LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC/MS)

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INTRODUCTION

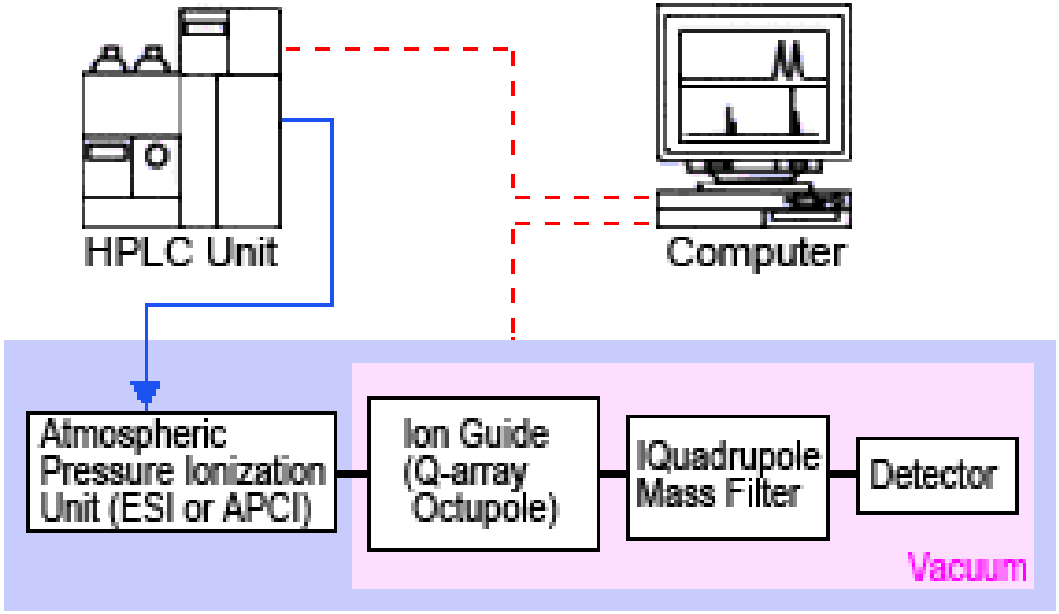
- PRINCIPLE:
- **LC/MS is a technique that combines physical separation capabilities of liquid chromatography with mass analysis capability of Mass spectrometry.**
- **It is a method that combines separation power of HPLC with detection power of Mass spectrometry.**
- **In LC-MS we remove the detector from the column of LC and fit the column to interface of MS.**
- **In the most of the cases the interface used in LC-MS are ionization source.**

Theory of LC/MS

- HPLC is a method for separating a complex mixture in to its components.
- High sensitivity of mass spectroscopy provides the information for identification of compounds or structural elucidation of compounds.
- Combination of these two techniques is LC-MS
- As the metabolites appear from the end of the column they enter the mass detector, where the solvent is removed and the metabolites are ionized.

LC-MS System Components

- Mass spectrometers work by ionizing molecules and then sorting and identifying the ions according to their mass-to-charge (m/z) ratios.



PROBLEMS IN COMBINING HPLC AND MS

HPLC

- Liquid phase operation
- 25 - 50 deg. C
- No mass range limitations
- Inorganic buffers
- 1 ml/min eluent flow is equivalent to 500 ml/min of gas

MS

- Vacuum operation
- 200 - 300 deg. C
- Up to 4000 Da for quadrupole MS
- Requires volatile buffers
- Accepts 10 ml/min gas flow

MOBILE PHASE

The mobile phase is the solvent that moves the solute through out column.

General requirements:-

(1) Low cost, UV transparency, high purity.

(2) Low viscosity, low toxicity, non flammability.

(3) Non corrosive to LC system component.

Solvent strength and selectivity:-

It is the ability of solvent to elute solutes from a column.

COLUMN

- The use of di-functional or tri-functional silanes to create bonded groups with two or three attachment points leading to phases with higher stability in low or higher pH and lower bleed for LCMS

- Most widely used columns for LC-MS are:-

  (1) fast LC column.

  the use of short column. (15-50mm)

  (2) Micro LC column.

  the use of large column. (20-150mm)

Sample preparation

- Sample preparation generally consists of concentrating the analyte and removing compounds that can cause background ion or suppress ionization.
- Example of sample preparation include:-

1. On Column concentration -to increase analyte concentration.
2. Desalting - to reduce the sodium and potassium adduct formation that commonly occurs in electro spray.
3. Filtration- to separate a low molecular-weight drug from proteins in plasma, milk, or tissue.

INTERFACES

- LC-MS systems include a device for introducing samples (such as an HPLC)an interface for connecting such device, an ion source that ionizes samples, an electrostatic lens that efficiently introduces the generated ions, a mass analyzer unit that separates ions based on their mass-to-charge (m/z) ratio, and a detector unit that detects the separated ions.

- In an LC-MS system, however, if the LC unit is simply connected directly to the MS unit, the liquid mobile phase would vaporize, resulting in large amounts of gas being introduced into the MS unit.
- This would decrease the vacuum level and prevent the target ions from reaching the detector. So interfaces are to be used.

TYPES OF INTERFACES

- It is difficult to interface a liquid chromatography to a mass-spectrometer cause of the necessity to remove the solvent.

- The commonly used interfaces are:-

  (1) Electrospray ionization (ESI)

  (2) Thermospray ionization (TSI)

  (3) Atmospheric pressure chemical ionization (APCI)

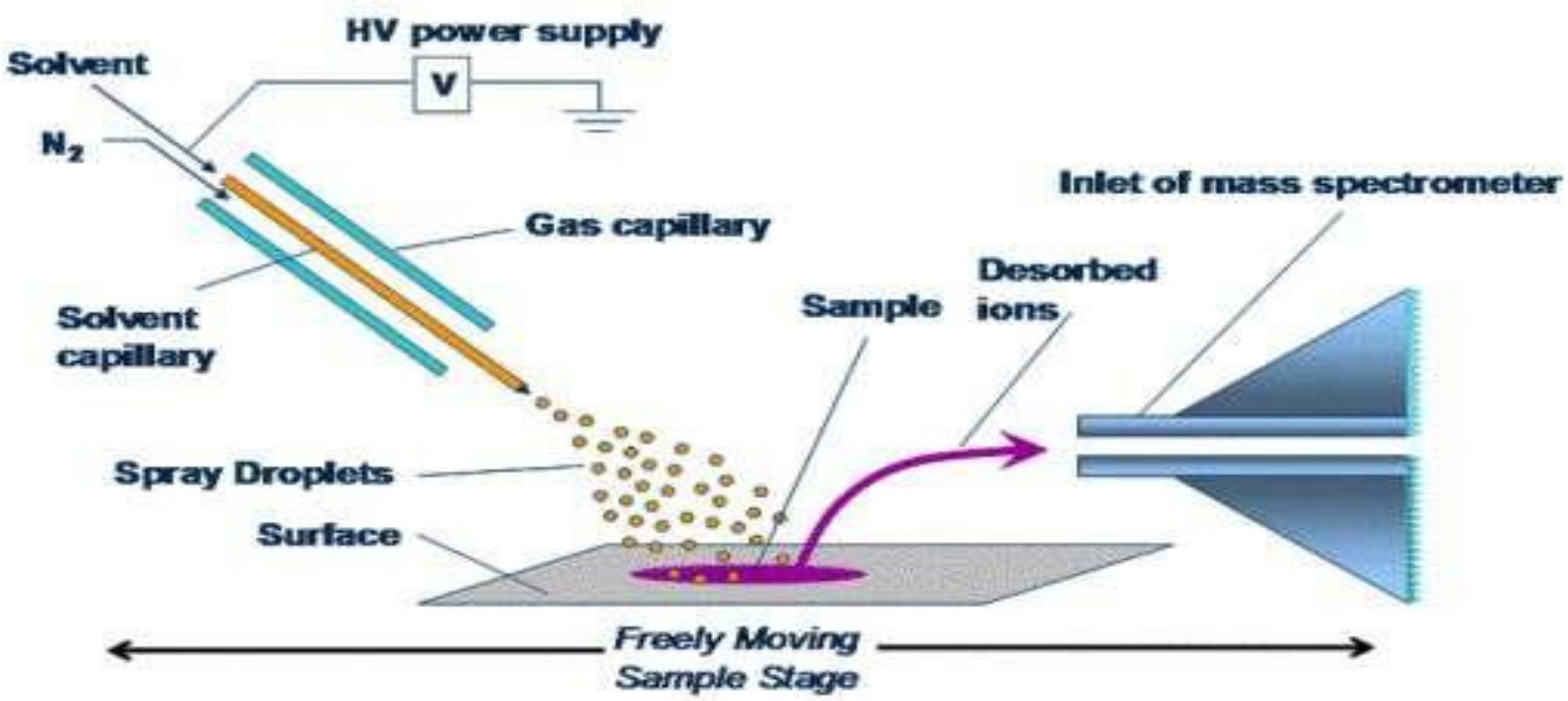
  (4) Atmospheric pressure photoionization(APPI)

Electro Spray Ionization (ESI)

- ESI draws sample solutions to the tip of a capillary tube, where it applies a high voltage of about 3 to 5 kV.
- A nebulizer gas flows from outside the capillary to spray the sample. This creates a fine mist of charged droplets with the same polarity as the applied voltage.
- As these charged particles move, the solvent continues to evaporate, thereby increasing the electric field on the droplet surface. When the mutual repulsive force of the charges exceeds the liquid surface tension, then fission occurs.
- As this evaporation and fission cycle is repeated, the droplets eventually become small enough that the sample ions are liberated into the gas phase

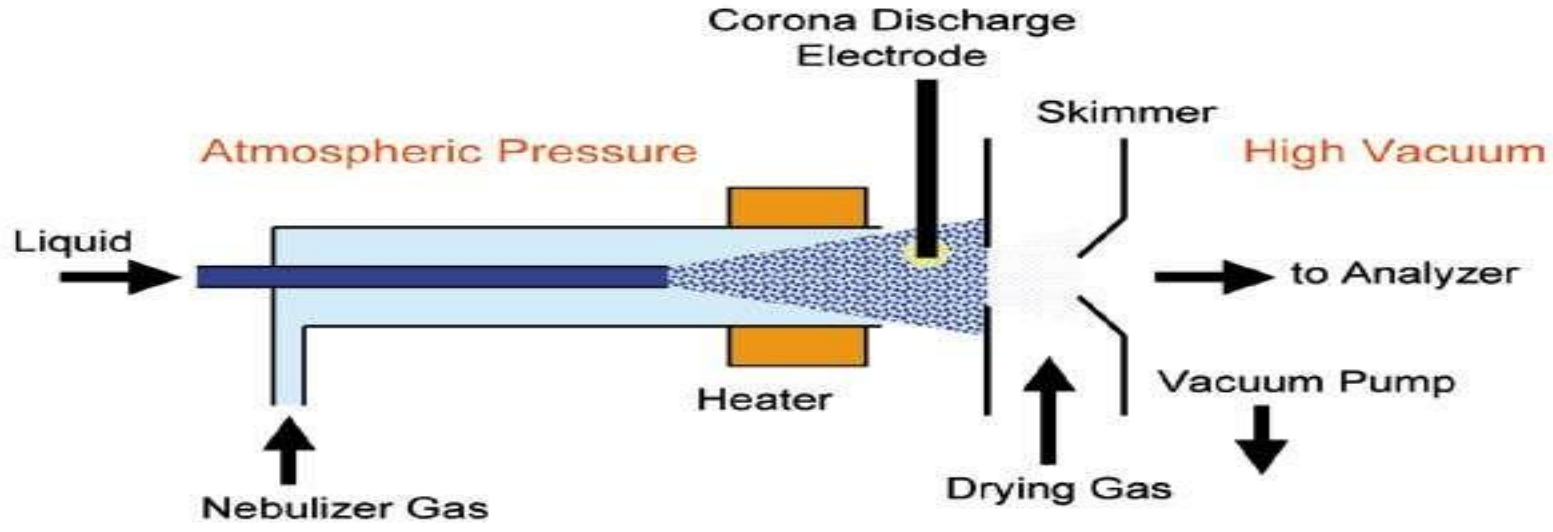
Electro Spray Ionization (ESI)

- ESI provides the softest ionization method available, which means it can be used for highly polar, least volatile, or thermally unstable compounds.



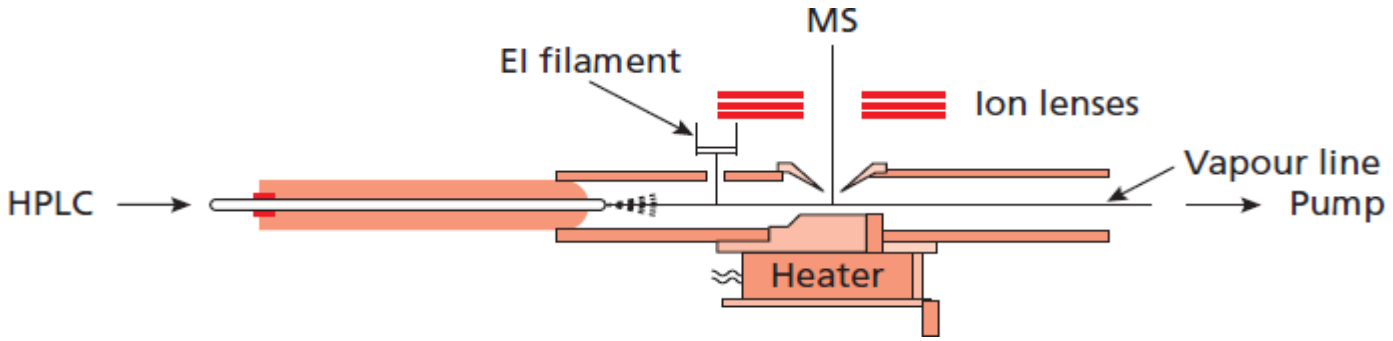
Atmospheric pressure chemical ionization (APCI)

- APCI vaporizes solvent and sample molecules by spraying the sample solution into a heater (heated to about 400 C) using a gas, such as N2.
- Solvent molecules are ionized by corona discharge to generate stable reaction ions.

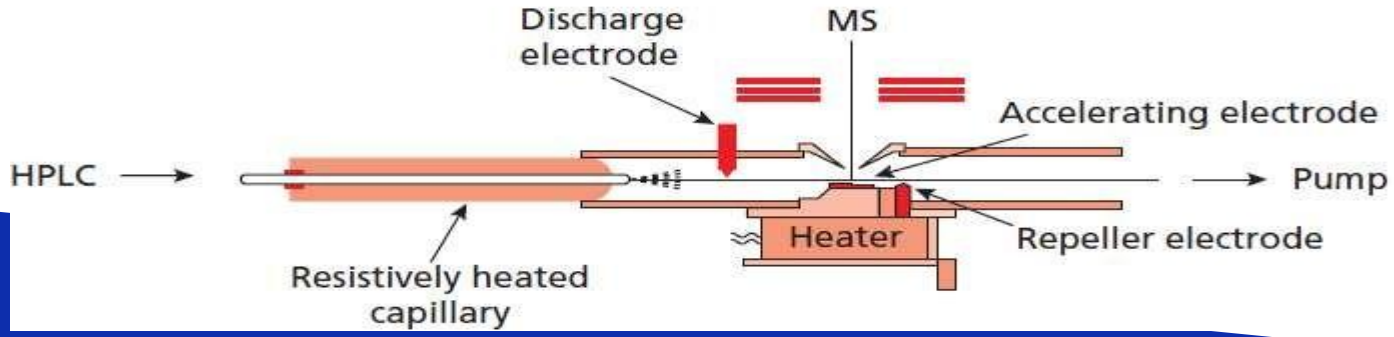


Thermospray ionization (TSI)

- They are of 2 type:
- a) Real-TSP ionization

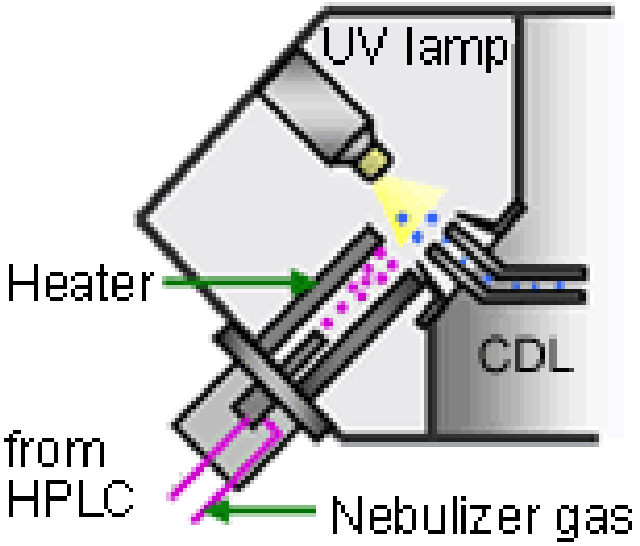


- b) Discharge electrode for external ionization and repeller electrode



Atmospheric pressure photoionization(APPI)

- The LC eluent is vaporized using a heater at atmospheric pressure. The resulting gas is made to pass through a beam of photons generated by a discharge lamp (UV lamp) which ionizes the gas molecules.



Mass Analyser

- They deflect ions down a curved tubes in a magnetic fields based on their kinetic energy determined by the mass, charge and velocity.
- The magnetic field is scanned to measure different ions.

➢ Types of mass analyzer:-

(1) Quadrapole mass filter.

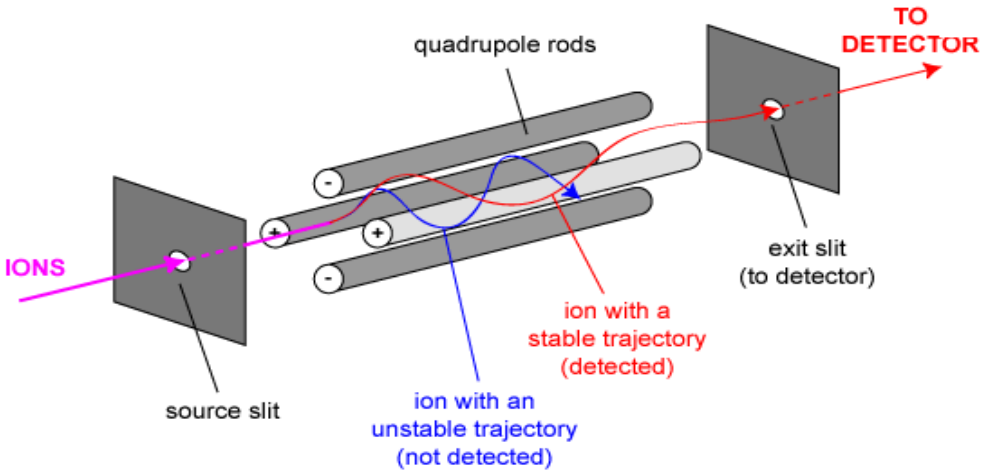
(2) Time of flight

(3) Ion trap

(4) Fourier transform ion cyclotron resonance (FT-ICR)

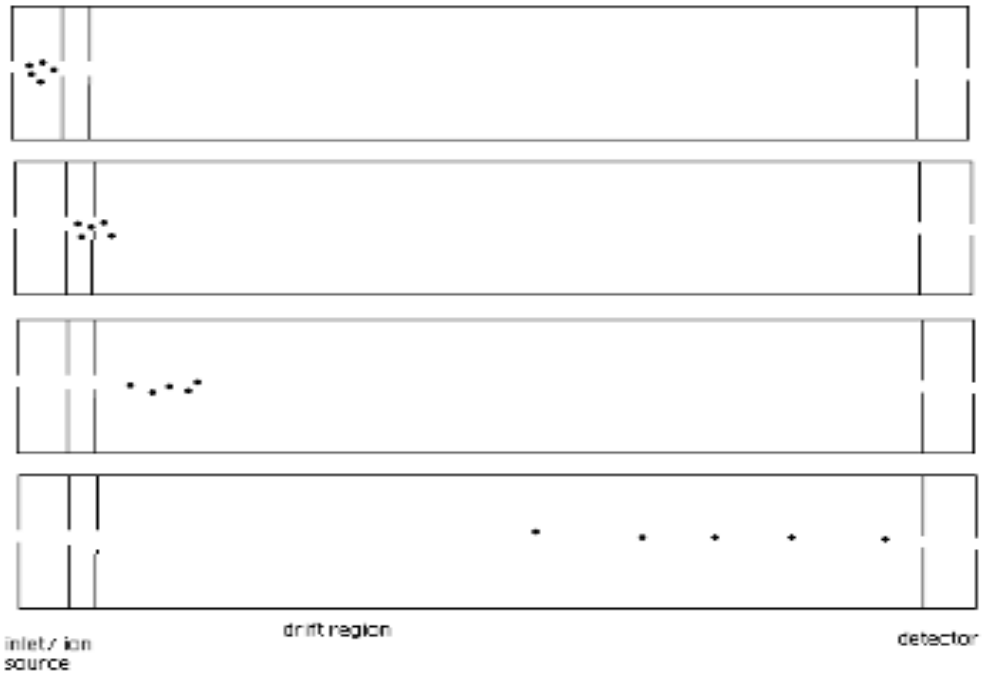
Quadrupole Mass Analyzer

- A Quadrupole mass filter consists of four parallel metal rods with different charges
- Two opposite rods have an applied + potential and the other two rods have a - potential
- The applied voltages affect the trajectory of ions traveling down the flight path
- For given DC and AC voltages, only ions of a certain mass-to-charge ratio pass through the quadrupole filter and all other ions are thrown out of their original path



TOF (Time of Flight) Mass Analyzer

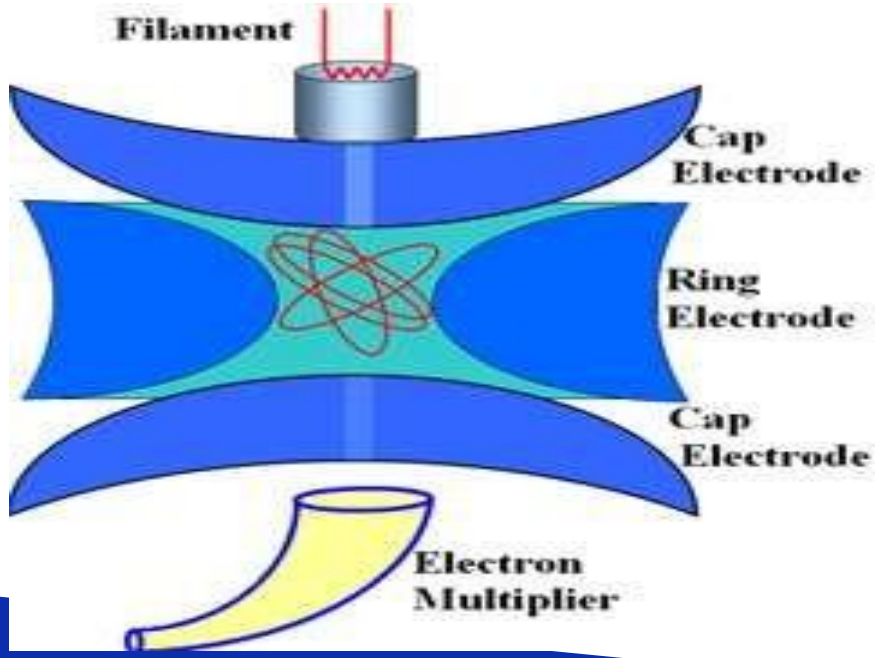
- TOF Analyzers separate ions by time without the use of an electric or magnetic field.
- In a crude sense, TOF is similar to chromatography, except there is no stationary/ mobile phase, instead the separation is based on the kinetic energy and velocity of the ions.



TOF system. as time goes by, the ion are separated

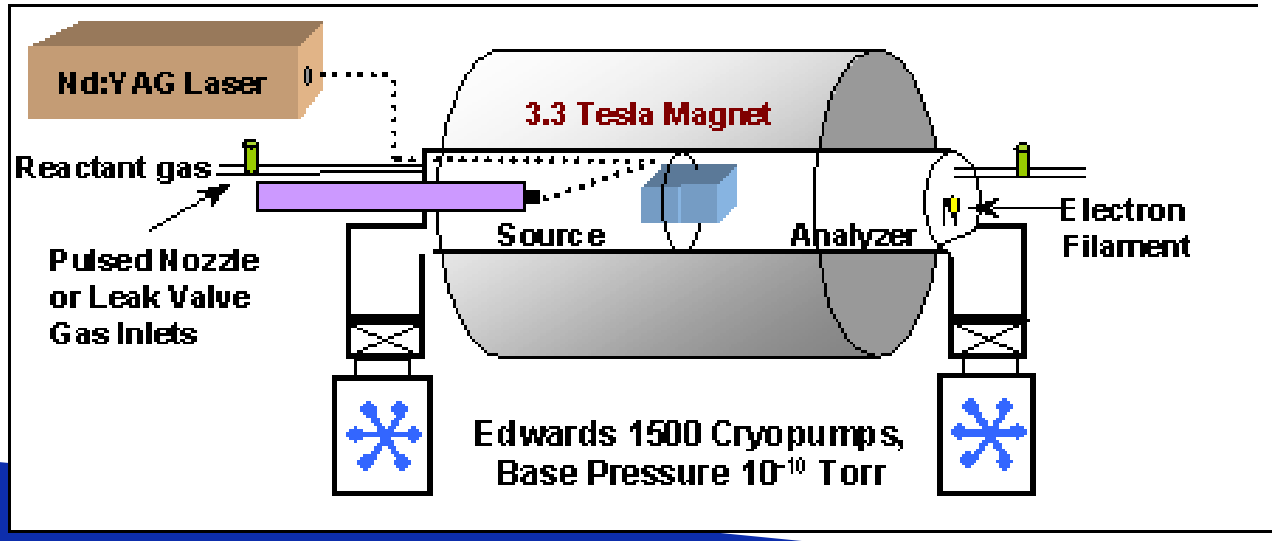
Ion Trap Mass Analyzer

- It uses an electric field for the separation of the ions by mass to charge ratios.
- The electric field in the cavity due to the electrodes causes the ions of certain m/z values to orbit in the space.



Fourier transform ion cyclotron resonance (FT-ICR)

- Uses a magnetic field in order to trap ions into an orbit inside of it.
- In this analyzer there is no separation that occurs rather all the ions of a particular range are trapped inside, and an applied external electric field helps to generate a signal.



Applications of LC-MS

- ***Pharmaceutical Applications:***
- ***Rapid chromatography of benzodiazepines***
- ***Identification of bile acid metabolite***

- ***Biochemical Applications:***
- ***Rapid protein identification using capillary LC/MS/MS and database searching.***

- ***Clinical Applications:***
- ***High-sensitivity detection of trimipramine and thioridazine***

Applications of LC-MS

- ***Food Applications:***
- ***Identification of aflatoxins in food***
- ***Determination of vitamin D3 in poultry feed supplements***

- ***Environmental Applications:***
- ***Detection of phenylurea herbicides***
- ***Detection of low levels of carbaryl in food***

- ***Forensic Applications:***
- ***illegal substances, toxic agents***
- ***Explosives***

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