

Introduction

Mass Spectrometry is the generation, separation and characterization of gas phase ions according to their relative mass as a function of charge. The requirement is that the sample be able to be vaporized (similar limitation to GC), but modern ionization techniques allow the study of such non-volatile molecules as proteins . The technique is a powerful qualitative and quantitative tool, routine analyses are performed down to the femtogram (10^{-15} g) level and as low as the zeptomole (10^{-21} mol) level for proteins .

Though organic mass spectrometry is used along with IR, NMR and UV for structural analysis it's theory is different. In mass spectrometry no absorption of radiation is involved as in the case of the others .

The Mass Spectrometer

-1 A mass spectrometer needs to perform three functions:

Creation of ions – the sample molecules are subjected to a high energy beam of electrons, converting some of them to ions.

Separation of ions – as they are accelerated in an electric or magnetic field, the ions are separated according to mass-to-charge ratio (m/z).

Detection of ions – as each separated population of ions is generated, the spectrometer needs to qualify and quantify them.

-3 Common to all is the need for very high vacuum to prevent interaction of analyte's ions with atmosphere's Components.

Components Of A Mass Spectrometer



Ionisation (Ion source)

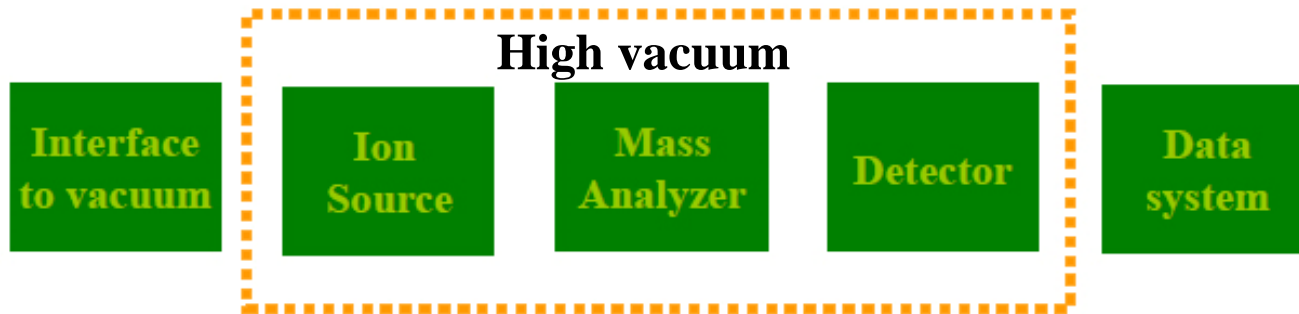
- Electron Ionisation (EI)
- Chemical Ionisation (CI)
- Fast Atom Bombardment (FAB)
- Electrospray Ionisation (ESI)
- Matrix-Assisted Laserdesorption/
Ionisation (MALDI)

Ion Separation (Mass Analyzer)

- Quadrupole
- Magnetic Sector Field
- Electric Sector Field
- Time-Of-Flight (TOF)
- Ion Trap

Ion Detection ((Detector

- Electron Multiplier
- Multichannel plate
- Faraday Cup



Principle of Mass Spectrometry

A small quantity of sample is injected and vaporized under high vacuum . The sample molecules or atoms are then bombarded with electrons having 25-80 eV of energy.

The molecules or atoms are ionized and broken up into many fragments, some of which are positive ions.

Ions (+) are accelerated using a (-) anode towards the focusing magnet.

Each kind of ions have a particular ratio of mass to charge i.e. m/e . For most ions ,the charge is one and thus , m/e is the molecular mass These ions are separated by deflection in magnetic or electric field according to their m/e ratio .For a given charge , the deflection is less for a heavy particle as compared to that of a light one of the ion .

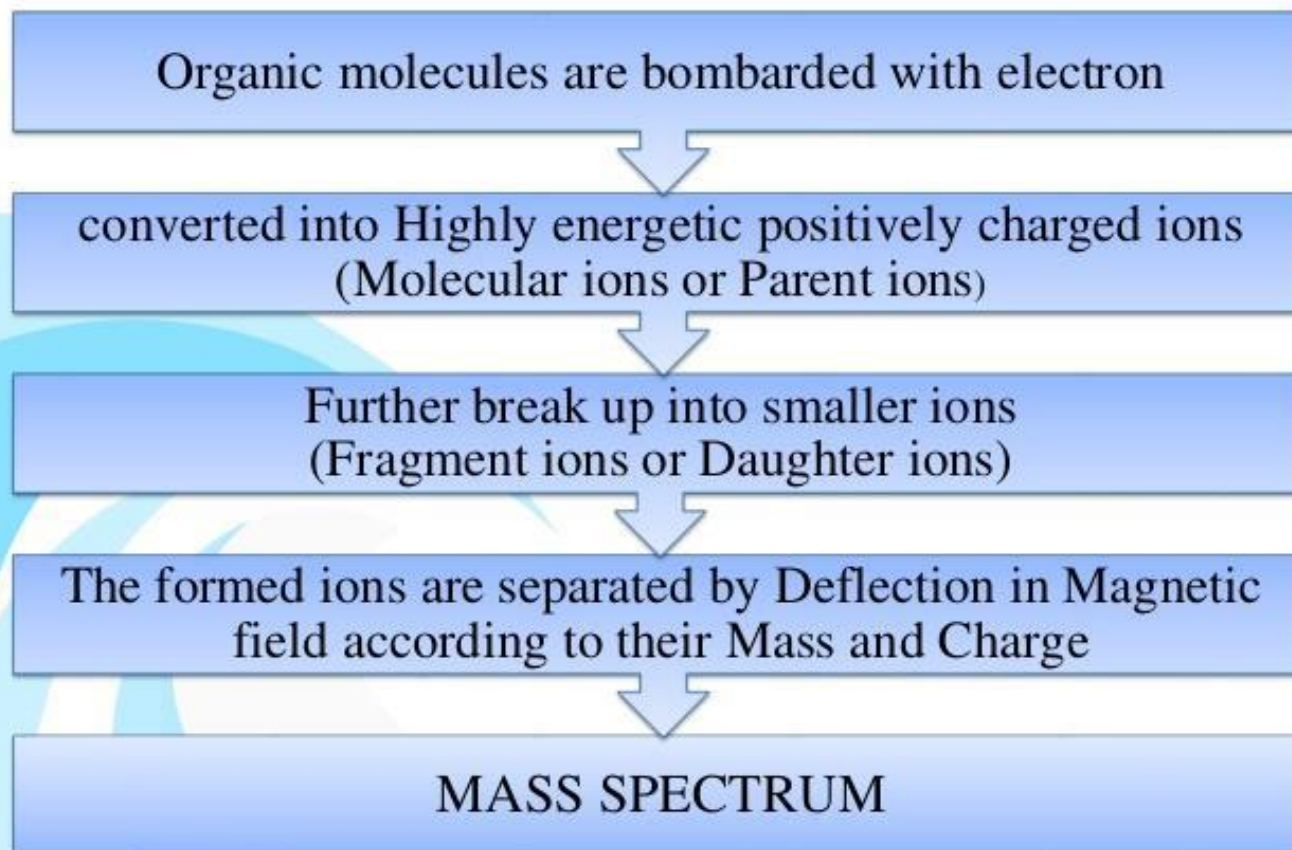
Principle of Mass Spectrometry ... Cont'd

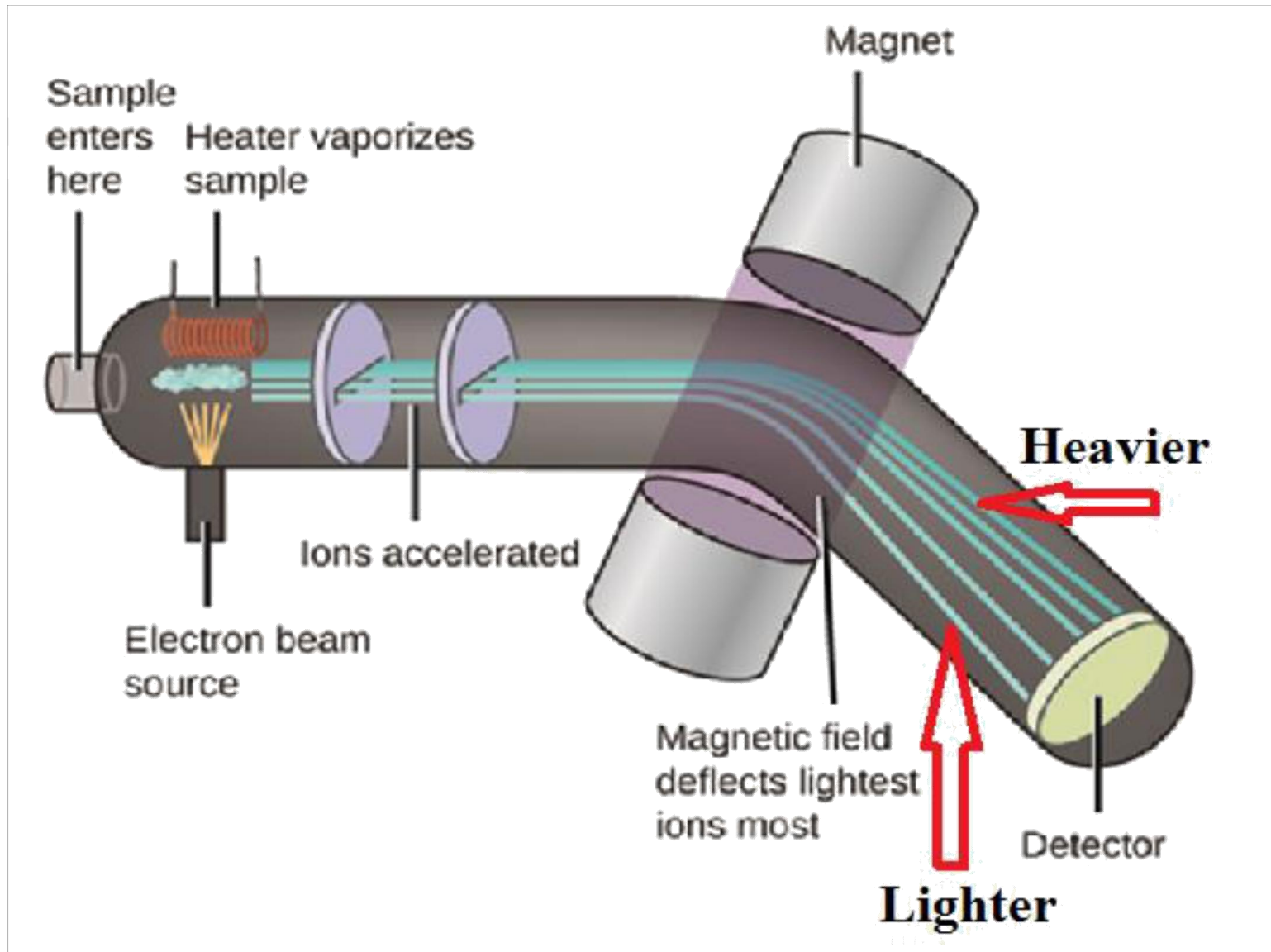
Thus , a number of beams each containing ions with the same m/e values are obtained .Not that ions with 2 (or more) positive charges are deflected more than ones with only one positive charge

These beams are then made to strike against a photographic plate where not only they appear as separate lines but the intensity of each peak is also recorded . Photomultiplier tube can be used instead of a photographic plate where the detector is basically a counter, that produces a current proportional to the number of ions that strike it. The ion-currents corresponding to the different ions are amplified and either displayed on an oscilloscope or a chart-recorder, or are stored in a computer.

The main interferences are encountered when 2 analytes have the same mass or when matrix species combine with the analyte and reduce the analyte signal.

BASIC PRINCIPLES





MASS SPECTRUM

The mass spectrum of a compound helps to establish the structure of a new compound in several different ways :

1- It can give the exact molecular mass .

2- It help in finding of elemental composition of parent ion and fragment ions .

3- It can give a molecular formula or it can reveal the presence of certain structure units (e.g. functional group) in a molecule. Thus, the mass spectrum of each compound is unique and can be used as a chemical fingerprint to characterize the sample .

The visual presentation of a mass spectrum is obtained by plotting m/e value vs relative abundance , assigning the most abundant ion (base peak) in the spectrum as 100 per cent .

MASS SPECTRUM

Fragmentation process :

Bombardment of molecules by an electron beam with energy 10 – 15 eV results in the ionization of molecules by removal of one electron To produce what are called **molecular ions** .

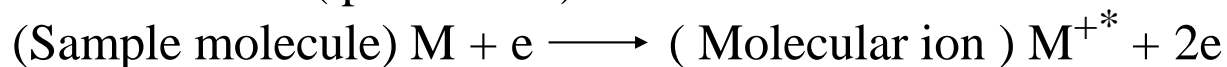
When the energy of electron beam is increased 50 – 70 eV , these molecular ions will break down into various fragments .

Therefor there are two main types of ions :

- 1- Molecular ion (parent ion) .
- 2- Fragment ions .

MASS SPECTRUM

Molecular ion (parent ion) :



The order of energy required to remove electrons is as follows :

(electrons > non-conjugated π > conjugated π > non-bonding electrons

In other words , the parent ion may reach the detector or may disintegrate to fragments ions depending on the stability of the analyte molecules which is in the following order :

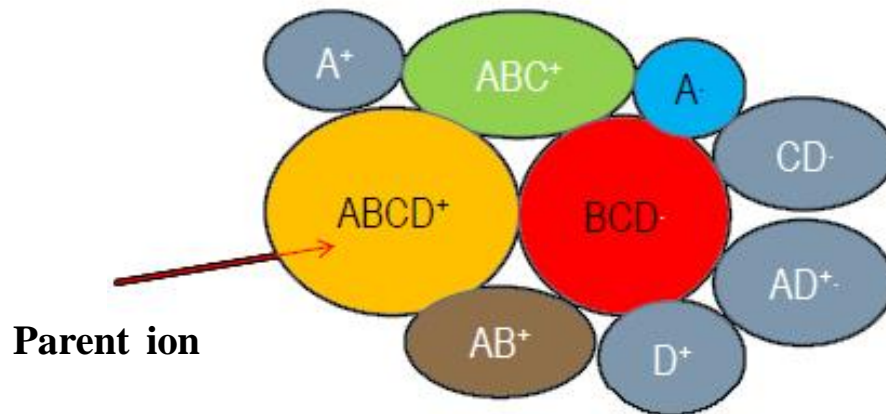
Aromatic > n-hydrocarbons > ketones > ethers > alcohols

i.e. double bonds and aromatic rings stabilize the parent ion and thus increase the probability of it's appearance in the spectrum .

MASS SPECTRUM

Example of fragmentation :

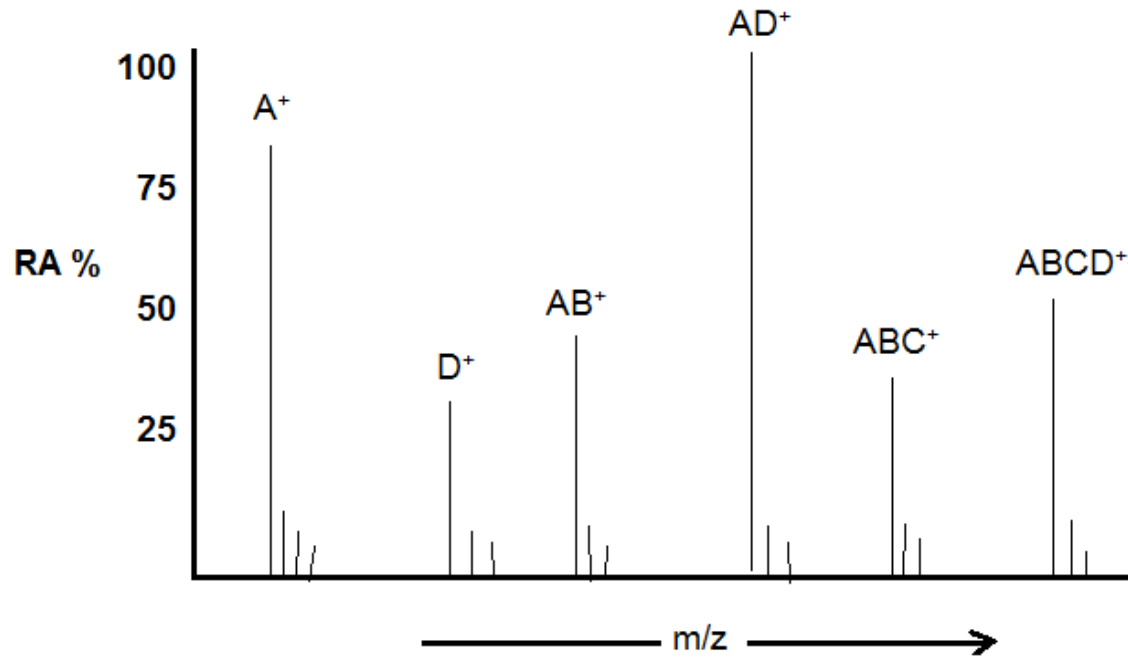
Ions produced in the ion source (ionization) of ABCD compound



MASS SPECTRUM

Hypothetical electron impact mass spectrum of a compound ABCD
(Mass spectrometer measures only positive ions)

RA = Relative Abundance



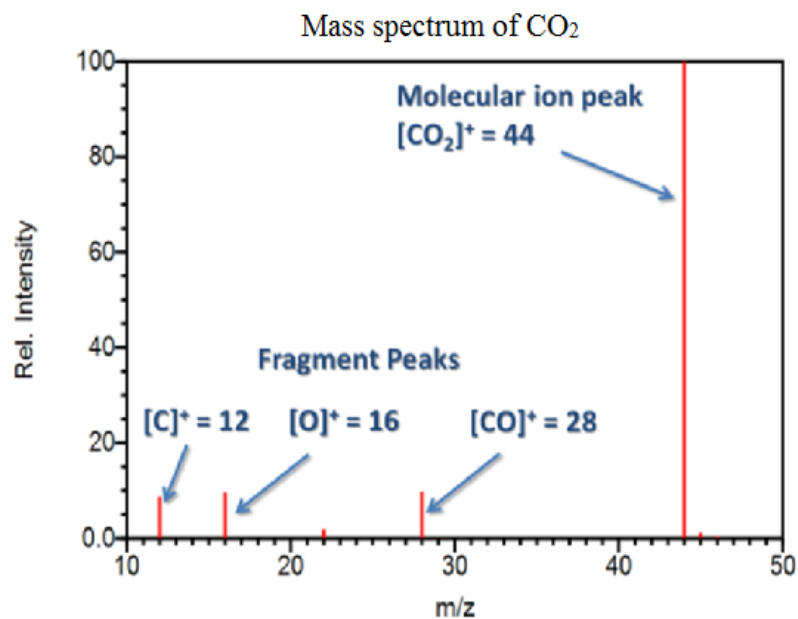
MASS SPECTRUM

The significance of parent ion peak that it gives the molecular weight of the compound .

Example : m/e of parent ion $\text{CO}_2^+ = 34 = \text{mw}$ of CO_2

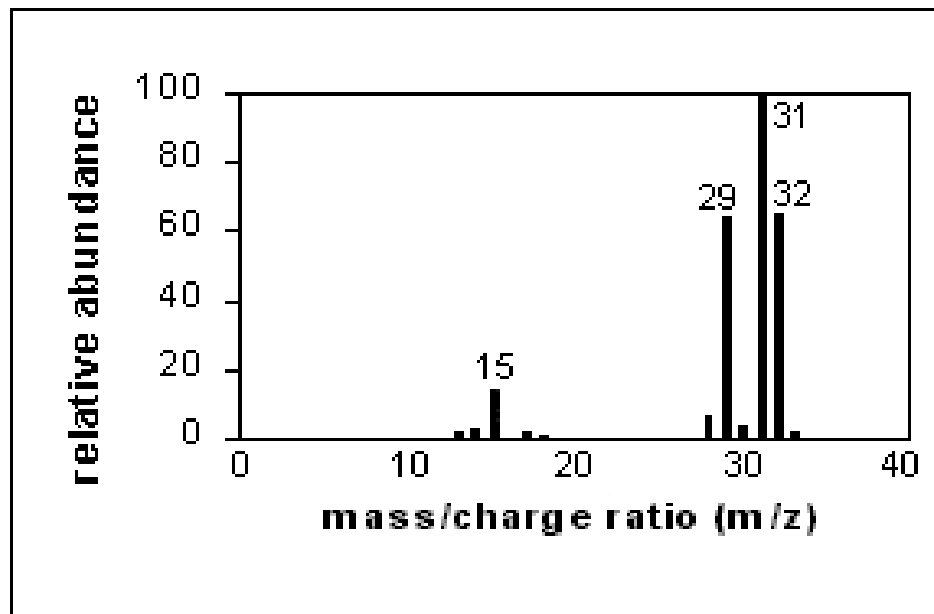
See the following mass spectrum of CO_2 .

Mass spectrum of CO_2 . Note that the molecular ion appears at $m/z = 44$ ($\text{C} = 12$, $\text{O} = 16$). Fragment ions appear at m/z values of 28, 16, and 12. These correspond to CO^+ , O^+ , and C^+ , respectively



Other example of Interpreting spectra

A simple spectrum, that of **methanol**, is shown here. **CH₃OH⁺** (the molecular ion) and fragment ions appear in this spectrum. Major peaks are shown in the table next to the spectrum. The x-axis of this bar graph is the increasing m/z ratio. The y-axis is the relative abundance of each ion, which is related to the number of times an ion of that m/z ratio strikes the detector. Assignment of relative abundance begins by assigning the most abundant ion a relative abundance of 100% (CH₂OH⁺ in this spectrum). All other ions are shown as a percentage of that most abundant ion. For example, there is approximately 64% of the ion CHO⁺ compared with the ion CH₂OH⁺ in this spectrum. The y-axis may also be shown as abundance (not relative). Relative abundance is a way to directly compare spectra produced at different times or using different instruments



ions	m/z
CH_3OH^+	32
$\text{H}_2\text{C}=\text{OH}^+$	31
$\text{HC}\equiv\text{O}^+$	29
H_3C^+	15

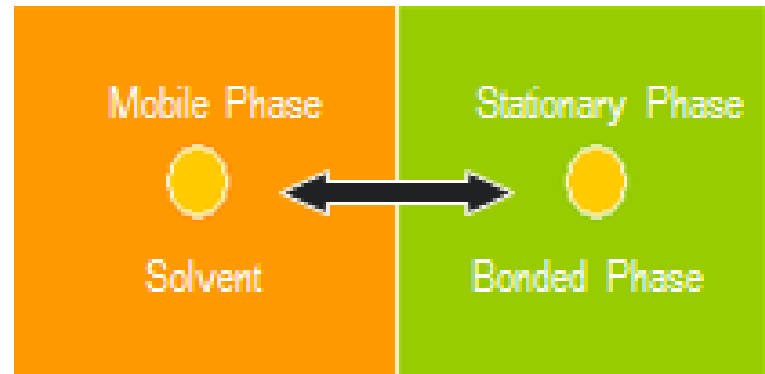
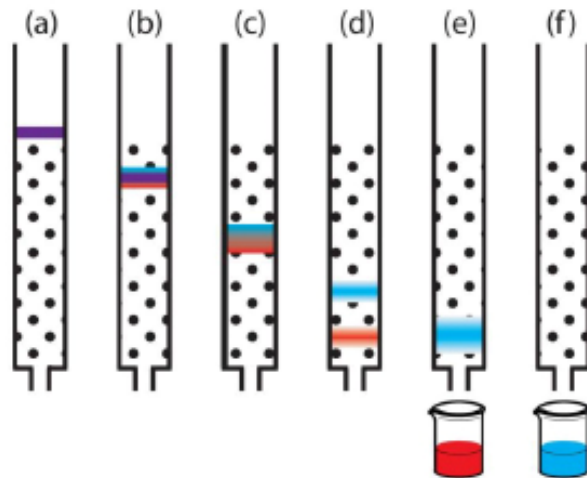
APPLICATIONS

Solid , liquid or gas samples can be analyzed . Mass spectrometry has special advantages such as high sensitivity and accuracy and the widely used coupling of mass spectrometry with ICP or chromatographic techniques such as Gas chromatography GC or high performance liquid chromatographic HPLC as we will see in the next slides . Mass spectrometry has wide applications in the analysis of various samples in food (pesticides) , clinical samples (drugs in blood and urine) ... etc. . Mass spectrometry is used to provide information about the elemental composition of samples of matter; the structures of inorganic, organic, and biological molecules; the qualitative and quantitative composition of complex mixtures;

Chromatography

In general, chromatography is used to separate mixtures of chemicals into individual components. Once isolated, the components can be evaluated individually. In all chromatography, separation occurs when the sample mixture is introduced (injected) into a mobile phase. In liquid chromatography (LC or HPLC), the mobile phase is a solvent or a mixture of solvents. In gas chromatography (GC), the mobile phase is an inert gas such as helium . The mobile phase carries the sample mixture through what is referred to as a stationary phase. The stationary phase is a usually chemical that can selectively retain components in a sample mixture. The stationary phase is usually contained in a tube called a column .

The mixture of compounds in the mobile phase interacts with the stationary phase. Each compound in the mixture interacts at a different rate. Those that retained weakly in the stationary phase will exit (elute from) the column first. Those that retained strongly will exit the column last. By changing characteristics of the mobile phase and the stationary phase, different mixtures of chemicals can be separated.

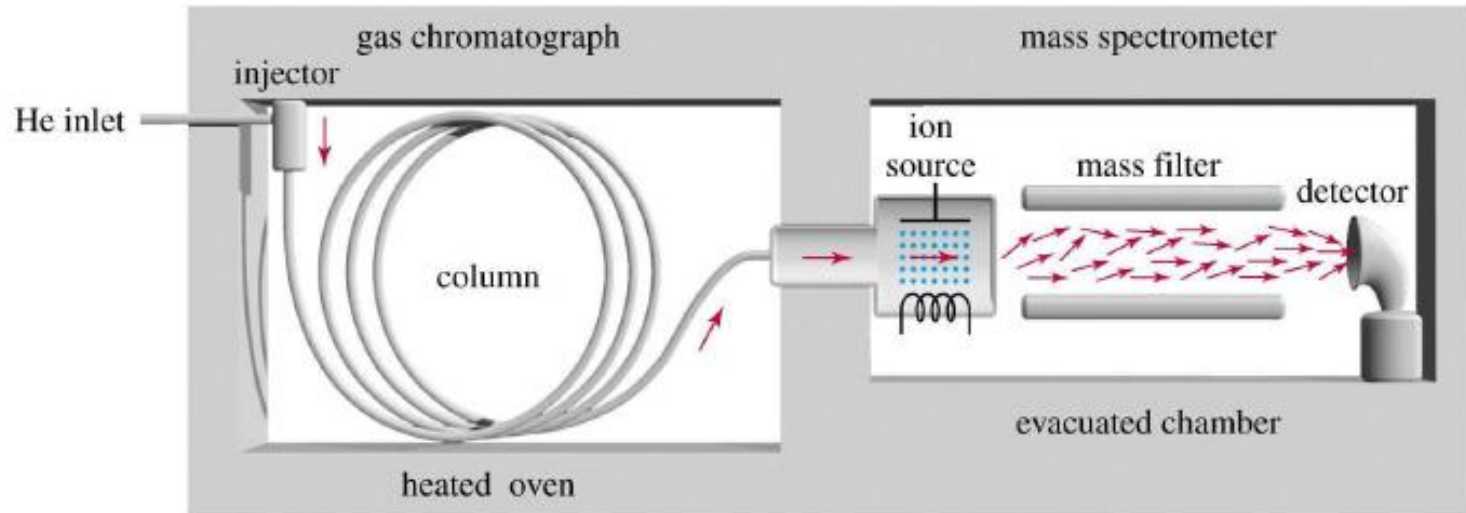


Gas Chromatography - Mass Spectroscopy (GC-MS)

GC - MS is actually two techniques that are combined to form a single method of analyzing complex mixtures of chemicals. Gas chromatography separates the components of the mixture after converting them to gases and mass spectroscopy characterizes each of the components individually. By combining the two techniques, an analytical chemist can both qualitatively and quantitatively evaluate a solution containing a large number of compounds .

The uses for GC-MS are numerous. They are used extensively in the medical, pharmacological, environmental, and many more fields .

Figure 1 : GC – MS Combination



Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification.

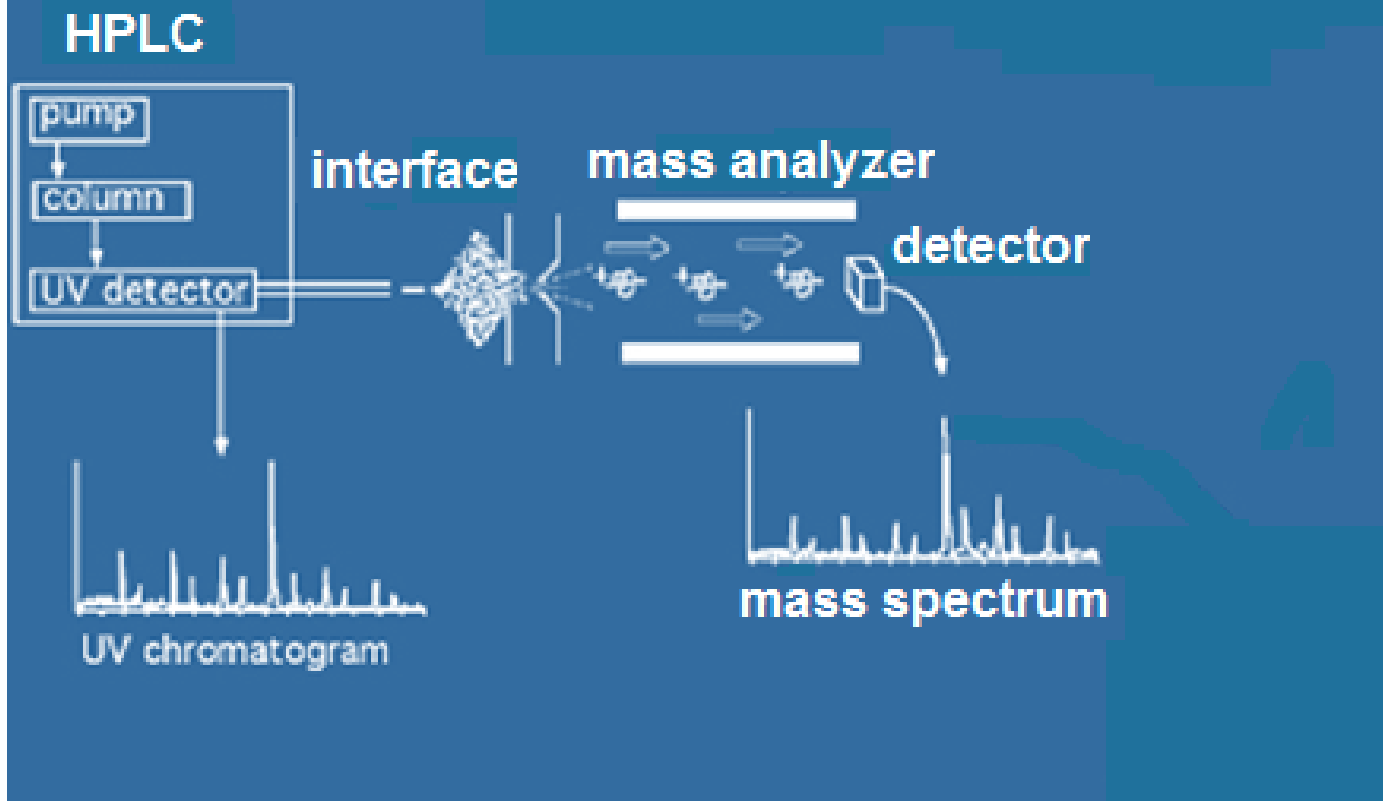
GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample. A non-specific test merely indicates that a substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the substance, this could lead to false positive identification.

Disadvantages: Limited to laboratory settings

High Performance Liquid Chromatography - Mass Spectroscopy(HPLC – MS)

A HPLC-MS is a system with a mass spec detector. The HPLC separates chemicals in the liquid phase by passing them through a column. Then they enter the mass detector, where the solvent is removed and the analytes are ionized, because the detector can only work with positive ions, not neutral molecules, so removal of the solvent is a vital first step. The mass detector then scans the molecules it sees by mass and produces a full high-resolution spectrum, separating all ions that have different masses. HPLC-MS is similar to GC-MS except that we apply the first for the analysis of nonvolatile analytes (mobile phase is mixture of solvents) where as the second is used for the analysis of volatile analytes (mobile phase is a gas).

Figure 2 : HPLC - MS Combination



ICP – MS Technique

The ICP source converts the atoms of the elements in the sample to ions. These ions are then transferred via an interface into the mass spectrometer where they are separated and detected. ICP – MS is the same as ICP-AES except using MS instead of AES. The interface blocks the radiations coming from the ICP torch, the electrons and the negative ions allowing only positive ions to pass into the MS.

ICP-MS has some limitations as to the amount of total dissolved solids in the samples, which must not be more than 0.2% ,otherwise the interface will become blocked .

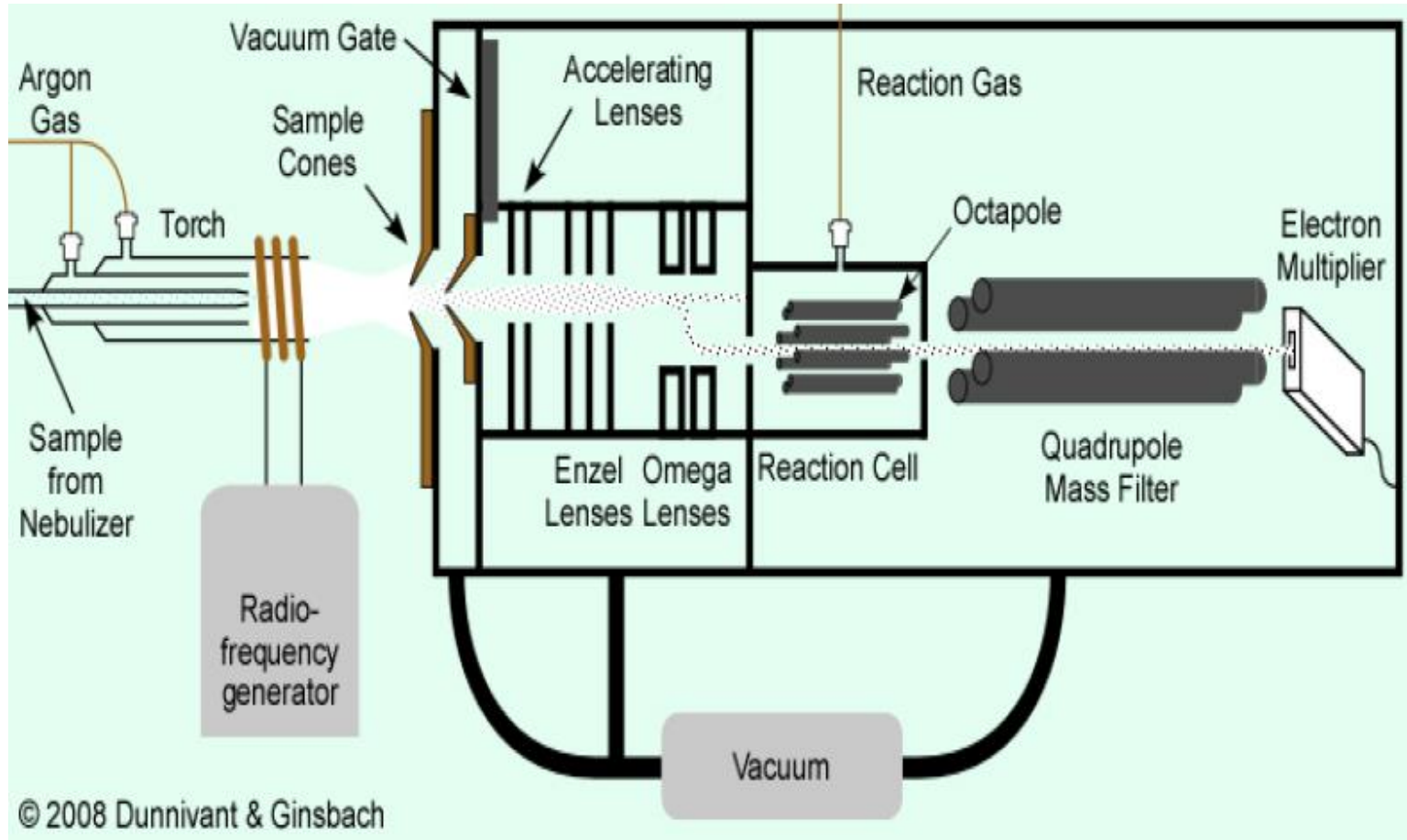
ICP-MS has many advantages .

-Detection limits for most elements equal to or better than those obtained by Graphite Furnace Atomic Absorption Spectroscopy or ICP-AES.

- The ability to handle complex matrices with a minimum of matrix interferences.

- ICP-MS is a fast, multi-elemental technique .But ICP-MS instrument is very expensive .

-ICP-MS is an analytical technique used for elemental determinations in various fields.



Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry which is capable of detecting metals and several non-metals at concentrations as low as one part in 10^{12} (part per trillion). This is achieved by ionizing the sample with inductively coupled plasma and then using a mass spectrometer to separate and quantify those ions.

-Compared to atomic absorption techniques, ICP-MS has greater speed, precision, and sensitivity. However, analysis by ICP-MS is also more susceptible to trace contaminants from glassware and reagents. In addition, the presence of some ions can interfere with the detection of other ions.

-The variety of applications exceeds that of ICP-OES and includes isotopic speciation. Due to possible applications in nuclear technologies, ICP-MS hardware is a subject for special exporting regulations

ICP-AES vs. ICP-MS

ICP-AES is an atomic emission technique – the inductively coupled plasma (ICP) serves as a means of exciting atoms and ions so that they emit characteristic wavelengths of energy. ICP-MS is a mass spectrometric technique – the ICP serves only as a means of generating ions for the mass spectrometer.

ICP-AES Robust and cheap Dependable Student Proof (sort of) Good for routine analyses.

ICP-MS Delicate and expensive Finicky Student phobic Capable of extraordinary performances .