Mass Spectroscopy

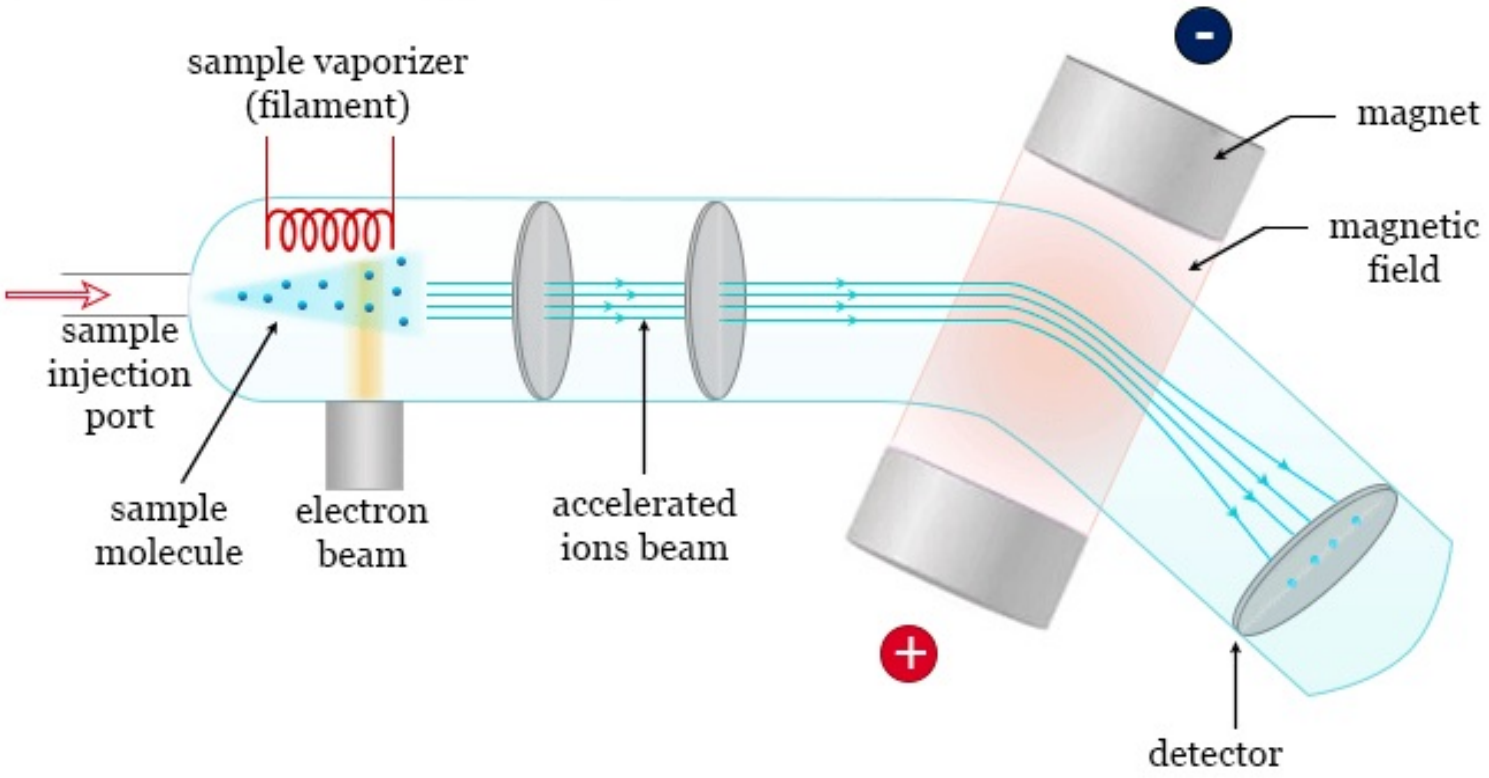
Mass spectroscopy is an analytical technique used to identify compounds based on their mass.

It helps us determine:

The molecular mass of a substance.
The structure of its molecule (by analyzing fragmentation patterns).
The atomic or isotopic composition.

Mass spectrum is widely used in pharmaceutical analysis, biotechnology, forensic science, food testing, and environmental monitoring.

Mass spectrometry



Mass Spectrometry- is a powerful analytical technique used to identify and characterize compounds by measuring their mass-to-charge (m/z) ratios.

1 *Sample Injection Port:*

Introduction of Sample:

The sample (solid, liquid, or gaseous) is first introduced into the mass spectrum through the injection port.

Vaporization:

Inside, a sample vaporizer (with a filament) converts the sample into a gaseous form, which is a crucial step for subsequent ionization.

2 *Electron Beam (Electron Bombardment):*

Source of Electron Beam:

An electron gun emits a stream of high-energy electrons (typically 70 electron volts).

Formation of Ions:

The electron beam collides with the sample molecules, knocking out electrons and creating positively charged ions.

This process is called electron ionization (EI).
Often, a parent or molecular ion (M+) forms, alongside various fragment ions due to break-up under electron collision.

3 *Acceleration of Ions:*

The ions are then accelerated by an electric field (generated by applying a voltage).
This forms a thin, collimated ion beam that moves toward the mass analyzer.

4 *Magnetic Field (Mass Analyzer):*

The ion beam enters a region with a strong magnetic field, typically perpendicular to its trajectory.

According to Lorentz Force, ions are deflected in their path — the degree of deflection depends on their mass-to-charge ratio (m/z).
Heavier ions (higher m/z) are deflected less, while lighter ions (lower m/z) are deflected more.

5 *Detector:*

The ions eventually reach the detector, which converts their impact into an electrical signal.

The detector measures:

The number of ions (intensity) at each m/z

This forms a mass spectrum — a graph of ion abundance vs m/z.

◆ *Principle of Mass Spectroscopy* ◆

Mass spectroscopy operates on the following principle:

Charged particles are deflected by electric or magnetic fields in proportion to their mass-to-charge (m/z) ratio.

The main steps involved are:

1. Vaporization: The sample is first vaporized (typically under vacuum).
2. Ionization: Atoms or molecules are ionized, usually by electron bombardment (forming positive ions).
3. Acceleration: The ions are accelerated by an electric field.
4. Deflection: The ions are passed through a magnetic field, which deflects them based on their mass-to-charge ratio (m/z).
5. Detection: The ions reach a detector (such as electron multipliers), generating a signal.
6. Analysis: The mass spectrum is displayed, showing peaks at m/z values. Each peak corresponds to a particular ion.

Different types of ionization like electron impact, chemical, field, FAB and MALDI, APCI, ESI, APPI

1 Electron Impact Ionization (EI) 🔷

Principle:

An electron beam (typically 70 eV) is directed toward gaseous sample molecules.

This knocks out an electron, forming a positive molecular ion (M+·).

Application:

Small, volatile, and thermally stable compounds (drugs, pesticides).

Features:

Often produces extensive fragmentation — helpful for structure determination.

Standard method; widely used; extensive spectral libraries exist.

2️⃣ *Chemical Ionization (CI) -*

Principle:

Reagent gas (methane, isobutane, ammonia) is first ionized by electron impact.

This forms reactive ions, which then react with the sample to produce predominantly protonated ions ([M+H]+).

Application:

Less volatile or less stable compounds where EI might cause extensive fragmentation.

Features:

Soft ionization — yielding a strong [M+H]+ peak with less fragmentation.

3 *Field Ionization (FI)-*

Principle:

A strong electrostatic field (~10^8 volts/cm) is applied to a metal needle.

The high field pulls electron(s) directly from nearby molecules.

Application:

Mainly non-polar compounds.

Features:

Few or no fragmentation; predominantly forms molecular ions.

4 *Field Desorption (FD)*

Principle:

An electrostatic field is applied to a metal needle with the sample deposited upon it.

The high field assists in desorption and ionization directly from the surface.

Application:

Large, nonvolatile, or thermally labile compounds.

Features:

Soft ionization with little or no fragmentation.

5️⃣ *Fast Atom Bombardment (FAB) -*

Principle:

Neutral fast atoms (typically Ar or Xe) collide with a liquid matrix that contains the sample.

Application:

Large, nonvolatile, or thermally labile compounds (peptides, sugars).

Features:

Mainly forms [M+H]+ ions with little fragmentation.

🔷 6️⃣ *Matrix-Assisted Laser Desorption Ionization (MALDI)*

Principle:

The sample is co-crystallized with a matrix that absorbs laser energy.

A pulse laser (typically UV) desorbs and ionises both the matrix and the analyte.

Application:

Large biomolecules — proteins, polymers, oligonucleotides.

Features:

Mostly singly protonated ions; very low fragmentation; high mass range (up to 100,000 Da).

7 *Atmospheric Pressure Chemical Ionization (APCI) -*

Principle:

Liquid solution is sprayed into a heated nebulizer. solvent vaporises; a corona discharge ionises solvent molecules.
This forms reactive ions, which then transfer a proton to the analyte.

Application:
Less polar compounds; small- to medium-molecular weight compounds.
Features:
Mainly forms [M+H]+ ions with low fragmentation.

8 *Electrospray Ionization (ESI) -*

Principle:
Liquid solution is forced through a needle under high voltage.
Droplets form; solvent evaporates, and ions are left in solution.

Application:
Large biomolecules — proteins, peptides, nucleotides.
Features:
Mainly forms multiple protonated ions ($[M+nH]^{n+}$).
Soft ionization — retaining molecular structure.

9 *Atmospheric Pressure Photoionization (APPI) -*

Principle:
UV light is used to photoionize a dopant (such as toluene).
This forms reactive ions, which subsequently ionize the analyte.

Application:
Less polar compounds, medium-molecular-weight compounds.
Features:
Mainly forms $[M+H]^+$ or $[M-H]^-$ with low fragmentation.

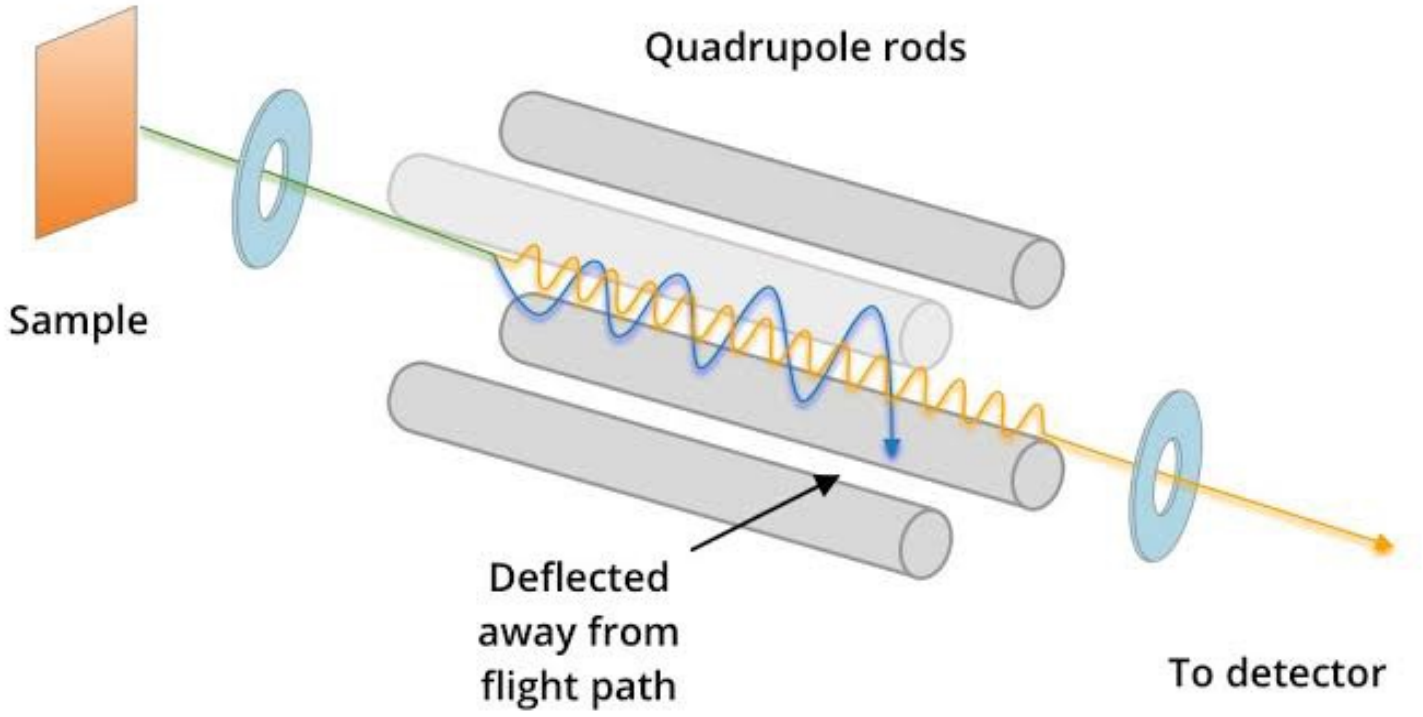
Mass Analyzers

1 Quadrupole Mass Analyzer

Principle:
Consists of 4 metal rods placed in parallel.
Radiofrequency (AC) and DC voltages are applied to these rods.

How it works:
Ions travel down the center.
Only ions with a specific m/z ratio have stable trajectory; rest are destabilized and collide with the rods.

Features:
Allows for scanning mass range.
Reliable, simple, low-cost, frequently used in GC-MS, LC-MS.



🔷 *Step 1 — Ion Source (Sample Introduction) -*

The sample is first introduced and ionized (typically by electron impact or electrospray).

This forms ions (with a positive charge) that are fed into the mass analyzer.

Step 2 — Entrance into Quadrupole Rods

The ions enter between the four metal rods placed in parallel — these are called quadrupole rods.
The rods are typically arranged in pairs, with alternating radiofrequency (AC) and direct current (DC) voltages applied.

🔷 *Step 3 — Electric Field Action -*

The combination of AC and DC fields generates a complex trajectory for the ions. Some ions will remain stable and travel forward toward the detector, while others will become unstable and deviate from their path — striking the rods or being removed.

Step 4 — Mass Filter Principle -

The stability of each ion's trajectory is influenced by its mass-to-charge (m/z) ratio.
The quadrupole can be tuned by changing the voltages to allow specific m/z ions to pass through while filtering out the rest.

🔷 *Step 5 — Detection -*

The ions that successfully pass through reach the detector at the far end of the quadrupole.
The detector converts the ions into an electric signal, which forms a mass spectrum.

2 *Time of Flight (TOF) Mass Analyzer-*

Principle:
Ions are accelerated by a voltage pulse.
All ions have same kinetic energy, but their velocity varies with mass to charge ratio (m/z).

How it works:
Lighter ions reach the detector faster, heavy ions arrive later.

Features:
Large mass range (up to 100,000 Da).
Fast and high-resolution.
Often used in MALDI-TOF for large biomolecules.

Mass Fragmentation and Rules-

When a molecule is ionized, it can break into fragments. Some general rules and mechanisms for fragmentation include:

✅ Cleavage at weak bonds (such as C–C, C–O, or C–N).

✅ Formation of more stable ions or radicals (such as benzyl, acylium ions).

✅ Rearrangements (like McLafferty rearrangement in carbonyl compounds).

✅ Heterolytic cleavage (forming a cation and a radical) under electron impact.

Meta-stable Ions -

Definition:
Ions that are formed in the ion source with sufficient energy to be unstable, but do not break immediately.
Instead, they decompose after traveling a certain distance in the mass analyzer.

Appearance in Mass Spectrum:
Meta-stable ions appear as diffuse or broad peaks.

Importance:
Provide information about fragmentation mechanisms and structures of ions.

Isotopic Peaks -

Atoms exist in different isotopic forms (with different number of neutrons).
This results in additional peaks at m+1 or m+2 alongside main peak.

Examples:
Carbon: 12C (100%) and 13C (~1.1%) → m+1 peak

Chlorine: 35Cl (~75%) and 37Cl (~25%) → m+2 peak

Bromine: 79Br (~50%) and 81Br (~50%) → m+2 peak with nearly equal intensity.

Applications of Mass Spectroscopy

1. Pharmaceutical Applications

a). Drug Discovery and Development:
Determining molecular mass, structure, and purity of new drugs.

b). Pharmaceutical Quality Control:
Detecting impurities, degradation products, or counterfeit drugs.

c). Metabolite Identification:
Analyzing metabolic products in plasma, urine, or other fluids.

2. Clinical Applications -

a). Proteomics and Peptide Mapping:
Studying protein structures and their modifications.

b). Hormone and Serum Drug Levels:
Quantitative determination for therapeutic drug monitoring.

c). Biomarker Discovery:
Detection of disease-related biomolecular patterns.

3. Environmental Applications -

a). Pollutant Detection:
Pesticides, heavy metals, industrial waste, toxic compounds in water, soil, or air.

b). Environmental Impact Assessments:
Analyzing soil, water, or sediments for traces of contaminants.

4. *Forensic Applications -*

a). Forensic Toxicology:
Detecting drugs of abuse, poisons, or alcohol in human fluids.
b). Evidence Analysis:
Trace identification of compounds at a crime scene (fiber, paint, explosive residues).

5. *General Research Applications -*

a). Protein Sequencing and Structural Elucidation:
Determining amino acid sequences.
b). Polymer Analysis:
Polymer structure, branching, and mass distribution.
c). Isotope Ratio Determination:
Applications in geochronology and metabolic studies.