

Mass spectrometry

Principles, equipment, usage

Principles of method

- Physico-chemical method for determination of the mass of atoms, molecules and molecular fragments after their transformation into ions.
- Possibility to characterize the structure of analyzed compounds.
- Connection of mass spectrometer with modern separation techniques to carry out quantitative and qualitative analysis of trace compounds and complex matrix.
- Availability of modern ion sources spread application areas of MS to high molecular non-volatile compounds (biochemical, clinical research).

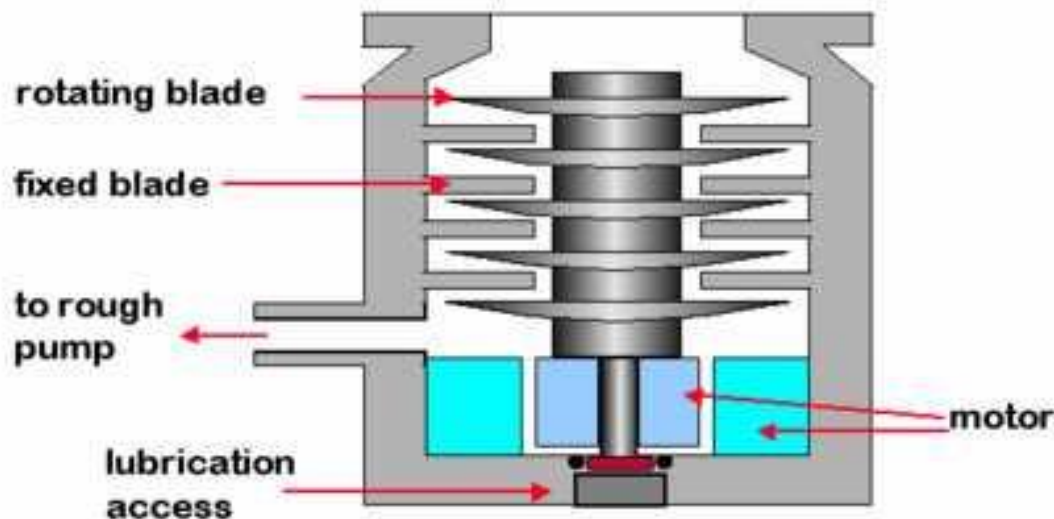
Usual arrangement of apparatus

- Basic building blocks:
 - *Ion source*
 - *Mass analyzer*
 - *Detector*
 - *Driving unit*
- Work in high vacuum.
- Ion source - transfer of analyzed compound into ionized state
 - In the space of ion source majority of fragmentation reactions take place
- Mass analyzer – allows the separation of a mixture of ions with different mass/charge ratios in space or time
- Detector – dependent on the number of dropping ions

- A part of each mass spectrometer is a powerful, usually two-stage vacuum generating system, allows to hold sufficient vacuum for the working conditions.

Turbomolecular pump

A turbomolecular pump relies on a series of blades or airfoils that spin at 30,000 - 90,000 RPM. This tends to deflect gas molecules down and out the outlet.



Ionisation and ion sources in MS

- All information given by mass spectrometry is concerned only with the charged particles (ions).
- Energetic demands depends on the type of the analyzed compound (7–16 eV).
- Used character of ionization substantially affects the application of a method.
- Development of new types of ion sources spread the applications into the area of non-volatile compounds with high molecular weight.
- Ionization yield in the majority of the techniques only exceptionally overcomes 0,01 %, the process of ionization limits the sensitivity and achieved limit of detection of the used method.

- According to the quantum of added energy – ionization techniques
 - **soft**
 - **hard**
- **Soft techniques** – excess of energy added to the molecule is low and probability of fragmentation of the primarily produced ion is low.
- **Hard techniques** – energy is enough to induce the fragmentation of the primarily produced ion.
- The most common are techniques of ionization from **gas phase** – analyzed compound is in advance vaporized into vacuum.
- Techniques of **ionization from condensed phase** are available for analysis of non-volatile compounds

Ionization

A number of ionization techniques exist.



Electron impact (EI)

Chemical ionization (CI)

Fast atom bombardment (FAB)

Field ionization

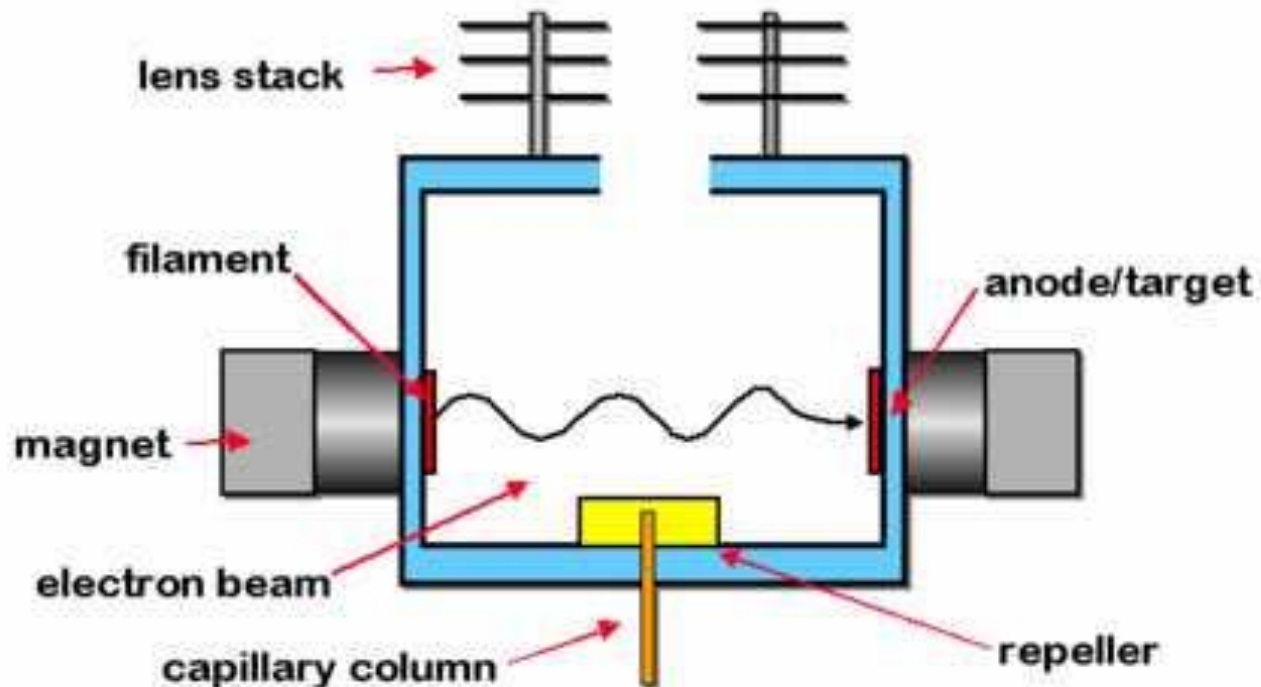
Plasma desorption

Ionization by electron impact (EI)

- Hard ionization technique from gas phase.
- Common and well described way of ionization.
- Analyzed compound interacts with a beam of accelerated electrons to produce radical-cation.
- Possibility of formation of radical-anion in respect to the high vacuum is deeply suppressed.
- Source of electrons – electrically heated rhenium or tungsten fiber
- The beam of electrons is directed through the space of the ion source to the anode.
- Formed ions from the space are repelled with the help of additional electrode - repeller.
- Usage: structural info about small molecules

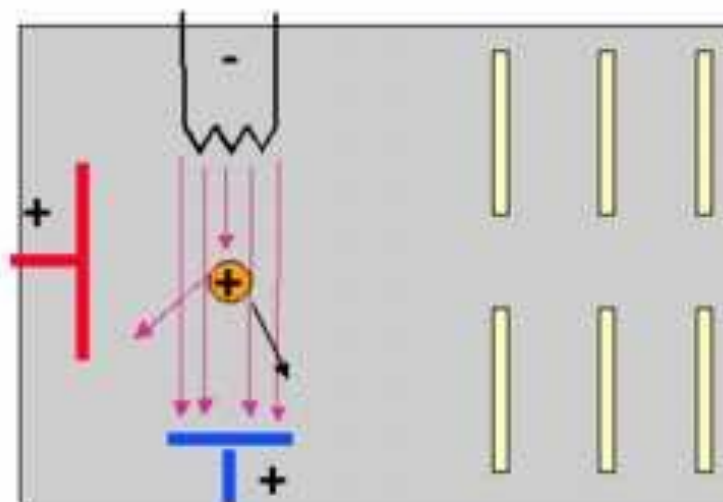
Electron impact source

HP type EI source showing direct capillary interface.



Electron impact source

When a sample molecule enters the source, it passes through the electron beam and is ionized.



Chemical ionisation (CI)

- Soft ionization technique from gas phase.
- Primary source of energy – beam of accelerated electrons.
- Energy of electrons is transferred to the analyzed compound via the reaction medium (**methane**, propane, hydrogen, water, ammonia, hydrogen/helium)
- Reaction medium is in the ionization chamber under pressure
- Reaction cations (CH_5^+ a C_2H_5^+) interact with the molecule of the analyte M to give rise to quasi-molecular ion $[\text{M}+\text{H}]^+$ or $[\text{M}-\text{H}]^-$
- Construction of ion source for CI is very similar to source of EI.

Ionization using fast atom bombardment (FAB)

- accelerated atoms of rare gases (Ar, Xe) are used for the ionization process
- Soft ionization technique from condensed phase.
- Impact of atoms causes desorption and ionization.
- Low fragmentation – intensive peaks of molecular ions – determination of molecular weight.
- Usage for analysis of thermally unstable compounds.

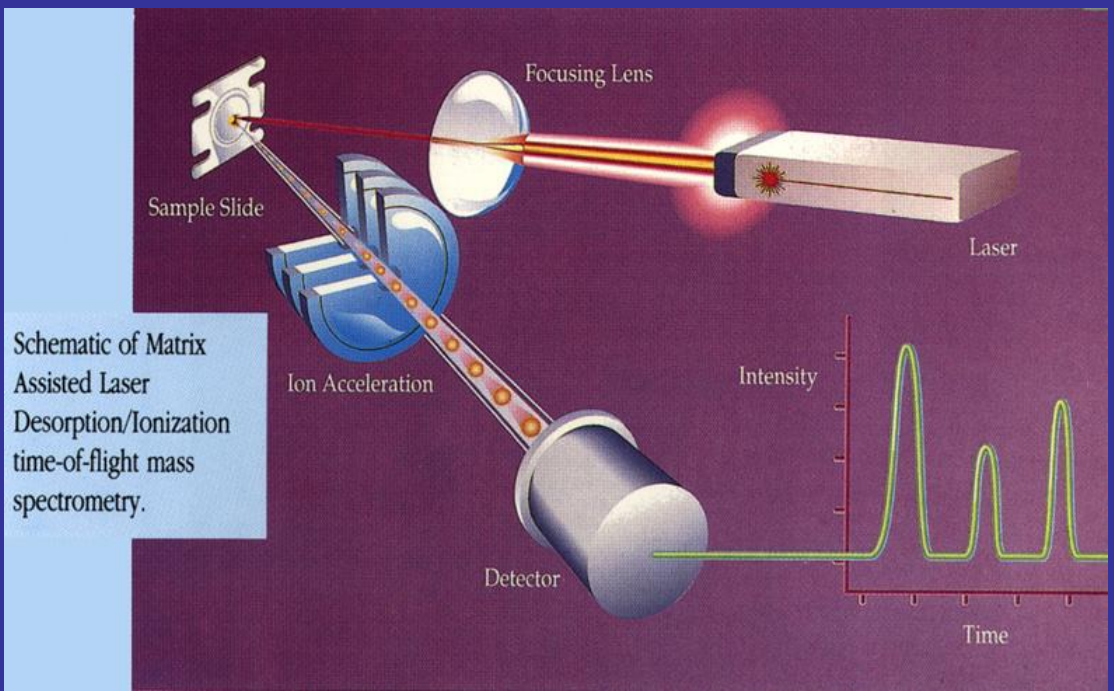
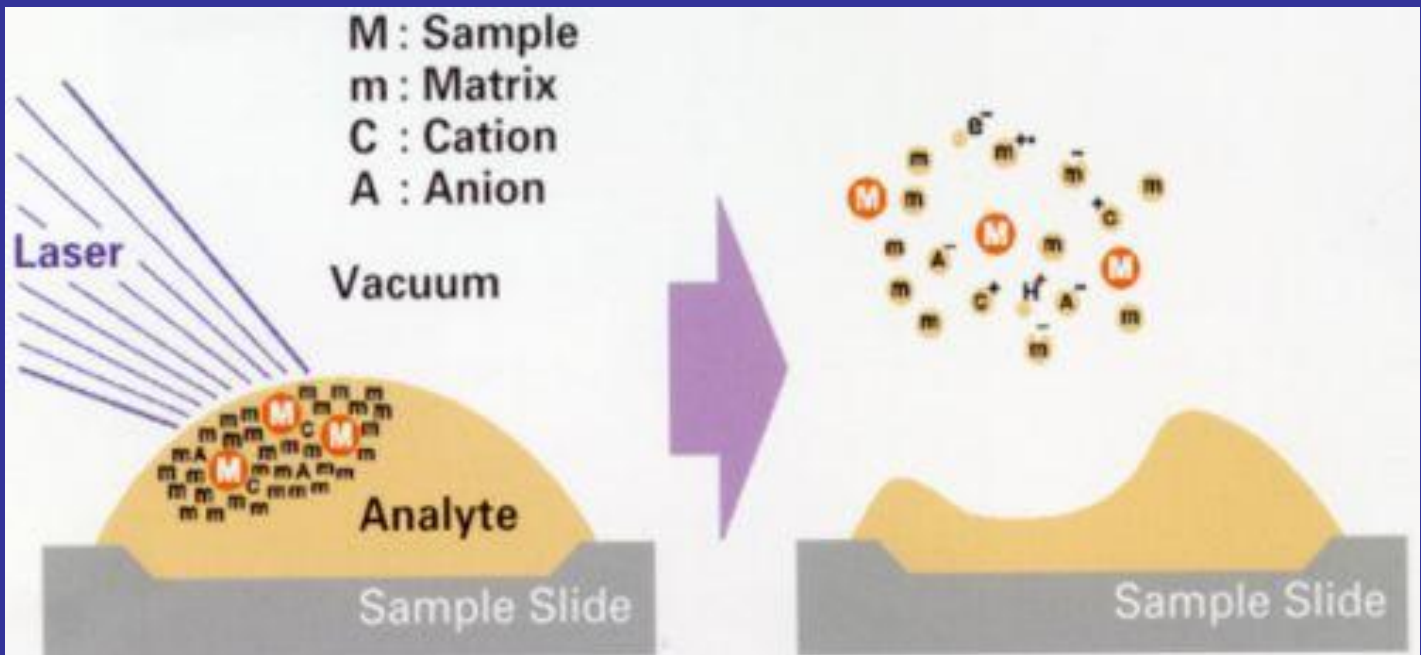
Ionization using fast ions (SIMS)

Secondary-ion bombardment mass spectrometry

- bombardment of analyzed molecules with accelerated ions Cs is used for the ionization
- Soft ionization technique from condensed phase.
- Used for analysis of thermally unstable non-volatile compounds.

Desorption photoionization (MALDI)

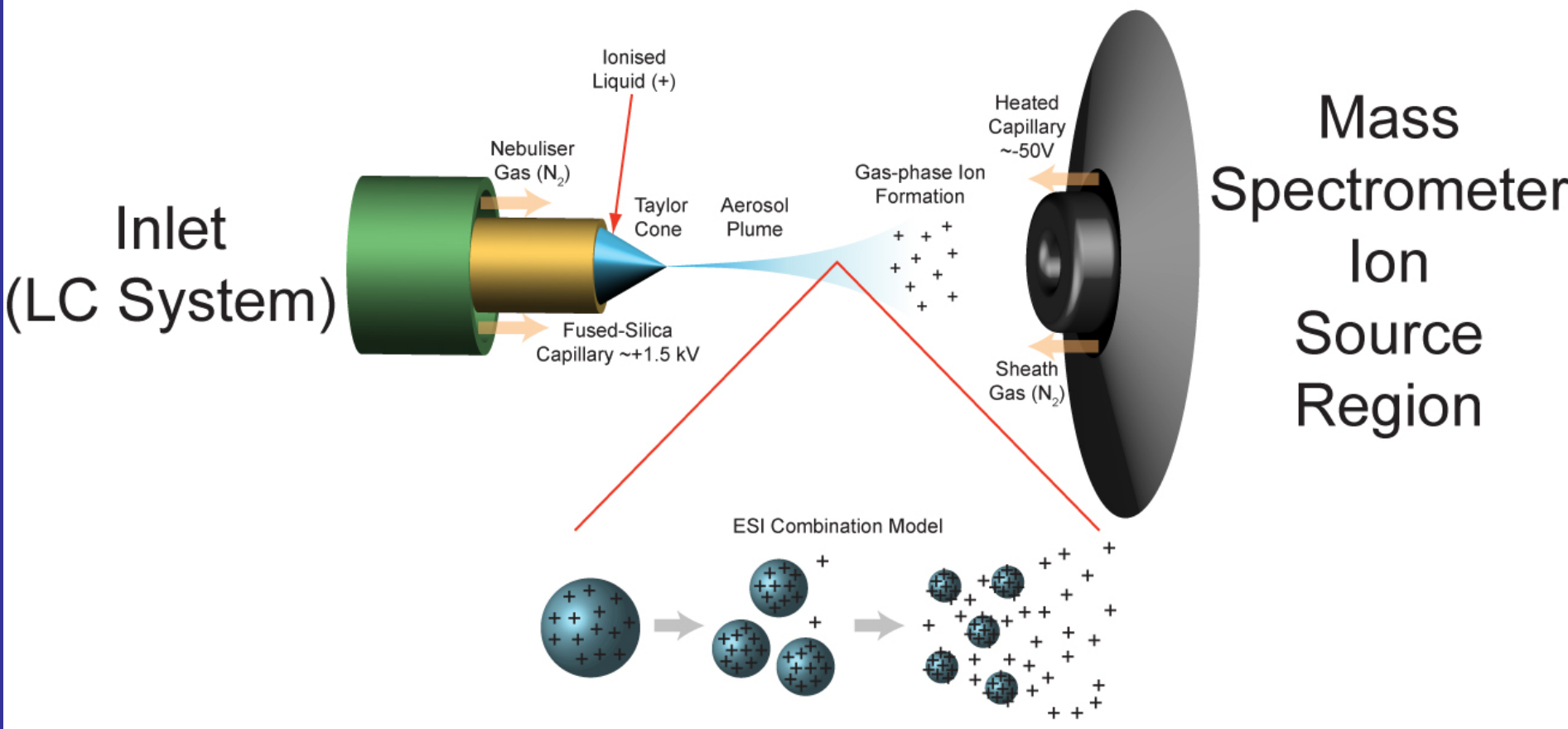
- Technique of ionization from condensed phase using laser radiation with suitable wavelength and intensity.
- Matrix – for example nicotinic or salicylic acid .
- Lasers working in infrared or ultraviolet spectrum are used as source of radiation
- MALDI is used mostly in combination with time-of-flight analyzer.
- Desorption photoionization is used for example for biopolymer analyses.



Spray ionization

- Soft ionization techniques from liquid phase.
- **For example : thermospray (TSI) and electrospray (ESI).**
- Transfer of ions from liquid into vacuum is caused by rapid drying of droplets.
- If the process of drying is fast enough, surface charge of droplets is high enough to achieve the transfer of ions into the vacuum without the addition of an external quantum of energy.
- Spray ionization techniques are often connected with combination of MS with flow-based analytical techniques (HPLC-MS).

Electrospray Ionisation (ESI) and Ion Source Overview



Mass analyzers

- Use for the dispersion or filtration of ions according to m/z (mass to charge ratio), or according to their kinetic energy.

Magnetic mass analyzer

- The oldest but one of the most perfect analyzer regarding the mass range and resolution.
- Enables space division of monoenergetic ion beam according to the m/z values.
- Construction electromagnetic sector tube, in which pass ions

- Ions accelerated in ion source possess kinetic energy:

$$E_k = m \times v^2 / 2 = z \times V$$

- m ... weight of ion
- v ... velocity of movement of ion
- z ... ion charge
- V ... acceleration voltage of ion source

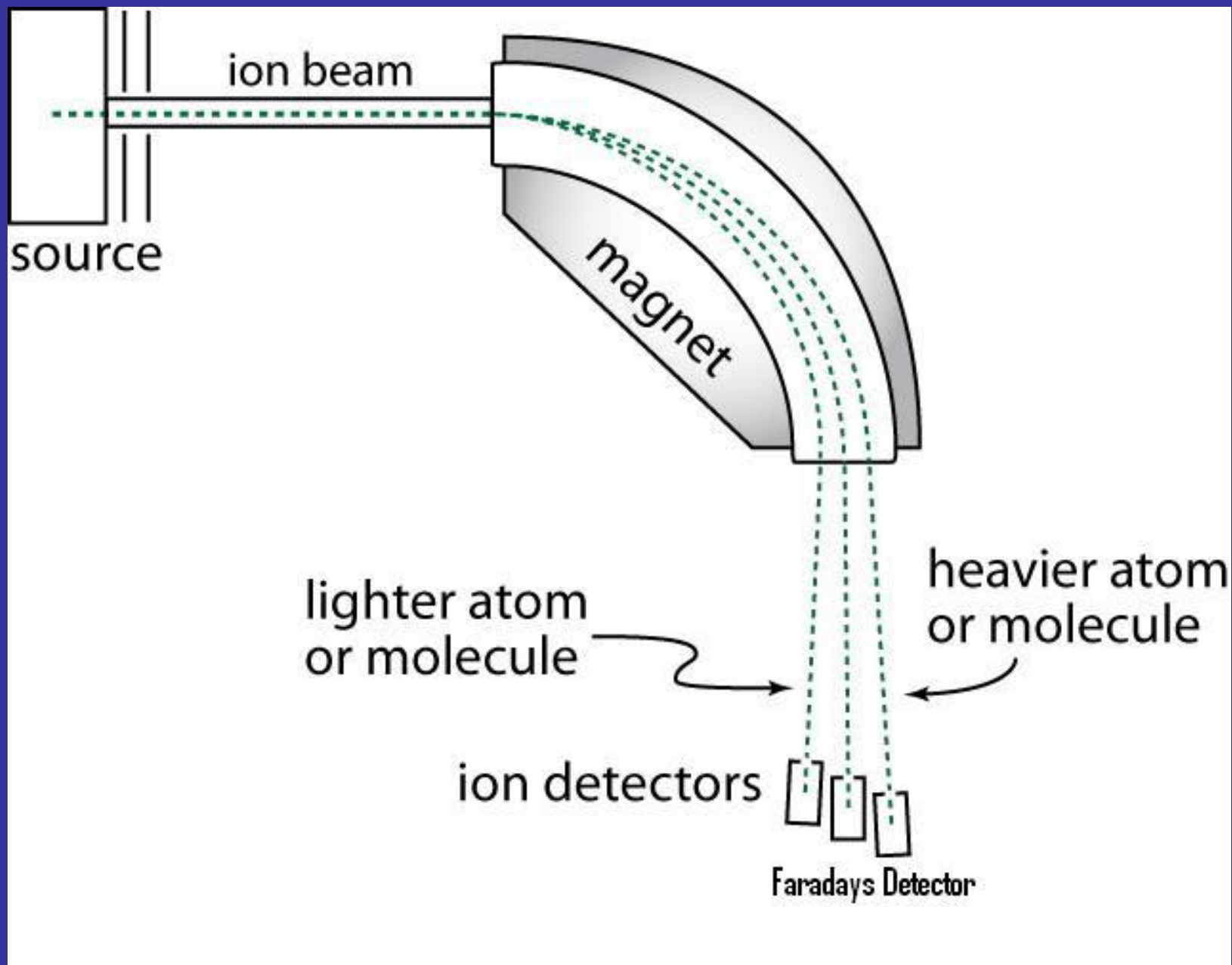
- In a homogenous magnetic field, ions affected by radial Lorentz force in balance with centrifugal force:

$$B \times z \times v = m \times v^2 / r$$

- B ... magnetic induction
- r ... radius of ion path
- Solution of quotations will give you a relation for mass spectrometer in form:

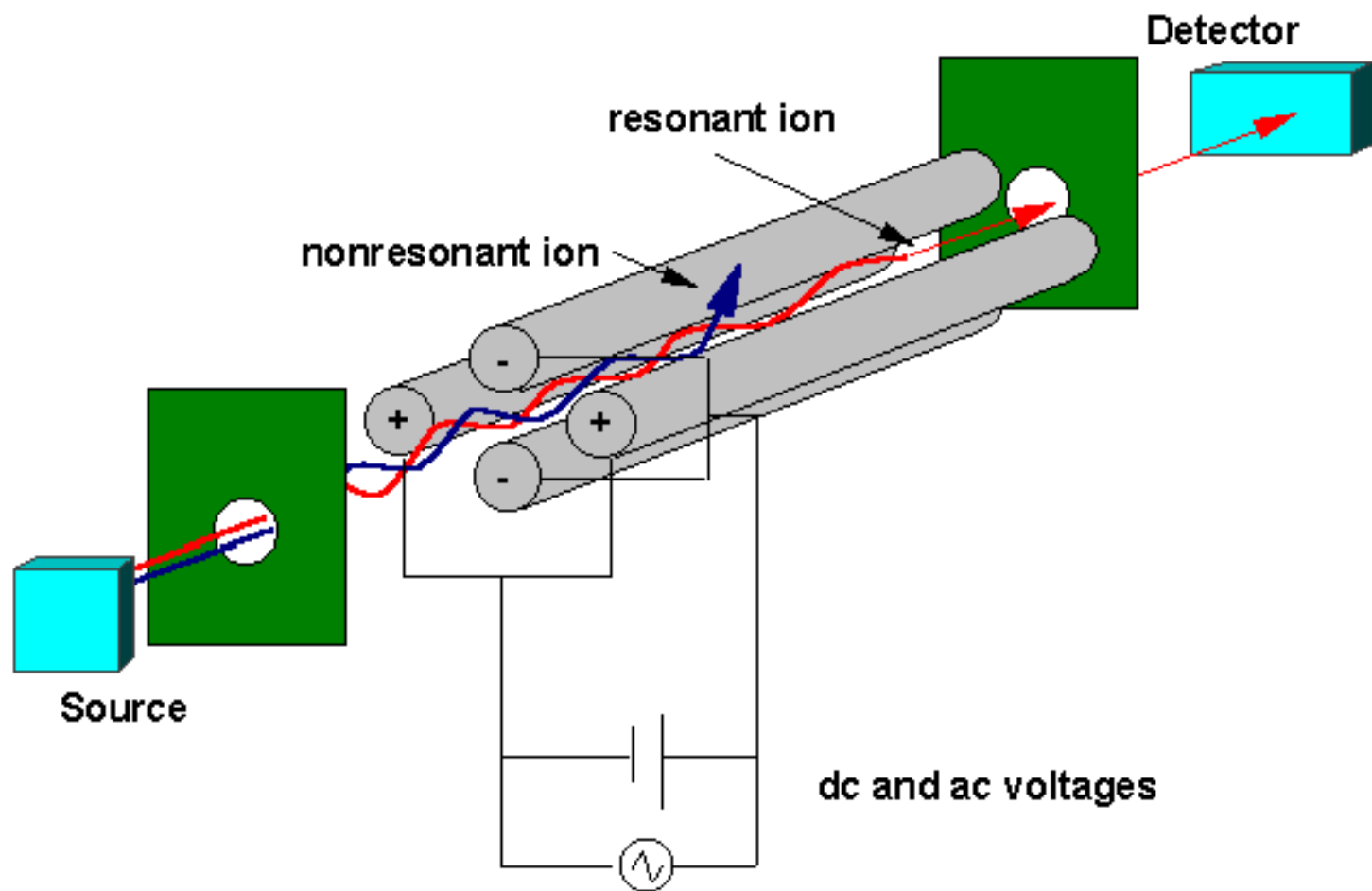
$$m/z = B^2 \times r^2 / (2 \times V)$$

- Ions with different ratio of m/z possess path with different diameters and therefore they are dispersed into space according their weight



Quadrupole analyzer (filter)

- Quadrupole filter is often a part of low resolution mass spectrometers connected with GC and HPLC
- Ions entering the space between rods enter alternate electromagnetic field and start to oscillate
- Proper relation of alternate and direct current and their values enable to pass ions with certain m/z
- Equipment reacts as filter with setting to certain value of m/z
- Changing of voltage allows to pass through the filter ions with large scale of m/z

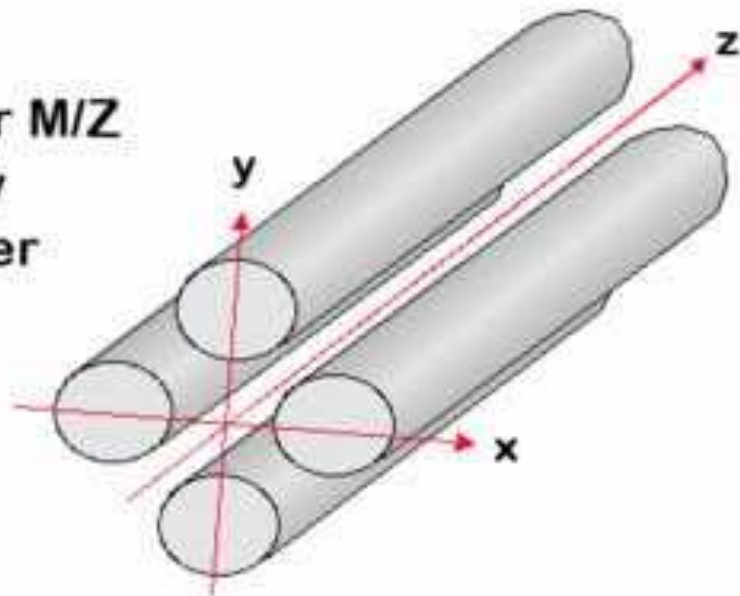


Quadrupole

This analyzer consists of four rods.

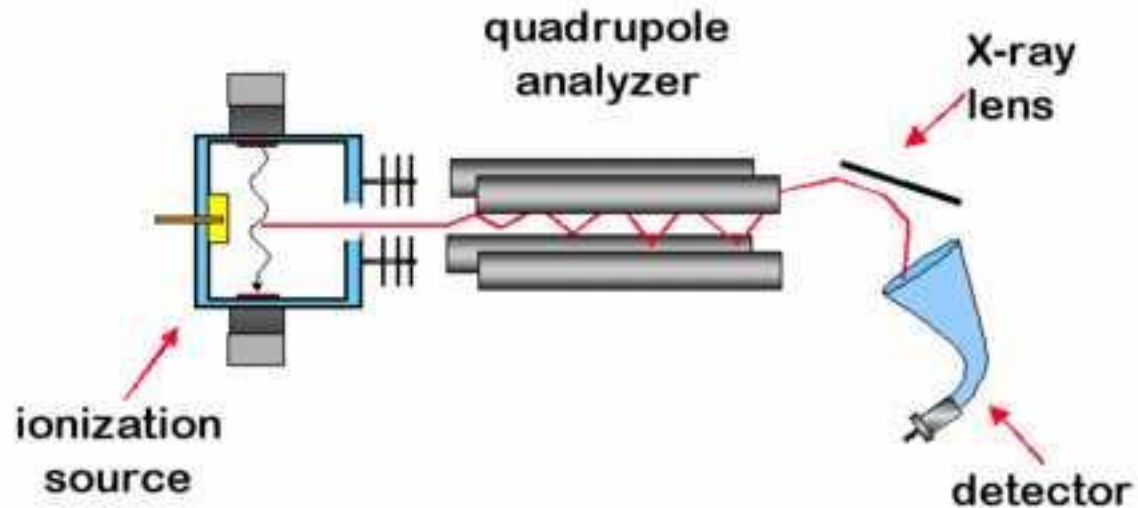
Rods operate in pairs (X or Y) and each carries a voltage.

Only ions of the proper M/Z value can successfully traverse the entire filter (Z axis)



Quadrupole

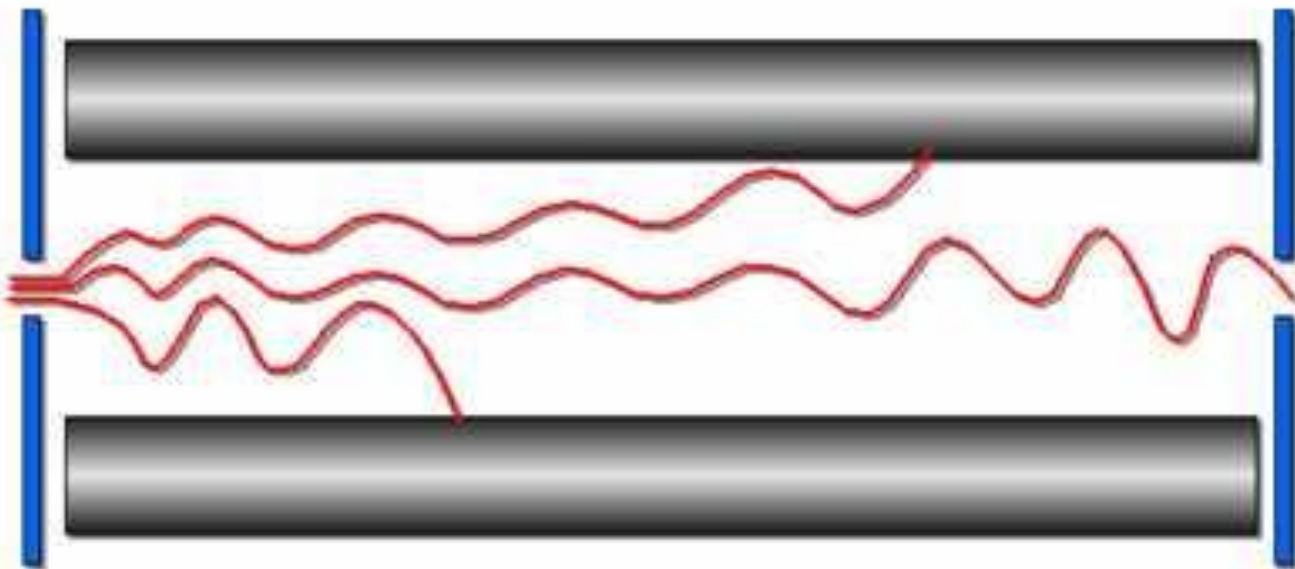
Schematic of a quadrupole MS system.



Quadrupole

At any set of conditions, only ions of a specific M/Z can successfully travel through the entire filter.

Others are drawn into the rods.

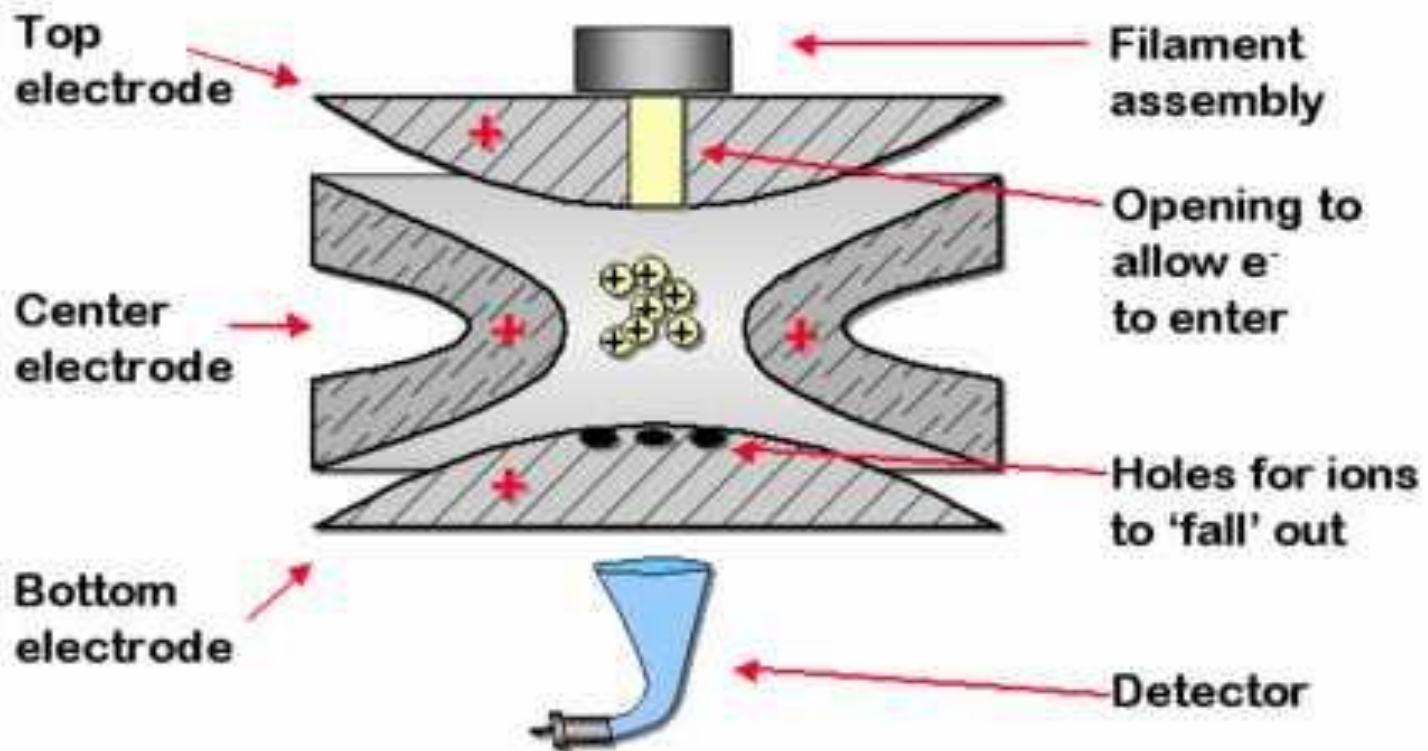


Ion trap

- Equipment using electromagnetic field to close ions in limited space
- Classical ion trap is constructed from entrance and outlet electrodes with cylindrical shape and central ring electrode
- Entrance and outlet electrodes are earthed, central electrode is equipped with high-frequency voltage with changing amplitude
- Ions are enforced to move inside the trap in closed circular pathways
- With increasing amplitude of voltage are ions with increasing m/z enforced to enter instable trajectories and are leaving the space of ion trap directed to detector

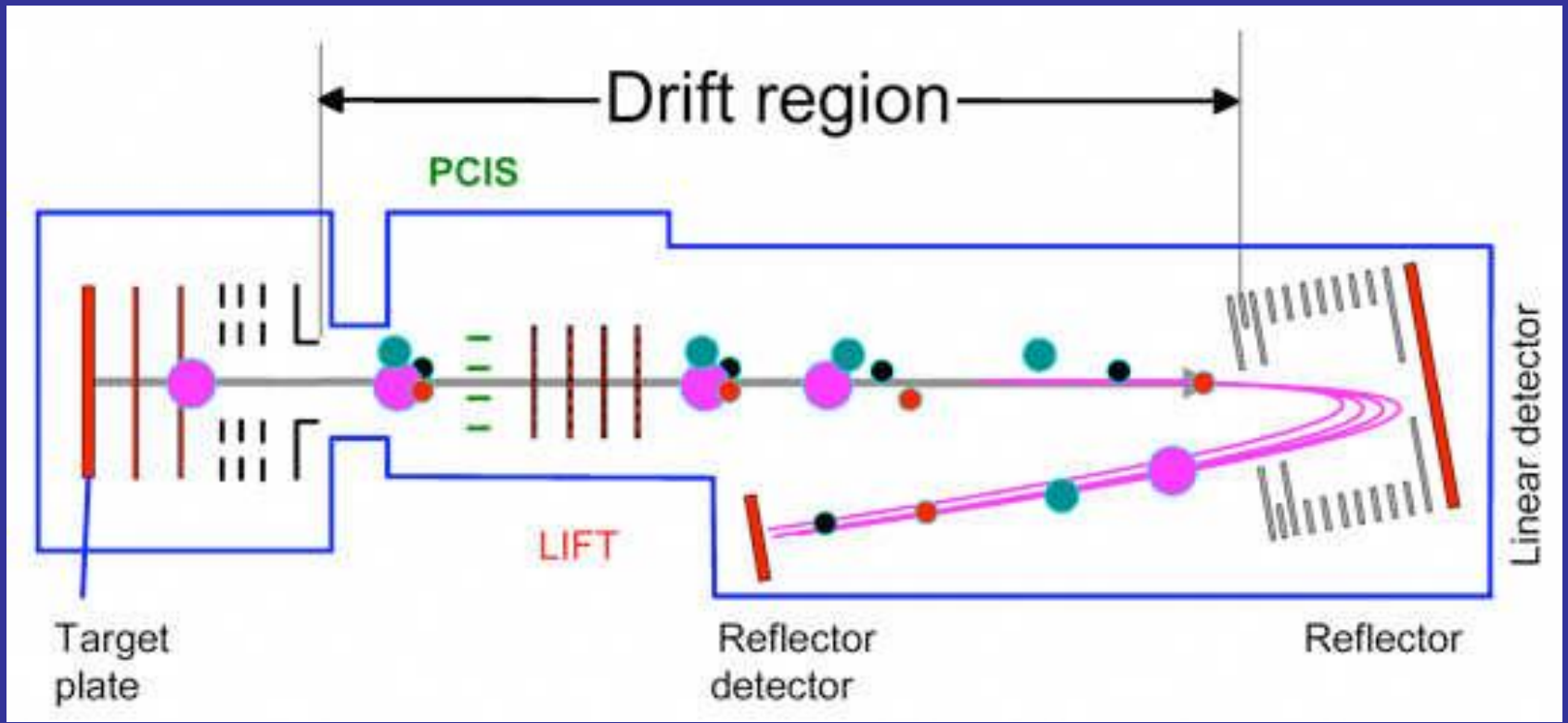
Ion trap

Cross-sectional view of an ion trap.



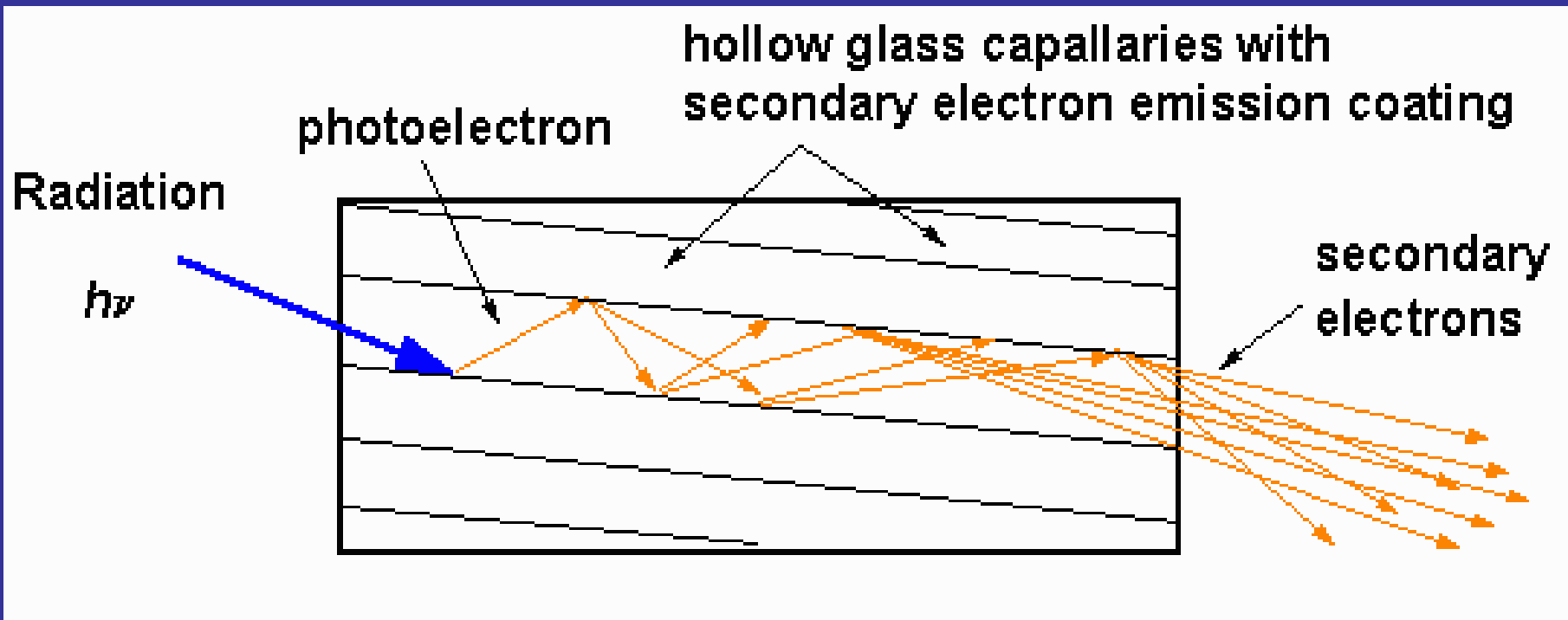
Time of Flight analyzer (TOF)

- The simplest mass analyzer, in principle constructed as empty tube only
- The time dependent separation ions according m/z is based on different time of their flight from ion source into detector
- Ions with greater m/z move with lower velocity than ions with lower and enter detector in later time
- Obtained resolution is dependent on length of path, which ions fly in TOF
- TOF needs ion source working in pulse mode



Detectors in mass spectrometry

- ***Detectors for direct measurements*** – detecting the electric current formed by direct impact of analyzed ions.
- ***Multiplier detectors*** - using effect of multiplication of electrons released from primary conversion dynode after impact of analyzed ions.
- Detectors for direct measurement are used for precise isotopic composition of elements, for example for age analysis. Usually part of unique systems.
- Multiplier detectors are common type of detector for classical MS measurement, they are able to supply a good signal for the analysis of single ions.



Mass spectrum

- Mass spectrum represents the dependence of detector response intensity on value of m/z
- Mass spectra are often transformed into normalized form (%)
 - The most intense peak value is assigned as 100 %
- In mass spectrum we often observe peak corresponding to **molecular ion** and peaks corresponding to **fragment ions**

Molecular ion

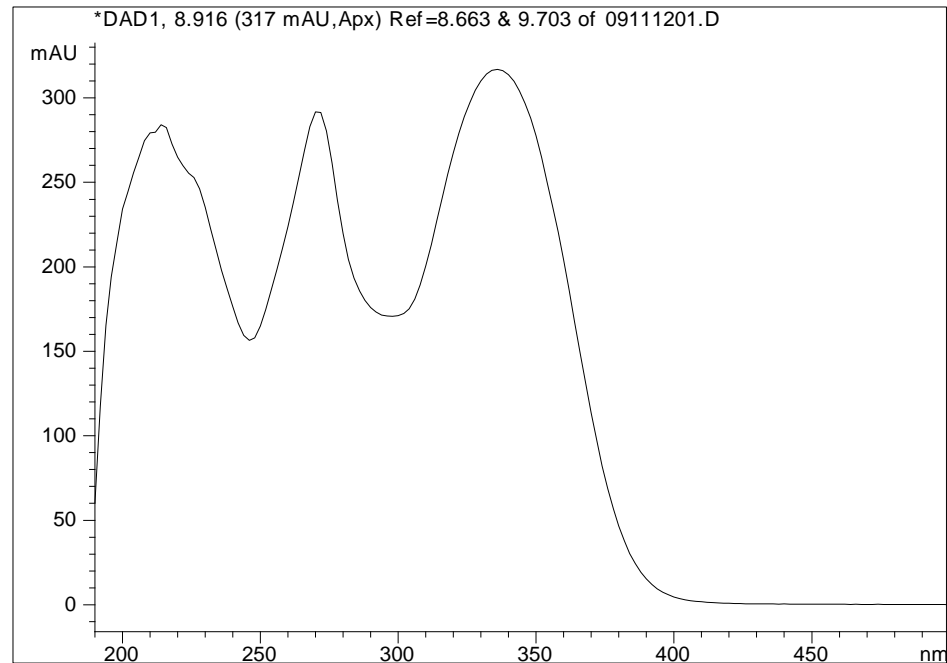
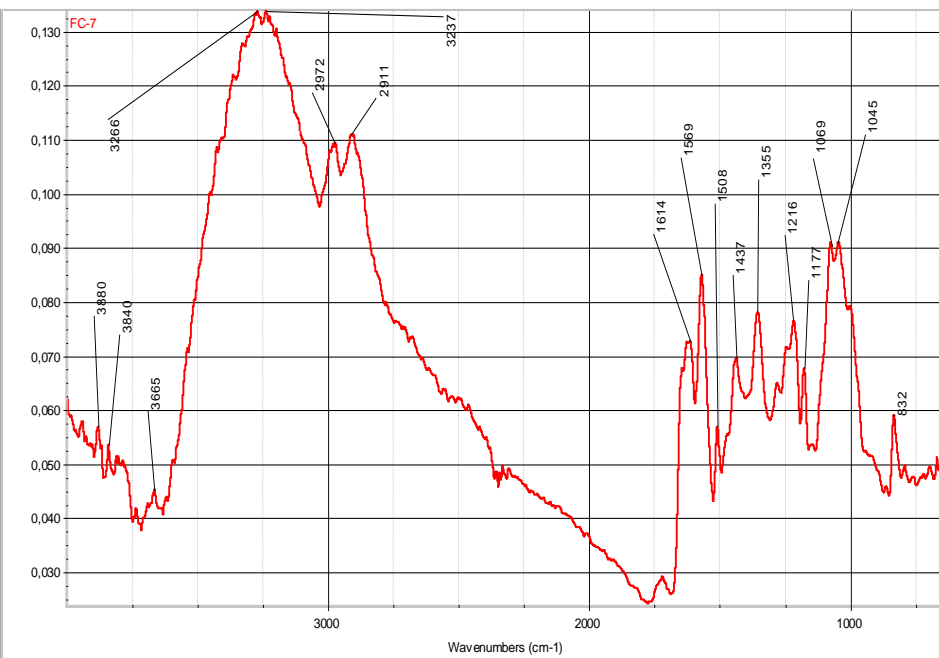
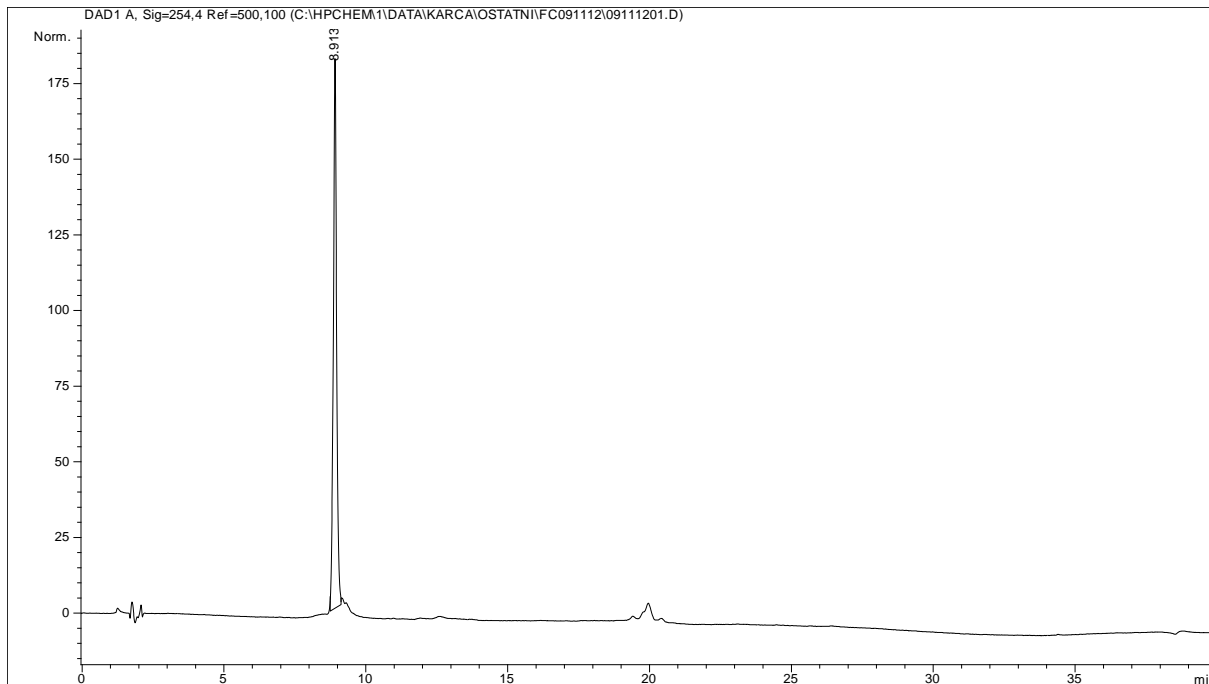
- Molecular ion possesses very important function in elucidation of structure of analyzed compound
- Molecular ion possesses the highest value in the spectrum (with exception of isotopic and quasi-molecular ion peaks)
- Molecular ion corresponds with other ion peaks in spectrum
- Its relative elementary abundance it must correspond with postulated structure
- Intensity of molecular ion peak possesses direct relation to structure of analyzed compound
- With increasing number of double bonds and cycles in structure intensity of molecular ion peak increases
- Peak corresponding to molecular ion can be dominant in the spectrum for some compounds (strychnine) , for others, it can be very insignificant

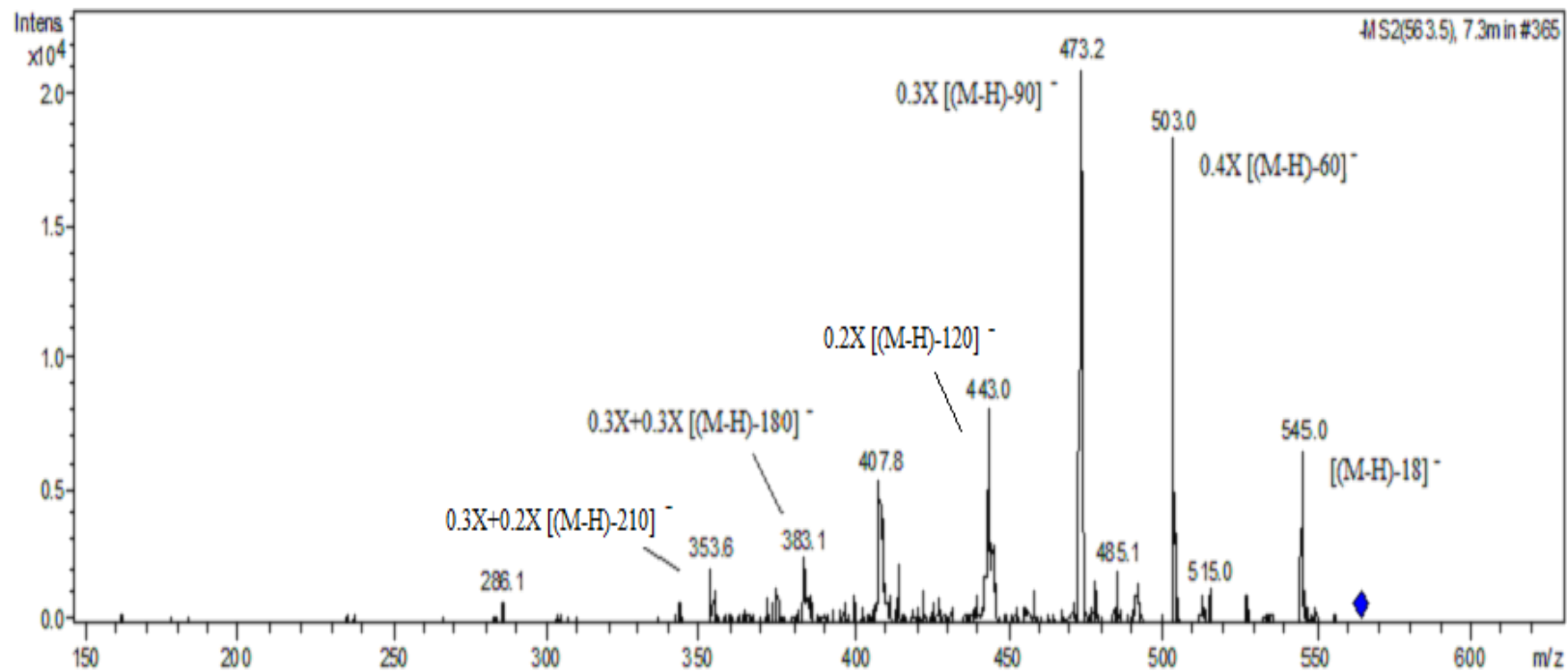
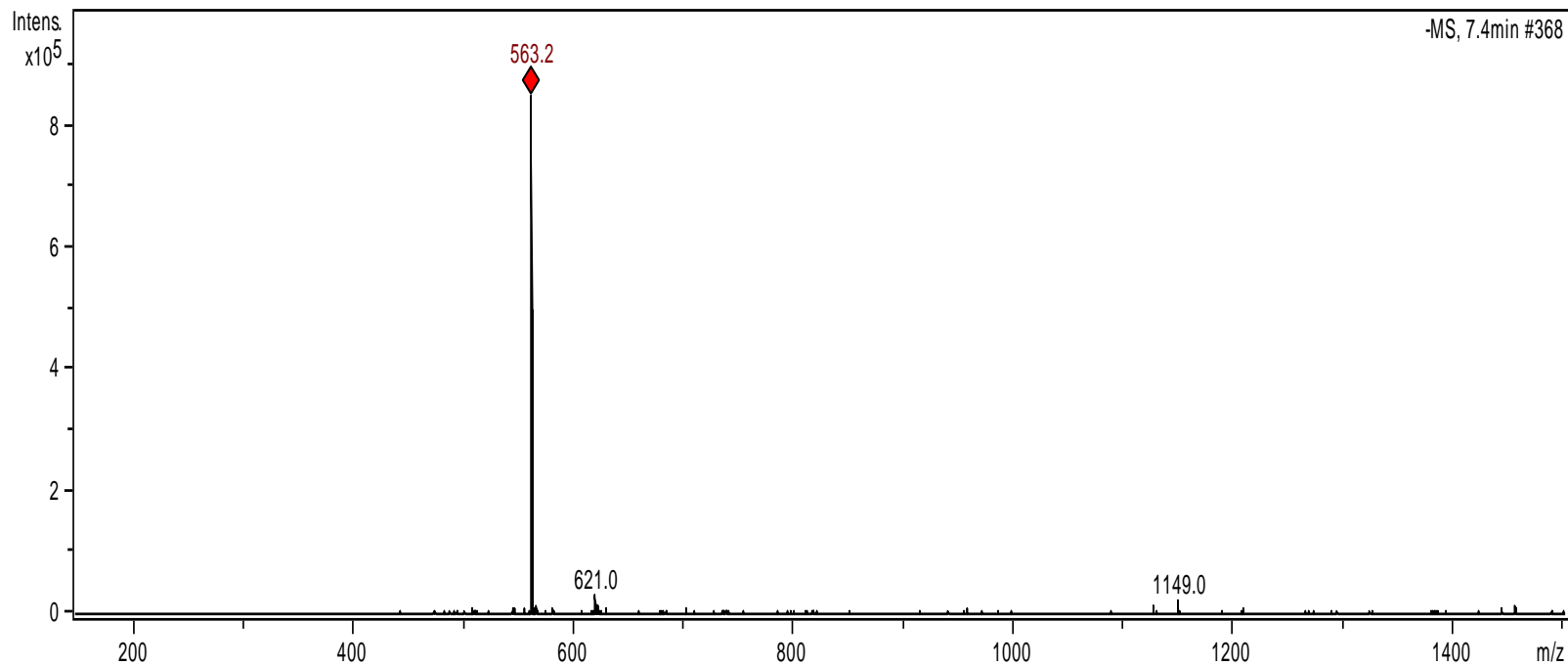
Fragmentation in mass spectrometry

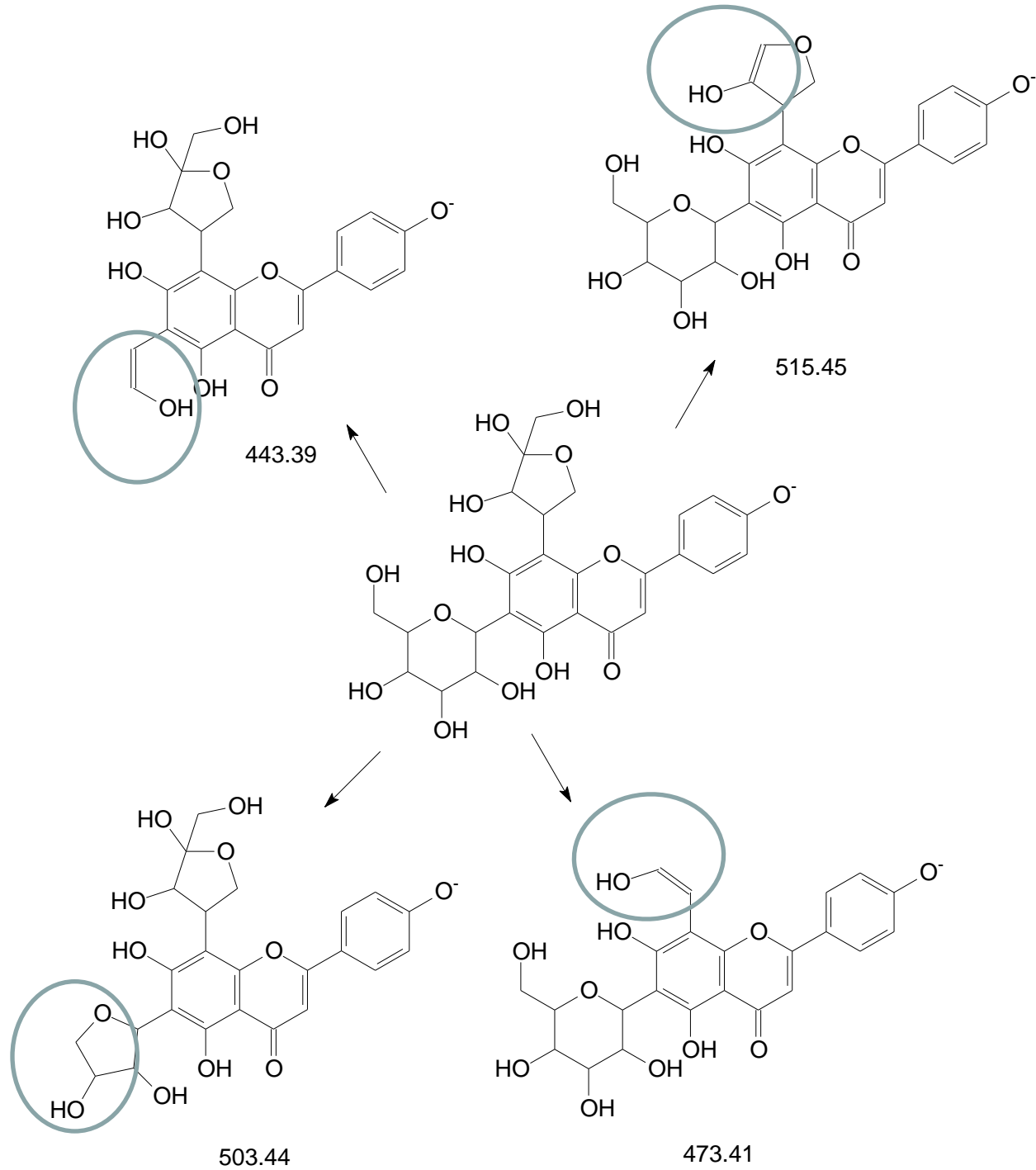
- Possibility of detailed recognition of the process of fragmentation is limited by the low knowledge of structures of the formed daughter ions
- If a molecular ion that possesses the exceeding internal energy, it is cleaved to produce ion fragment and usually electro-neutral particle
- Possibility of fragmentation of molecular ion rises with increasing weight, double bonds increase the stability of the molecular ion
- Rate of fragmentation depends on surplus of energy used for ionization
- Mechanism of ionization depends on the type of apparatus
- EI - in the process of ionization is the most probable cleavage of electron with the lowest ionization energy, from energetically highest bonding molecule orbitals or non-bonding orbital
- Because of the low pressure during EI ionization procedure, the reactions that take place in the ion source are almost completely monomolecular
- Relative abundance of the ion in the spectrum is based on the level of fragmentation leading to its rise and on its stability
- Fragment ions can further decompose because of surplus of energy

Important mechanisms of fragmentation

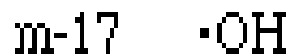
- fission of σ -bond (initiated by radical center)
- a fission initiated by radical center
- fission initiated by charge center
- fission of cyclic structures connected with cleavage of several bonds
- overlaps initiated by radical center
- overlaps initiated by charge center



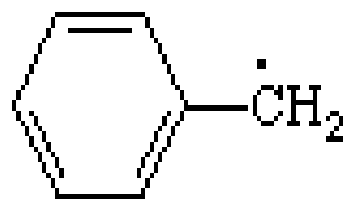




Commonly Lost Fragments



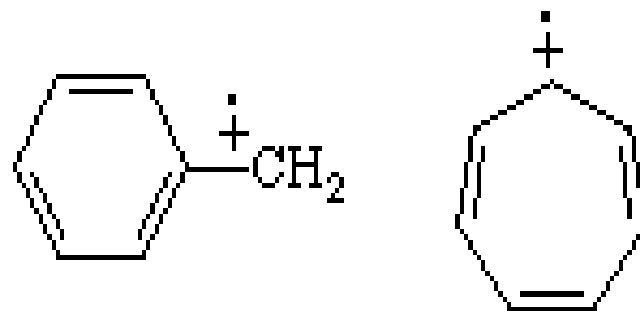
m-91



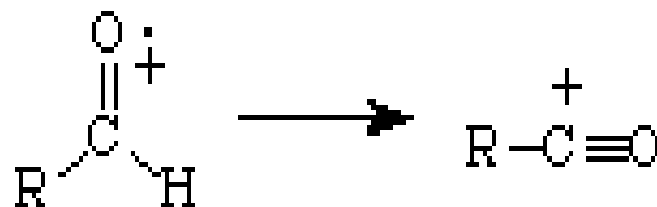
Common Stable Ions

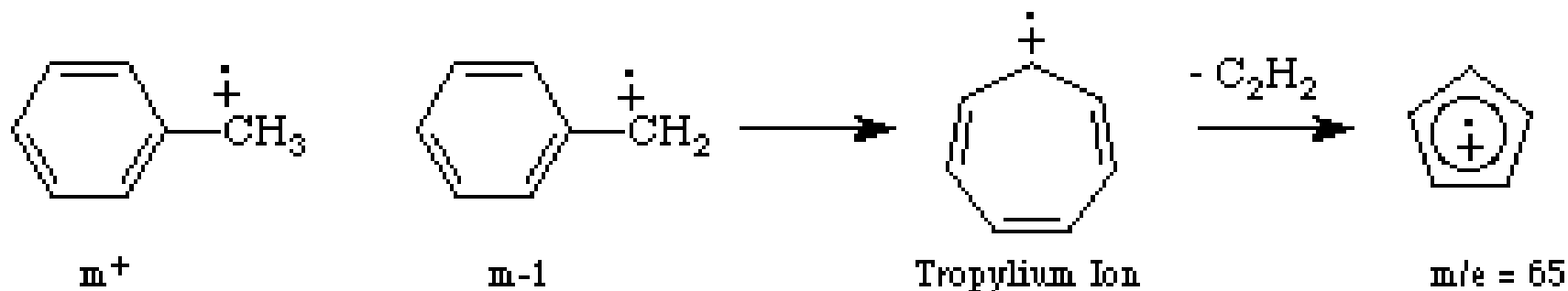
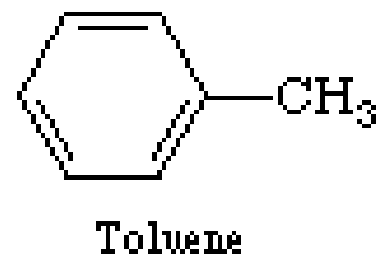
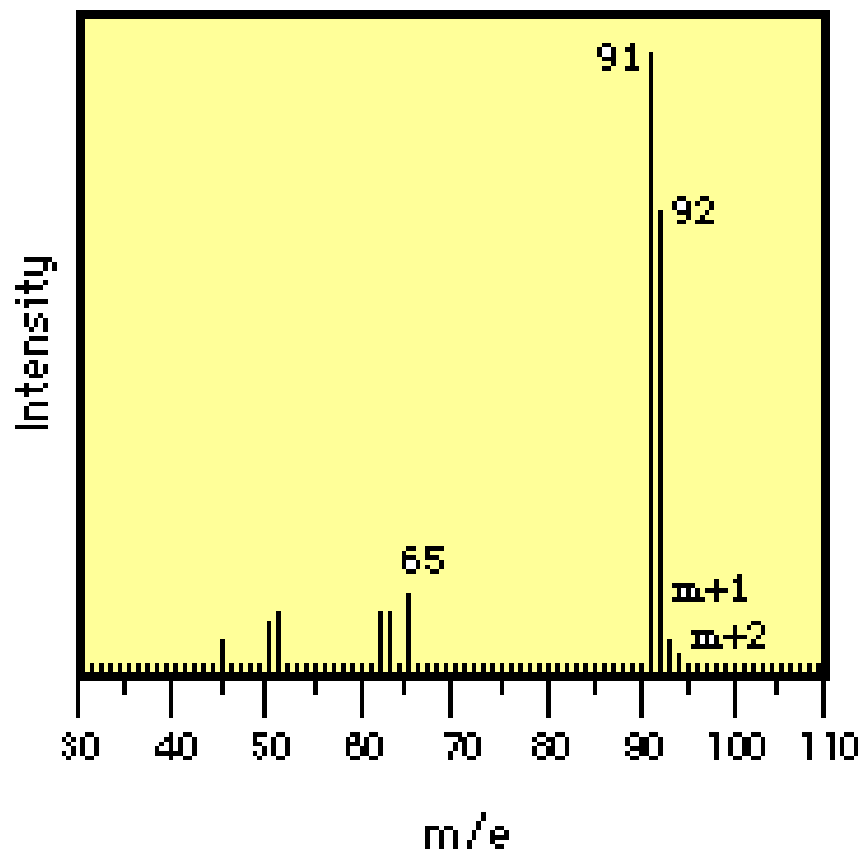


m/e = 91

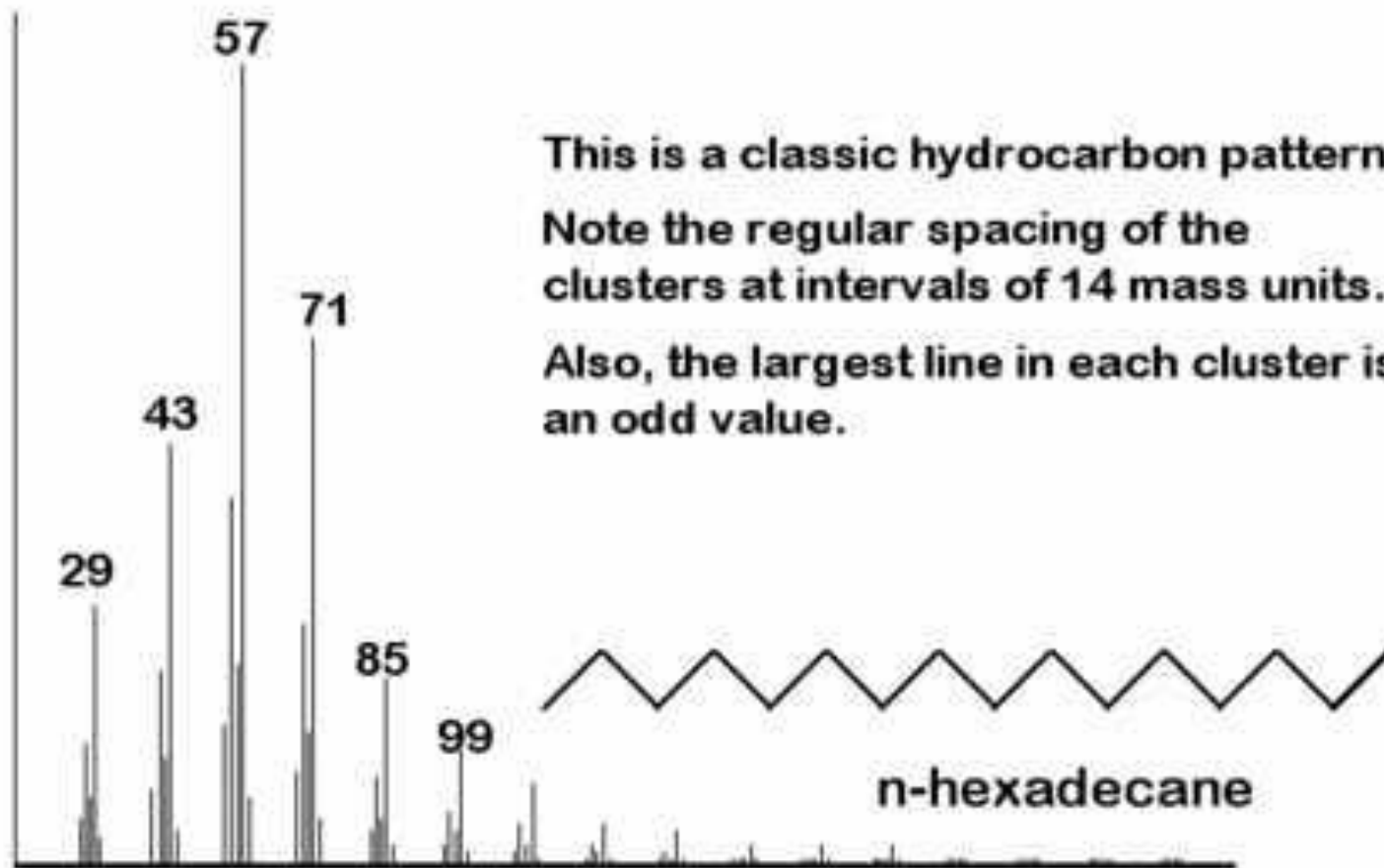


m/e = m-1





Saturated hydrocarbons



Usage of mass spectrometry

- Structural analysis and identification of chemical compounds
- Information about functional groups present in molecules of analyzed compound
- Possibility of determination of isotopic composition
- Fast identification of chlorinated or brominated compounds on the base of presence of isotopes (^{79}Br , ^{81}Br ; ^{35}Cl , ^{37}Cl)
- Usage of MS apparatus as detector for GC and HPLC
- Trace analysis of gaseous mixtures of organic and inorganic origin
- Determination of impurities in very pure substances
- Possibility of application in quantitative analysis on the base of linear dependence between ion current and compound concentration
- Analysis of surfaces of solid compounds using secondary ions mass spectrometry