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Paper : P-12 Biochemical Techniques

Module : GC-MS

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Development Team

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Description of Module	
Subject Name	Biochemstry
Paper Name	Biochemical techniques
Module Name/Title	**GC-MS**

Introduction

Gas chromatography mass spectrometry (GC-MS) is an analytical tool to identify the unknown compound. It is the combination of gas chromatography and mass spectrometry. It has ability to identify the trace elements present in the sample and very popular in forensic sciences.

History

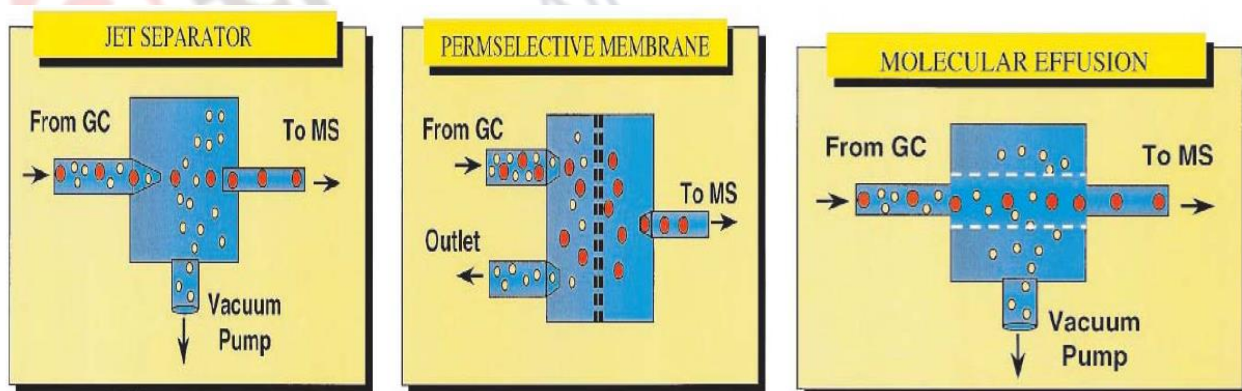
The coupling of mass spectrometer and gas chromatography was first time developed by Roland Gohlke and Fred McLafferty in late 1950s. Later on in 1996 the combination of these two instruments were paired with the computer that allowed the easy and fast analysis of sample. It could now analyze the single component of fire accelerants within 90 sec, while for the same analysis the first-generation GC-MS needed minimum of least 16 minutes. There are two major building blocks i.e the gas chromatograph and the mass spectrometer. The gas chromatograph consists of a capillary column. The separation of molecules in the mixture depends on the column's measurements (length, diameter, film thickness) and on the phase properties (e.g. 5% phenyl polysiloxane). Depending upon the chemical properties of molecules the sample pass through the length of the column and they took different amounts of time to come out of the gas chromatograph, this is known as retention time. After this the molecule goes into the mass spectrometer in order to capture, and this allows the mass spectrometer downstream to identify the molecule after ionization. Mass spectrometer senses these molecules by their mass to charge ratio. However, it is not the accurate way to identify a particular molecule by gas GC-MS alone, other techniques are also need to verify the results obtained from GC-MS. This is because sometimes two unlike molecules may have similar pattern of ionized fragments in a mass spectrometer.

Principle

In GC-MS gas chromatograph is used as a separator and mass spectrometer is acting as a detector as it ionizes all the molecules. It was challenge to combine these two instruments as they need variable operating conditions. Gas chromatogram needs high pressure while mass spectrometer works under high vacuum. The carrier gas is needed to run the gas chromatogram but this gas could collapse the

vacuum of mass spectrometer. Therefore, carrier gas has to be removed before sample reaches the mass spectrometer. Since, the carrier gas such as nitrogen is lighter than the eluted molecules; they can be removed by effluent chamber easily. This provides the easy passage of molecule to the mass spectrometer without producing any hindrance in the vacuum creation. To, perform aforesaid activity a precisely aligned supersonic jet/orifice system is used. Effluent oozes from the carrier gas chromatograph is squeezed through a fine orifice followed by its sudden expansion into a vacuum chamber. Due to this expansion, the lighter carrier gas which has higher diffusion rate concentrated at the core of the gas stream, which is directed towards a second orifice. Alignment of these two orifices the first one for expansion and the second one for collection is very critical, their distance must be change with the change of flow rate (Fig 1). Another thing is also mentioned to separate the molecule and the carrier gas after the elution from gas chromatogram and i.e membrane separator which is made up of dimethyl silicon having thickness of 0.025- 0.040 mm. Carrier gas has low permeability for dimethyl silicon while other molecules directly passed to the vacuum chamber of mass spectrometer (Fig 1).

Fig1: GC-MS coupling



Instrumentation and working of GC-MS

The introduction of GC-MS instrumentation begins with the carrier gas. They are highly purified gases eg. Helium, hydrogen and nitrogen. These gases pushes the evaporated sample into column and help them to travel through the column. Consequently, choice of carrier gas in separating the sample plays important role. Therefore, in more than 90% of the GC-MS instruments helium gas is used due to its inert nature.

After, the carrier gas here comes the role of column in the separation of sample. GC columns are either filled with silica particles or they are hollow capillary columns having the stationary phase encrusted onto the inner wall. GC columns are fine capillary with 0.10- 0.50 mm diameter and 10 to 120 m length. Selecting the right column for the analysis is very important. There are following things that need to consider before selecting column:

1. Selection of stationary phase – the selection of the stationary phase depends upon the polarity and phenyl content. Polarity depends on the structure of stationary phase and it effects the separation of molecules and hence the peak separation.

2. Dimensions of column: The dimensions of column controls the factors such as solute retention, efficiency, carrier gas flow rates and head pressure.

- Column diameter: The diameter of column influence five different parameters viz; retention, efficiency, carrier gas flow rate, pressure, and capacity. There is an inverse relationship between column efficiency (N/m) and column diameter i.e smaller diameter column has high efficiency. Similarly, retention time and pressure is also inversely proportional to column diameter. Contrary, to this, if we keep pressure constant then, carrier gas flow rates increase with increase in column diameter. The larger diameter columns are usually used with those samples that need high flow rate. If columns with smaller diameters were used for aforesaid samples then they need special considerations such as use of ovens or split injectors.

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- Column length: Column length affects three major components viz; efficiency, retention (analysis time) and carrier gas pressure. Column efficiency, retention time and carrier gas pressure increases with increase in column length.

3. Temperature: In total there are four major regions where temperature has to be controlled i.e injector, oven, injector and MS vacuum chamber. The injector is always set at 20°C higher that the final oven temperature. The oven temperature can be set at isothermal mode or it can be programmed. Usually, the initial oven temperature is set 10-15°C below the boiling temperature of solvent to concentrate the sample at the start of column and hold at this point in case of split less mode of injection and this was followed by more than one temperature ramps and finally set at high temperature ramp to elute the remaining analytes that may not be of our interest. Then comes the detector which is always set at constant temperature which is 10-15°C above the oven temperature. The detector is always set at a constant temperature 15 to 20 degrees above the maximum temperature of the oven. Both the injector and detector are set at higher temperature just to prevent recondensation of analytes. This also helps in reducing the cross contamination of samples. Finally the MS vacuum chamber is set below the oven's lower temperature.

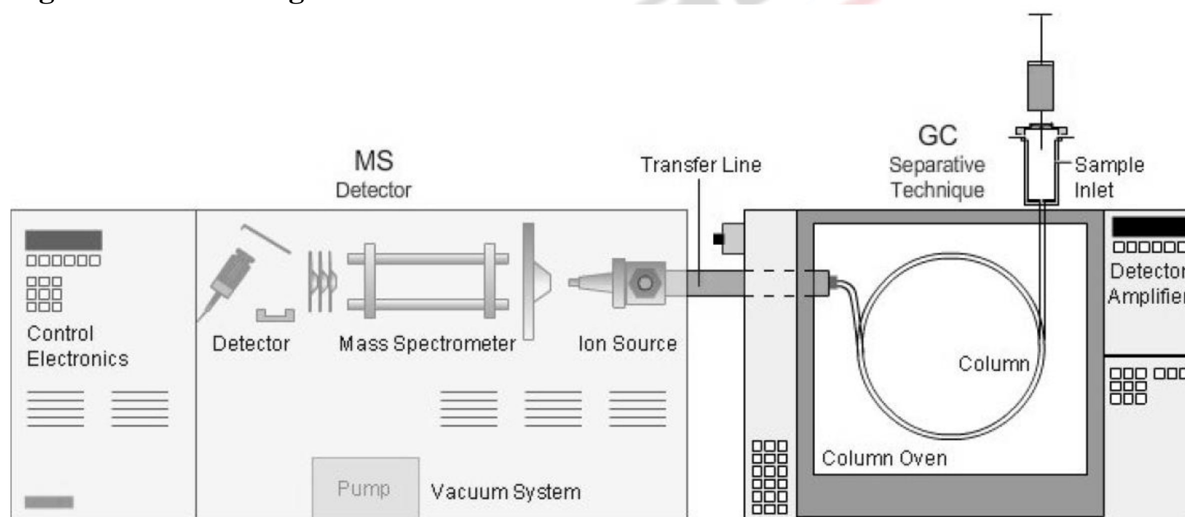
4. Mass Spectrometer: Mass spectrometer helps in the separation of ions in order to differentiate them according to the mass/charge ratio. It first accelerate the ions through magnetic field and then break them into charged fragments. Finally, a spectral plot is generated which displays the mass of each fragment. From

5. Mass analyzer: mass analyzer separates the species on the basis of mass to charge ratio.

6. Detector: It detects the ion beams emerge from the mass analyzer. It generates the signals by inducing current or by generating secondary electrons these signal came in the form of chromatogram. There are many type of detectors viz; TCD (thermal conductivity detector), FID (flame ionization detector, ECD (electron capture detector), FPD (flame photometric detector), PID (photoionization detector) and PFPD (pulsed flame photometric detector) etc.

- TID: These detectors have ability to sense any kind of changes in thermal conductivity of the effluent coming from the column and compare them with the reference carrier gas.
- FID: It measures the concentration of organic species in the analyte and compares it to the reference.
- FPD: It measures the volatile sulphur and phosphorus compounds.
- PID: It uses the ultra-violet light to ionize gas molecule and used to detect the volatile organic compounds.

Fig 2: Schematic diagram of GC



Application of GC-MS

GC-MS has found application in various unrelated fields such as in forensic sciences, analysis of environment, pharma and drug analysis, bio-pesticides, clinical toxicology, geology etc. Forensic analysis includes analysis of drugs, explosives, arson etc. There are certain software available for these kind of analysis viz; Chromato Probe, Open Probe and ultra fast GC-MS analysis is best for forensic GC-MS system. The Supersonic GC-MS could be used to investigate the CSI (Crime Scene Investigation). For this analysis the sample is allowed to touch and push it inside the GC-MS will provide the results about the type of mixture in few seconds.

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