

Gas Chromatography and Its Hyphenated Techniques

Saba Yousuf*

Department of Pharmaceutical Analysis, Mesco College of Pharmacy, Telangana, India, 500006

Corresponding Author: Saba Yousuf*

Email: sabayousuf456@gmail.com

Abstract:

Gas chromatography (GC) is a common chromatographic technique used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Classic applications of GC include testing the purity of a substance and separating the different components of a mixture. Hyphenated techniques combine chromatographic and spectral methods to utilize the advantages of both. Chromatography isolates pure or nearly pure components in a mixture, while spectroscopy provides selective information for identification using standards or library spectra. In this article, a detailed study of gas chromatography and the coupling of GC to different detectors, such as GC-MS and GC-AED, for solving complex analytical problems is discussed.

Keywords: Chromatographic analysis, Mass spectrometry, Atomic emission detection, Analytical separation

I. Introduction

History of Gas Chromatography

At the early 1900s, gas chromatography (GC) was introduced by Mikhail Semenovitch Tsvett as a technique to separate compounds. In organic chemistry, liquid-solid column chromatography is commonly used to separate organic compounds in solution. Among the various types of gas chromatography, gas-liquid chromatography is primarily employed to separate organic compounds. The combination of gas chromatography and mass spectrometry is an essential tool for the identification of molecules. A gas chromatograph consists of an injection port, a column, carrier gas flow control equipment, ovens and heaters for regulating the temperatures of the injection port and column, an integrator or chart recorder, and a detector.

Principle of Gas Chromatography

Gas chromatography is widely used in analytical chemistry to separate and analyze compounds. Typical applications include testing the purity of a specific substance or separating the different components of a mixture. In preparative chromatography, GC is often used to isolate pure compounds from a mixture.

Alternate Names

Gas chromatography is also referred to as vapor-phase chromatography (VPC) or gas-liquid partition chromatography (GLPC). These alternative names and their respective abbreviations are frequently utilized in scientific literature.

Working Mechanism

In GC, compounds in a mixture are separated by introducing a gaseous or liquid sample into a mobile phase, called the carrier gas, which flows through a stationary phase. The mobile phase is usually an inert gas, such as helium, argon, nitrogen, or hydrogen. The stationary phase is a thin layer of a liquid or solid support inside a glass or metal column. The column is placed in an oven where the temperature can be regulated, and the eluent

is monitored by a detector. Gas chromatographs are often hyphenated to mass spectrometers (GC-MS) to enable chemical component identification [1,2].

Sample Injection and Separation

To separate compounds in gas-liquid chromatography (GLC), a solution containing the target organic compounds is injected into the sample port, where it is vaporized. The vaporized sample is carried by an inert gas through a glass column packed with silica coated with a liquid. Materials that are less soluble in the liquid elute faster than those with greater solubility. This method provides an understanding of separation and measurement techniques as well as their applications.

Stationary Phase in GLC

In GLC, the liquid stationary phase is adsorbed onto a solid inert packing or immobilized on the capillary tubing walls. The column is considered packed if it contains small spherical inert supports. The liquid phase adsorbs onto the surface of these beads in a thin layer. In capillary columns, the tubing walls are coated with the stationary phase or an adsorbent layer capable of supporting the liquid phase. However, gas-solid chromatography (GSC) has limited laboratory applications due to severe peak tailing and the semi-permanent retention of polar compounds within the column [3].

II. Principle

➤ The Chromatographic Process

Gas chromatography is a separation technique in which the components of a sample partition between two phases:

1. The stationary phase.
2. The mobile gas phases.

Gas chromatography is categorized based on the nature of the stationary phase. There are two main types: gas-solid chromatography (GSC), where the stationary phase is solid, and gas-liquid chromatography (GLC), which uses a liquid as the stationary phase. GLC is far more commonly used than GSC. In the process of gas chromatography, the sample is first vaporized and then transported through the column by the mobile gas phase, which is referred to as the carrier gas. The separation of different components happens due to their varying vapor pressures and their interactions with the stationary phase. The tendency of a substance to interact with the stationary phase can be expressed as a chemical equilibrium constant known as the distribution constant, or partition coefficient. This constant, denoted as K_c , is calculated by comparing the concentration of compound A in the stationary phase, $[A]_s$, with its concentration in the mobile phase, $[A]_m$

$$K_c = [A]_s / [A]_m$$

Criteria for Compounds to Be Analyzed by Gas Chromatography

Two important criteria are

1. **Volatility:** Unless a compound is volatile. It cannot be mixed with mobile phase. Hence volatility is important.
2. **Thermostability:** All the compounds will not be in the form of vapor. There will be solid and liquid samples. Hence to convert them to a vapor form, they have to be heated to a higher temperature. At that temperature, the compounds have to be thermostable. [5]

III. Instrumentation of Gas Chromatography

➤ Sample Injection

A sample port is needed to introduce the sample at the top of the column. In modern injection methods, heated sample ports are commonly used, allowing the sample to be injected and vaporized almost at the same time. A calibrated micro syringe is used to introduce a small amount of sample, typically a few microliters, through a rubber septum into the vaporization chamber. Most analytical processes require only a small part of the original sample, and a sample splitter is used to send the extra sample to waste. Many commercial gas chromatographs offer both split and split less injection options, which are useful when switching between packed and capillary columns. The vaporization chamber is usually set to a temperature 50 °C higher than the lowest boiling point of the sample, and then the vaporized sample is mixed with the carrier gas before being carried into the column.

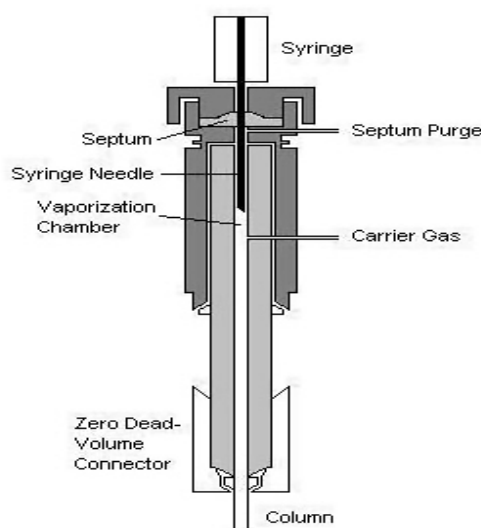


Figure 1: A cross-sectional view of a micro flash vaporizer direct injector.

➤ Carrier Gas

The carrier gas is a crucial element in gas chromatography and can differ depending on the system used. It must be dry, free from oxygen and chemically inert, as it serves as the mobile phase. Helium is the most commonly used carrier gas because it is safer than hydrogen while offering similar efficiency, has a broader range of flow rates, and works well with many detectors. Nitrogen, argon, and hydrogen are also used, depending on the desired performance and the type of detector. Hydrogen and helium, frequently used in traditional detectors such as Flame Ionization Detector (FID), Thermal Conductivity Detector (TCD), and Electron Capture Detector (ECD), allow for faster analysis times and lower elution temperatures due to their higher flow rates and low molecular weight. For example, hydrogen or helium provides the highest sensitivity with TCD because the difference in thermal conductivity between the organic vapor and hydrogen/helium is greater than with other carrier gases. Other detectors like mass spectrometry, however, often use nitrogen or argon, which have higher molecular weights and offer better advantages than hydrogen or helium. Carrier gases are supplied in pressurized tanks, and pressure regulators, gauges, and flow meters are used to precisely control the gas flow. Most gas supplies should have a purity level between 99.995% and 99.9995%, with a low level of oxygen and total hydrocarbons, typically less than 0.5 ppm. The carrier gas system includes a molecular sieve to remove

water and other impurities. Traps can also be used to maintain system purity, sensitivity, and to eliminate traces of water and other contaminants. A two-stage pressure regulation system is necessary to minimize pressure fluctuations and to monitor the gas flow. Flow or pressure regulators are also required for both the gas tank and the chromatograph's inlet, and the type of regulator can vary depending on the gas used. The carrier gas is preheated and filtered through a molecular sieve to remove impurities and water before being introduced into the vaporization chamber. A carrier gas is essential in a GC system as it flows through the injector and carries the gaseous components of the sample to the GC column, ultimately reaching the detector. [6]

➤ Column Oven

The thermostatted oven is used to control the temperature very precisely, within a few tenths of a degree, which is necessary for accurate work. The oven can be used in two ways: isothermal programming or temperature programming. In isothermal programming, the column temperature remains constant throughout the whole process. The best temperature for isothermal operation is around the middle of the sample's boiling range. But isothermal programming works well only when the boiling range of the sample is narrow. If a low temperature is used with a wide boiling range, then the low boiling parts are well separated, but the high boiling parts move slowly and there is a lot of band broadening. If the temperature is set higher, closer to the boiling point of the high boiling compounds, the high boiling parts come out as sharp peaks, but the low boiling parts move too quickly and there is no proper separation.

In temperature programming, the column temperature increases either gradually or in steps as the process moves forward.

This method is good for separating samples with a wide boiling range. The process starts at a lower temperature to separate the lighter components and the temperature is increased as the separation continues. Typical temperature increase rates are around 5 to 7 degrees Celsius per minute.

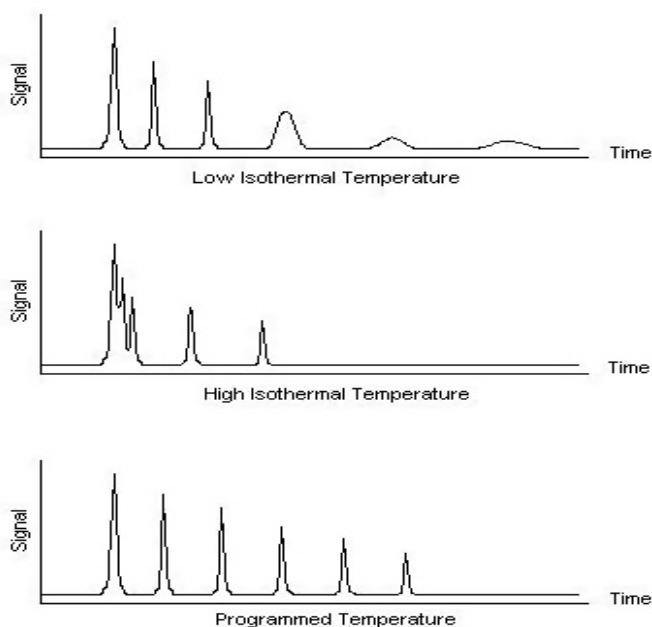


Figure 2: The effect of column temperature on the shape of the peaks.

- **Open Tubular Columns and Packed Columns**

Open tubular columns, also known as capillary columns, come in two primary types. The first type is the wall-coated open tubular (WCOT) column, which consists of a narrow capillary tube coated with a thin layer of the stationary phase on its inner walls. The second type is the support-coated open tubular (SCOT) column. In SCOT columns, the inner walls are initially coated with a thin layer, about 30 micrometers thick, of an adsorbent solid like diatomaceous earth. This material is made from the remains of single-celled sea plants. Following this, a liquid stationary phase is applied. Even though SCOT columns can hold a greater amount of stationary phase and thus have a higher sample capacity, WCOT columns generally offer better performance.

Packed columns are made from glass or metal tubes that are densely packed with a solid support material such as diatomaceous earth.

Due to the difficulty in evenly filling the tubing, these columns tend to be thicker in diameter and shorter in length compared to open tubular columns. This results in packed columns typically achieving only about half the efficiency of a similar WCOT column. Additionally, the diatomaceous earth used in packed columns may gradually lose its effectiveness as impurities are absorbed into the column over time. In contrast, FSWC open tubular columns are designed to prevent these adsorption issues and are largely free from such problems.

Stationary Phase Column Packing

Packed columns have a packed stationary phase while the stationary phase of the capillary column is coated on the inner surface. This is a major difference between packed column and capillary column.

- **Detection Systems**

The detector is the device placed at the end of the column, which gives a precise measurement of the different parts of a mixture as they come out of the column along with the carrier gas. In theory, any characteristic of the gaseous mixture that differs from the carrier gas can be used to detect the components. These detection methods are categorized into two types: bulk properties and specific properties. Bulk properties, also called general properties, are features that both the carrier gas and the sample share, but to varying extents. Specific properties, such as those used in nitrogen-phosphorus detectors, have more limited uses but offer greater sensitivity. Each detector consists of two main components that work together to act as transducers, changing the detected changes into an electrical signal that is recorded as a chromatogram.

The first part is the sensor, which is positioned as close to the column outlet as possible to ensure optimal detection. The second part is the electronic system used to convert the analog signal into a digital one, allowing a computer to process the recorded chromatogram. Converting the analog signal to digital as quickly as possible improves the signal-to-noise ratio, since analog signals are prone to various types of interference.

An ideal gas chromatography detector has several key features.

First, it should have enough sensitivity to produce a clear signal for all components in the mixture. However, this is an ideal scenario because a sample with such a small volume would require the detector to have infinite sensitivity to detect it. In modern instruments, detector sensitivities typically range from 10^{-8} to 10^{-15} grams of solute per second. Additionally, the amount of sample injected must be consistent, as many columns can distort peaks if too much sample is introduced. An ideal column should also be chemically inert, meaning it doesn't alter the sample in any way. Optimized columns can endure temperatures ranging from $-200\text{ }^{\circ}\text{C}$ to at least $400\text{ }^{\circ}\text{C}$. They should also have a short response time that remains consistent regardless of flow rate, covering a wide range of values. Moreover, the detector should be dependable, predictable, and easy to use. It is not feasible for a detector to meet all these requirements.

The following sections will explore some of the more commonly used types of gas chromatography detectors, along with their respective strengths and weaknesses.

- **Mass Spectrometry Detectors**

Mass Spectrometer (MS) detectors are considered the most effective among all gas chromatography detectors. In a GC/MS setup, the mass spectrometer continuously scans the different masses during the separation process. Once the sample leaves the chromatography column, it travels through a transfer line and enters the mass spectrometer's inlet. The sample is then ionized and broken down into fragments, usually through an electron-impact ion source. During this process, the sample is exposed to high-energy electrons, which strip electrons from the molecules due to electrostatic forces. This causes the molecules to become ions. Further exposure to these electrons leads to the fragmentation of the ions. The resulting ions are then sent into a mass analyzer, where they are separated based on their mass-to-charge ratio (m/z). Most of these ions carry a single electrical charge.

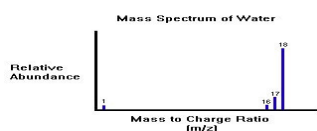


Figure 3: Mass Spectrum of Water

GC/MS systems are beneficial because they enable the direct measurement of the analyte's mass and help identify components that may not be fully separated. These systems are durable, user-friendly, and can process samples almost as fast as they are separated. However, mass spectrometry detectors have some drawbacks, such as the risk of sample degradation due to high temperatures before detection, and the fact that the sample is completely broken-down during analysis. [8]

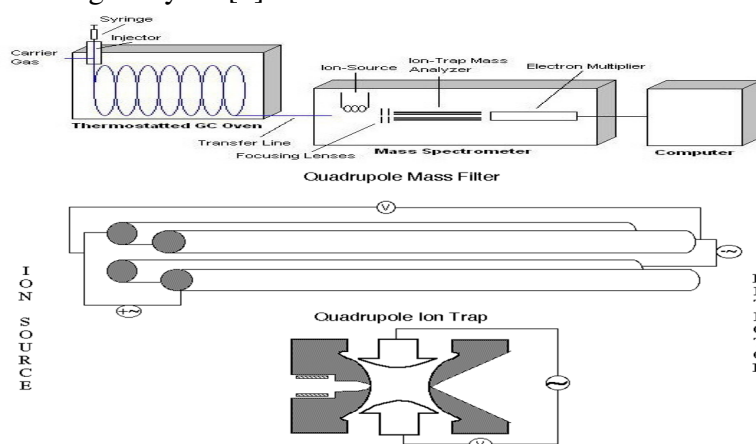


Figure 4: Arrangement of the poles in Quadrupole and Ion Trap Mass spectrometers

- **Flame Ionization Detectors**

Flame ionization detectors, or FIDs, are the most versatile and commonly used type of detector. When the sample exits the column, it is directed into an air-hydrogen flame. Due to the high temperature of the flame, the sample undergoes pyrolysis, which is a chemical breakdown caused by intense heating. During this process, the hydrocarbons break down into ions and electrons that carry an electrical current. A high-impedance picoammeter measures this current to track the sample as it elutes.

Using a FID is beneficial because it is not affected by flow rate, noncombustible gases, or water. These qualities make the FID highly sensitive and produce low noise. The detector is also reliable and relatively easy to operate. However, this method requires a flammable gas and results in the destruction of the sample.

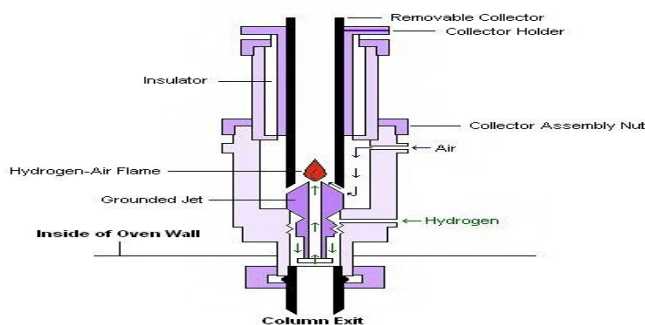


Figure 5: Schematic of a typical flame ionization detector.

- **Thermal Conductivity Detectors**

Thermal conductivity detectors (TCDs) were among the first detectors developed for use with gas chromatography. The TCD functions by measuring changes in the thermal conductivity of the carrier gas, which occurs when the sample, having a different thermal conductivity, is present. The design of the TCD is straightforward and involves an electrically heated source that remains at a constant power level. The temperature of this source is influenced by the thermal conductivities of the surrounding gases. Typically, the source is a thin wire made of platinum or gold. The resistance of the wire varies with temperature, which in turn is affected by the thermal conductivity of the gas.

The benefits of TCDs include their ease of use, wide applicability to both inorganic and organic compounds, and the capability to collect the analyte after separation and detection.

However, the main disadvantage of TCDs is their relatively low sensitivity compared to other detection methods, as well as their dependence on flow rate and concentration.

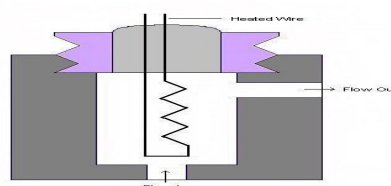


Figure 6: Schematic of thermal conductivity detection cell

- **Electron-capture Detectors**

Electron-capture detectors (ECDs) are highly selective instruments often used for analyzing environmental samples. The device is designed to specifically identify certain organic compounds that contain specific functional groups like halogens, peroxides, quinones, and nitro groups. It does not respond significantly to other types of compounds. Because of this, ECDs are especially useful in situations where very small amounts of chemicals, such as pesticides, need to be detected, and other chromatographic techniques are not practical.

One key benefit of ECDs is their high sensitivity and selectivity for organic compounds with electronegative functional groups.

However, there are some drawbacks. The detector has a limited range of signal response and can be hazardous due to the presence of radioactive materials. Additionally, the signal-to-noise ratio is affected by the natural decay of radioactive substances and the presence of oxygen inside the detector.

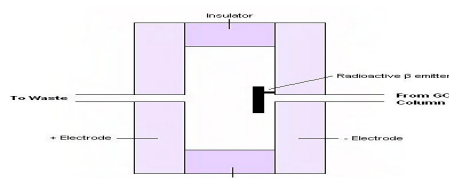


Figure 7: Schematic of an electron-capture detector.

• **Atomic Emission Detectors**

Atomic emission detectors (AEDs) are a newer tool used by gas chromatographers. They are element-specific detectors that use plasma, which is a gas that has some charged particles, to turn all the elements in a sample into atoms and make them emit light at certain wavelengths. AEDs are very useful because they can detect a wide range of elements by looking at the light they emit. There are three main ways to make plasma: Microwave-Induced Plasma (MIP), Inductively Coupled Plasma (ICP), and Direct Current Plasma (DCP). MIP is the most common type and uses a diode array that can check the light emissions from multiple elements at the same time. The parts of an AED include: 1) a part that connects the capillary GC column to the plasma chamber, 2) a microwave chamber, 3) a cooling system, 4) a diffraction grating, and 5) a photodiode array that can move and is connected to a computer.

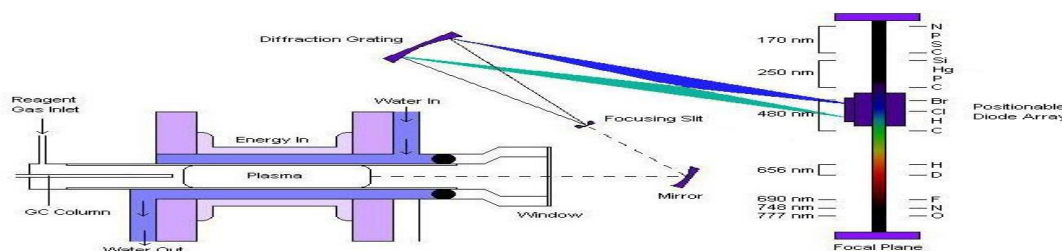


Figure 8: Schematic of atomic emission detector.

• **GC Chemiluminescence Detectors**

Chemiluminescence spectroscopy (CS) is a method that can be used to find out both the type and amount of a substance by looking at the light that comes from chemicals that are excited. It works in a way that is similar to atomic emission spectroscopy (AES), but instead of using light from excited atoms, it uses light from molecules that have energy. Chemiluminescence can happen in liquids or gases, while AES only works with gases. The light in chemiluminescence comes from chemical reactions that create light as part of their process. This light is used directly, instead of needing another light source like a beam.

Like other techniques, chemiluminescence has some limits.

One main issue is related to the photomultiplier tube (PMT) used to detect the light. A PMT needs a dark current to properly see the light from the sample.

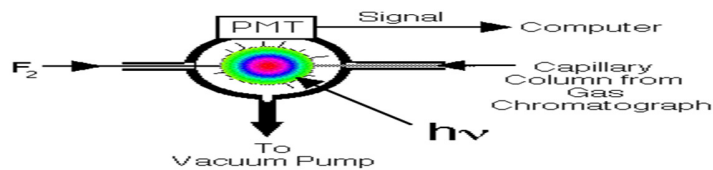


Figure 9: Schematic of a GC Chemiluminescence Detector

- **Photo ionization Detectors**

Another type of detector used with gas chromatography is the photoionization detector, which uses chemiluminescence spectroscopy. A photoionization detector, or PID, is a portable device that can detect vapors and gases. It is good at identifying aromatic hydrocarbons, organo-heteroatom compounds, inorganic substances, and other organic materials. The PID includes a UV lamp that sends out light, which is absorbed by the compounds in an ionization chamber connected to the GC column. Only a small part of the molecules get ionized, and this process doesn't destroy the sample, which allows for further analysis by other detectors. PIDs come in portable handheld versions and different types of lamps. Results are usually quick. PIDs are often used to detect volatile organic compounds in soil, sediment, air, and water. They're commonly used to find pollutants in the air and soil. However, one downside is that they can't detect certain low molecular weight hydrocarbons like methane and ethane.

- **Limitations**

1. Not suitable for detecting semi-volatile compounds
2. Only indicates if volatile organic compounds are presents.
3. High concentration so methane is required for higher performance.
4. Frequent calibration is required.
5. Units of parts per million range
6. Environmental distraction, especially water vapor.
7. Strong electrical fields Rapid variation in temperature at the detector and naturally occurring compounds may affect instrumental signal. [10]

- **Recorders and Integrators:**

- Recorders are used to record the responses obtained from detectors after amplification, if necessary.
- Integrators: Integrators are improved version of recorders with some data processing capabilities. They provide more information on peaks than recorders.

- **Temperature Control Devices:**

- Preheaters:

Preheaters are used to convert the sample into its vapor form and mix them with the mobile phase. These are present along with injecting devices.

- Thermo statistically controlled oven:

Since partition coefficient as well as solubility of a solute depends upon temperature, temperature maintenance in a column is highly essential for efficient separation. These ovens are highly accurate and can maintain temperature nearest to 0.1 degrees Celsius.

➤ **Derivatization of Sample**

Derivatization is a technique of treatment of the sample to improve the sample of separation by column or detection by detector.

- Precolumn derivatization: With this technique the components are converted to more volatile and thermo stable derivatives. Improved separation can be seen after such treatment.

This is done when:

- ✓ The component is less volatile
 - ✓ The components are thermo labile
 - ✓ To improve separation factor.
- Post column derivatization: this is done to improve the response shown by detector. [11]

IV. How Does Gas Chromatography Work?

GC uses a carrier gas to separate chemicals. This gas acts as the mobile phase, carrying the sample molecules through the system without interfering with the sample or the instrument parts.

The sample is added to the gas chromatograph (GC) either with a syringe or from an auto-sampler that might also extract chemicals from solid or liquid samples.

The sample is injected into the GC inlet through a septum, which keeps the mobile phase from escaping. Connected to the inlet is the analytical column—a long, narrow tube (0.1 to 0.53 mm in diameter) made of fused silica or metal, with a stationary phase coated on its inner walls. The column is placed in an oven that heats up during analysis, helping to release the less volatile components. The column's end connects to a detector, which reacts to the chemicals coming out of the column and creates a signal. This signal is recorded by software on a computer, making a chromatogram.

After being injected into the GC inlet, the sample's chemicals are first turned into gas, if they aren't already. For samples with low concentration, the whole vapor goes into the analytical column, known as splitless mode. For high concentration samples, only part of the sample goes into the column in split mode, and the rest is removed through a split line to avoid overloading the column.

Once in the column, the different chemicals separate based on how they interact with the stationary phase. Choosing the right column depends on the volatility and chemical structure of the sample. Liquid stationary phases are mostly either polyethylene glycol (PEG) or polydimethylsiloxane (PDMS). PDMS has different functional groups like dimethyl, diphenyl, or mid-polar groups, such as cyanopropyl phenyl. Non-polar columns work well with non-polar substances, while columns with phenyl groups are good for molecules that form π - π interactions. For chemicals that make hydrogen bonds, such as acids and alcohols, PEG columns are usually best unless they have been modified to be less polar.

The last step is detecting the chemicals as they come out of the column.

There are various types of detectors. Some, like the flame ionization detector (FID), respond to C-H bonds. Others detect specific elements, such as sulfur, nitrogen, or phosphorus. Still others sense specific molecule properties, such as the ability to capture an electron, as used in the electron capture detector (ECD). [2]

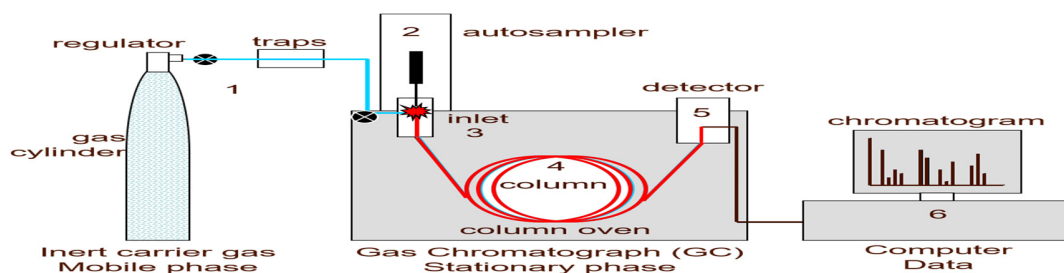


Figure 10: A simplified diagram of a gas chromatograph showing: (1) carrier gas, (2) auto sampler, (3) inlet, (4) analytical column, (5) detector and (6) PC.

V. How Do You Read a Chromatogram and What Does It Tell You?

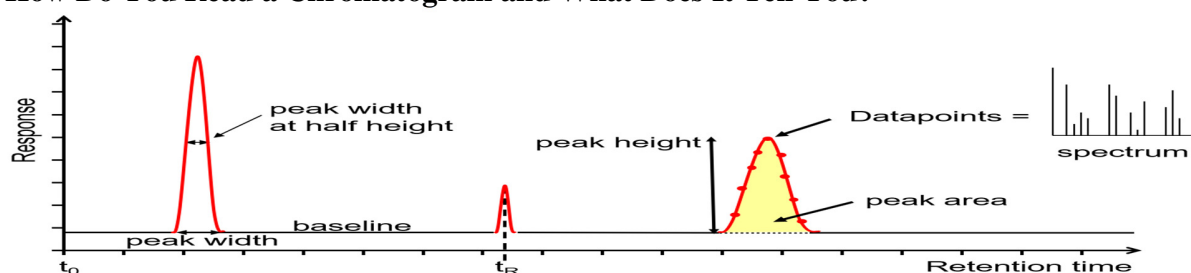


Figure 11: Chromatogram output from a GC or GC-MS.

A lot of information can be learned from the chromatogram about the health of the GC or GC-MS system and the data needed for qualitative or quantitative analysis. The x-axis shows the retention time, which is the time from when the sample was injected into the GC (t_0) until the end of the run. Each peak from an analyte starts at the apex of the peak, like t_R . The y-axis shows the response of the detector to the analyte peak. The baseline is the signal from the detector when no analyte is coming out of the column, or if it is below the detection limit. The baseline can come from electrical noise, which is usually low, and chemical noise, like impurities in the carrier gas, bleed from the column’s stationary phase, or system contamination. If the baseline is higher than it should be, that suggests a problem or that maintenance is needed. You can take several measurements from the peak, such as the width at the baseline, width at half height, total height, and area. The last two are related to concentration, but the area is used for quantitation because it is less affected by band broadening. These measurements can help calculate the amount of band broadening and how the analyte molecules spread out on the column. Narrower, sharper peaks mean better sensitivity and better resolution between peaks. The peaks are usually shaped like a Gaussian curve. However, if a peak tailed (the right side is wider), that might mean there is activity or a dead volume in the system. If a peak fronted (the left side is wider), that means the column is overloaded. The accuracy of measurements can be affected by how many data points are across a peak. The ideal number of data points is 15 to 25. Too few makes the peak look like a child’s dot-to-dot drawing, which can impact peak area, resolution, and deconvolution in GC-MS. Too many data points lower the signal-to-noise ratio, which can reduce sensitivity.

Strengths and limitations of gas chromatography

- ✓ GC is a common method used in many different industries. It's used for everyday testing as well as for research, and it can analyze a small number up to hundreds—or even thousands with GC x GC—of

substances in various types of samples, including solids and gases. It's a strong and reliable technique that works well with other methods like mass spectrometry.

- ✓ GC can only analyze substances that are volatile, meaning they can turn into a gas. These substances usually have a boiling point lower than water and a molecular weight up to about 1250 u. Some substances break down when heated, so it's important to use cold injection methods or keep the temperature low to prevent this. Also, very polar substances might not move through the system properly or could be lost, so the system should be kept clean and well-maintained, or the substances should be chemically changed to make them easier to analyze. [2]

VI. Common problems with gas chromatography

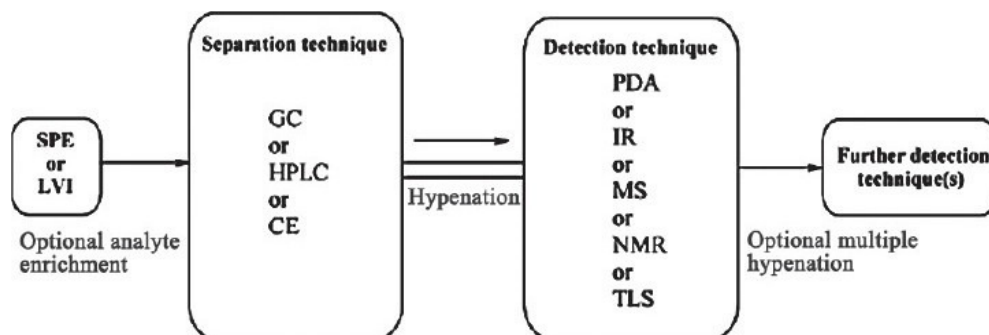
- ✓ The biggest issue in gas chromatography is leaks. Since the mobile phase is a gas and moves through the whole system, it's very important to install all parts and supplies correctly and to check for leaks regularly.
 - ✓ Another common problem is activity, especially with more polar substances, especially those present in very small amounts. Silanol groups on the glass parts of the column and the inlet, as well as dirt buildup in the system, can lead to tailing peaks, sticky adsorption, or chemical breakdown. The inlet is the most problematic part because this is where the sample is added, turned into vapor, and sent into the column. So, keeping the inlet clean and using the right materials, like a deactivated inlet liner, is very important for the system to work properly. [2]

VII. Applications of Gas Chromatography

- ✓ Gas chromatography is a way to separate mixtures that can turn into gas. It is used in many areas like medicine, beauty products, and checking for harmful substances in the environment. Because the samples need to be able to vaporize, it's easy to test things people breathe, their blood, saliva, and other body fluids that have many volatile chemicals. Understanding how much of each substance is present helps scientists learn more about how these chemicals affect health and the environment.
- ✓ Air samples can also be tested with gas chromatography. Usually, air quality teams use gas chromatography with a flame ionization detector (FID) to find out what's in the air. Even though other types of detectors are helpful, FID is best because it's very sensitive and can detect small amounts of substances.
- ✓ Another method called GC/MS helps find out what's in a mixture by looking at how long each part stays in the system and how much of it is present. This technique is used in many medicine areas to check the amount of chemicals in medicines. It is also used by beauty product makers to make sure they know exactly how much of each chemical is in their products. [5]

VIII. Hyphenated Techniques

Hyphenated techniques mix chromatography and spectroscopy to use the strengths of both. Chromatography separates mixtures into pure or almost pure parts. Spectroscopy gives specific information to identify chemicals using known standards or stored spectra. A few decades ago, Hirschfeld first used the word "hyphenation" to describe combining a separation method with one or more spectroscopic methods. This technique, which comes from joining a separation method with a spectroscopic technique, is now called a hyphenated technique.



In recent years, hyphenated techniques have become very popular for solving difficult analytical problems. Over time, combining separation methods with spectroscopy has been shown to help both find and measure unknown compounds in complex natural product mixtures. To learn about the structure of compounds in raw samples, methods like liquid chromatography (often HPLC), gas chromatography (GC), or capillary electrophoresis (CE) are connected to spectroscopic techniques like FTIR, PDA UV-VIS absorption, fluorescence, mass spectrometry (MS), and NMR. This has led to many modern hyphenated methods, such as CE-MS, GC-MS, LC-MS, and LC-NMR. HPLC is the most commonly used method for finding and measuring compounds in natural products. When HPLC is connected to MS or NMR, it helps better understand the structure of complex natural products. Because of its higher sensitivity, LC-MS is used more than LC-NMR. Hyphenated techniques don't always have to be between two methods; sometimes more than one separation or detection method is used, like LC-PDA-MS, LC-MS-MS, LC-NMR-MS, or LCPDA-NMR-MS. When trace analysis is important and the sample needs to be enriched, on-line coupling with SPE, solid-phase micro extraction, or LVI can be used to make a more powerful system, like SPE-LC-MS or LVI-GC-MS. In natural product research, two main goals are isolating and purifying compounds from raw extracts or fractions and accurately identifying them. For this, the online characterization of secondary metabolites in raw natural product samples needs a high level of skill, rich structural information, sensitivity, and selectivity. The development of many hyphenated techniques has given researchers powerful tools that provide excellent separation and online complementary spectroscopic data on specific peaks in a complex mixture. [13]

Available Hyphenated Techniques

- GC-MS

With MS being the main way to detect things, and single- and triple quadrupole, ion trap, and time-of-flight (TOF) mass spectrometers being the most commonly used instruments, both LC-MS and GC-MS are the most widely used techniques today. GC-MS is a technique created by combining GC and MS, and it was the first of its kind to be useful for research and development. The mass spectra from this technique offer more information about the structure of compounds based on how they break apart. The fragments with different amounts can be compared with library spectra. Compounds that are volatile enough, small, and don't break down at high

temperatures can be analyzed easily with GC-MS. Sometimes, polar compounds, especially those with a lot of hydroxyl groups, need to be treated with a chemical called a derivatizing agent before being analyzed. The most common method is turning the compound into a trimethylsilyl derivative. In GC-MS, a sample is injected into the GC, heated up, separated in the column, and then analyzed by the MS detector. The time between injection and when a component comes out is called the retention time (RT). The equipment used in GC-MS has an injection port at one end of a metal column (often filled with a material that looks like sand to help separate compounds) and a detector (MS) at the other end. A carrier gas like argon, helium, nitrogen, or hydrogen moves the sample through the column. The GC separates the parts of a mix over time, and the MS helps find out what each part is made of. GC-MS columns come in two types: capillary columns and macro bore or packed columns. The following things need to be considered carefully about the GC-MS interface.

First, the interface should move the sample from the GC to the MS efficiently.

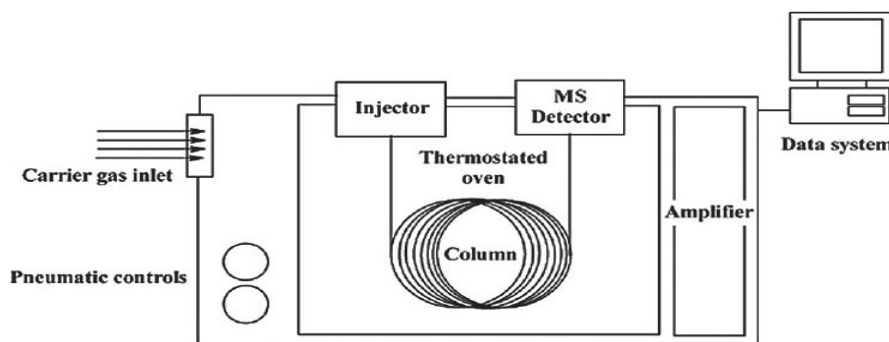
Second, the compound should not freeze in the interface.

Third, it should not break apart before reaching the MS ion source.

Fourth, the amount of gas entering the ion source should not be too much for the MS to handle.

The most commonly used interfaces in GC-MS are electron impact ionization (EI) and chemical ionization (CI).

But in newer GC-MS systems, other types are also used that help find the molecular ion. For example, an orthogonal TOF mass spectrometer connected to GC is used to check the purity and identity of substances by measuring their exact mass and calculating their elemental makeup. Today, GC-MS systems are often connected with online MS databases that have references for various compounds, which helps match spectra for identifying the separated components. [1]



- **GC -AED (Atomic Emission Detector)**

Gas Chromatography–Atomic Emission Detector, or GC-AED, is used to analyze gasoline, diesel, oil, environmental pollutants in soil, water, and wastewater, as well as Volatile Organic Compounds (VOCs) in water. It uses Atomic Emission Spectroscopy to detect elements as they come out of the gas chromatograph's column.

The AED uses helium as the carrier gas to move the sample through the GC.

The helium needs to be very pure because water and oxygen can mix with the stationary phase and cause problems like baseline noise and column bleed, which make the results unreliable. The AED also uses a helium plasma, which is very sensitive, so the helium must be extremely pure. Sometimes a reagent gas is used to make the AED more sensitive by stopping soot from building up on the lamp or discharge tube. Helium is also used at the end of the column to improve the flow rate into the detector. Regular calibration with a standard mixture is part of the routine.

The AED measures the energy released at specific wavelengths by atoms in the helium plasma (200–250 mL per minute) to find out how many atoms are present in each peak from the chromatography. When combined with the GC's ability to separate the compounds, this helps determine the exact amount of each substance.

The helium from the GC, at a flow rate of 3.5 mL per minute, travels through the capillary column in the heated transfer line at 250°C to reach the AED.

The helium plasma chamber is also kept at 250°C. The gases, including helium from the GC, hydrogen at 12 psi or 0.83 bar, and oxygen at 15 psi or 1.03 bar, flow through a fused silica tube and are turned into a plasma by microwave energy. In the high-energy plasma, the sample compounds from the GC are broken into free radicals, ions, and atoms. As they go back to their normal state, they emit light at specific wavelengths that are unique to each element. This light goes through a fused silica lens and a narrow slit, then hits a fixed mirror that reflects it onto a holographic grating. The grating spreads the light into different wavelengths across a plane. This light then hits two charge-coupled devices (CCDs) to measure the intensity. There's a gap between the two CCDs, which leaves a 7 nm gap in the 183–190 nm range of the spectrum. The CCDs turn the light into electrical signals that are recorded by the AED III software. The software automatically translates the light intensity into wavelength data using a calibration table before each measurement. After each run, the data is stored with 0.01 nm resolution. [14]

IX. Conclusion:

One of the main downsides of gas chromatography is that it works only with compounds that are volatile or can be made volatile through chemical changes without breaking down. When a mix of unknown substances is injected, the components that stick to the column move slowly through it. Sometimes, increasing the temperature during the process helps, but the column has a maximum temperature it can handle, depending on its type. This is another drawback of gas chromatography. Even though it has these limitations, gas chromatography is widely used in many areas of the pharmaceutical and clinical fields, both in research and quality control. It is used for things like ensuring product quality, manufacturing, developing pilot plants for active pharmaceutical ingredients, bulk drugs, and drug formulations. It also helps identify impurities during drug production, in pharmacognosy, pharmaceutical process control, and biotechnology because it has a high sensitivity and can clearly separate compounds. When gas chromatography is combined with other spectral techniques, it becomes even more powerful for solving complex analysis problems.

X. References:

1. Wikipedia contributors. Gas chromatography. Wikipedia, The Free Encyclopedia; 2025 Sep 30 [cited 2025 Oct 6]. Available from: https://en.wikipedia.org/wiki/Gas_chromatography
2. Technology Networks. Gas chromatography: How a gas chromatography machine works, how to read a chromatograph, and GCxGC. Technology Networks; 2020 [cited 2025 Oct 6]. Available from: <https://www.technologynetworks.com/analysis/articles/gas-chromatography-how-a-gas-chromatography-machine-works-how-to-read-a-chromatograph-and-gcxc-335168>
3. Air Products. Gas chromatography – atomic emission detector. Air Products Analytical Laboratories; 2023 [cited 2025 Oct 6]. Available from: <http://www.airproducts.ca/industries/Analytical-Laboratories/analytical-lab-applications/product-list/gc-with-atomic-emission-detector-gc-aed-analytical-laboratories.aspx?itemId=F63C60220EDA4615903A0FA3243BEAEB>

4. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed. Belmont (CA): Thomson Brooks/Cole; 2007.
5. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis. 5th ed. Mumbai: Himalaya Publishing House; 2013.
6. Krugers J. Instrumentation in Gas Chromatography. Eindhoven: Centrex Publishing Company; 1968.
7. Hubschmann HJ. Handbook of GC/MS: Fundamentals and Applications. Weinheim (Germany): Wiley-VCH Verlag; 2001.
8. Scott RPW. Chromatographic Detectors: Design, Function, and Operation. New York: Marcel Dekker Inc.; 1996.
9. Driscoll JN. Review of photoionization detection in gas chromatography: the first decade. J Chromatogr Sci. 1985;23(11):488-92.
10. Boer H. Vapor phase chromatography. In: Desty DH, editor. Gas Chromatography. London: Butterworths Scientific Publications; 1957. p.169.
11. Holler FJ, Crouch SR, West DM. Fundamentals of Analytical Chemistry. 9th ed. Belmont (CA): Brooks/Cole; 2014.
12. Wilson ID, Brinkman UA. Hyphenation and hyphenation: the practice and prospects of multiple hyphenation. J Chromatogr A. 2003;1000(1-2):325-56.