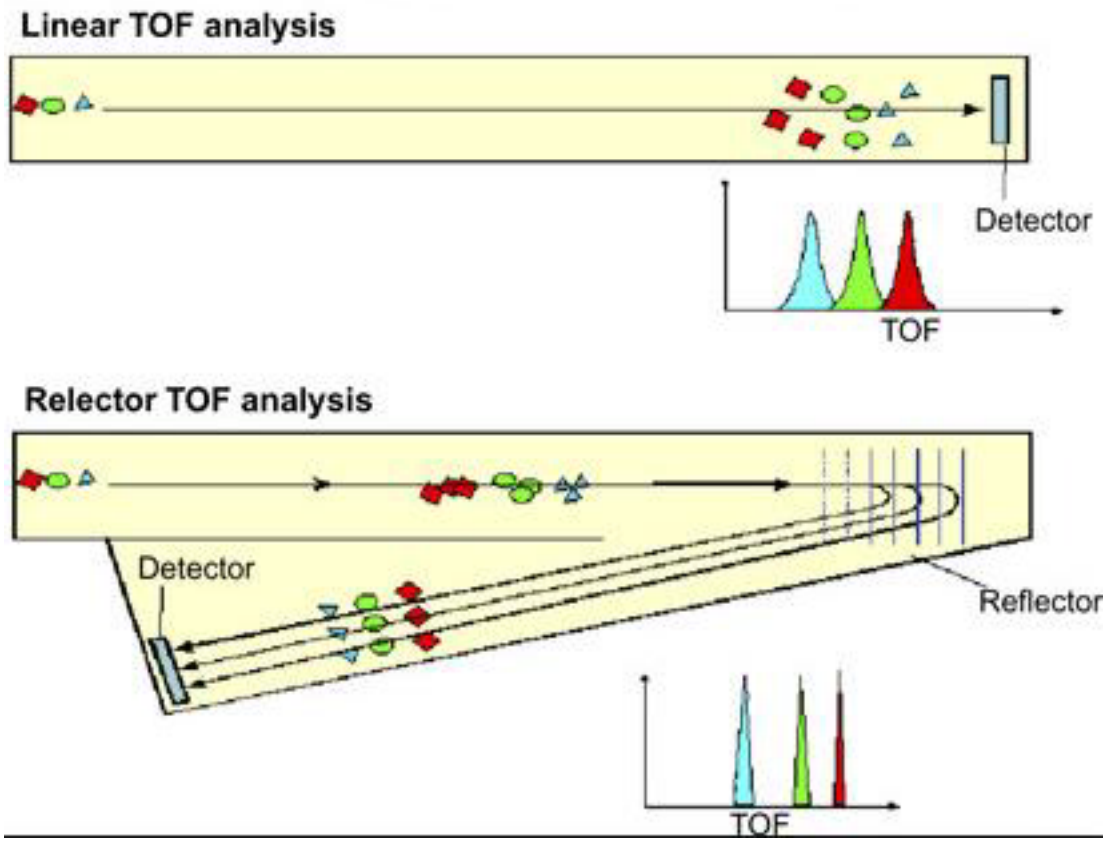
$$W_k = \frac{mv^2}{2} = qU \qquad v = \sqrt{\frac{2qU}{m}} \qquad t_D = \frac{D}{\sqrt{2qU \times \frac{1}{m}}}$$

W_k = kinetic energy v = velocity t_d = drift time

U = extraction voltage q = charge D = field-free drift region

m = molecular mass

Linear vs Reflector TOF analysis



Linear detector

- up to 350 kDa
- high sensitivity
- low resolution

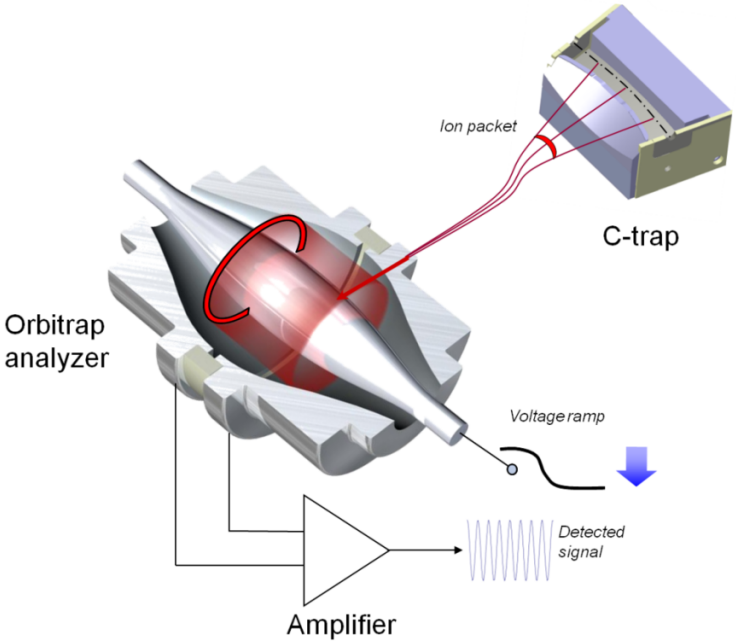
Reflector detector

- up to 5000 Da
- low sensitivity
- high resolution

Orbitrap

Originally described in 1920 (Kingdon trap), but it was developed into a mass analyzer only in the late ’90’s by Alexander Makarov.

Ions are injected into the Orbitrap where they are electrostatically trapped, while rotating around the central electrode and performing axial oscillation.
Only the axial frequency is completely independent on energy and position of ions, therefore it can be used for mass analysis.



The axial oscillation frequency follows the formula

Where ω = oscillation frequency

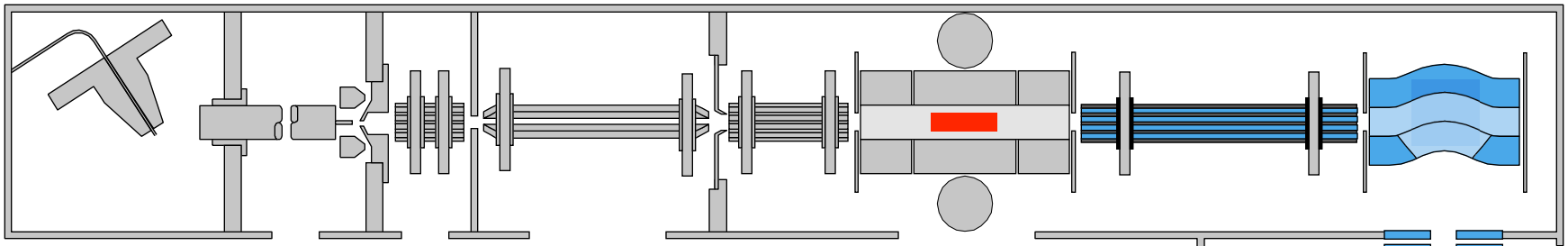
k = instrumental constant

m/z = …. well, we have seen this before

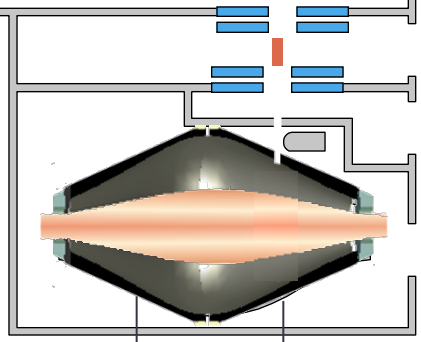
$$\omega = \sqrt{\frac{k}{m/z}}$$

LTQ Orbitrap operation principle

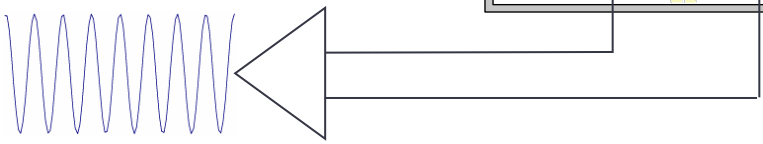
1. Ions are stored in the Linear Trap
2. …. are axially ejected
3. …. and trapped in the C-trap
4. …. they are squeezed into a small cloud and injected into the Orbitrap
5. …. where they are electrostatically trapped, while rotating around the central electrode and performing axial oscillation



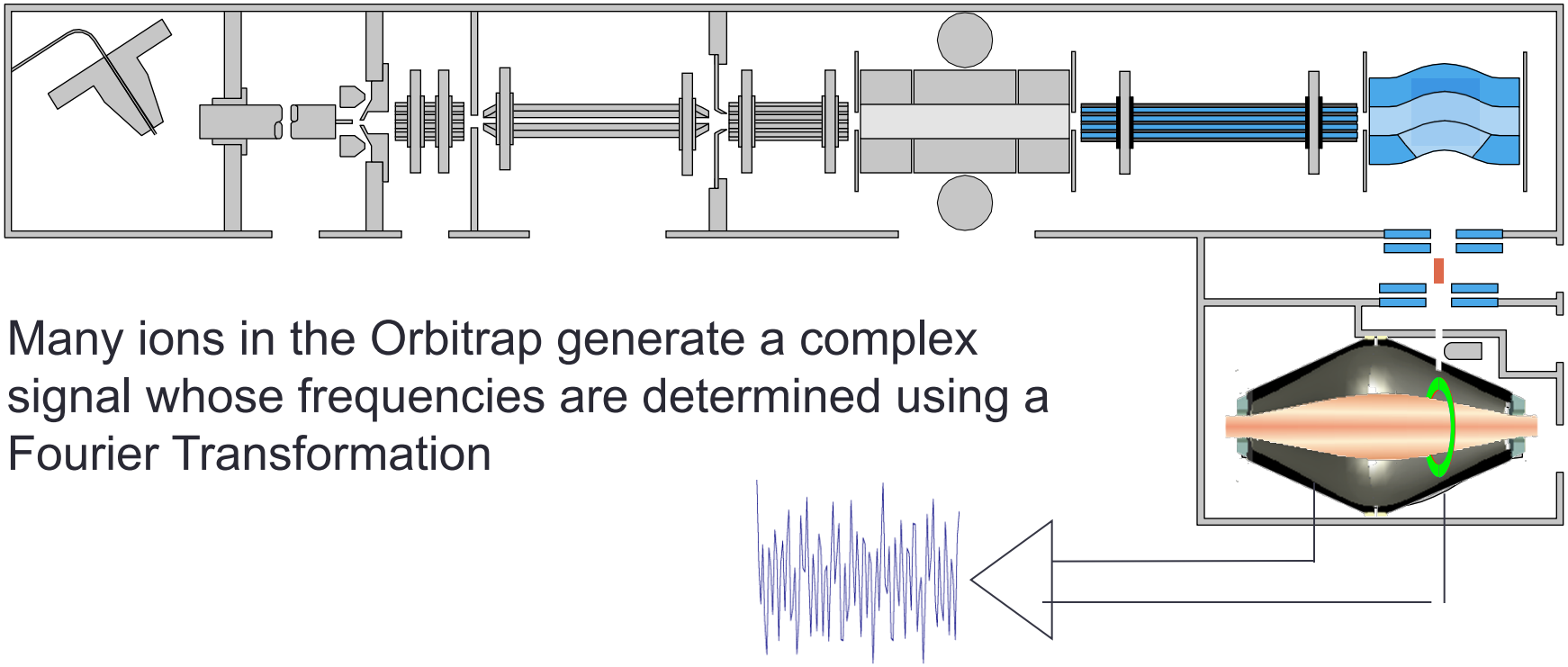
The oscillating ions induce an image current into the two outer halves of the orbitrap, which can be detected using a differential amplifier



Ions of only one mass generate a sine wave signal



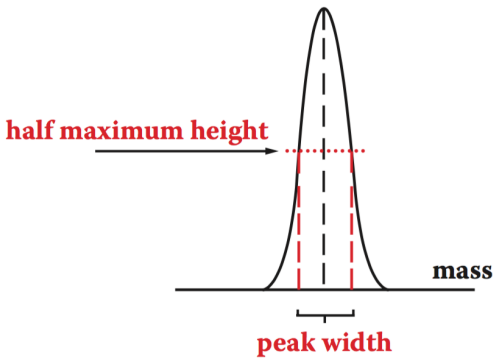
LTQ Orbitrap operation principle



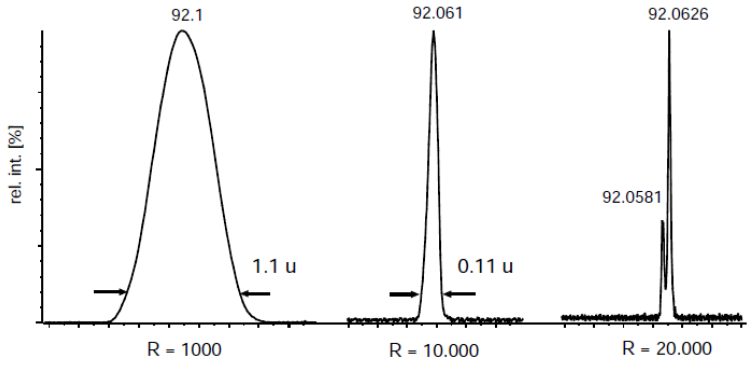
Many ions in the Orbitrap generate a complex signal whose frequencies are determined using a Fourier Transformation

Characteristics for measuring the mass analyzer performance

- Mass range limit (limit of *m/z* over which the mass analyzer can measure)
- Analysis speed (scan speed, the rate at which the mass analyzer measures over a particular mass range)
- Transmission (ion losses)
- Mass accuracy ($m_{theoretical}$-$m_{measured}$)
- Resolution, resolving power (the ability of analyzer to yield distinct signals for two ions with a small *m/z* difference, R= *m/Δm)*



$$\text{Resolution} = \frac{\text{mass}}{\text{width at half maximum height}}$$



A mixture of $^{13}CC_6H_7^+$ and $C_7H_8^{+\bullet}$ at different resolution settings.

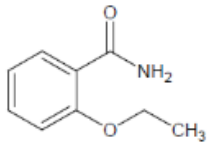
Comparison of mass analyzers

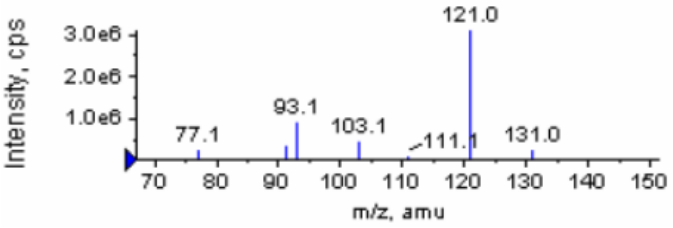
	Quadrupole	Ion trap	TOF	Orbitrap	FTICR
Mass limit	4 000	6 000	1000 000 (10 000)*	6 000	30 000
Resolution	2000	4000	5000 (20 000)*	140 000	500 000
Mass accuracy	100 ppm	100 ppm	200 ppm (10 ppm)*	< 5ppm	< 5ppm
Sensitivity	10^{-15} g	10^{-15} g	10^{-12} g	10^{-15} g	10^{-12} g

* TOF reflectron

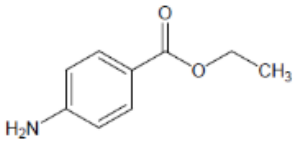
Is it enough to measure only mass?

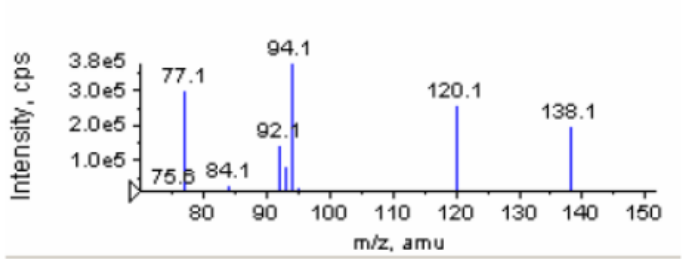
Ethenzamide





Benzocaine



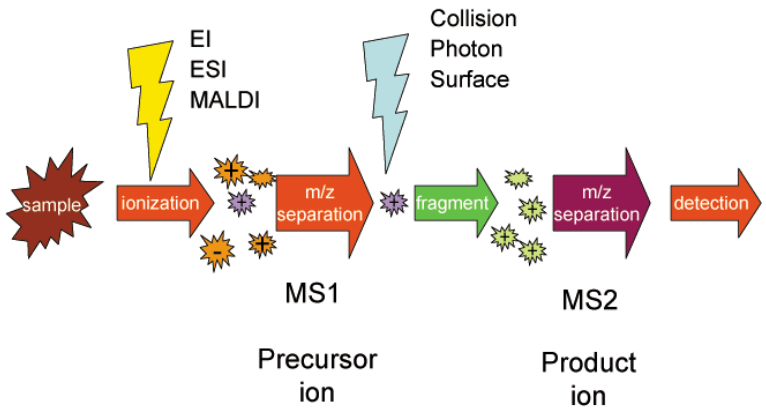


Formula: $C_9H_{11}NO_2$
Molecular mass: 165.189 g/mol

Tandem mass spectrometry *(MS/MS)*

A technique to **break down selected ions** (*precursor ions*) **into frag**ments (*product ions*).
The fragments then reveal aspects of the chemical structure of the precursor ion.

In a tandem mass spectrometer, ions are separated by mass-to-charge ratio in the first mass analyzer (MS1). Precursor ions of a specific m/z are selected and fragment ions (product ions) are created by some fragmentation process. The resulting ions are then separated and detected in a second mass analyzer (MS2).
Examples: QqQ, QTOF, Qtrap, TOF-TOF



Sample complexity in biological samples – the challenge

- Body fluids (urine, blood, cerebrospinal fluid (CSF))
- Tissue samples (biopsy, tumor, etc.)
- Other complex mixtures of various samples

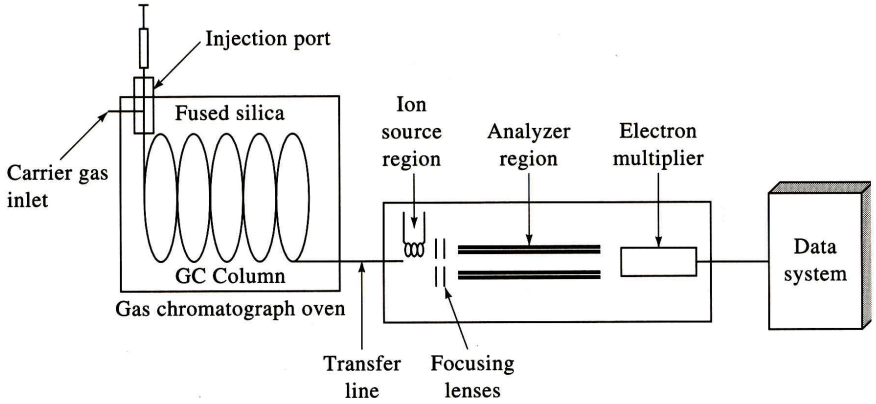
The solution is **separation**.

Off-line or on-line coupled separation.

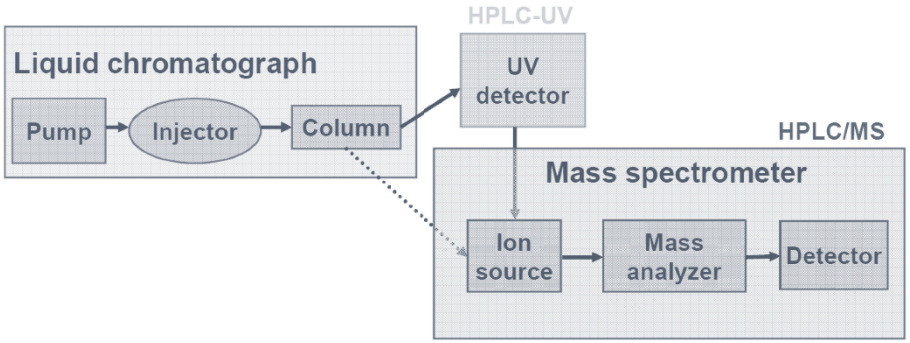
Chromatography	- gas chromatography - liquid chromatography (size-exclusion, ion-exchange, reversed-phase, hydrophobic interaction, etc.)
Electrophoresis	- gel electrophoresis (1D/2D-PAGE, DIGE) - capillary electrophoresis

Coupled systems

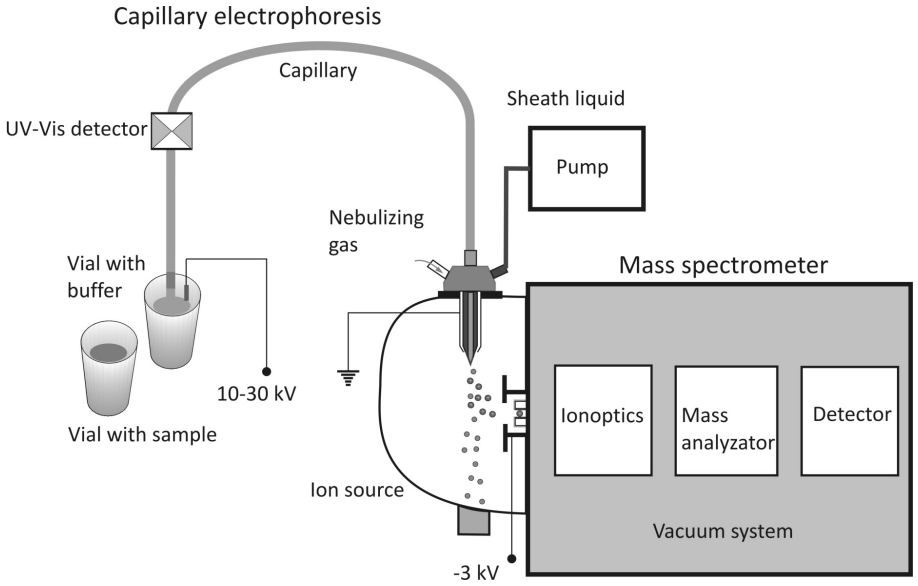
GC-MS system



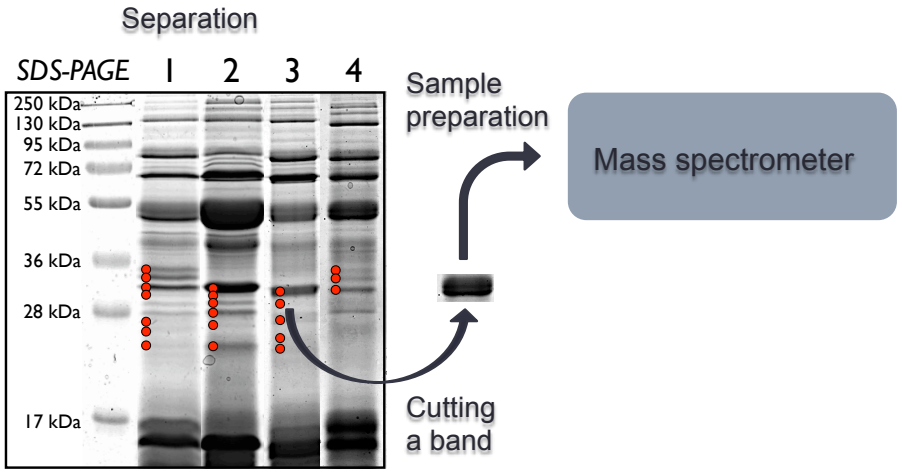
LC-MS system



CE-MS system



Off-line separation



Thank You!