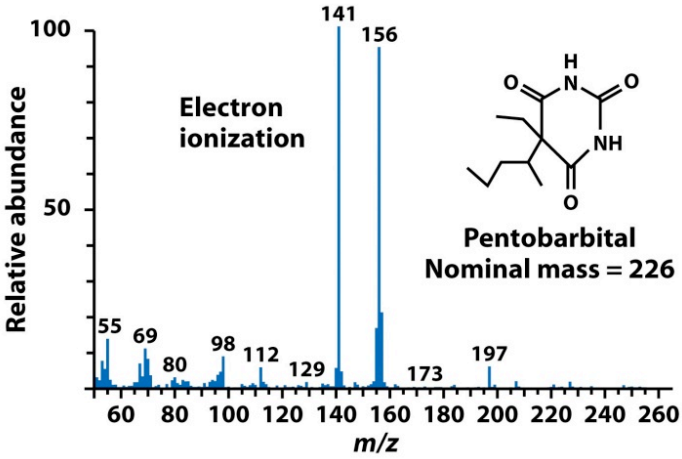
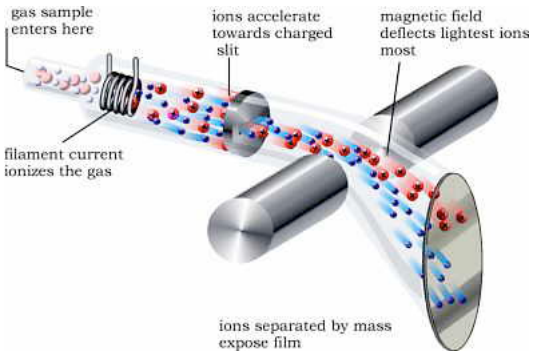
Chapter 21. Mass Spectrometry





- (left) Mass spectrum of small organic molecules
- (right) Mass spectrometer differentiate ions of different m/z by manipulating them (pulling them and bending their flight path) with an applied electric/magnetic field.

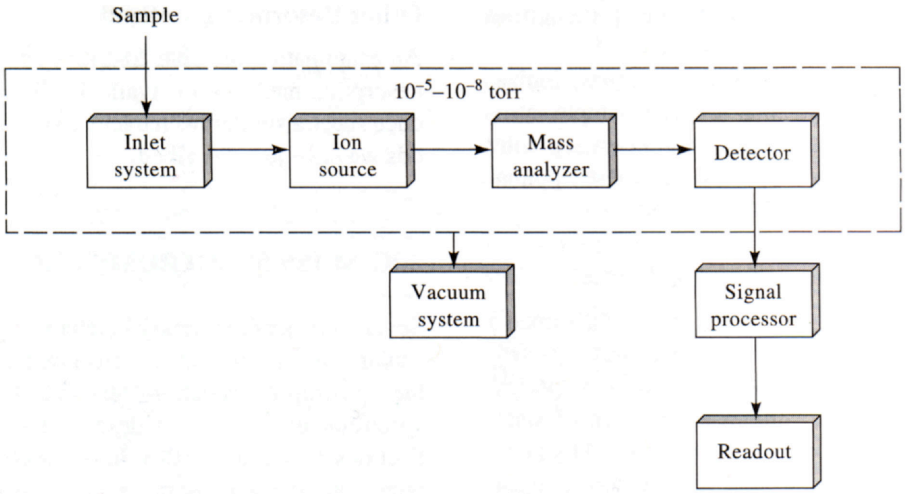
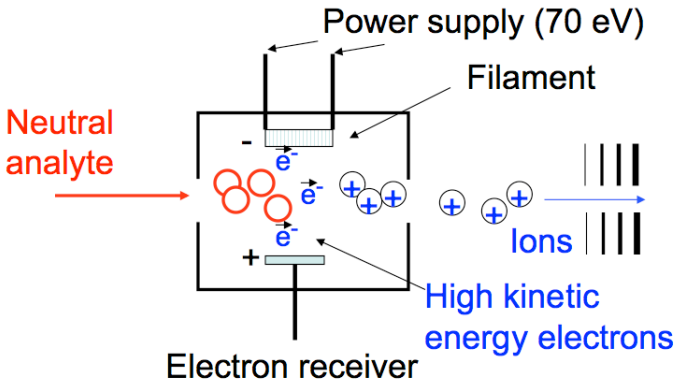


Figure 20-10 Components of a mass spectrometer.

Ionization Methods

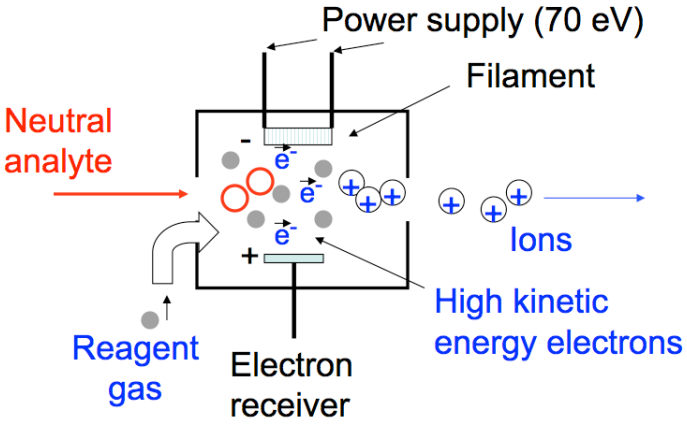
1. Electron Impact Ionization (EI) - analyte molecules collided with a beam of energetic elections. This is a hard ionization method that is very effective in ionization, but also in fragmentation of analyte molecules.

$$M + e^- (70\ eV) \rightarrow M^{+\cdot} + e^- + e^-$$



$$M^{+\cdot} \rightarrow \text{fragment ions}$$

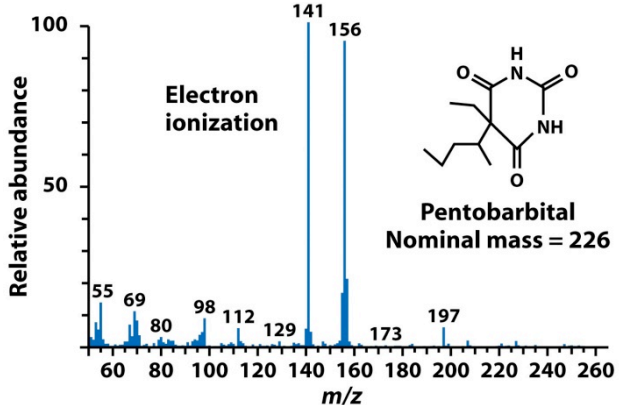
2. Chemical Ionization (CI) - electron impact ionization source is filled with reagent gas. Most electrons are absorbed by the gas. Collision with ionized reagent gas results in ionization of analyte. A softer ionization method, less fragmentation.

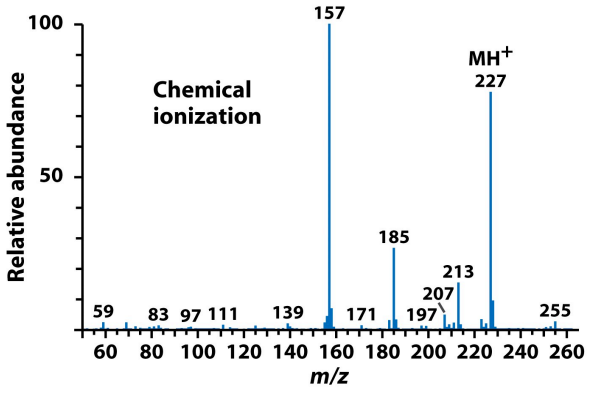


$CH_4 + e^- \rightarrow CH_4^{+\cdot} + 2\ e^-$ EI

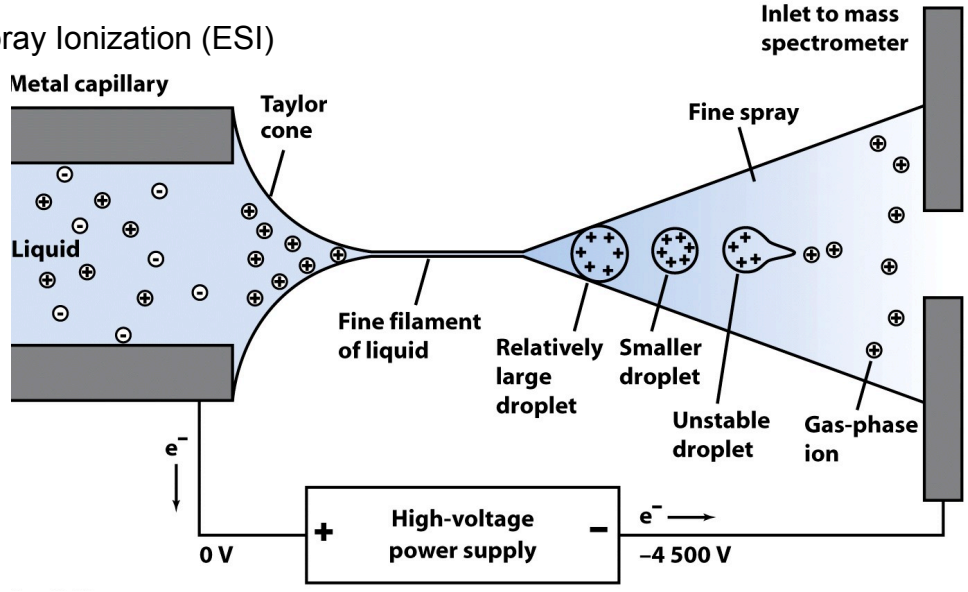
$CH_4^{+\cdot} + CH_4 \rightarrow CH_5^+ + CH_3^\cdot$ Ion/Molecule Rxn

$CH_5^+ + M \rightarrow MH^+ + CH_4$ Proton transfer to analyte



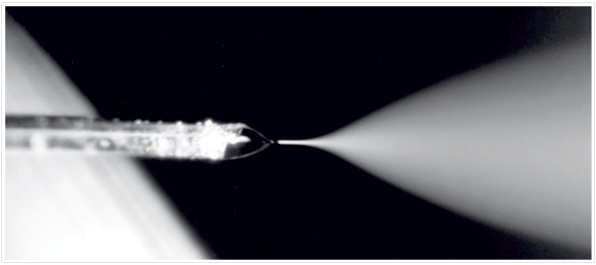


3. Electrospray Ionization (ESI)

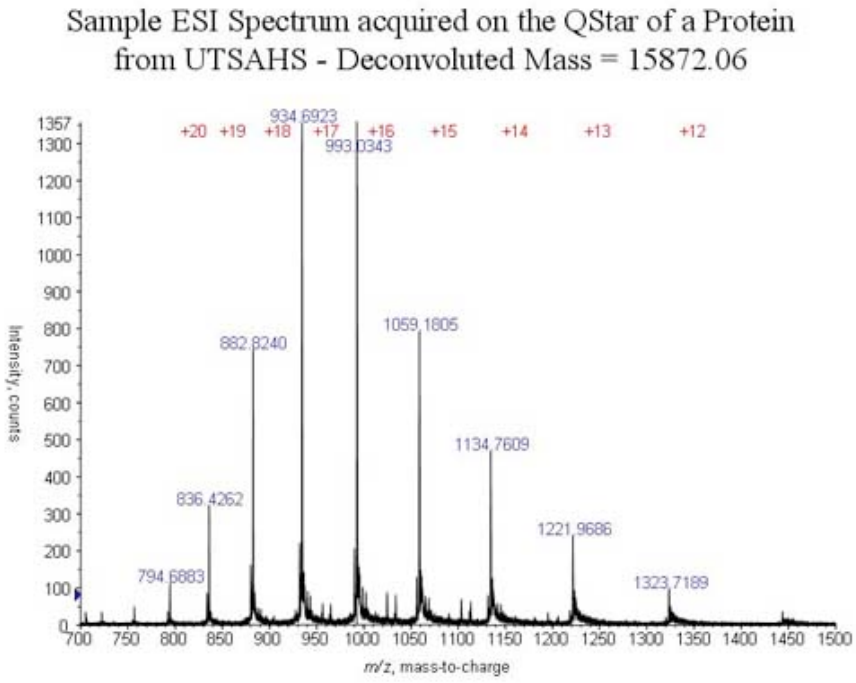


A charged droplet shrinks to ~ 1 mm by solvent evaporation until the repulsive force of the excess charge equals the cohesive force of surface tension.

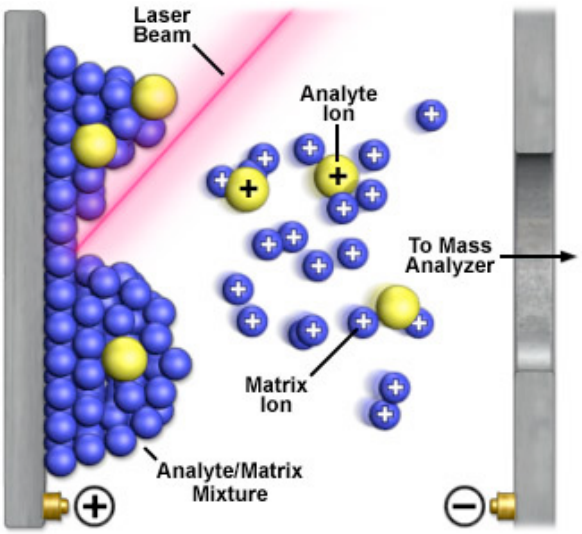
The droplet breaks up by ejecting tiny droplets with diameters of ~ 10 nm. These droplets are still charged. The remaining solvents in the charged tiny droplet evaporate, leaving the analyte ions in the gas phase.



ESI ionizies molecules without thermal-induced decomposition. And the molecular ions are stable and usually do not dissociate into fragment ions.



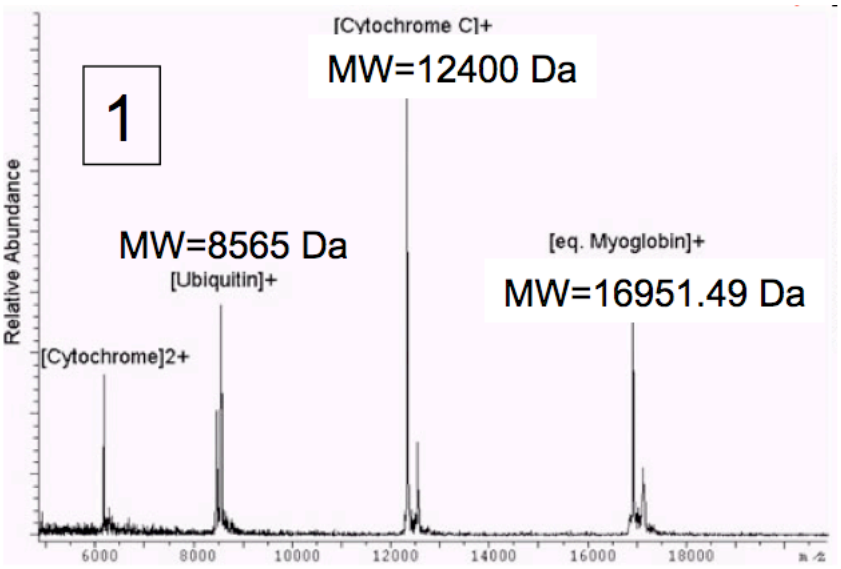
4. Matrix-Assisted Laser Desorption/Ionization (MALDI)



UV MALDI Matrix List

Compound	Other Names	Solvent	Wavelength (nm)	Applications
2,5-dihydroxy benzoic acid[1]	DHB, Gentisic acid	acetonitrile, water, methanol, acetone, chloroform	337, 355, 266	peptides, nucleotides, oligonucleotides, oligosaccharides
3,5-dimethoxy-4-hydroxycinnamic acid[2][3]	sinapic acid; sinapinic acid; SA	acetonitrile, water, acetone, chloroform	337, 355, 266	peptides, proteins, lipids
4-hydroxy-3-methoxycinnamic acid[2][3]	ferulic acid	acetonitrile, water, propanol	337, 355, 266	proteins
α-cyano-4-hydroxycinnamic acid[4]	CHCA	acetonitrile, water, ethanol, acetone	337, 355	peptides, lipids, nucleotides
Picolinic acid[5]	PA	Ethanol	266	oligonucleotides
3-hydroxy picolinic acid[6]	HPA	Ethanol	337, 355	oligonucleotides

The ionization is triggered by a laser beam (normally a nitrogen laser). A matrix (dried small organic molecules) is used to protect the biomolecule (analyte) from being destroyed by direct laser beam and to facilitate vaporization and ionization.



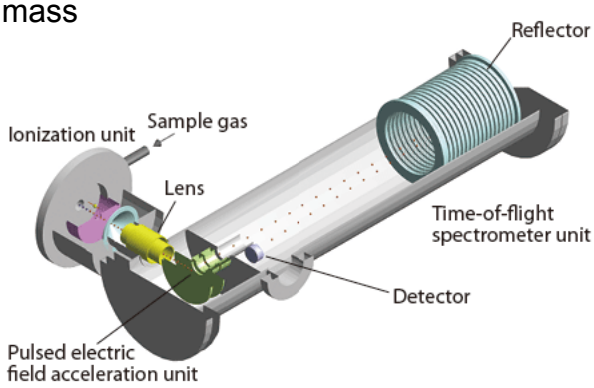
Typical MALDI mass spectrum of proteins

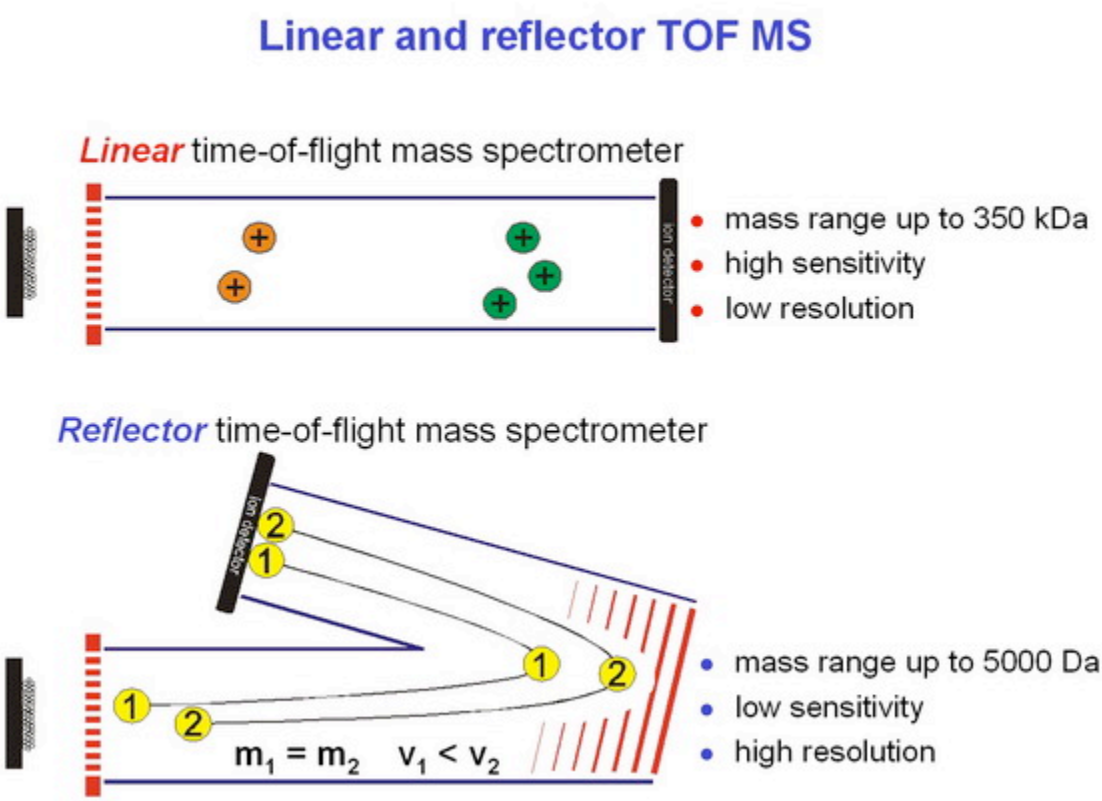
Mass analyzer – separates ions based on their mass-to-charge (m/z)

- variety of methods
- different size, cost, speed and resolving power
- time of flight (TOF)
- quadrupole (Q, mass filter)
- Ion Cyclotron Resonance or Fourier Transform
  (ICR-MS) (FT-MS)
- May perform more than one m/z separation
  (Q-TOF, triple quad, TOF/TOF)

1. Time-of-flight (TOF)

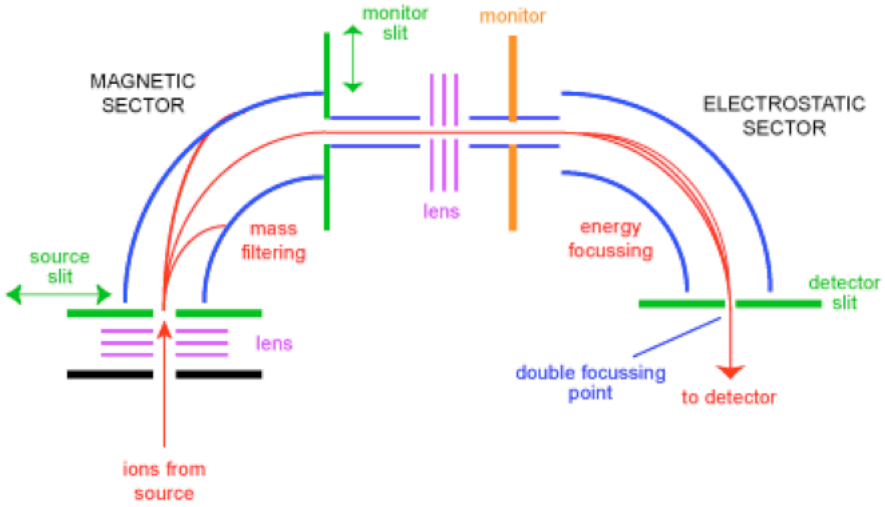
- prior the entering the TOF region, all ions are accelerated with a fix kinetic energy
- the resulting velocity is a function of the ions' mass
- measure the drift time in a field free region
- the use of reflector will lengthen the TOF distance for enhanced resolution





2. Sector mass analyzer

A static electric or magnetic sector or some combination of the two is used as a mass analyzer.

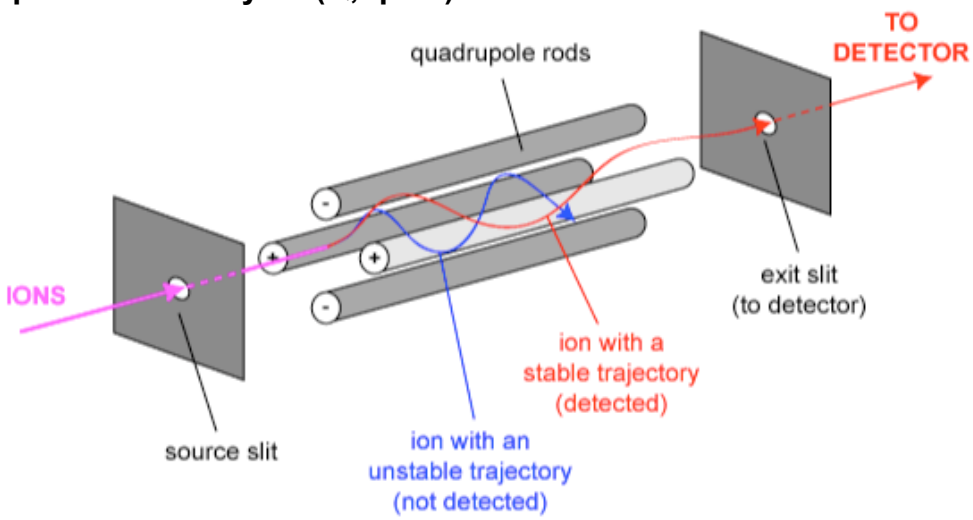


In both type of sectors, the (electric or magnetic) field bends the migration path of ions. The angle of deflection depends on the m/z of the ions. Varying the field of the sector allows scanning of ions based on m/z.

The first sector is to mass select the ion of interest. The second sector is to focus the ion zone into a very narrow pulse for detection at very high mass resolution.

Most modern sector instruments are double focusing instruments in that they focus the ion beams both in direction and velocity.

2. Quadrupole mass analyzer (Q, quad)



The quadrupole consists of four parallel metal rods.

Each opposing rod pair is connected together electrically, and a radio frequency (RF, or alternating current AC) voltage is applied between one pair of rods and the other. A direct current (DC) voltage is then superimposed on the RF voltage.

Ions travel down the quadrupole between the rods. Only ions of a certain mass-to-charge ratio m/z will reach the detector for a given ratio of voltages: other ions have unstable trajectories and will collide with the rods.

This permits selection of an ion with a particular m/z or allows the operator to scan for a range of m/z-values by continuously varying the applied voltage.

4. Ion Trap mass analyzer

A) Linear Ion Trap (quadrupole) - uses a set of quadrupole rods to confine ions radially and a static electrical potential on-end electrodes to confine the ions axially. The linear form of the trap can be used as a selective mass filter, or as an actual trap by creating a potential well for the ions along the axis of the electrodes. Advantages of the linear trap design are increased ion storage capacity, faster scan times, and simplicity of construction.

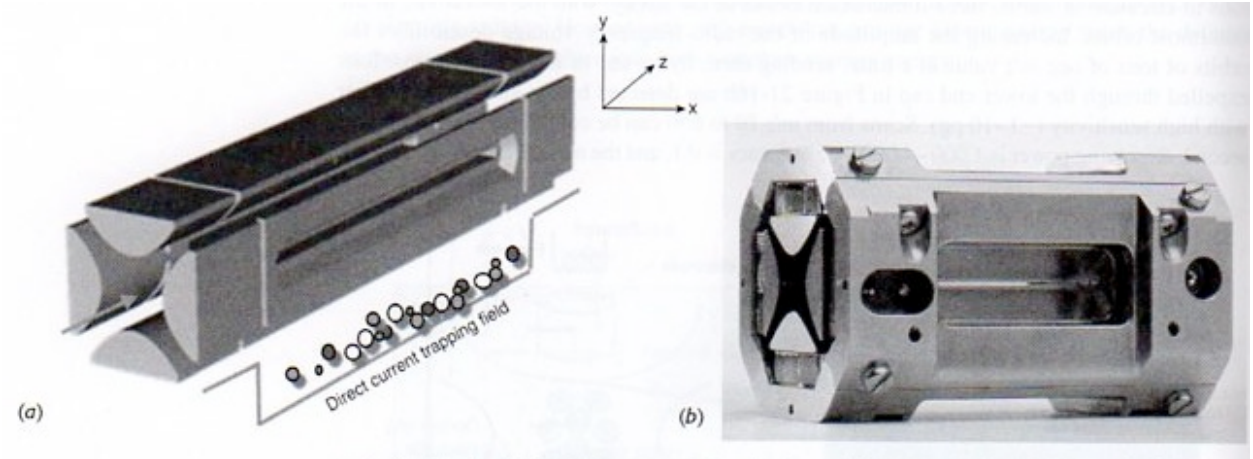
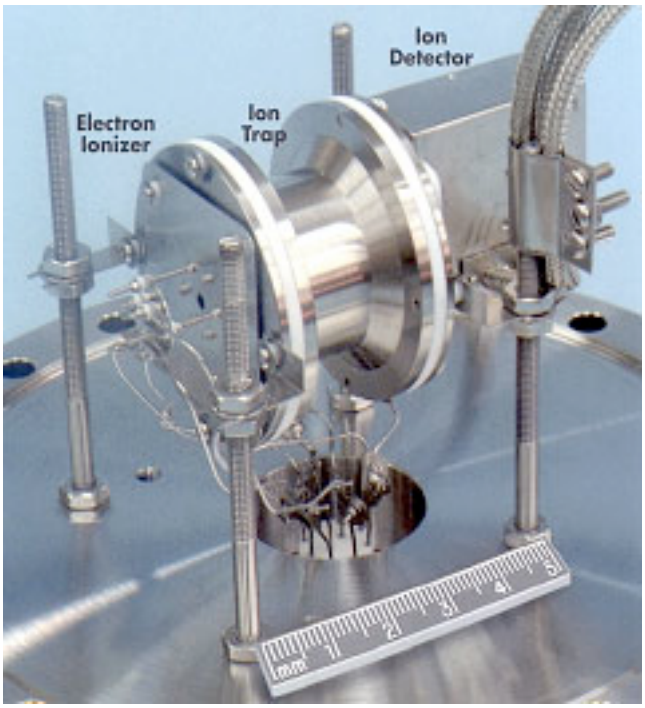
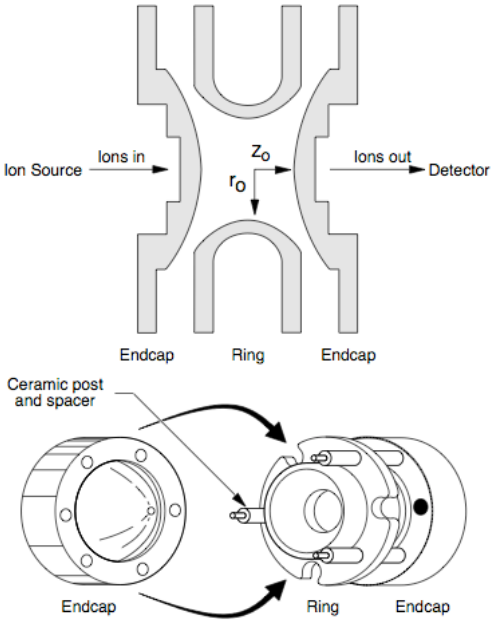


FIGURE 21-17 Linear ion trap. [Drawing from Z. Ouyang, G. Wu, Y. Song, H. Li, W. R. Plass, and R. G. Cooks, "Rectilinear Ion Trap: Concepts, Calculations, and Analytical Performance of a New Mass Analyzer," *Anal. Chem.* **2004**, *76*, 4595. Photograph of LTQ XL linear ion trip courtesy Thermo Fisher Scientific, San Jose, CA.]

B) 3D Ion Trap - ions are trapped within the system of three electrodes-a ring electrode and two end-cap electrodes of hyperbolic cross-section.

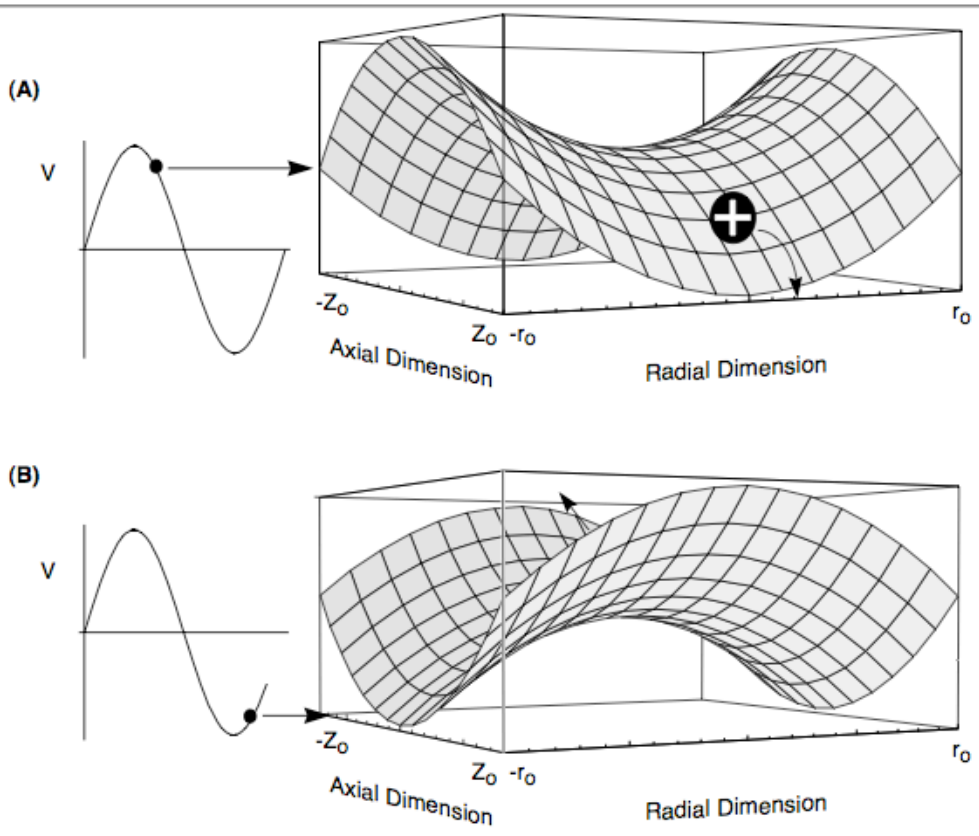
Specific m/z trapped in circular orbit by a specific radio-frequency (RF). Increasing strength of RF causes specific m/z orbit to increase, eventually exit the ion trap and collide with end electrode (detector).





F4

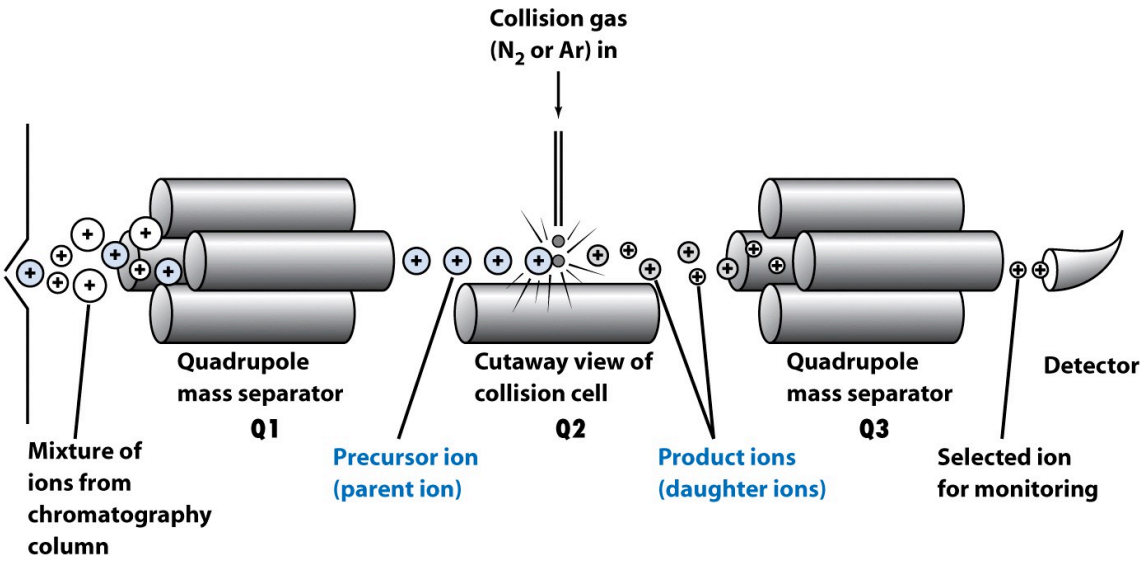
Potential used for trapping ions (A) in the radial direction and (B) in the axial direction. An ion in the position shown is accelerated away from the trap center in the axial direction at the rf phase shown in (A) and towards it in (B) (Adapted from reference 13).



Tandem Mass Spectrometry or MS/MS

1. For enhanced resolution. The instrument still operates to obtain full scan acquisition of a wide m/z range. Examples are sector analyzer, quadruple-TOF (Q-TOF) with Q to allow all ions to pass.

2. Obtain mass information of fragment ions from selected precursor ions of interest.



Selected Reaction Monitoring (SRM)

MS + Collision Induced Dissociation (fragmentation) + MS:

MS/MS spectra - obtain fragment ions of selected precursor ions:
Q1) Use mass analyzer to select a specific m/z
Q2) Fragment that specific m/z
Q3) Scan and detect fragment ions

Scan through the precusor ions that produce certain selected fragment ions:
Q1) Use mass analyzer to scan through the entire m/z range
Q2) Fragment all sequentially
Q3) Separate and look for fragment ions of interest

Selected Reaction Monitoring (SRM) or Multiple Reaction Monitoring (MRM)
Q1) Resolving and find selected precursor ions
Q2) Fragment selected precursors
Q3) Resolving and find specific fragment ions from selected precursors ions

High Resolution MS

- usually refers to Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS); also can apply to Orbitrap FT MS
- based on ion trap rather than “crashing” ions out to detect them, the current caused by the orbiting ions is measured;
- higher the magnetic field, the faster a detectable image current is obtained
- well known for exceptionally high resolving power and m/z over 100,000

FT-ICR MS

Orbitrap FT-MS