
Mass Spectrometry

Facts and Basics

Mass Spectrometry

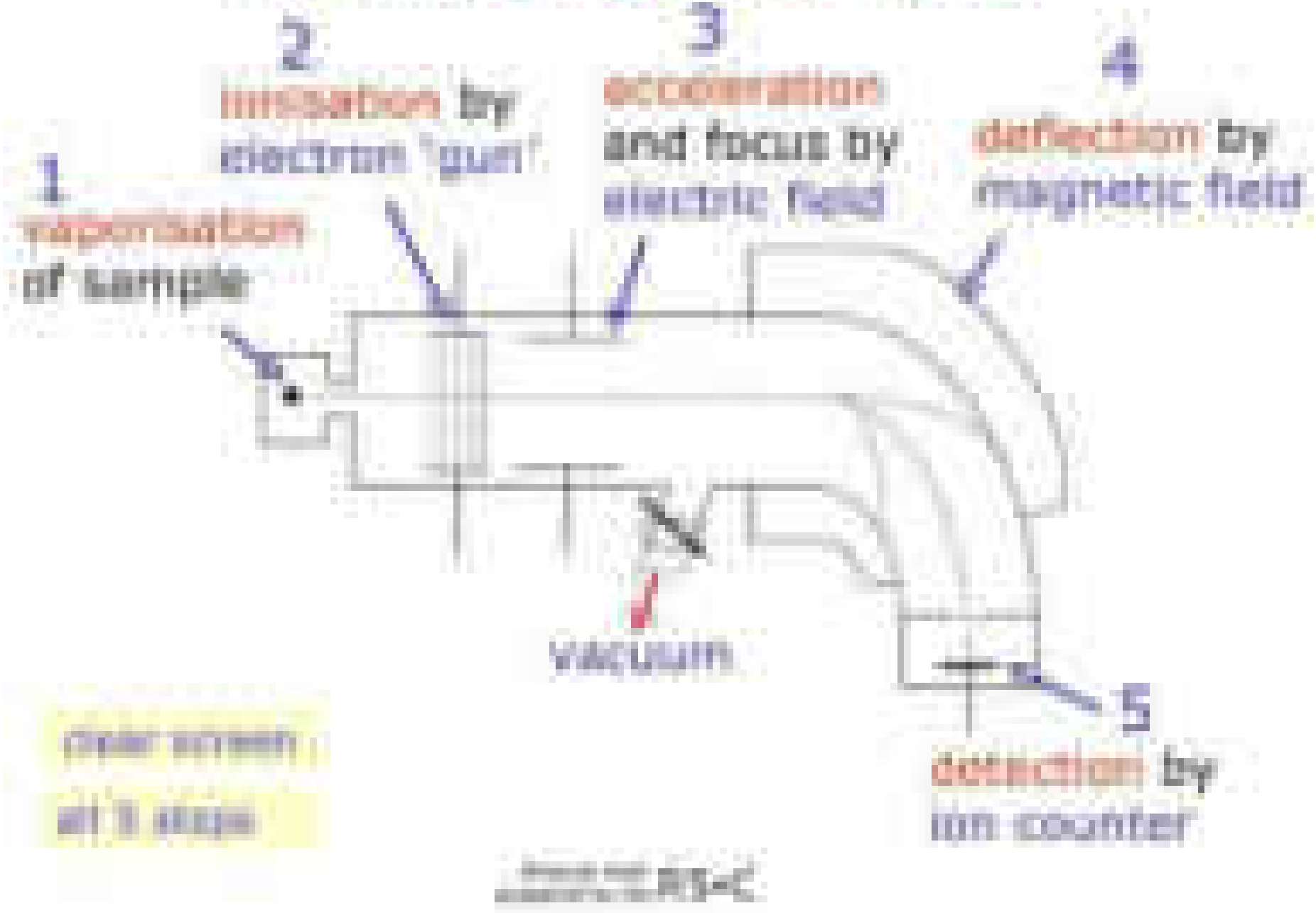
A technique for measuring and analyzing molecules, that involves introducing enough energy into a (neutral) target molecule to cause its ionization and disintegration. The resulting primary ions and their fragments are then analyzed, based on their mass/ charge ratios, to produce a "molecular fingerprint."

PRINCIPLE

- It is also called as positive ion spectra or [illegible]
- Sample is bombarded with the high electron beam produce the positive ions.
- They travel in straight path.
- When a magnetic field or electric field is applied then travels in curved path
- The fragments of different masses are seperated based on the radius of curvature.
- m/e $\propto r^2$

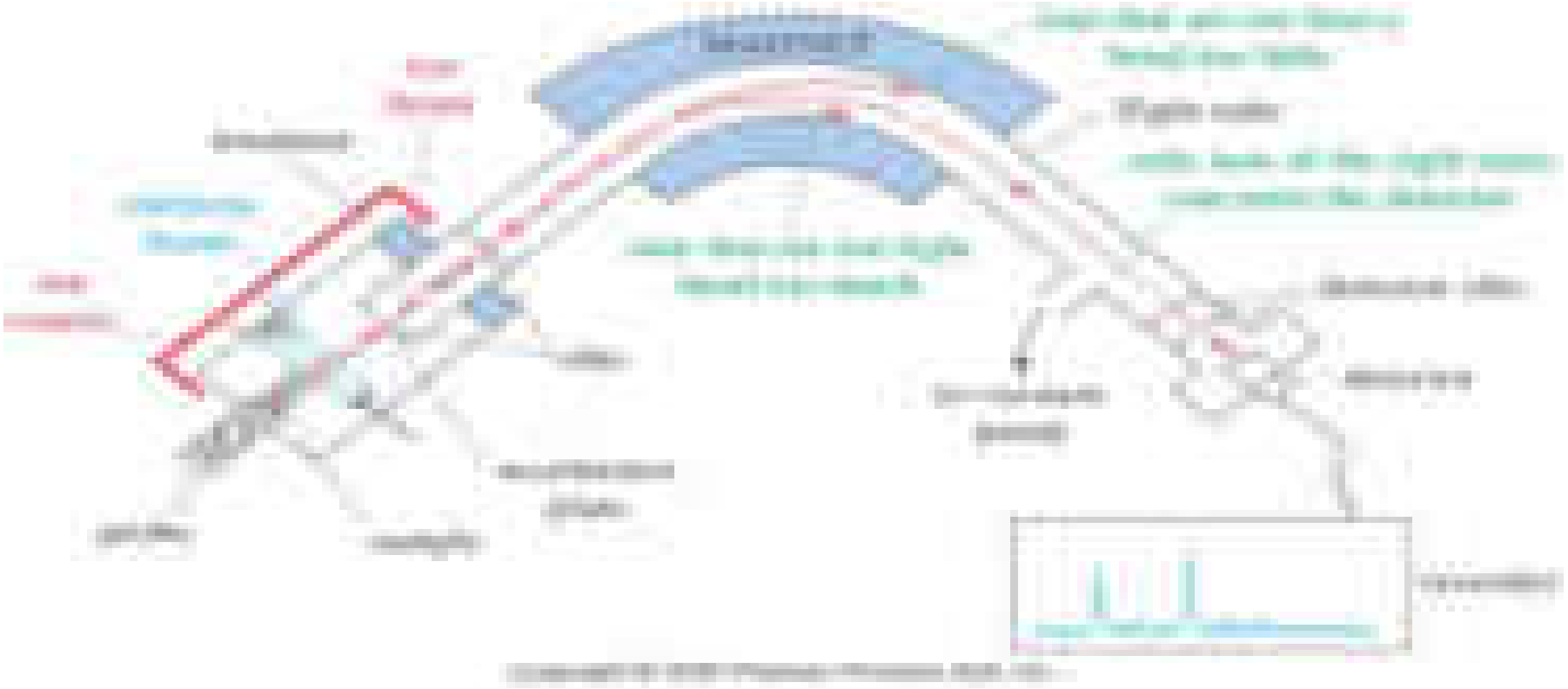
Instrumentation :

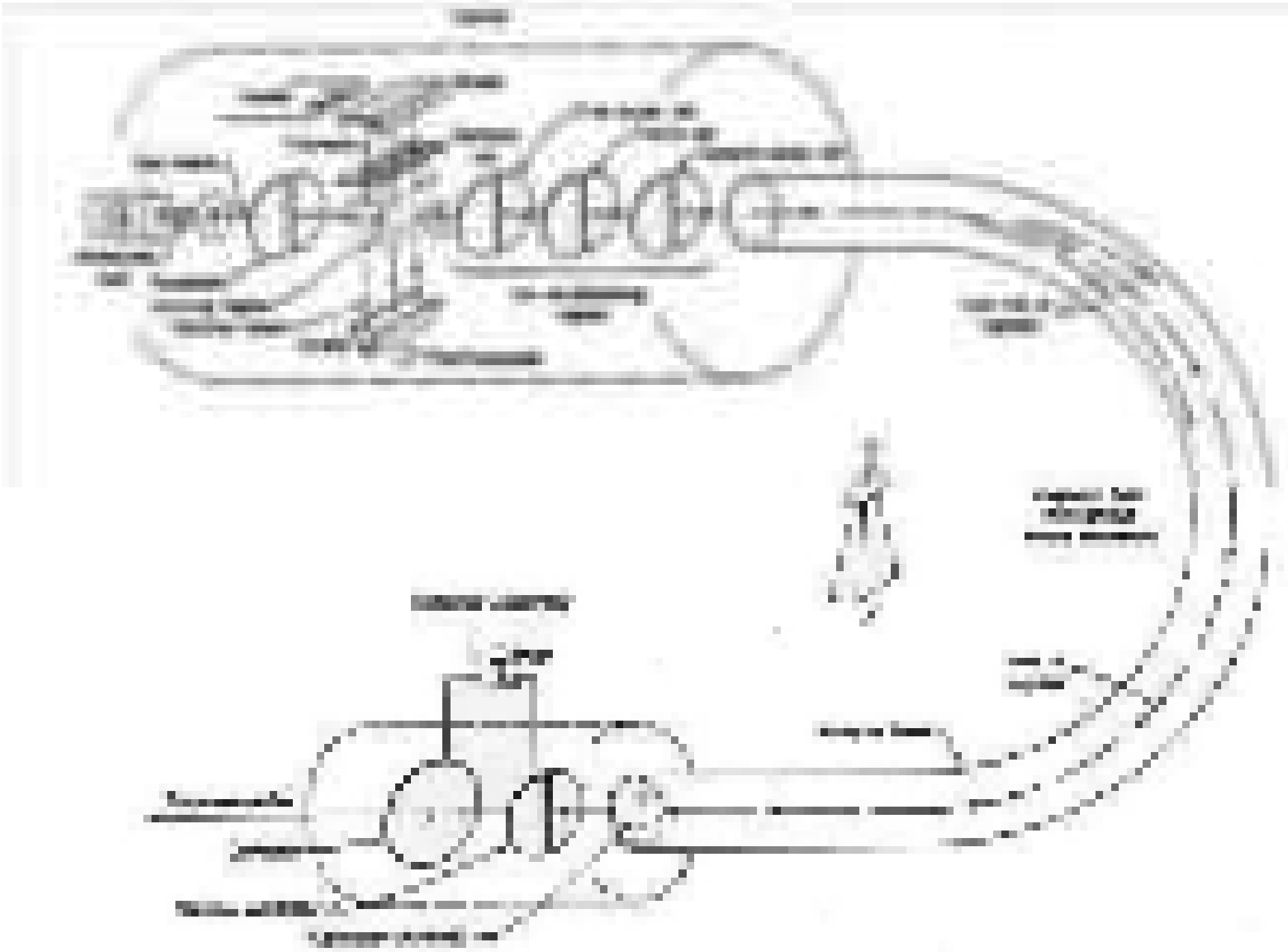
The Mass Spectrometer



Background

- The cations that are formed are separated by magnetic deflection.





Components Of A Mass Spectrometer

Ionisation

Ion Source

Electron Ionisation (EI)

Chemical Ionisation (CI)

Fast Atom Bombardment (FAB)

Electrospray Ionisation (ESI)

Matrix-Assisted Laserdesorption/ Ionisation (MALDI)

Ion Separation

Mass Analyser

Quadrupole

Magnetic Sector Field

Electric Sector Field

Time-Of-Flight (TOF)

Ion Trap

Ion Detection

Detector

Electron Multiplier

Multichannel plate

Faraday Cup

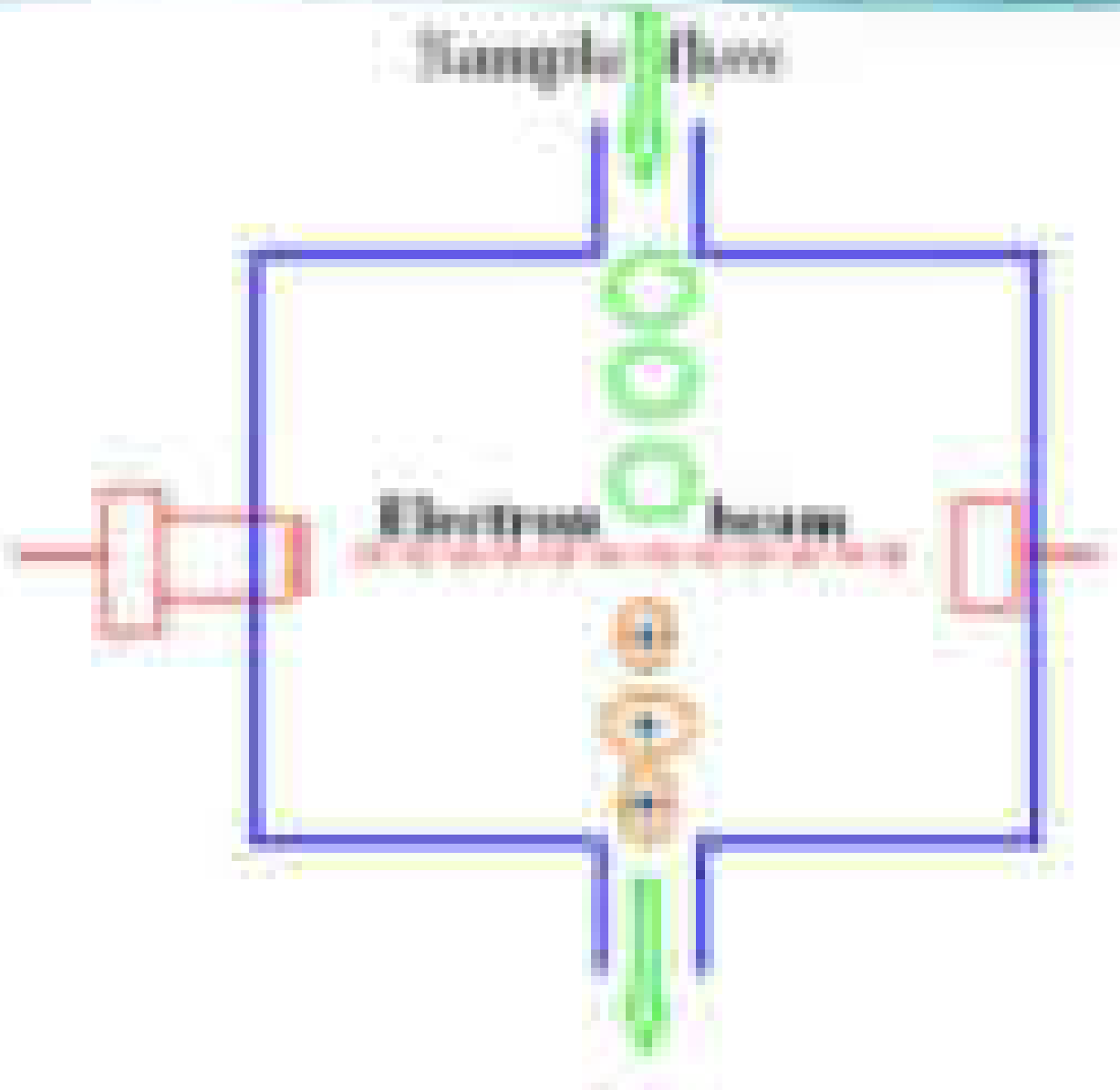
- **SAMPLE HANDLING SYSTEM:**
- Different types of samples having the different sample inlet systems.
- Heated inlet system
- gases and less volatile liquids, the liquids vaporized externally an then slowly introduced into the ionization source.
- Direct inlet system
- Solids, nonvolatile liquids, unstable compounds directly [illegible] source [illegible] [illegible]
- Non volatile liquids : steroids, carbohydrates polymeric substances etc

a) ION SOURCE

- TYPES:
  - ELECTRON IMPACT TECHNIQUE (EI)

i) ELECTRON IMPACT:

- Electrons are produced from electrically heated tungsten. These electrons are accelerated by an electric field to an average electron beam energy of about 70eV.
- It is sufficient to the ionization of the sample.
- The vapour of the sample molecule introduced at right angles to the electron beam.
- The sample pressure is about [illegible]
- Drawback: sample need to be vaporized. It may cause the thermal decomposition of the compound.



a) CHEMICAL IONIZATION:

In this reagent gas is used normally methane.

On electron impact gives primary ion like CH_4^+, CH_3^+

These react with excess of CH_4 to give secondary ions.

$CH_4 + e \rightarrow CH_4^+ + 2e$

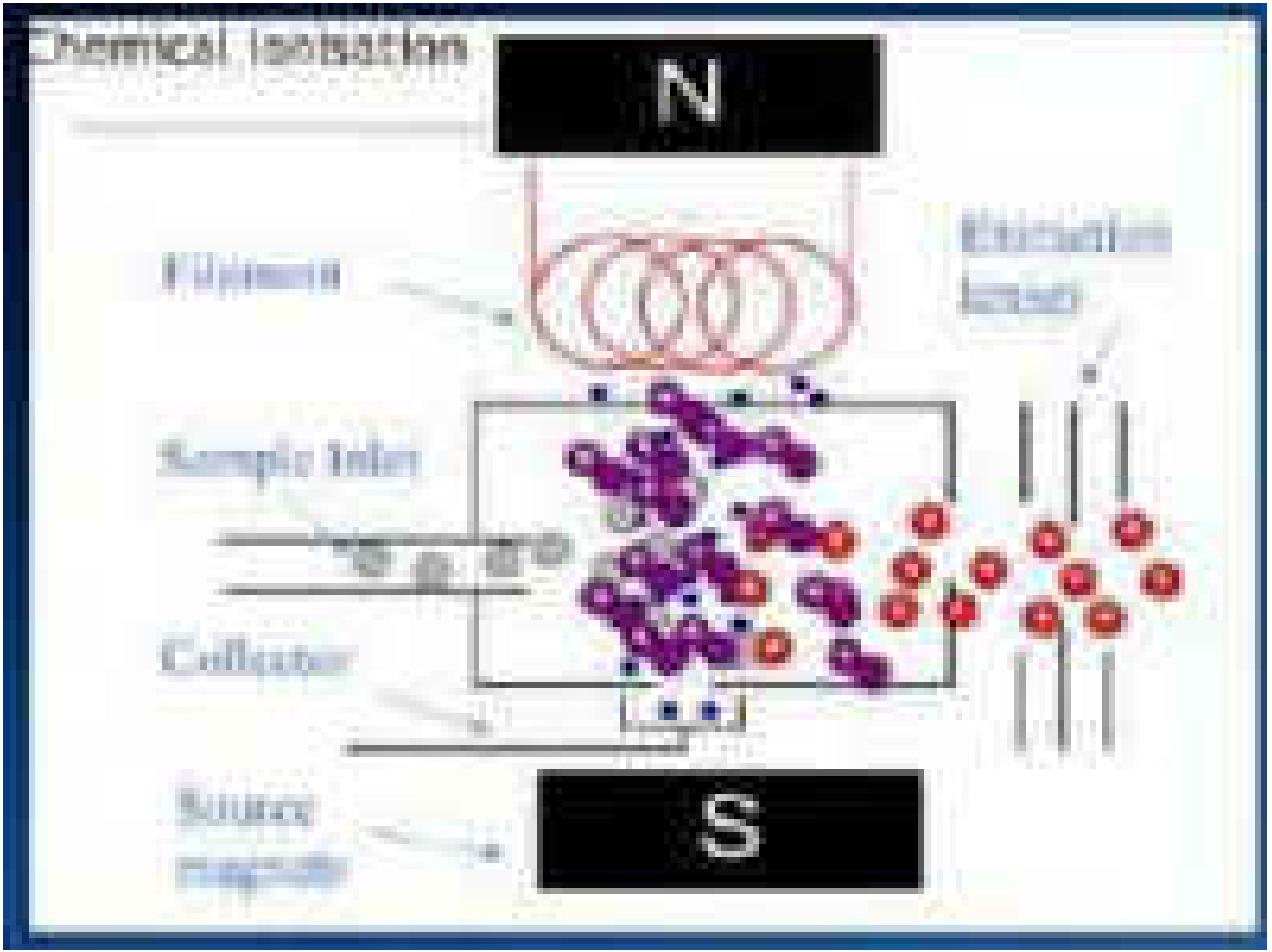
$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3$

$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$

These secondary ions react with analyte molecule (M)

$CH_5^+ + M \rightarrow CH_4 + MH^+$

$C_2H_5^+ + M \rightarrow C_2H_4 + MH^+$


Chemical Ionization
N
S

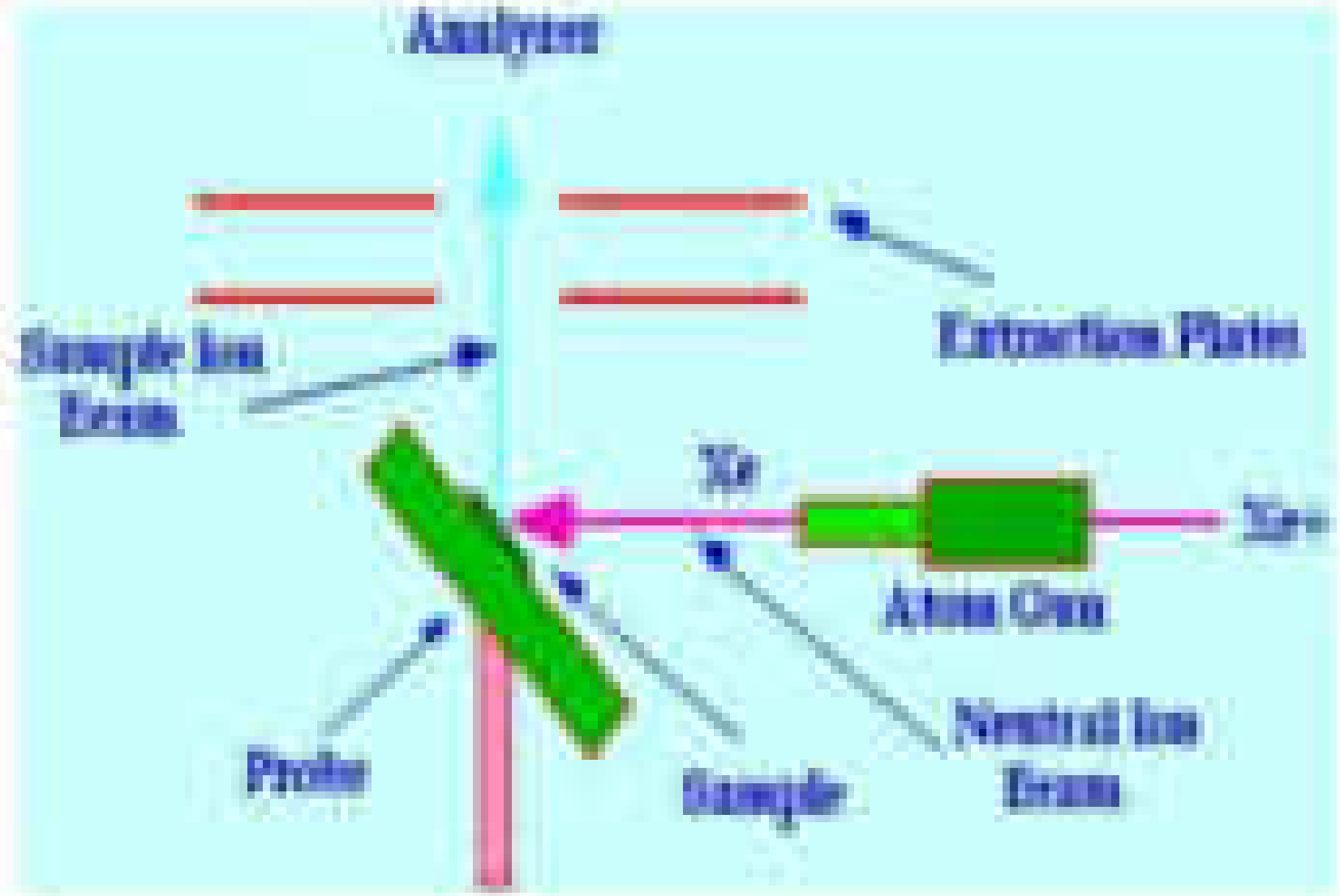
g) FAB:

- Few µg of sample is dissolved in few µl of glycerol as matrix.
- this solution is bombarded by a beam of fast atom atoms.
- These fast atoms are prepared by accelerating xenon ions to an energy of 6–9 keV, these ions are transferred to the xenon gas ,where these ion get the electrons and forms the high energy xe atom.

- After the impact of fast [illegible] into the solution, the [illegible] by momentum transfer.
- The beam of sample ion is analyzed in mass spectrometer.

- ADVANTAGES:-
- High resolution, rapid & simple
- Tolerant to variations in sampling

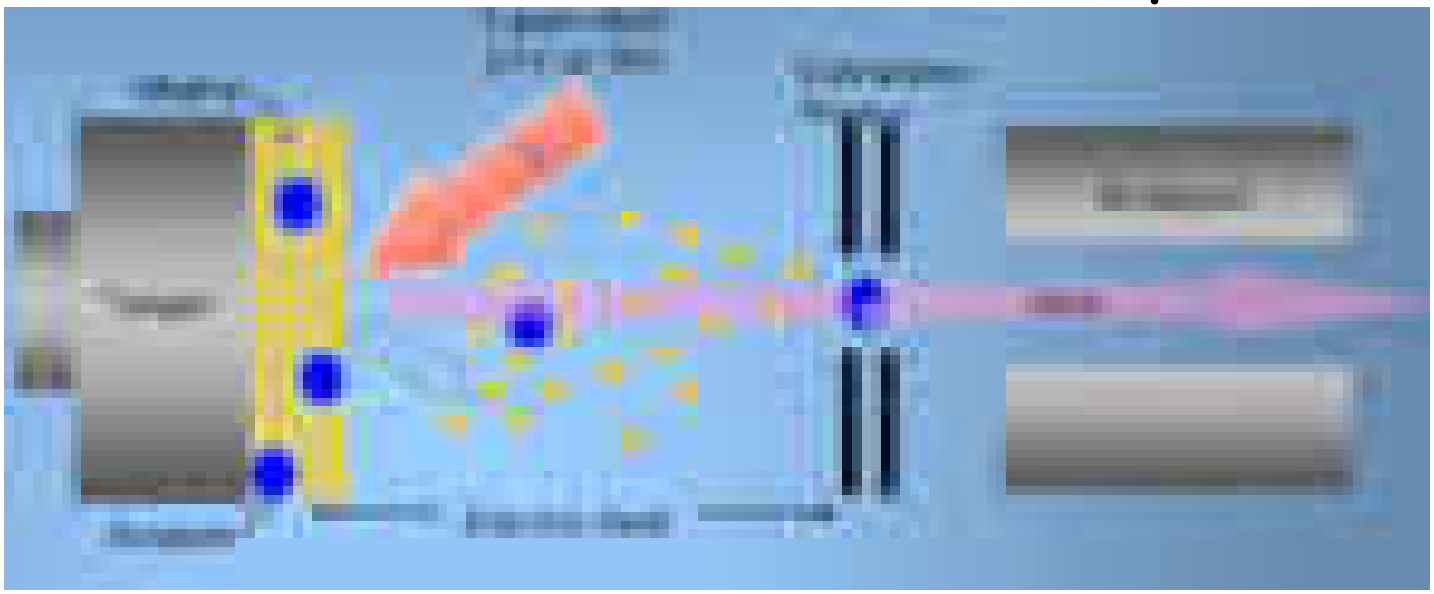
- DISADVANTAGES:-
- matrix also forms ions on bombardment which complicates the spectrum

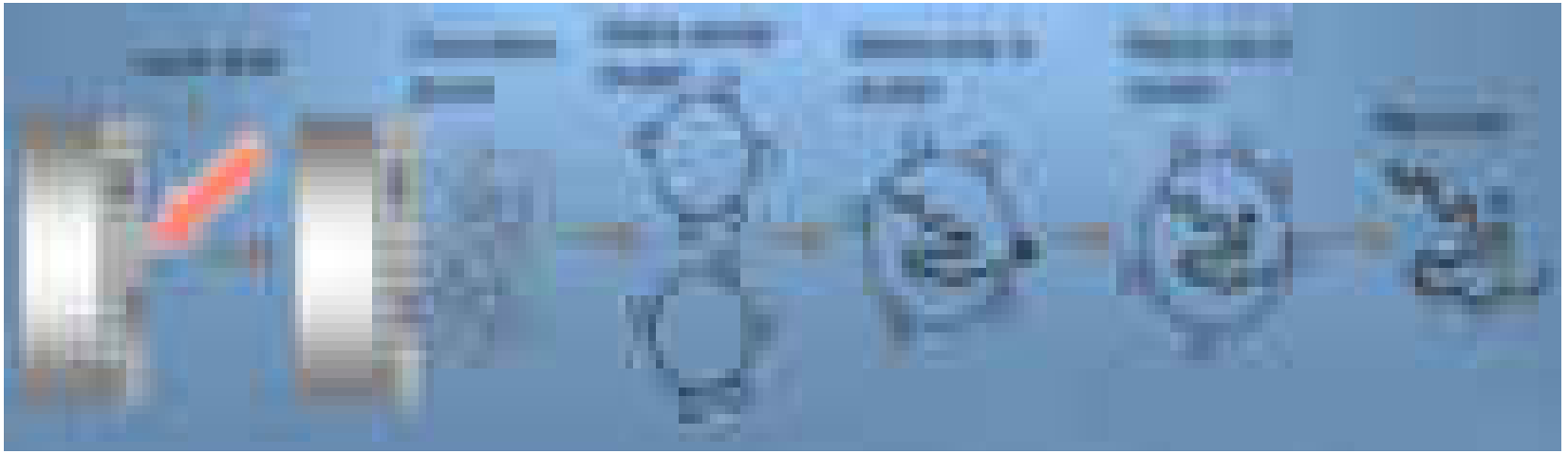

Analyzer
Extraction Plate
Xe
Xe+
Atom Gun
Neutral Ion Beam
Probe
Sample

MATRIX ASSISTED LASER DESORPTION

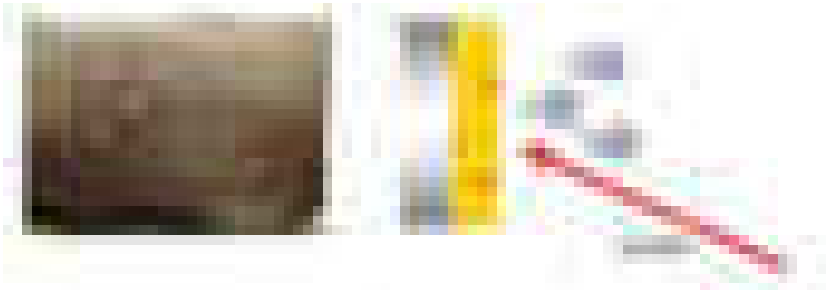
- It is new ionization method, which shows accurate molecular weight information of compounds ranging in molecular weight from few thousands to several hundred thousand Daltons
- In this technique low concentration of the analyte is uniformly dispersed in a solid or liquid matrix deposited on the metal plate.
- The metal plate put in vaccum chamber and laser beam focussed on the sample.
- Then matrix and the sample strongly absorb the laser radiation. Then the sample gets ionized.

Matrix Assisted Laser Desorption





Matrix Assisted Laser Desorption



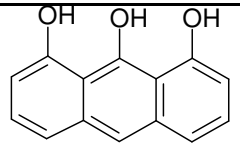
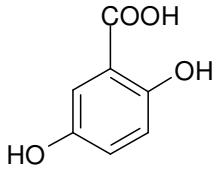
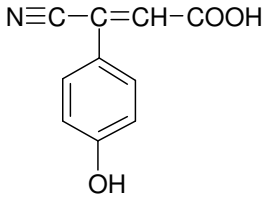
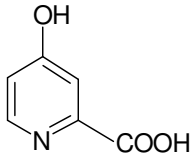
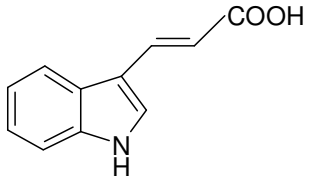
TOF Parameters

Simple, cheap (in theory), robust, sensitive.

A good modern TOF should give:

- >10k Resolving power
- ~1-10 fmol sensitivity (single scan)
- ~10 ppm mass accuracy internally calibrated (5 ppm if the peak is particularly large or clean).
- >1000 scans/second
- Unlimited mass range

Matrices

Matrix		
1,8,9-Trihydroxyanthracen (Dithranol)		polymers
2,5-Dihydroxy-benzoic acid (DHB)		proteins, peptides, polymers
α-Cyano-4-hydroxycinnamic acid		peptides, (polymers)
4-Hydroxypicolinic acid		oligonucleotides
Trans-Indol-3-acrylacid (IAA)		polymers

The most common type of mass analyser used with the is the time of flight analyser

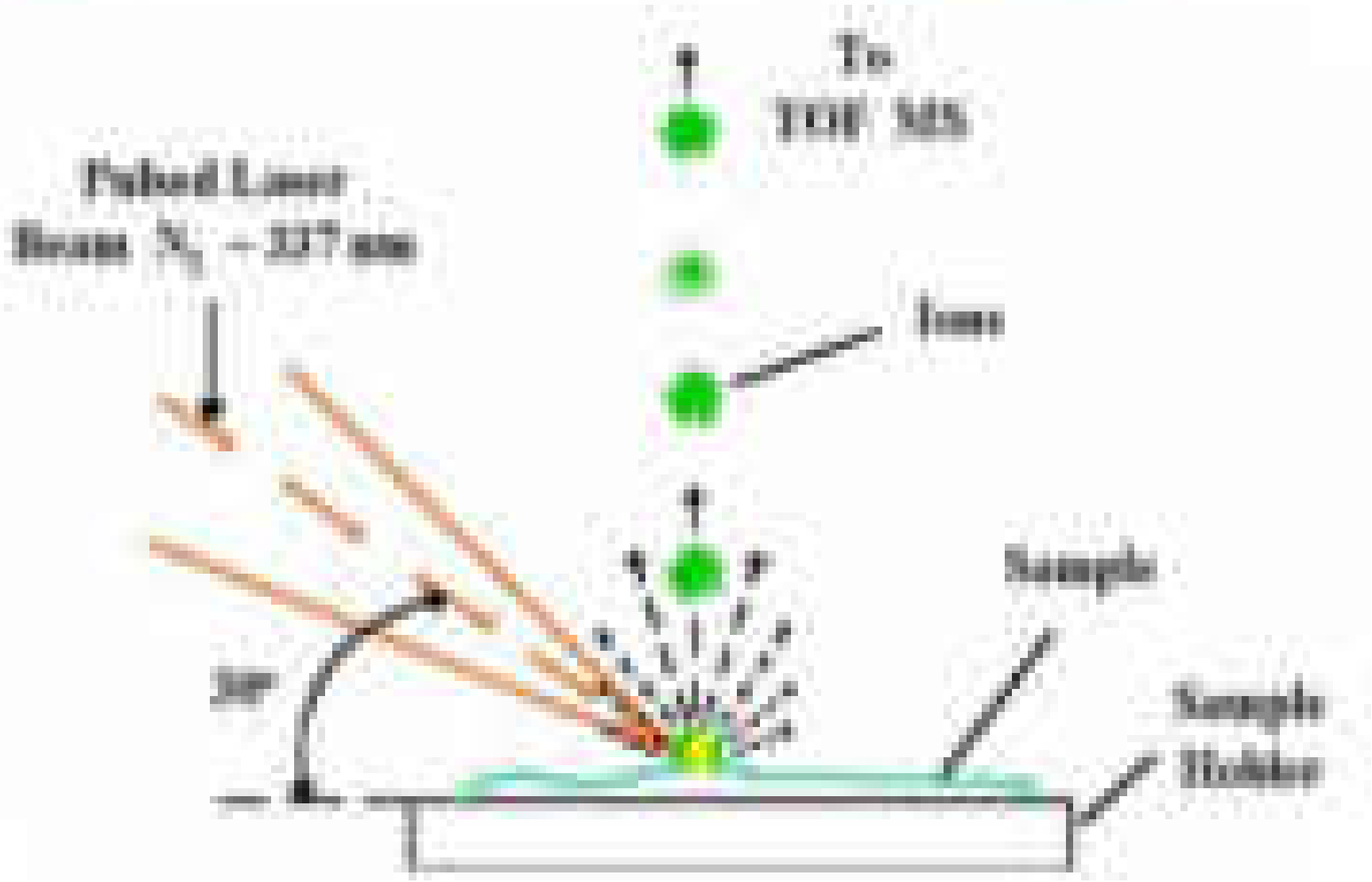
Various types of matrix

Nicotinic acid matrix – to analyse the proteins glycoproteins

Ferulic acid matrix – to analyse the proteins and

Sinapinic acid matrix – to analyse the proteins

Succinic acid matrix – to analyse the proteins


To
TOF MS
Pulsed Laser
Beam N_2 ~ 337 nm
Ions
Sample
Sample
Holder

ELECTRON SPRAY IONISATION:

- A solution of the sample pumped through a stainless steel capillary needle.

↓

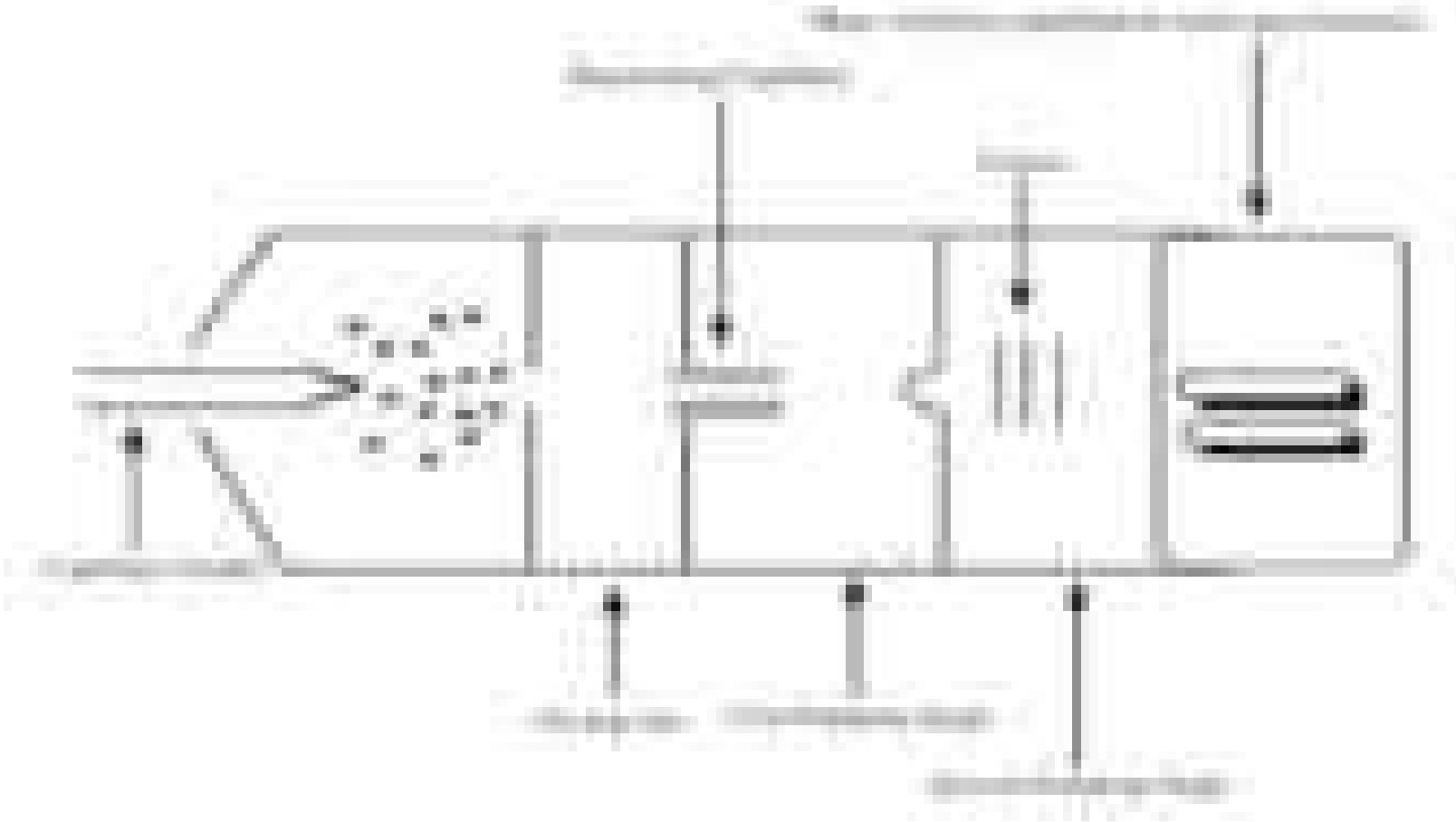
- The resulting charged spray of fine droplets pass through the desolvating capillary.

↓

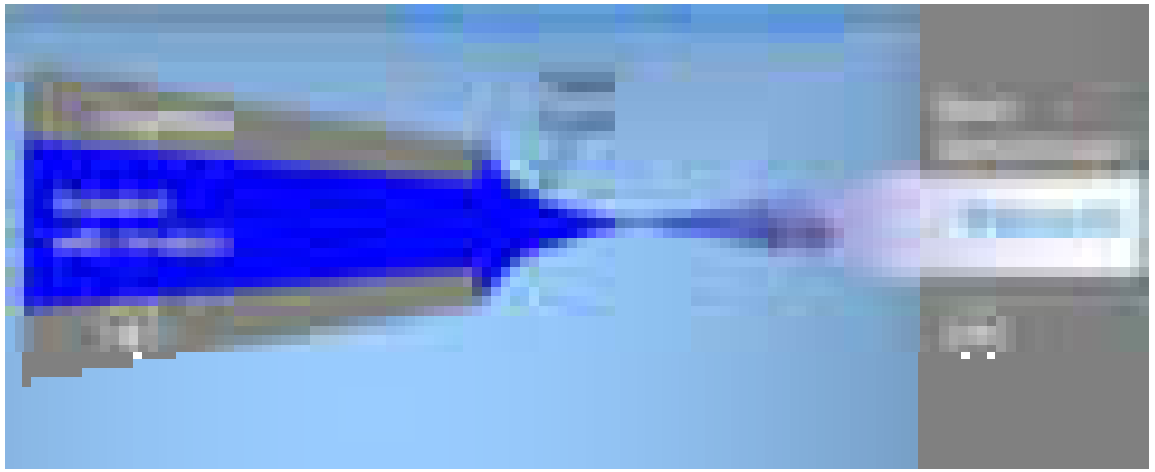
- where evaporation of the solvent attaining the charge in the molecules(desolvation)

↓

- desolvation process continues through various pumping stages as the molecular ion travels towards the mass analyzer.

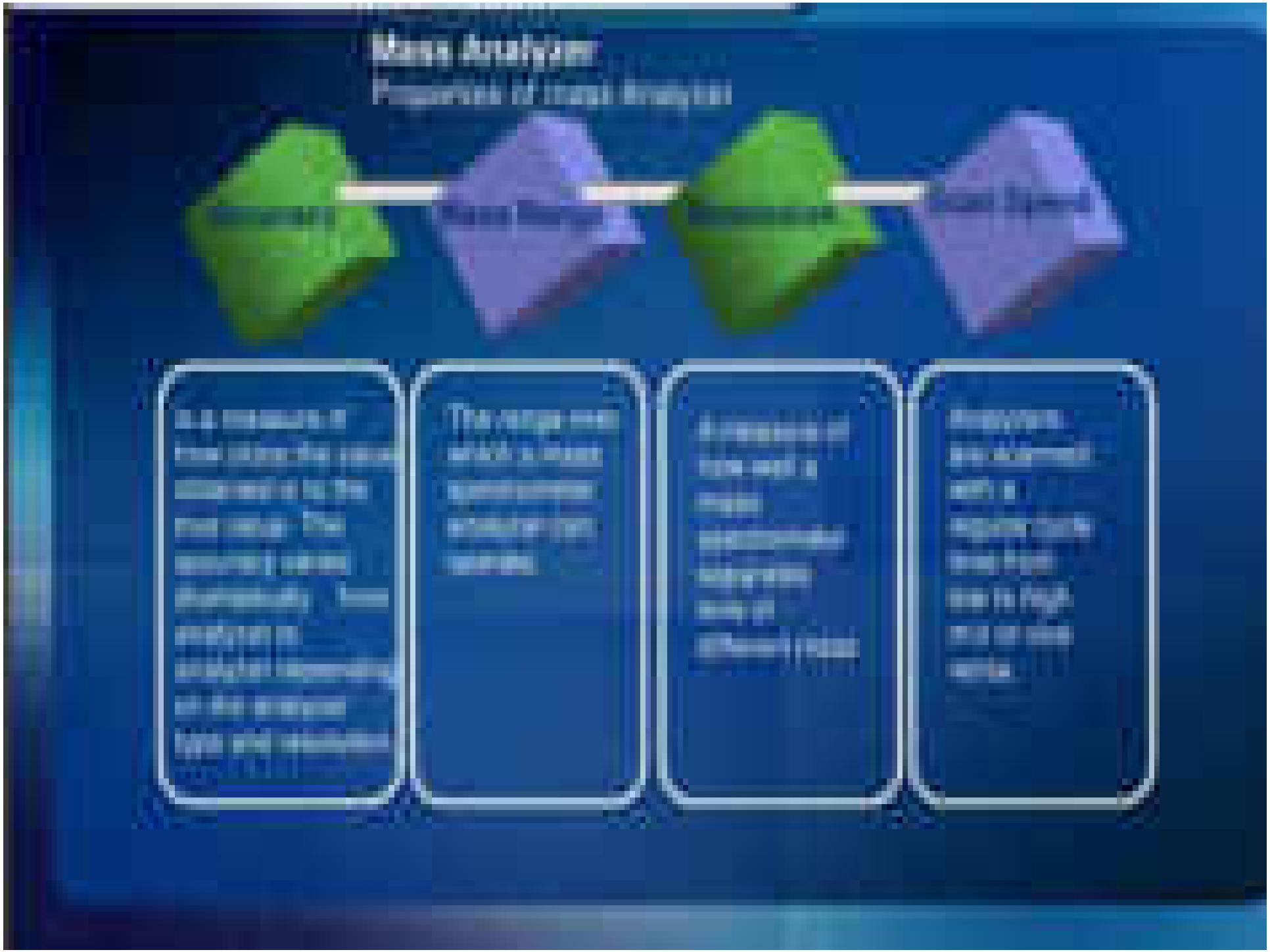


Electrospray (ESI)

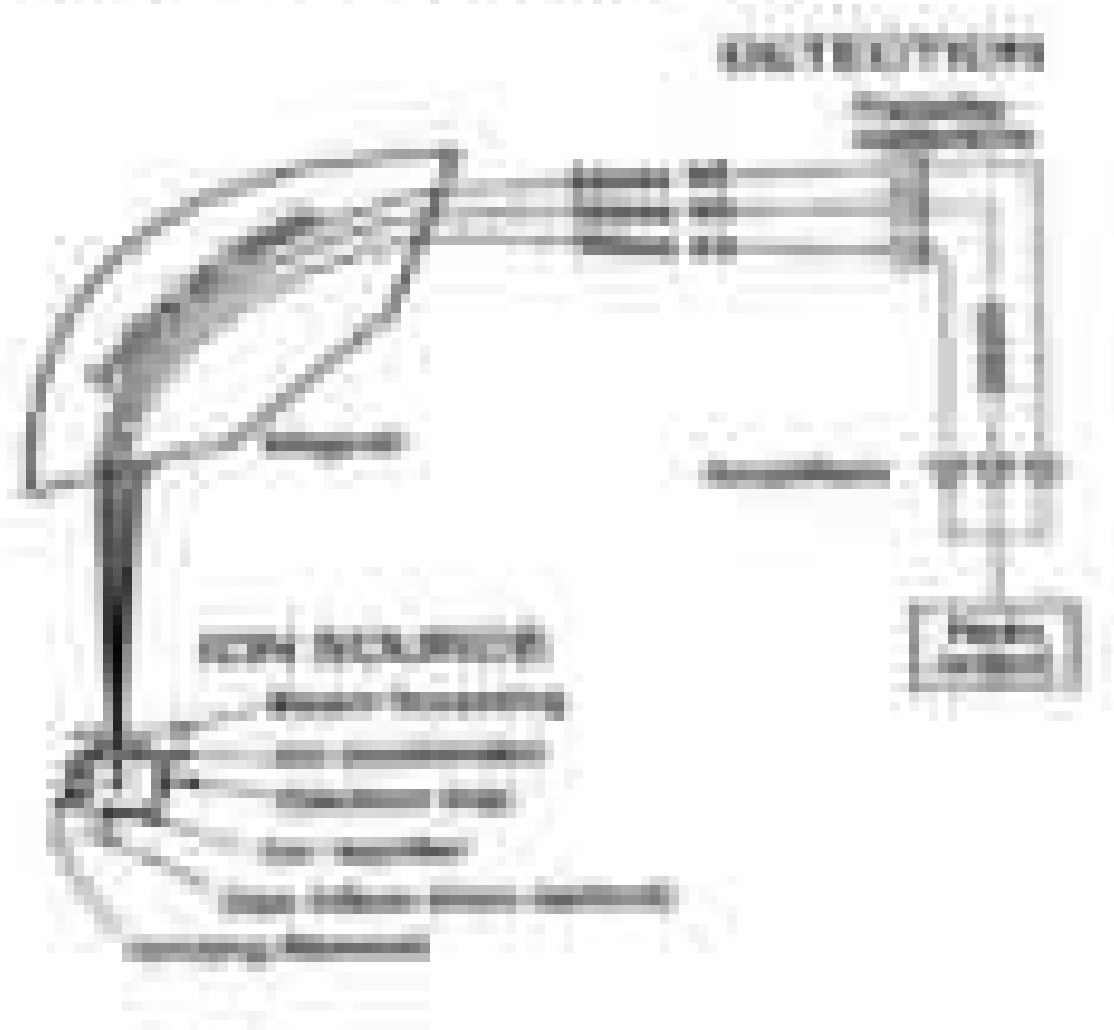


- MASS ANALYSERS: ion seperator

- SINGLE FOCUSSING ANALYSER
- DOUBLE FOCUSSING ANALYSER
- QUADRUPOLE ANALYSER
- TIME OF FLIGHT ANALYSER



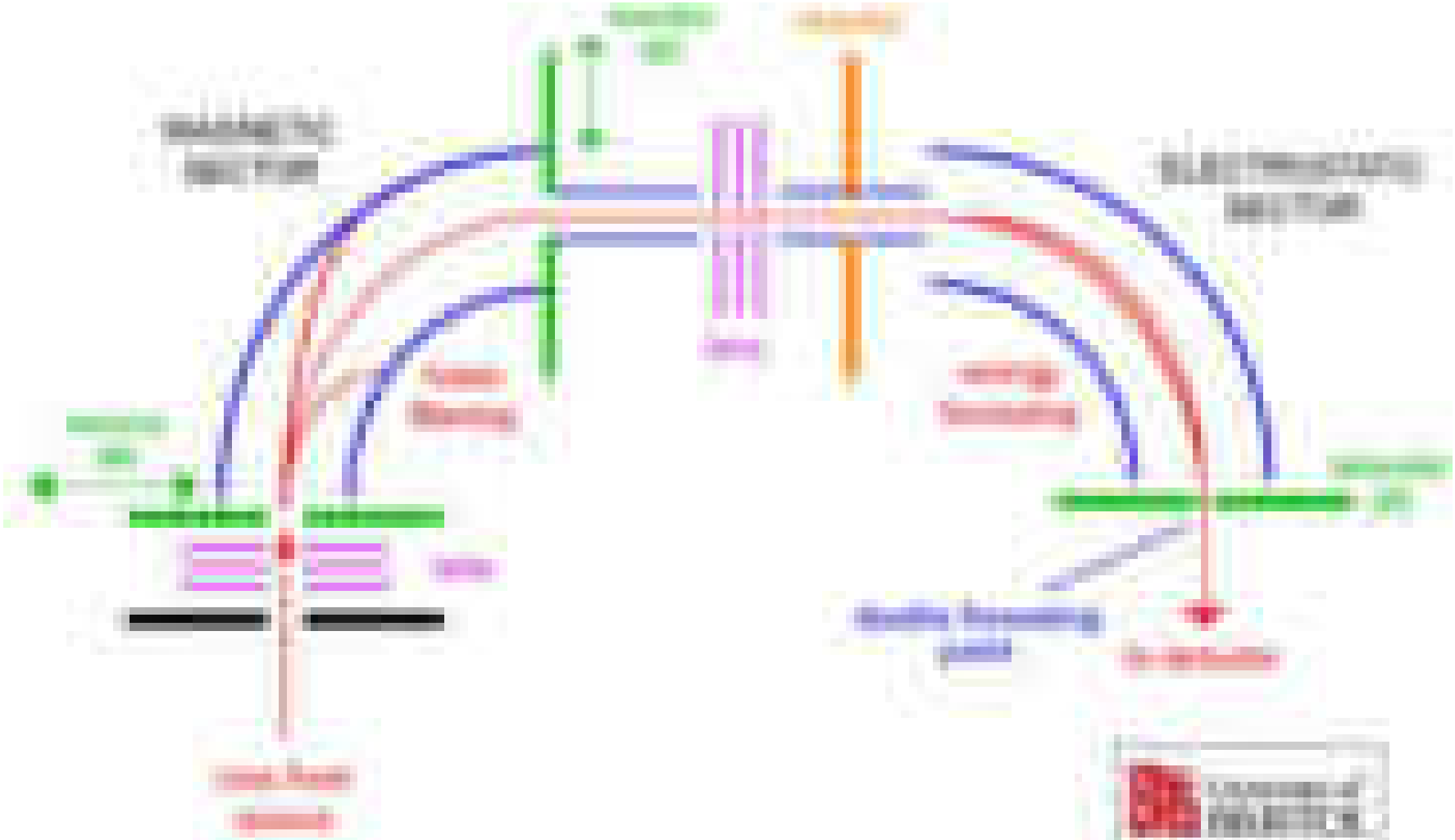
1. SINGLE FOCUSING ANALYSER:



- It has horse shoe shaped glass tube which is evacuated, consists of sample inlet, electron bombarding source, accelerating plates on one end & collector slit at other end.
- At curvature of tube there is provision to apply electric/magnetic field
- Sample in the form vapour is allowed through inlet and bombarded with electron beam at 70eV

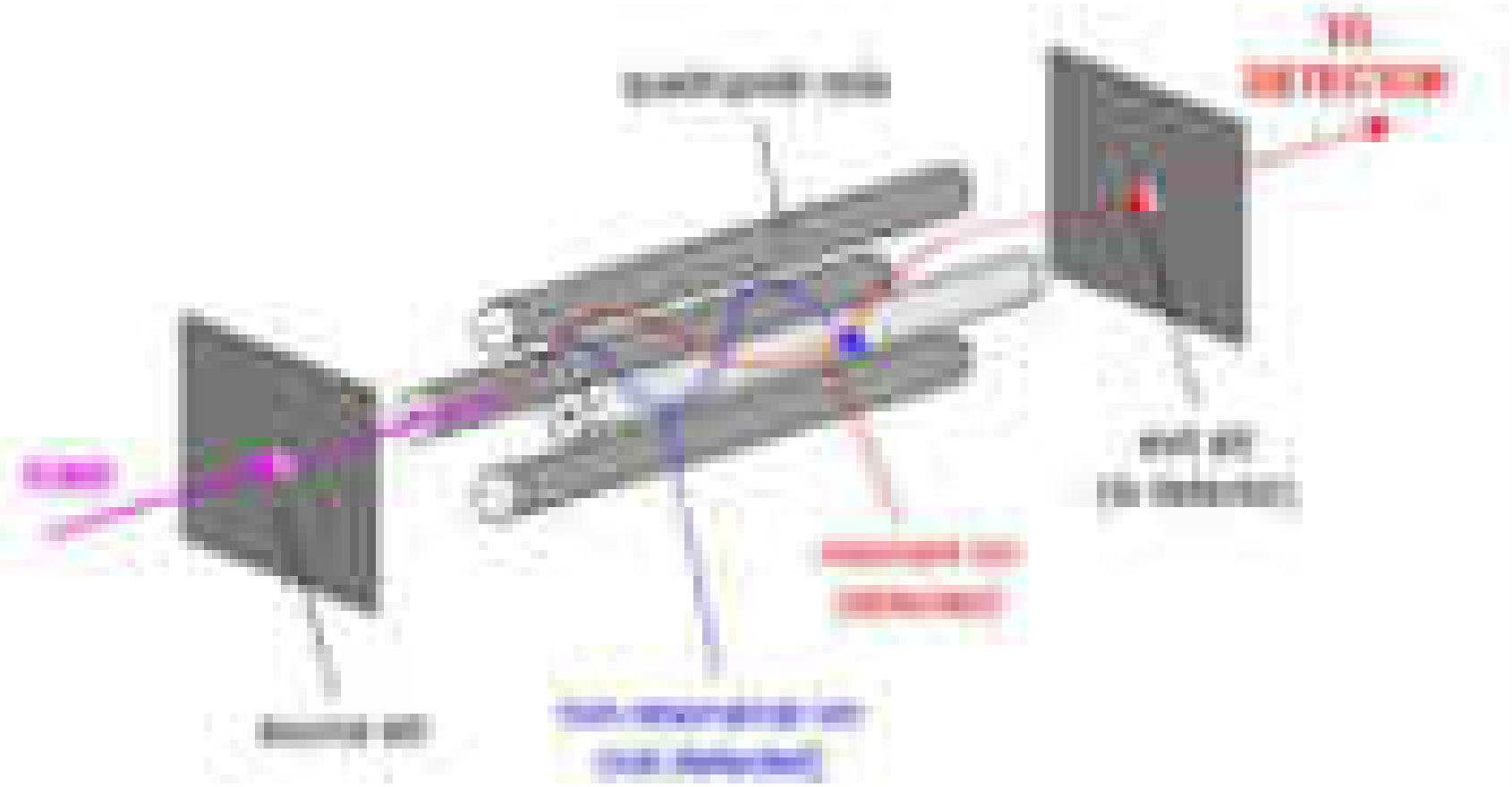
- It knocks off one electron from every molecule then they become +vely charged ion.
- As these molecules become +ve charged, they are accelerated by accelerating plates and travel in straight path.
- By application of electric or magnetic field they travel in curved path & molecular ions are separated according to their masses and collected
- Different fragments fall on detector then mass spectrum is recorded

DOUBLE FOCUSSING ANALYSER:



- It is used differentiate the small mass difference of the fragment.
- The provides the resolution as high as three
- To achieve better focusing, energy has to be reduced before ions are allowed to enter the magnetic field and increase resolving power can be obtained for mass

QUADRUPOLE MASS ANALYSER



Mass Analyzer: Quadrupole (Q)

Four parallel rods or poles through which the ions being separated are passed.
Poles have a fixed DC and alternating RF voltages applied to them.

- Depending on the produced electric field, only ions of a particular m/z will be focused on the detector, all the other ions will be deflected into the rods.
- Scanning by varying the amplitude of the voltages (AC/DC constant)

- It consists of 4 voltage carrying rods.
- The ions are pass from one end to another end
- During this apply the radiofrequency and voltage complex oscillations will take place.
- Here the single positive charge ions shows the stable oscillation and the remaining the shows the unstable oscillations.
- Mass scanning is carried out by varying each of the rf and voltage frequencies ratios keeping their ratios constant.
  - Quadrupole ion storage (ion trap)
  - It stores the unsorted ions temporarily, they released to the detector by scanning the electric field.

- TIME OF FLIGHT ANALYSER:

In this type of analyser the sorting of ions is done by absence of magnetic field.

The ions produced are acquiring different velocities depending on their masses

Here the particles reach the detector in the order of the increasing order of their masses

Here electron multiplier detector is used.

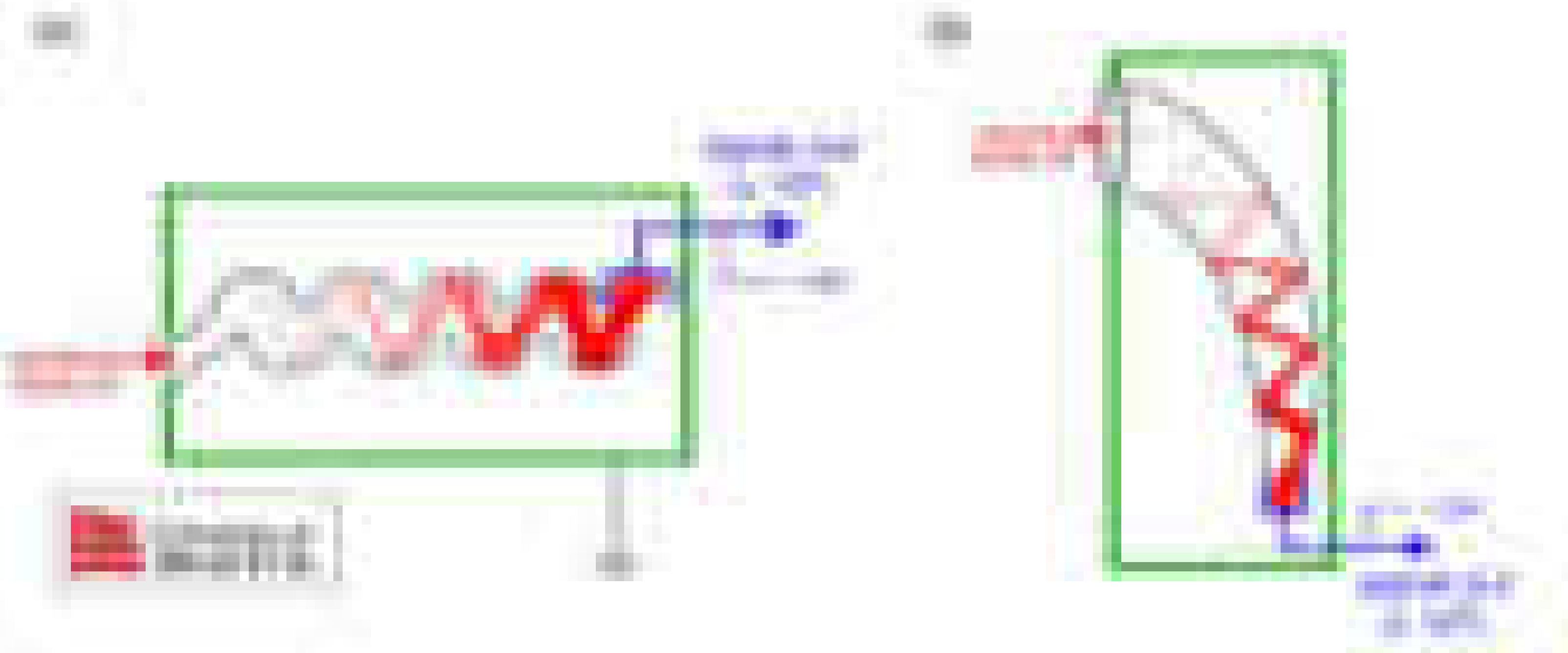
The resolution power of this is 500-600

- MASS DETECTORS:

The Faraday cup detector:

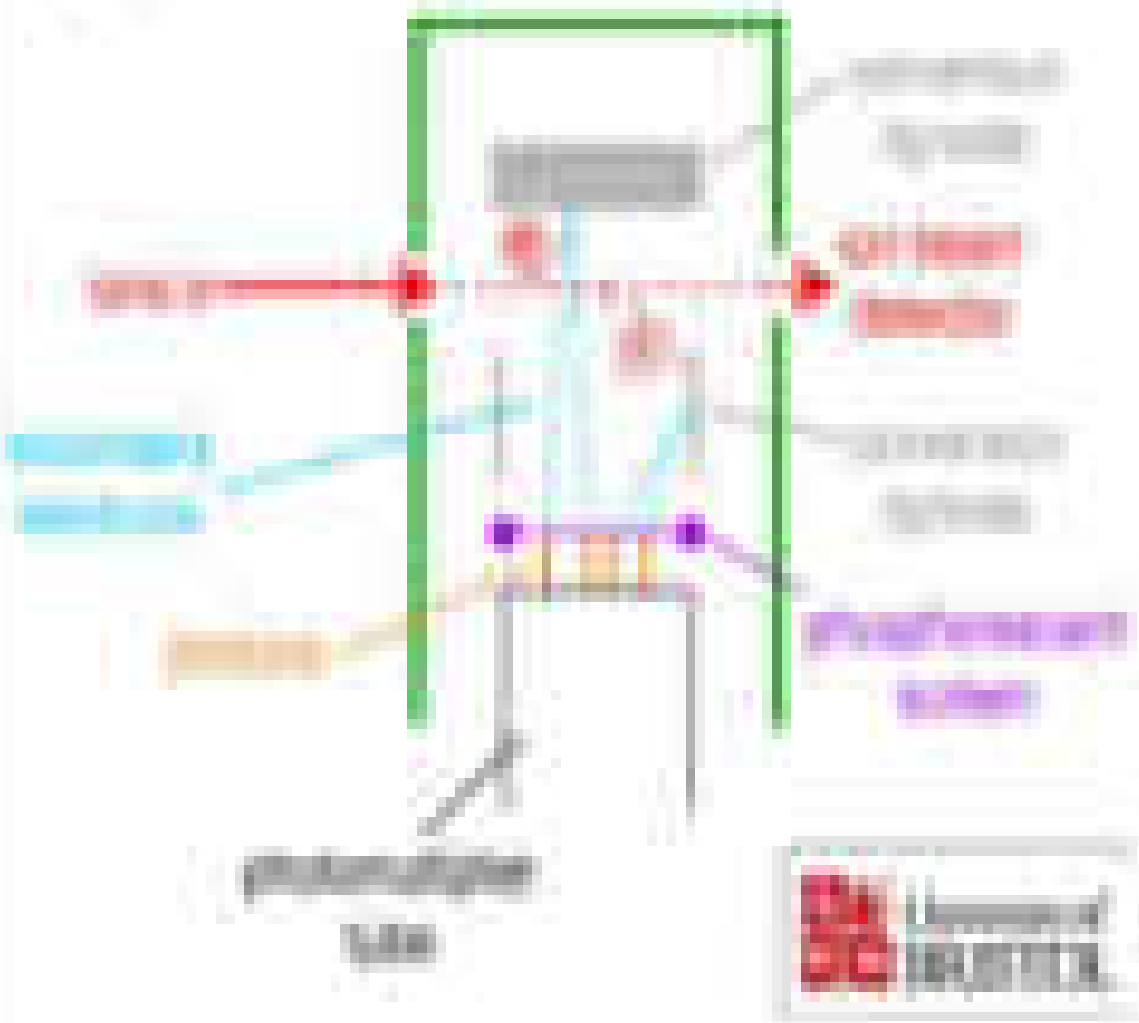
- The detector is very simple.
- The basic principle is that the incident ion strikes the dynode surface
- which emits electrons and induces a current which is amplified and recorded.
- The dynode electrode is made of a secondary emitting material like $CsSb_3$ or BeO.

Electron multiplier



The sensitivity of the detector is [illegible]
Two types of detectors: 1) series of dynodes are used; 2) single horn-shaped dynode. This is similar to the PMT

photomultiplier detector



Positive ion

Strike dynode

Release electrons

Fall on the phosphorescent screen

Produce the photons

Transfer to PMT

amplification

Types of ions produced:

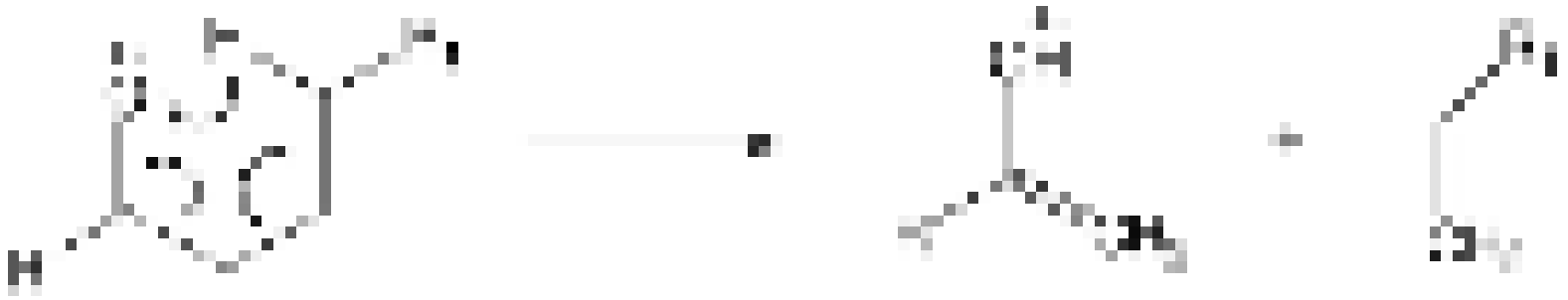
a) Molecular ion or parent ions
b) Fragment ion
c) Rearrangement ion
d) Metastable ions
e) Multiple charged ions
f) Isotope ions
g) Negative ions

- Molecular ion:
- If the electron beam energy is [illegible] than ionisation potential, electrons may be ejected from a lower lying molecular orbital. That type of ion are called molecular ion.
- The molecular ions are formed in the ground state, the yield of molecular ions can be increased by increasing the electron beamenergy

Fragment ion

- Rearrangement ions:
- This ions is produced by rearrangement of hydrogen atoms one part of the ion to another part.
- Rearrangement process common in the unsaturated compounds.

Ex : Allyl chloride rearrangement



- Metastable ion:

  Stable and unstable ion on fragmentation gives the sharp peaks, but intermediate stability ions gives the broad peaks

Multiple charged ion:

Loss of two or more electrons from a molecule with out fragmentation produces [illegible] molecular ions

- Isotope ions:

If the molecule having the F, Cl, Br, I, P produce the isotope peaks.

Ex; methyl bromide

CH_3Br^{79} gives one parent peak at m/e 94

CH_3Br^{81} gives one parent peak at m/e 96

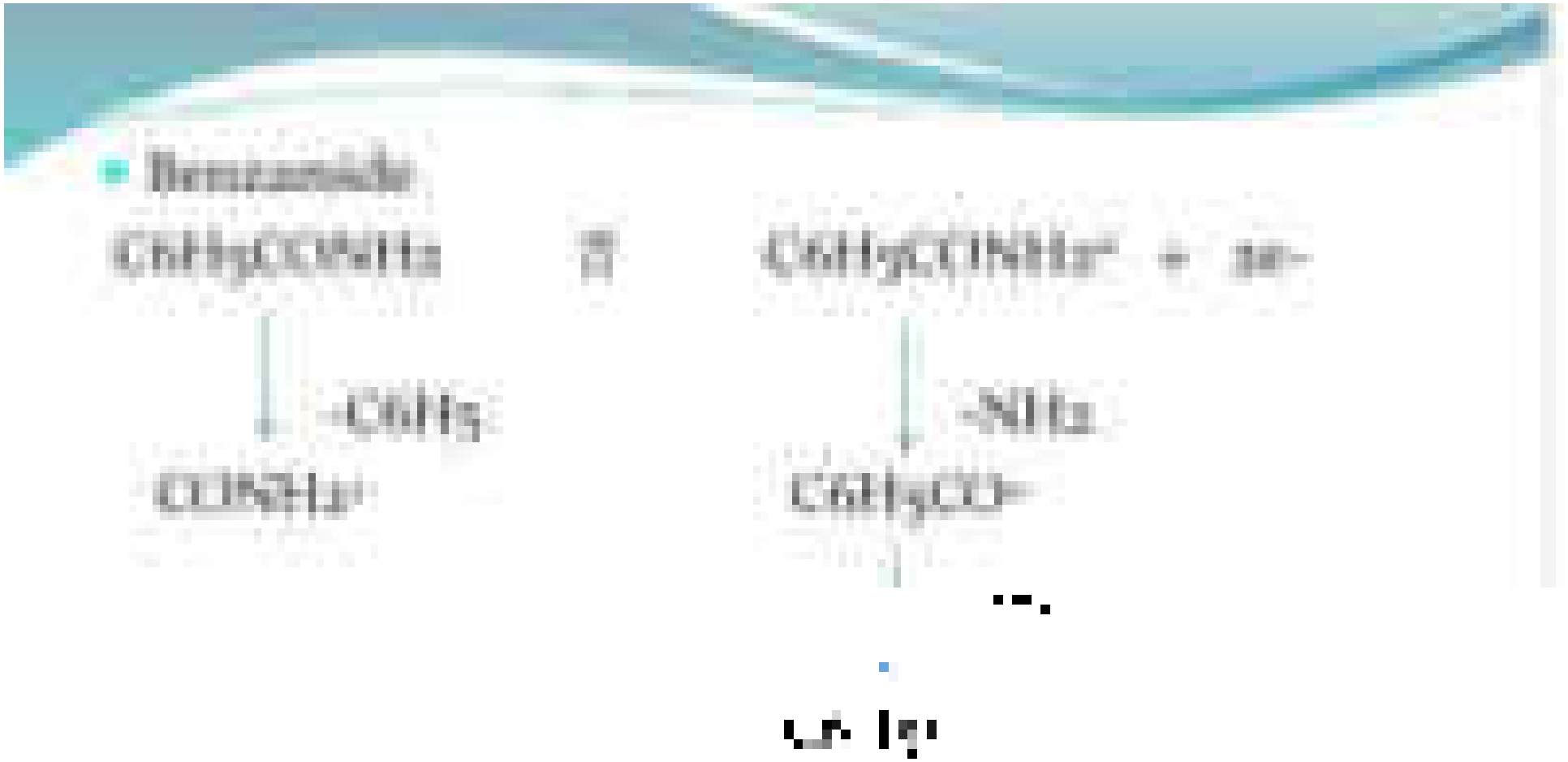
- Negative ions:

In few cases only negative ions are formed during the fragmentation.

These are formed by capture of the electron by the molecule during the collision.

FRAGMENTATION

- The process of breaking molecules/ions into fragments is known as fragmentation.
- This can be seen in the form of peaks in mass spectra
- Methanol can be divided in to fragments



- FRAGMENTATION RULES:
  - (a) Straight chain compound – relative height of molecular ion peak is great
    Branched chain – height decreases
  - (b) Molecular wt increases – height decreases

3)Cleavage is favoured at branched carbon atoms, more branched more likely the cleavage

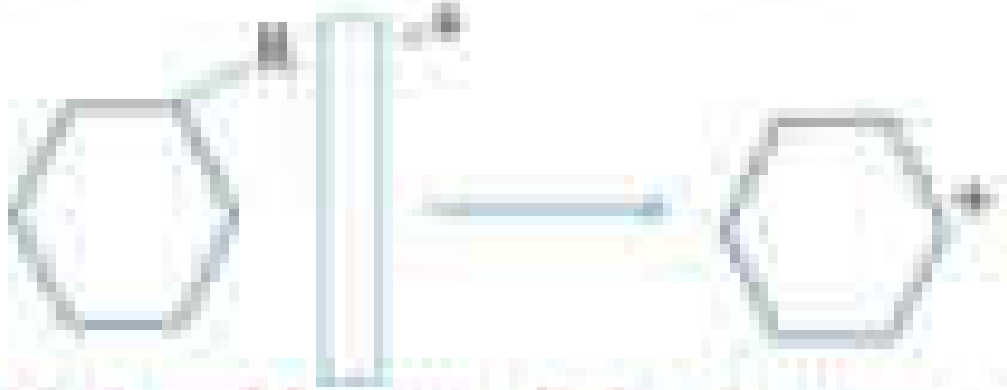
4) Cleavage occurs at alkyl substituted carbon atom, the more substituted, more likely is the cleavage.

Consequence of increased stability of 3° carbonium ion over a 2° which in turn more stable than 1°.

iii)In alkyl substituted aromatic compounds, cleavage occur at bond β to the ring

iv)Cleavage of c-x bond is difficult than c-c bond. It occur +ve charge is carried by carbon atom not by the heteroatom

7) Saturated ring (non alkyl side chain at α bond), +ve charge tends to stay with ring fragment.



8) Double bond (non allylic cleavage) is given resonance stabilized allylic carbonium ion.

$$CH_2{=}CH{-}CH_2{-}R \longrightarrow CH_2{-}CH{=}CH_2$$

FRAGMENTATION PATTERN

- Relative abundance of ions of various masses is characteristic of particular compound under the specified conditions of excitation, is known as fragmentation pattern.

- Strong peak of large mass number is taken as parent peak.

- Molecular peak of a compound depends up on stability of molecular ion & stability of radical ion
- Stability of ion can be justified by stabilization of charge
- Increased order of stability is
- aliphatic alcohols<acids<esters<ethers<aldehydes<ketones<cyclo alkenes<alkenes<conjugated polyenes<aromatic and hetero aromatic compounds

MCLAFFERTY REARRANGEMENT

- Rearrangement ions are fragments, they are formed due to the result of intermolecular atomic rearrangement during fragmentation
- To undergo this rearrangement the molecule must possess heteroatom, one double bond and hydrogen atom

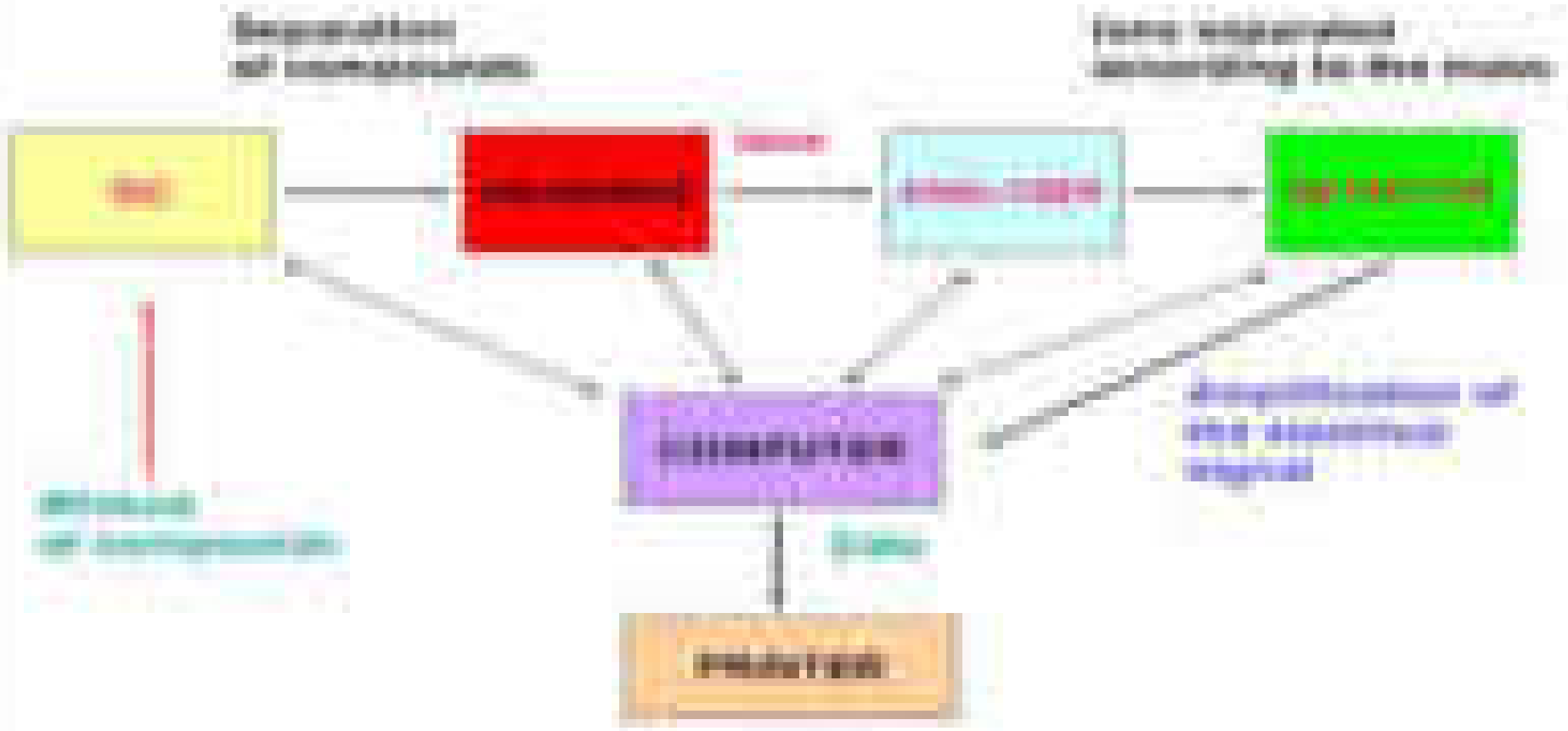
NITROGEN RULE:

- It is used for determination of molecular mass of compounds and its elemental composition
- Molecules having odd mass number contain odd number of nitrogen atoms.
- Molecules having even mass number contain even no of nitrogen atoms.

GC/MS

- GC is coupled to MS through an interface, in this complex mixtures of chemicals are separated, identified and quantified
- Compound to be analyzed should be volatile & thermally stable
- Sample solution is injected in to GC inlet there it is vaporized and swept on chromatographic column by carrier gas
- Sample flows through column and compounds in the sample mixture are separated by their interaction with column coating mixture and carrier gas

- That separated components are passed through the MS inlet, into the MS and there the compounds are analysed and detected.



LC/MS

- Liquid chromatography-mass spectrometry is a technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry.

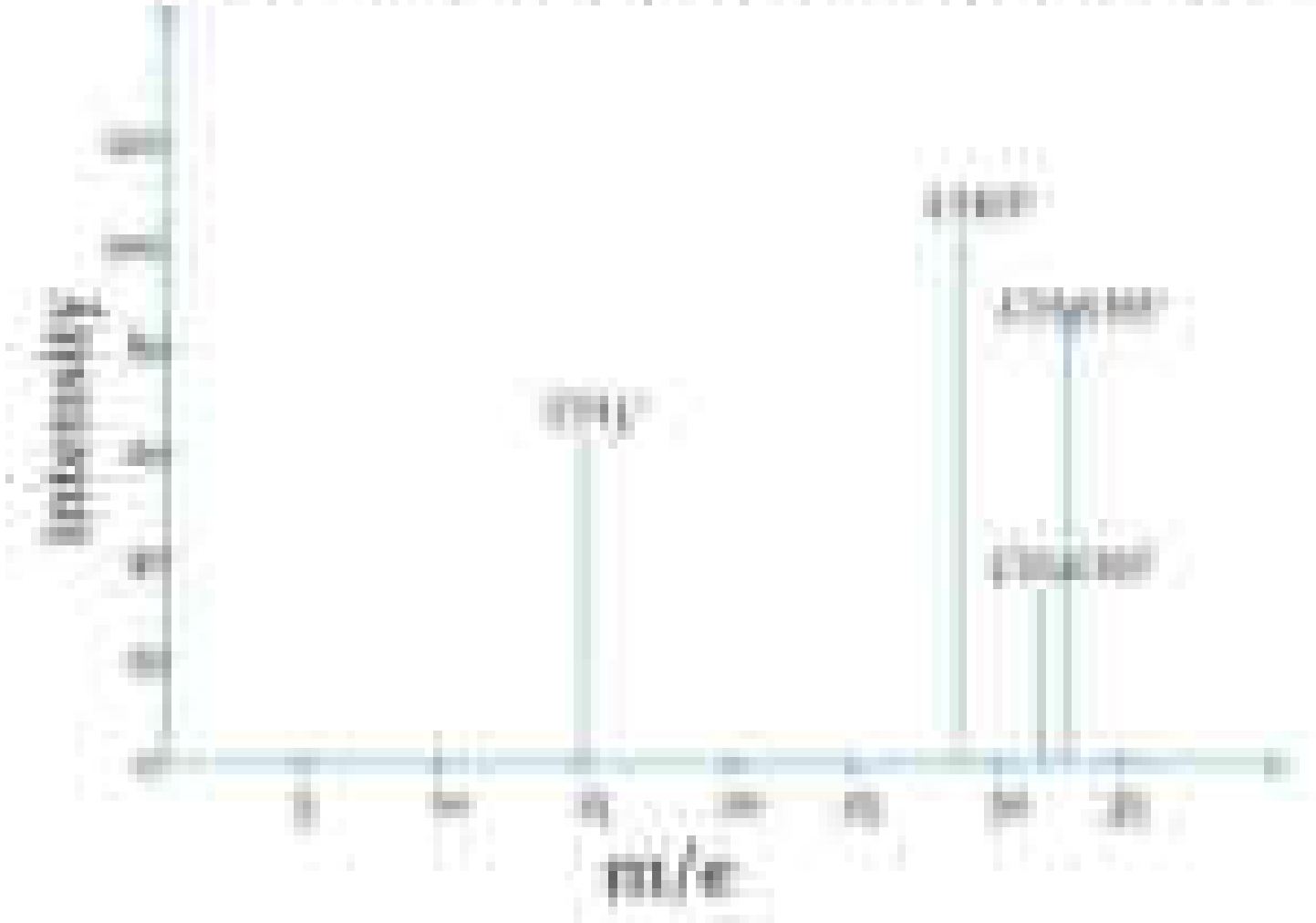
- In this Sample solution is injected in to HPLC column.

- These columns comprises of narrow stain less steel tube, packed with chemically modified silica particles.

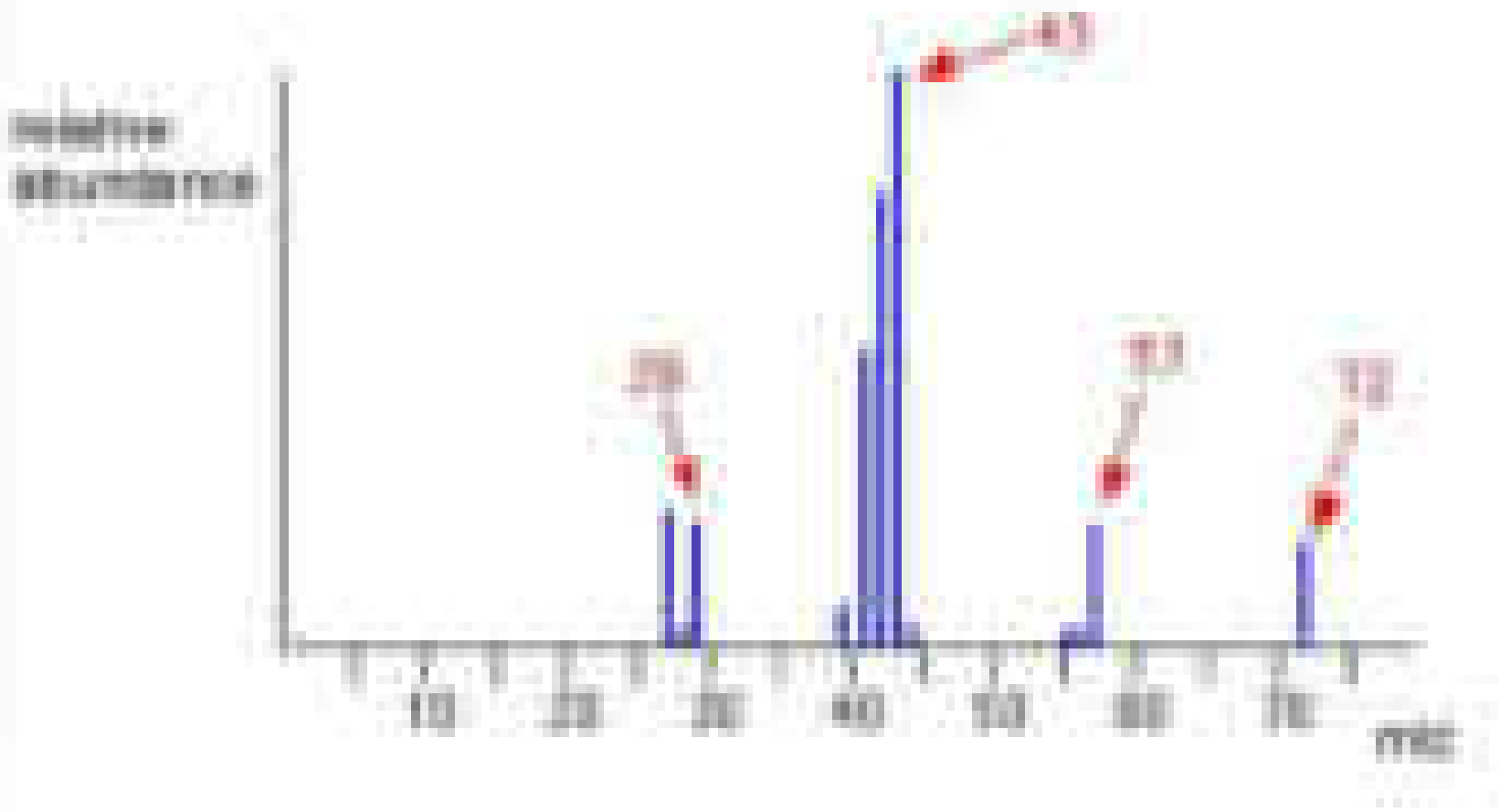
- Components eluting from the chromatographic column are then introduced to mass spectra via specialized interface.

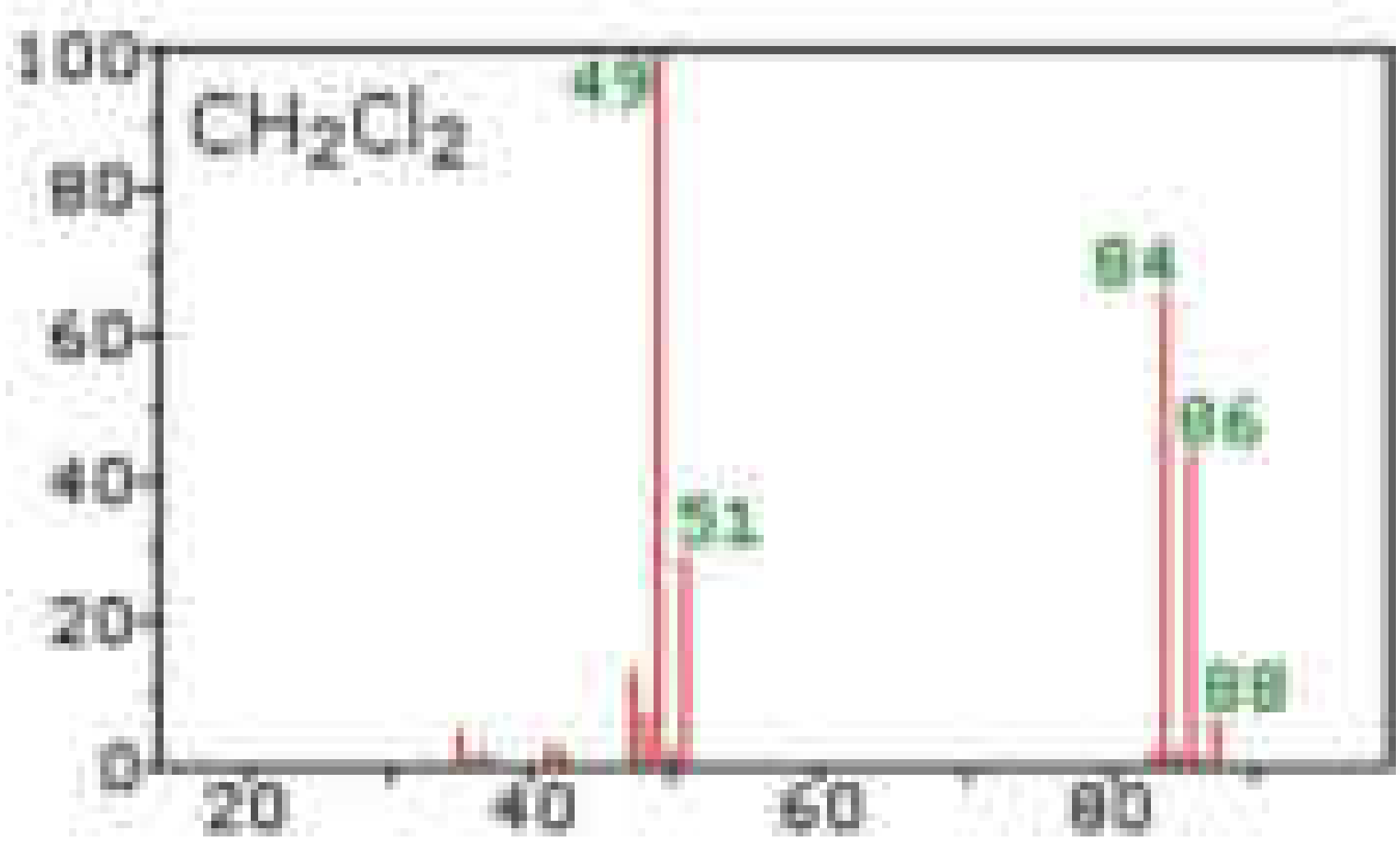
- The most commonly used interfaces are electrospray ionization, atmospheric pressure chemical ionization interfaces.

INTERPRETATION OF METHANOL



INTERPRETATION OF PENTANE




100
80
60
40
20
0
CH_2Cl_2
49
84
86
51
88
20
40
60
80

APPLICATIONS

- Determination of molecular mass & ionization potential
- Determination of elemental composition
- To know the reaction kinetics
- To elucidate chemical structure of molecule
- Detection of impurities
- Used in drug metabolism studies
- Determination of bond dissociation energies
- Determination of isotopic composition of elements in molecule

REFERENCES

- Spectrometric identification of organic compounds by Robert M. Silverstein.
- Instrumental methods of chemical analysis by Gurdeep, R.chatwal
- Organic spectroscopy by William kemp