Gas Chromatography-Mass Spectrometry (GC-MS): Working Principle and Applications

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1. Introduction

Gas chromatography-mass spectrometry (GC-MS) is a **hyphenated** technique consisting of two analytical procedures coupled in series that is a Gas Chromatography (GC) for separation of volatile compounds and Mass Spectrometry (MS) for identification and quantification of molecular weight of each separated component. The GC helps in separation of multiple components mixture so that they can reach the mass spectrometry one at a time. Thus, the molecular mass and other fragments mass values describe only for a single component at a time.

The GC-MS instrument has two independent techniques as follows:

1.1 Gas Chromatography (GC)

The GC is based on chromatographic method used in the separation of volatile organic and/or inorganic mixtures. It is broadly classified into two types depending upon mobile phase (MP) and stationary phase (SP) used. When MP is a gas and SP is a liquid called Gas-Liquid Chromatography (GLC) while when SP is a solid then called Gas-Solid Chromatography (GSC). The GC uses generally a high-resolution fused silica capillary column coated with SP as solid or nonvolatile liquid, housed in a temperature-controlled oven. Temperature of the oven is controlled to optimize the vaporization of mixture without decomposition which led to separation. When a sample solution is injected into the injection port where it get vaporized immediately because of the high temperature (upto ~300 ^{0}C) under low pressure (~10^{-4} -10^{-7} torr). Then, the sample (mixture) is transported through the column by continuous addition of MP. This process is called *elution*. As the sample travel through the column, the components physically interact with the SP as well as MP material of varying degrees depending on mixture components affinity. As a result, different compounds will travel with different speeds through the capillary column and will exit from the column after a distinct **retention time**. When the components present in mobile phase travels through the column and when reached to the detector, a signal is produced depending on the concentration and sensitivity of a particular component. A plot of signal vs. time generates a series of peaks in a chromatogram depending upon the number of components present in the sample. For example, Fig.1 shows that the mixture has five different components in it.

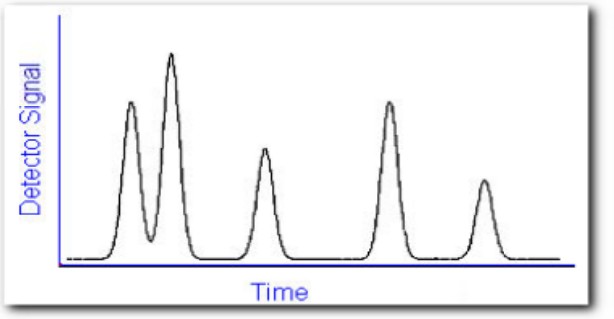


Fig. 1. GC-chromatogram

Thus, a GC chromatogram provides information like sample composition, retention time of the component and quantity from the heights of the peaks or the area under the peaks. In principle, the components would exit the column one after another depending upon retention time (shorter the retention time faster will be elution, i.e. less interaction with SP). However, sometime due to less interaction with MP (gas phase) and/or high interaction with SP results in peaks overlapping or peak broadening.

Instrumentation: There are five major parts as follow:

a. Injector : Samples are vaporized
b. Carrier gas: Nitrogen (N_2), Helium (He) or Hydrogen (H_2)
c. Oven: Isothermal or gradient temperature
d. Columns: Capillary Column and Packed column
e. Detectors: Different detectors are used depending upon selectivity and/or sensitivity of detection for specific compound. For example,

Flame-ionization detector (FID): FID is extremely sensitive with a large dynamic range. It has only disadvantage to destroy the sample.

Electron-capture detector (ECD): ECD is sensitive like FID, but has a limited dynamic range. Mostly used in the analysis of electronegative atoms containing organic molecules such as halogens, phosphorous and nitro groups.

Flame-photometric detector (FPD): The determination of sulfur or phosphorus containing compounds

Theory: The distribution of components between MP and SP is a dynamic equilibrium process which can be explained in terms of Gaussian probability distribution method. For example, analyte A is in equilibrium between the two phases:

$$A_{mobile} \rightleftharpoons A_{stationary}$$

The equilibrium constant (K) is termed as *partition coefficient* or *distribution constant* is defined as the degree of the solute molar concentration (C_M) distributes themselves between the mobile and stationary phases.

$$K = [C_M]_S / [C_M]_M$$

Where, $[C_M]_S$ is molar concentration of the analyte in the SP and $[C_M]_M$ is molar concentration in the MP. If K is constant over a wide range of analyte concentrations, then $[C_M]_S$ is directly proportional to $[C_M]_M$ and chromatographic peak is symmetrical, Gaussian distribution and retention time are independent of the amount of analyte injected.

Retention Times of an analyte is defined as the time it takes after sample injection for the analyte to reach the detector and elute. The time for non-interacted species with SP to reach the detector is defined as the dead time (t_D), if taken in volume called dead volume (V_D). Thus.the rate of migration of a non-interacted species is the same as the rate of motion of the mobile phase expressed as,

$$Rate_M = L / t_D \quad \text{.....(i)}$$

Where, L is the column length and t_D is the retention time of the mobile phase/ non-interacted species.

Similarly, the linear rate of a solute molecule is calculated by dividing the column length by the retention time t_R,

$$Rate_S = L / t_R \quad \text{..... .....(ii)}$$

The linear rate of a solute molecule can be expressed as a function of the rate of migration of the unretained species,

$$Rate_S = Rate_M \times S_M(t) \quad \text{.....(iii)}$$

Where, $S_M(t)$ is the fraction of time the solute spends in the mobile phase.

Generally, we need to know about movements of solutes through a gas chromatographic column. The efficiency of the column itself is described by the **height equivalent to theoretical plates (HETP)**,

$$HETP = L / N \quad \text{.....(iv)}$$

Where, N is the **number of theoretical plates**. Obtaining the number of theoretical plates requires some manipulation of the chromatogram,

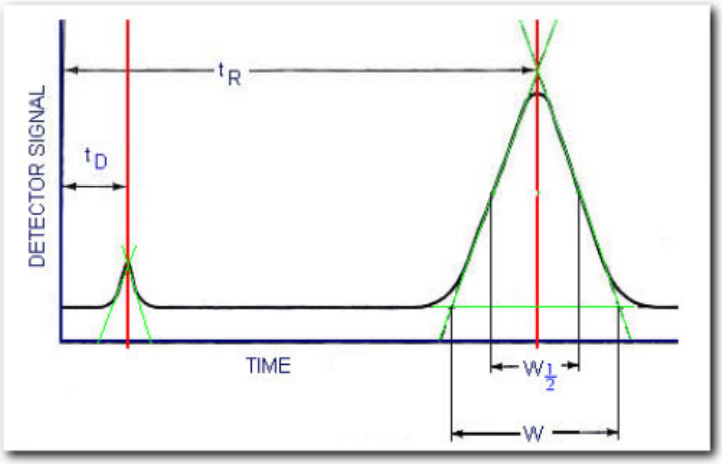


Fig. 2. GC-chromatogram

Determining the number of theoretical plates: From Fig. 2, the number of theoretical plates can be calculated,

$$N = 16\,(t_R / W)^2 = 5.55(t_R / W_{1/2})^2 \ldots\ldots\ldots\ldots (v)$$

Where, W is the peak width measured in the same units as t_R and $W_{1/2}$ is the peak width measured at half the peak height.

The **resolution** of a column is defined as its ability to separate a mixture of compounds. For example, compounds Ψ and Θ were separated by the GC column as shown in Fig. 3.

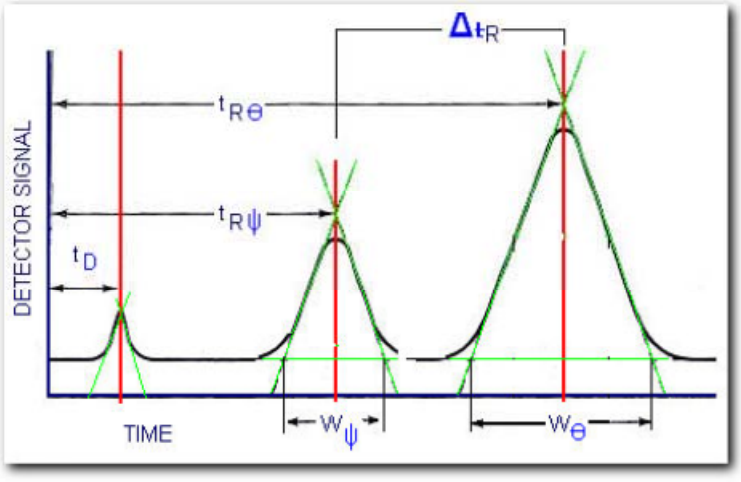


Fig. 3. GC-chromatogram

Determining the column resolution: After some mathematical manipulation, the required parameters to compute the column resolution is as follow:

$$R_S = (\Theta t_R - \Psi t_R) / (W_\Theta + W_\Psi) = \Delta t_R / (W_\Theta + W_\Psi) \quad \text{.....(vi)}$$

The chromatogram generally plotted in detector signal vs. time, gave Gaussian-shaped (or bell shaped) peaks. In reality, non-Gaussian peak shapes will often occur. The front side of a peak might be drawn out while the tail on the right is steep called **fronting**, might be due to large amount of a sample is introduced into the column or wrong combination of packing material. The right side of the peak is drawn out while the tail on the left is steep called **tailing** and usually occurs when the solute has a concentration dependent distribution coefficient. Fronting and tailing will result in less accurate quantitative analysis and peaks broadening.

Equipment calibration: The GC-MS instrument needed to be tuned before each analysis. The calibration consists of injecting a known volume of a standard and measuring the time between injection and elution. The retention time of a compound remained the same for a given set of variables (temperature, flow rate and column length). If the quantitative analysis is required, it might need to compare peak heights or areas of the peak with that of one of the standards. The most ancient method for quantitative analysis is the **internal standard calibration method** (ISTD) and involves the preparation of a series of standards that approximate the composition of unknown. In this procedure, a measured quantity of an internal standard is introduced into each standard and sample. Chromatograms for the standards are generated and peak areas are plotted as a function of analyte amount or concentration. Such plot should yield a straight line passing through the origin and gives qualitative and quantitative analysis of different components present in the mixture (Fig. 4).

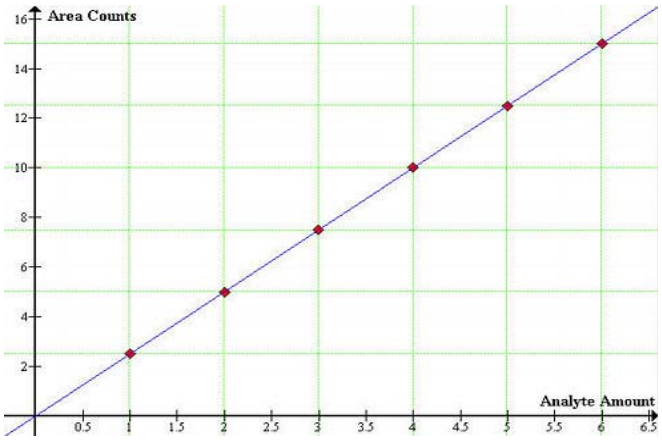


Fig. 4. An ISTD calibration curve (2.5 area counts = 1.0 units of compound amount)

1.2 Mass Spectrometry (MS)

In GC-MS technique, GC produces 'pure' fractions after separating a mixture. Followed by each component sends to the mass spectrometry where they undergo

ionization and fragmentation in ion source region, mass separation in ion analyzer and detection processes, respectively. For example, a mixture of three components got separated in GC followed by MS gave three mass spectra for each component (Fig. 5). GC and MS used together to give better understanding of substance in identification and composition independently.

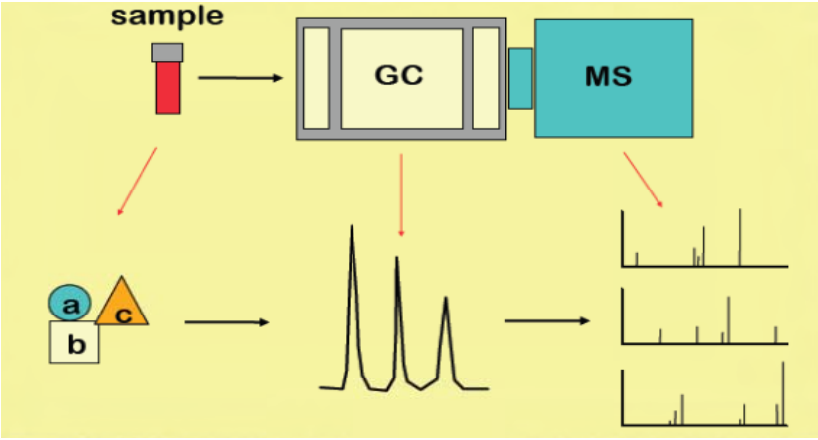


Fig. 5. GC and MS chromatograms

Principle: Independently, MS is a powerful analytical technique used to studies the effect of ionizing energy on molecules. It depends upon chemical reactions in the gaseous and ionic phases in which sample molecules are consumed during the formation of ionic and neutral species. The process involves the conversion of the sample into gaseous ions with or without fragmentation, which are characterized by their mass to charge (m/z) ratios and relative abundances (peaks intensity).

Instrumentation: All mass spectrometers have three major components (Fig. 6): (i) Ionizer (ion source), (ii) Ion/mass Analyzer and (iii) Detector

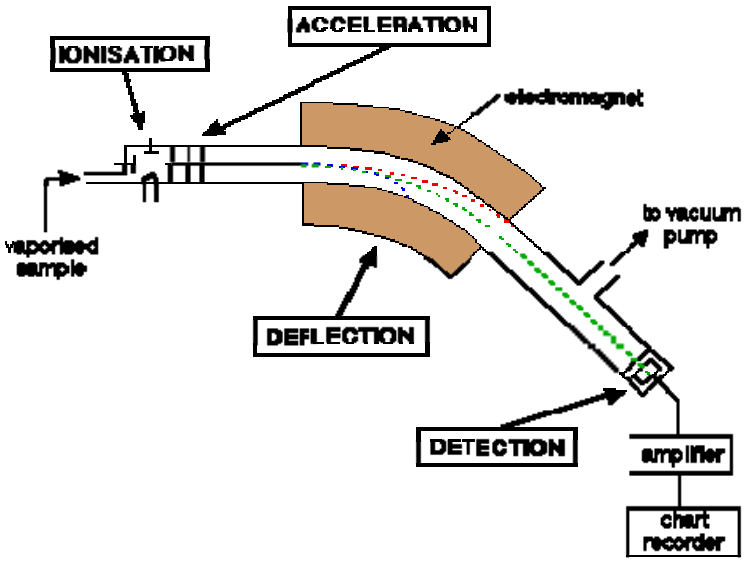


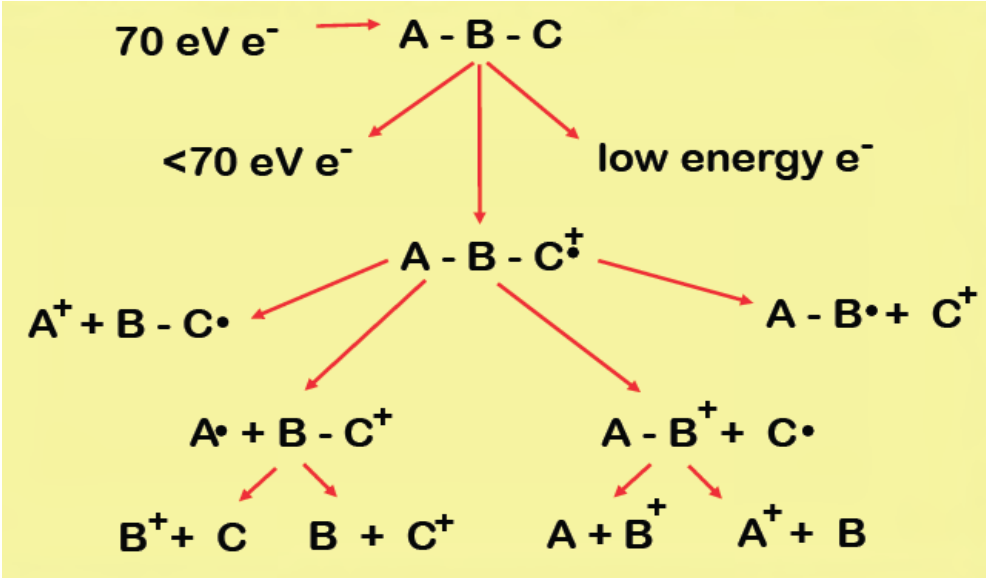
Fig. 6. MS instrument

(i) **Ionizer:** A sample vaporized at >400 ^{0}C and 10^{-3} Pa pressure is introduced in the mass spectrometer where molecule get ionized using different ionization methods that include electron impact (EI), In-beam (desorption) electron impact (DEI), Chemical ionization (CI), Alternate CI-EI (ACE), Rapid heating (desorption) Chemical ionization (DCI), Atmospheric pressure ionization (API), Atmospheric pressure chemical ionization (APCI), Electrospray ionization (ESI), Thermospray ionization (TSP), Laser ionization (Laser desorption), Matrix assisted Laser ionization (MALDI), Field ionization (FI) and Field desorption (FD), Fission fragment ionization or Plasma desorption mass spectrometry (PDMS), Secondary ion mass spectrometry (SIMS), Liquid SIMS, Fast atom bombardment (FAB), Photon and multiphoton ionization (MPI) and Resonance Ionization, etc. Mostly, MS ionizers work with positive ions (negative is rare). For example, in EI-MS technique, 70 eV electron energy is used for molecule ionization where atom knocked out one or more electrons off to give a positive ion (molecular ion) and lower the electron energy. The high energy radical cation (mol. ion) undergoes further fragmentation to minimize its energy (Scheme 1).

General reaction:

$$\text{A-B-C}_{(\text{vapor})} + e^- \xrightarrow{\text{70 eV}} \text{A-B-C}^{\cdot+} + 2e^-$$

(radical cation)



Scheme 1. Fragmentation of molecular/patent ion

In the ionization chamber, the vaporized molecules are bombarded with a stream of electrons emitted from electrically heated metal coil. Some of the collisions are energetic enough to knock out one or more electrons to make positive ions. Mostly, the positive ions formed carry single charge because it becomes much more difficult to remove further electrons from a positive ion. The free electron(s) are attracted towards the electron trap (+vely charged plate called electron collector). The ions produced in the ionization chamber travel forward direction only due to repeller ((+vely charged plate)

placed from other side. In ion source and mass analyzer sections, a high vacuum is required to avoid high energy electrons to strike with air molecules (Fig. 7).

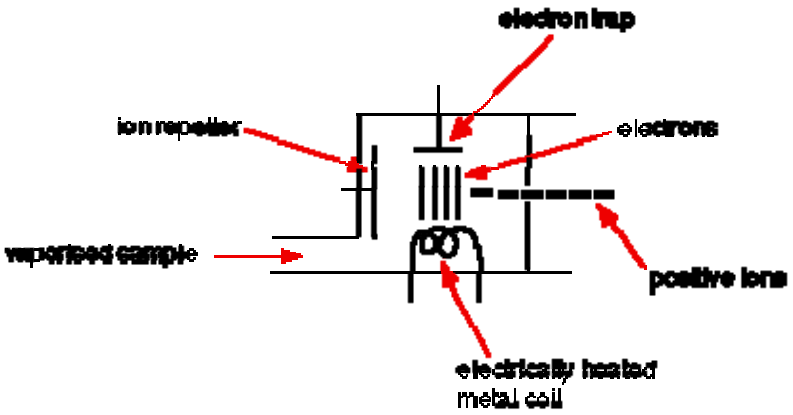


Fig. 7. Ionization Chamber

The positive ions are repelled away from the very positive ionization chamber and pass through three slits, having potential difference in decreasing order from + 10,000 to 0 volts. Therefore, all the ions are accelerated and have the same kinetic energy at the end of 0 volts (Fig. 8).

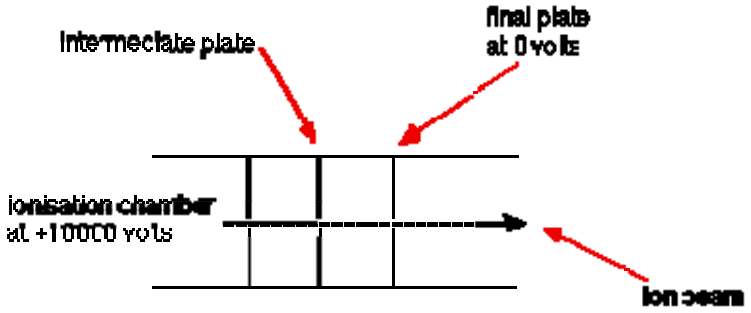


Fig. 8. Ions acceleration tube

(ii) **Mass/Ion Analyzer:** Advancement in GC-MS machine was obtained by using different mass analyzers for separating the ion beam into beams of different masses. For example,

a. magnetic sector analyzer (MSA): gave high resolution, exact mass
b. quadrupole analyzer (Q): gave low (1 amu) resolution, fast, cheap
c. Time-of-flight analyzer (TOF): has no upper m/z limit and high throughput
d. Ion trap mass analyzer (QSTAR): gave good resolution
e. Fourier Transformation Ion Cyclotron Resonance (FT-ICR): gave highest resolution, exact mass but costly.

Based on use of different type of ion source and mass analyzer, mass spectrometry has been developed such as ESI-QTOF (electrospray ionization source + quadrupole mass filter + time-of-flight mass analyzer), MALDI-QTOF (matrix assisted desorption ionization + quadrupole + time-of-flight mass analyzer), GC-MS (separates volatile compounds in gas column and ID's by mass), LC-MS (separates compounds in HPLC column and ID's by mass), MS-MS (Tandem mass spectrometry) (separate compound fragments by magnetic field and ID's by mass), LC/LC-MS/MS (Tandem LC and Tandem MS) (separates by HPLC, ID's by mass and AA sequence), etc.

In the mass analyzer, the accelerated ions are then entering into mass separation unit, depending upon their mass/charge (m/z or m/e) ratio. For example, magnetic sector analyzer (MSA) deflects ions by an electromagnetic field according to their masses (Fig. 9). Lighter ions are deflected more compared to heavier. The amount of deflection also depends on the number of positive charges on ion. The more the ion is charged, the more it gets deflected.

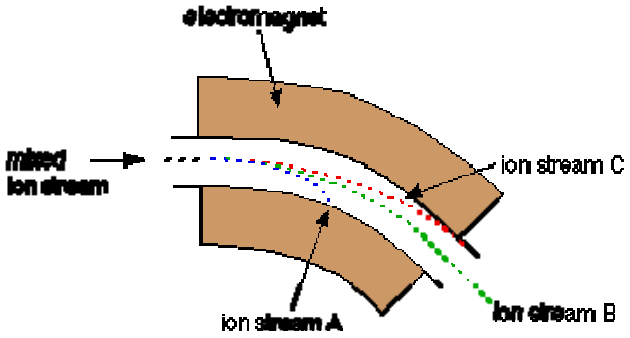


Fig. 9. Ions deflection in mass analyzer

In Fig. 9, ion stream A is most deflected because ions have minimum mass/charge ratio. Ion stream C is the least deflected because ions have maximum mass/charge ratio, assuming all are +1 ions. Thus, stream A has the lightest ions, stream B the next lightest and stream C the heaviest. Lighter ions are going to be more deflected than heavy ones. Only stream B ions will reach to the detector in this case. For stream A ions to be recorded, we need less electromagnetic field to be applied. Conversely, for stream C ions to be recorded, we need stronger electromagnetic field to be applied which can be done by changing the magnetic field.

(iii) Detector: Only stream B ions reach to the ion detector. The other ions collide with the walls where they will pick up electrons and be neutralized (uncharged). Finally, they get removed from the mass spectrometer by the vacuum pump. Ions detection is based on either charge or momentum value. In modern instruments, for large signals a Faraday cup is used to collect ions and measure the current. In old instruments, photographic plates are used to measure the ion abundance at each mass/charge ratio. Most detectors used amplifying the ion signal using a collector similar to a photomultiplier tube. These

amplifying detectors include: electron multipliers, channeltrons and multichannel plates. The gain is controlled by changing the high voltage applied to the detector. A detector is selected for its speed, dynamic range, gain and geometry. Some detectors are sensitive enough to detect single ions.

When an ion hits the metal surface, its charge is neutralized by an electron jumping from the metal on the ion (right hand diagram). That leaves a space amongst the electrons in the metal and the electrons in the wire shuffle along to fill it. A flow of electrons in the wire is detected as an electric current which can be amplified and recorded through computer software. Larger the ions knocking, the greater will be the current which indicate the intensity of the peak.

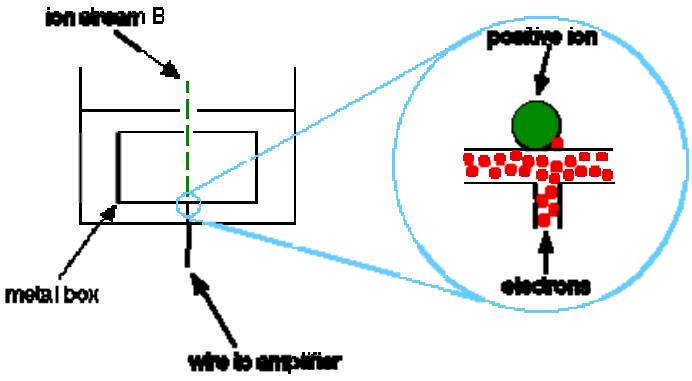
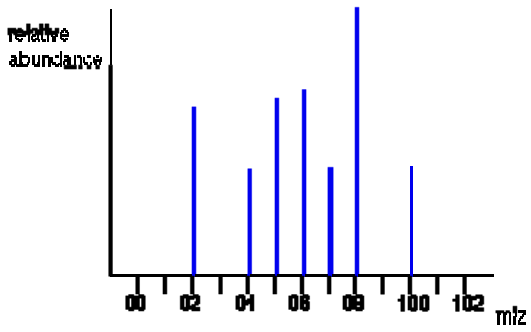
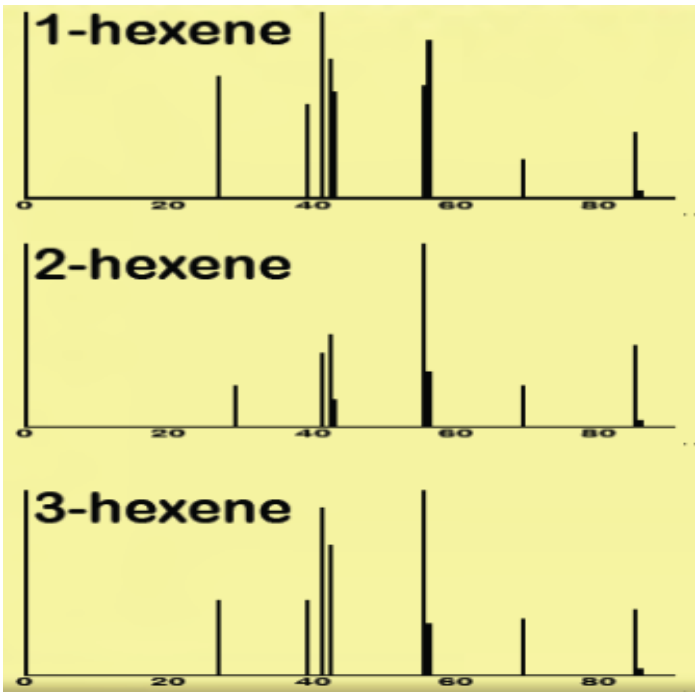


Fig. 10. Detection of steam B ions current

What the mass spectrometer output looks like?

The output from the chart recorder is usually simplified into a "stick diagram". This shows the relative current produced by ions of varying mass/charge ratio.





(a) (b)

Fig.11. Mass chromatogram (a) molybdenum atom (inorganic); (b) hexenes (organic) molecule

Chromatogram for molybdenum atom is recorded on y-axis as relative abundance/relative intensity vs. x-axis mass/charge ration. The vertical scale is related to the current received by the chart recorder (the number of ions arrived at detector). The greater the current, more will be abundance of the ion. The most abundant peak is called **base peak**, for example, mass/charge = 98. Other ions have mass/charge ratios of 92, 94, 95, 96, 97 and 100 indicate different isotopes of the molybdenum atom, assuming that the ions all have a charge of +1, that means that the masses of the 7 isotopes on the carbon-12 scale are 92, 94, 95, 96, 97, 98 and 100. Similarly, chromatogram for three hexane molecules (structural isomers) can be explained in mass spectroscopy. All the three isomers gave same molecular ion (Fig. 11b) due to same mass. However, base peak and other fragmentations are different which indicates different mode of fragmentation and mechanism. These informations are helpful in their structure determination as three isomers.

1.3 Applications

1.3.1 Water monitoring and analysis

GC-MS is highly sensitive analytical tool which can separate and quantify organic compounds at ppb level. Therefore, this can be used in water quality monitoring and

assessment for the total organic carbons (TOCs) that include pesticides, endocrine disruptors, chlorofluoro-compounds, protein degradation and radioactive products for drinking water. Very low levels of total organic carbon are essential with a typical resistivity of 18.2 MΩ. cm, a very low TOC value of less than 2 ppb and bacteria levels below 0.1 CFU/ml – is highly recommended for ultra pure water which can be achieved with GC-MS analysis.

1.3.2 Environmental monitoring and clean-up

GC-MS is used as the tool of choice for tracking organic pollutants in the environment. There are some compounds for which GC-MS is not sufficiently sensitive, including certain pesticides and herbicides, but for most organic analysis of environmental samples, including many major classes of pesticides, it is very sensitive and effective.

1.3.3 Criminal forensics

GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established. There is even an established American Society for Testing Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and results, used in court, need to be highly accurate.

1.3.4 Law enforcement

GC-MS is used for detection of illegal narcotics and supplant drug-sniffing dogs. It is also commonly used in forensic toxicology to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased.

1.3.5 Sports anti-doping analysis

GC-MS is the main tool used in sports anti-doping laboratories to test athletes' urine samples for prohibited performance-enhancing drugs, for example anabolic steroids.

1.3.6 Food, beverage and perfume analysis

Foods and beverages contain numerous aromatic compounds, GC-MS is extensively used for the analysis of these compounds. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful for human, for example pesticides.

1.3.7 Astrochemistry

Several GC-MS have left earth. Two were brought to Mars by the Viking program. Venera 11 and 12 and Pioneer Venus analyzed the atmosphere of Venus with GC-MS. The Huygens probe of the Cassini-Huygens mission landed one GC-MS on Saturn's largest moon, Titan. The material in the comet 67P/Churyumov-Gerasimenko will be analyzed by the Rosetta mission with a chiral GC-MS in 2014.

1.3.8 Medicine

Dozens of congenital metabolic diseases also known as "In born" errors of metabolism are now detectable by gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. It is now possible to test a newborn for over 100 genetic metabolic disorders by a urine test at birth based on GC-MS and compared with healthy condition.

In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of ^{13}C as the labeling and the measurement of ^{13}C-^{12}C ratios with an **isotope ratio mass spectrometer** (**IRMS**); an MS with a detector designed to measure a few select ions and return values as ratios.

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