Chapter N° 1:

Chromatographic methods

Chromatography is a general term used to define separation methods based on the distribution of a solute between two phases, one of which is mobile (a gas or a liquid), and the other is stationary (a solid or a liquid).

I.1. Definition of Chromatography

Chromatography is a technique used to separate the components of a mixture. It allows purification, identification, and quantification of compound.

Uses in pharmaceutical labs:

- Check the purity of raw materials and products.
- Follow the progress of a chemical reaction.
- Isolate and purify drug substances.

I.2. Historical Background

The term "chromatography" may have originated from the separation of colored compounds, as "Chroma" (χρῶμα) in Greek means color and graphein means write.

- 1906: Tswett discovered chromatography by separating plant pigments.
- 1931: Kuhn & Lederer revived the method (carotene/xanthophyll).
- 1940s: Martin & Synge developed chromatography → Nobel Prize (1952).
- 1952: Gas Chromatography (GC) was developed.
- 1968: High-Performance Liquid Chromatography (HPLC).
- 1979: First chiral separation with HPLC.

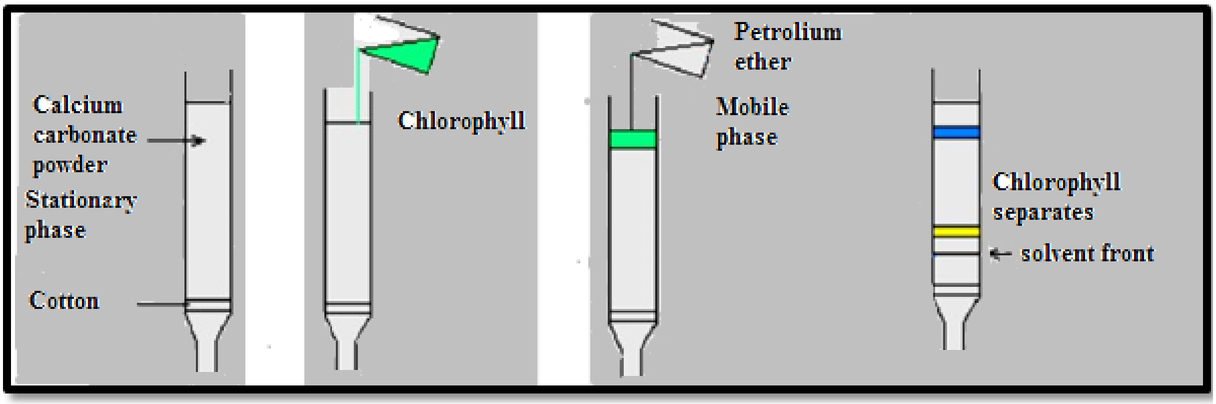


Fig 1. Representation of Tswett's experiment (**1906**)

I.3. Principle

- Separation is based on how substances distribute between **two phases**:
  - **Stationary phase** (fixed: solid or liquid on a support).

- **Mobile phase** (moving: liquid, gas, or supercritical fluid).

- Each compound moves at a different speed depending on its **affinity for the stationary phase** and its **solubility in the mobile phase** → this leads to separation.

I.4. General Chromatography Terminology

- **Solute (S)**: Any substance, component of a mixture, separated by chromatography.
- **Mobile Phase (PhM)**: The vector, whether liquid or gaseous, that moves the solute.
- **Stationary Phase (PhS)**: The substance that, due to its affinity for the solutes, allows their separation when the mobile phase displaces them.
- **Support**: An inert substrate that carries the stationary phase.
- **Chromatographic column**: A tube of variable diameter and length, made of glass, metal, or other materials, inside of which chromatographic separations occur.
- **Retention values**: Any data that quantifies the specific action of the stationary phase on the solute during the analysis (retention time, retention volume, etc.).
- **Chromatogram chromatography**: The set of successive responses from the detector during the elution of solutes from the column.
- **Elution**: A process that allows a compound adsorbed to be put into solution (referred to as the eluate) using a solvent called the eluent.
- **Eluent**: The solvent used for the separation of substances adsorbed on a support in chromatography separation process.
- **Eluate**: The mixture of solute and solvent that exits the column.

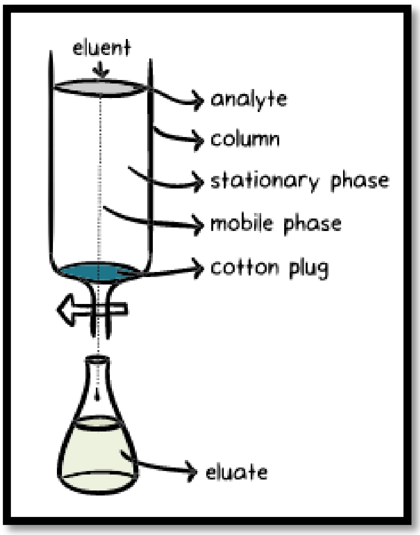


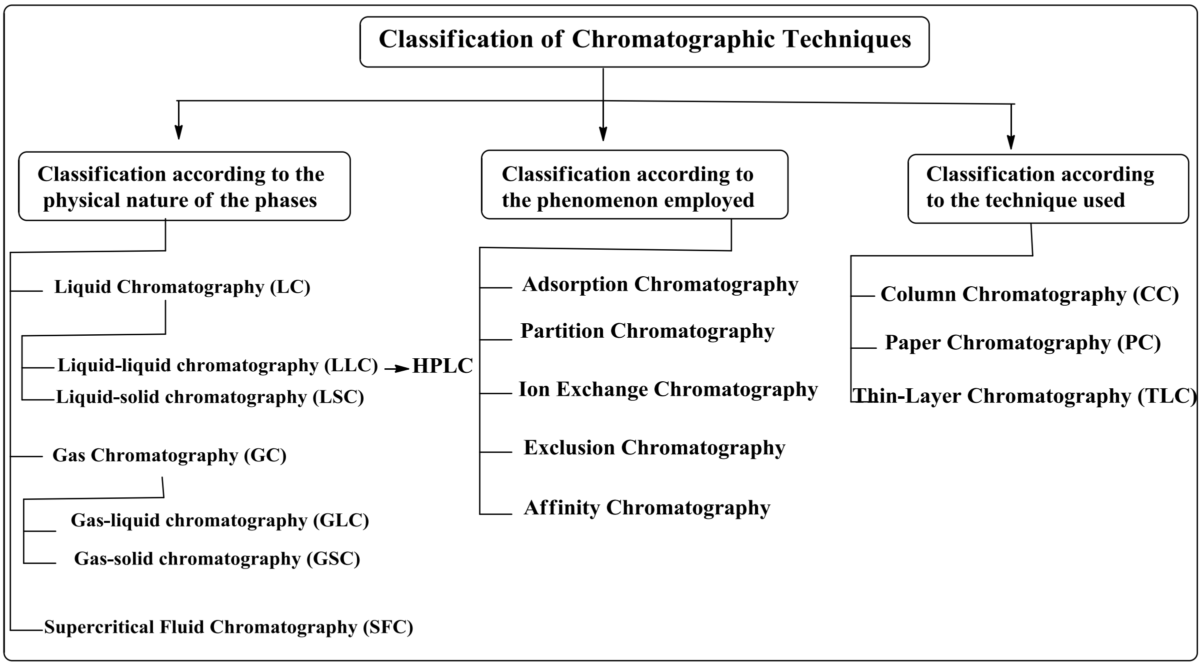
Fig 2. Column chromatography description

I.5. Classification of Chromatographic Techniques

Chromatography can be classified depending on how separation occurs. Several factors can influence separation: solubility, polarity, molecular size/shape, electrical charge, and specific interactions.

Under the term chromatography, a wide range of different techniques is grouped, which can be classified into three categories based on:

- Classification according to the physical nature of the phases.
- Classification according to the phenomenon employed.
- Classification according to the technique used.



I.5.1. Classification According to the Physical Nature of the Phases

In this classification:

I.5.1.1. Liquid Chromatography (LC)

If the mobile phase is a liquid, the stationary phase can either be a finely powdered solid or a liquid immobilized on a solid phase. The combination of these possibilities allows for the distinction of two types of chromatography:

- **Liquid-liquid chromatography (LLC).**
- **Liquid-solid chromatography (LSC).**

I.5.1.2. Gas Chromatography (GC) If the mobile phase is a gas (carrier), the stationary phase can either be a porous solid, reserved for the analysis of low-boiling gas mixtures, or a liquid immobilized on a solid support through impregnation or grafting. This leads to the distinction of two types of chromatography:

- **Gas-liquid chromatography (GLC).**
- **Gas-solid chromatography (GSC).**

I.5.1.3. Supercritical Fluid Chromatography (SFC)

SFC represents an intermediate case between **LC** and **GC**, as supercritical fluids possess properties that are between those of liquids and gases.

I.5.2. Classification According to Chromatographic Phenomena

The phenomena leading to separation depend on the nature of the stationary phases, and we consider:

I.5.2.1. Adsorption Chromatography

The stationary phase is a finely divided solid on which molecules adhere through both physisorption and chemisorption. The relevant physicochemical parameter is the adsorption coefficient.

I.5.2.2. Partition Chromatography

The stationary phase is a liquid immobilized on an inert solid support: either impregnated into a porous solid (risk of leaching) or grafted onto the solid (grafted phase). The separation is based on the partition coefficient of the solute between the two liquid phases.

I.5.2.3. Ion Exchange Chromatography

The stationary phase consists of macromolecules (resins) bearing acidic or basic functional groups that allow for the exchange of counter-ions with ions of the same charge from the sample. The separation relies on ionic distribution coefficients.

I.5.2.4. Exclusion Chromatography, or Gel Permeation, or Molecular Sieve Chromatography

The stationary phase is a porous solid: large particles are excluded from it, while small particles are included and diffuse into the pores of the gel, thus being delayed.

I.5.2.5. Affinity Chromatography

The stationary phase is an inert substrate onto which an "effector" is grafted that has an affinity for a solute from the sample to be analyzed (enzyme-substrate affinity, ligand-receptor, and antibody-antigen).

I.5.3. Classification According to the Procedure Used

Based on the immobilization of the stationary phase, we distinguish:

I.5.3.1. Column Chromatography (CC)

The stationary phase is contained within a cylindrical column made of glass or metal.

I.5.3.2. Paper Chromatography (PC):

A cellulose surface, considered as a support, maintains a liquid stationary phase by imbibition.

I.5.3.3. Thin-Layer Chromatography (TLC): In this case, the stationary phase is retained on a flat surface (glass, plastic, or aluminum foil) covered with a thin layer of 0.2 to 0.3 mm thick silica gel, cellulose, alumina, or even ion-exchange resin grains.

I.5.4. Choice of Technique

The different techniques are complementary rather than competitive. The choice of one technique over another depends on:

The nature of the solute such as: gas, volatile liquid, non-volatile liquid, solid, macromolecule, organic species, polar, ionic, etc.

Purpose of the Analysis: The analysis aims to identify components in a mixture, determine whether chromatography should be coupled with spectroscopic methods or mass spectrometry (e.g., GC/MS), and assess sample purity. Additionally, it serves to purify compounds using preparative columns, monitor reactions continuously to optimize parameters, and perform quantitative analysis (dosage).

I.6. Basic Theory - Fundamental Parameters

I.6.1. Chromatogram

The chromatogram is a curve that represents the variation over time of a parameter related to the instantaneous concentration of the solute exiting the column. The baseline corresponds to the trace obtained in the absence of an eluted compound (Figure 1). This graph is used for both qualitative and quantitative analysis.

Qualitative analysis: allows for the identification of compounds by the position of the peak.

Quantitative analysis: evaluates the concentration or mass of a compound using the area of the peaks.

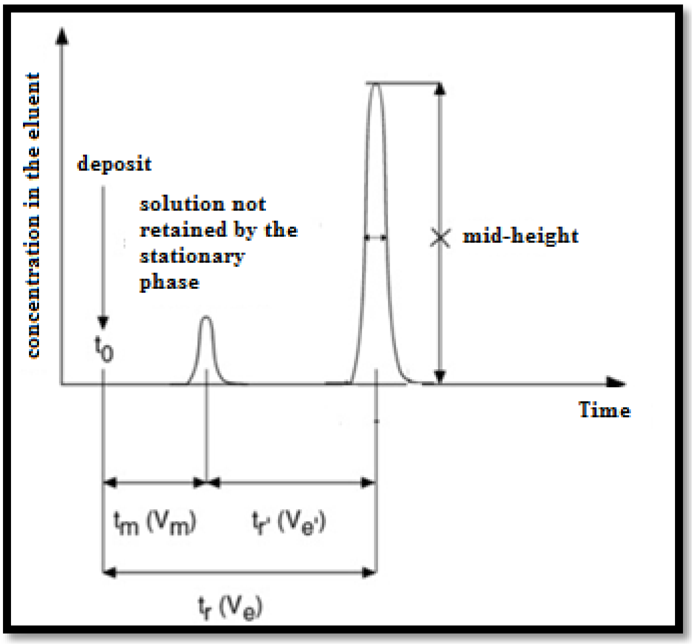


Fig 3. Diagram Showing the Characteristics of a Chromatogram

A chromatographic peak is characterized by:

I.6.1.1. Dead Time

Dead time (t_m) is the time taken by a compound that is not retained by the stationary phase of the column to travel from the entrance to the exit of the column (or the time taken by the mobile phase to traverse the column). The time **t_0** is the time at the beginning of the injection.

I.6.1.2. Retention Time

Retention Time is the time taken by the molecules of a compound to be analyzed to travel from the entrance to the exit of the column. A component is characterized by its retention time (**t_r**), which is the elapsed time between the moment of injection and the moment determined at the maximum of the corresponding peak on the chromatogram. Retention time is independent of:

- The amount injected,
- The nature and abundance of other constituents in the mixture.

However, it depends on:

- The mass of the stationary phase in the column,
- The flow rate of the mobile phase,
- The dead volume of the chromatograph (injector, detector, tubing, etc.),
- The nature of the stationary phase.

I.6.1.3. Corrected or Reduced Retention Time (tr')

This is the time taken by the solute or analyte molecules to traverse the distance from the entrance (inlet) to the exit of the column (outlet), representing the time spent in the stationary phase. It is obtained by subtracting the dead time from the actual retention time. It is solely related to the retention phenomenon:

$$\mathbf{t'_r = t_r - t_m}$$

I.6.1.4. Retention Volume

The retention volume of each solute represents the volume of the mobile phase needed to migrate it from one end to the other of the column. This volume corresponds on the chromatogram to the volume of the mobile phase that has flowed between the moment of injection and the moment corresponding to the peak maximum. If **D** is the flow rate, then:

$$\mathbf{V_r = t_r \times D}$$

I.6.1.5. Dead volume

The dead volume can also be calculated, corresponding to the volume of the mobile phase in the column (interstitial volume):$\mathbf{V_m = t_m \times D}$

Average Linear Velocity of the Solute and Mobile Phase

The average linear velocity of the solute (v) is given by:

$$\boldsymbol{\nu = \frac{L}{t_r}}$$

Where **L** : is the length of the column.

The average linear velocity of the mobile phase (u) is given by:

$$\boldsymbol{\mu = \frac{L}{t_m}}$$

I.6.2. Distribution Coefficient

In liquid chromatography, separations are based on the difference in distribution of species between two immiscible phases: one stationary (solid particles that is able to hold onto gas or liquid particles on its outer surface) and the other mobile (liquid). For a given chromatographic system, the distribution coefficient **K** (or partition coefficient) is defined by:

$$\boldsymbol{K = \frac{C_s}{C_m}}$$

Where:

$\mathbf{C_s}$: concentration of the solute in the stationary phase

$\mathbf{C_m}$: concentration of the solute in the mobile phase.

I.6.3. Retention Factor

Retention factor is also known as partition factor or capacity factor. When a compound is introduced into the column, its total mass $\mathbf{m_T}$ is divided into two quantities: $\mathbf{m_M}$ in the mobile phase and $\mathbf{m_S}$ in the stationary phase. These quantities remain constant during its migration in the column. They depend on $\mathbf{m_T}$ and **K**. Their ratio is fixed and is called the retention factor:

$$K' = \frac{m_s}{m_M} = \frac{C_s\, V_s}{C_M\, V_M} = K\, \frac{V_s}{V_M}$$

Where **k':** distribution coefficient.

With **Vs**: volume of the stationary phase, calculated as the difference between the total volume of the column and the volume of the mobile phase $\mathbf{V_M}$.

k' is not a constant but varies with operating conditions (temperature, composition of the mobile phase, etc.). It is the most important parameter in chromatography for defining the behavior of a column. Values of **k'** that are too high should be avoided to prevent extending the analysis time.

k' can be determined directly from the chromatogram:

$$\boldsymbol{K' = \frac{t_r - t_m}{t_m}} \qquad\qquad \boldsymbol{K' = \frac{t'_r}{t_m}}$$

This relationship is also often encountered in the form:

$$\boldsymbol{t_r = t_m \times (1 + K')}$$

I.6.4. Efficiency of Column

I.6.4.1. Number of Theoretical and Effective Plates

The efficiency of a chromatographic column, which depends on the peak broadening, is measured for each compound by the number of theoretical plates **N** of the column. This theory originated from the search for a static model to describe the functioning of a chromatographic column similarly to that of a distillation column.

Instead of considering the actual, continuous movement of the mobile phase, it is assumed that it progresses in successive jumps and equilibrates with the stationary phase between two transfers. This allows us to fictively divide the column of length **L** into **N** small fictitious disks of equal height **H**, numbered from **1** to **n**. For each of these, the concentration of the solute in the mobile phase is in equilibrium with the concentration in the stationary phase of that solute. At new equilibrium, the solute has progressed through one smaller disk in the column, called a theoretical plate (Figure 4).

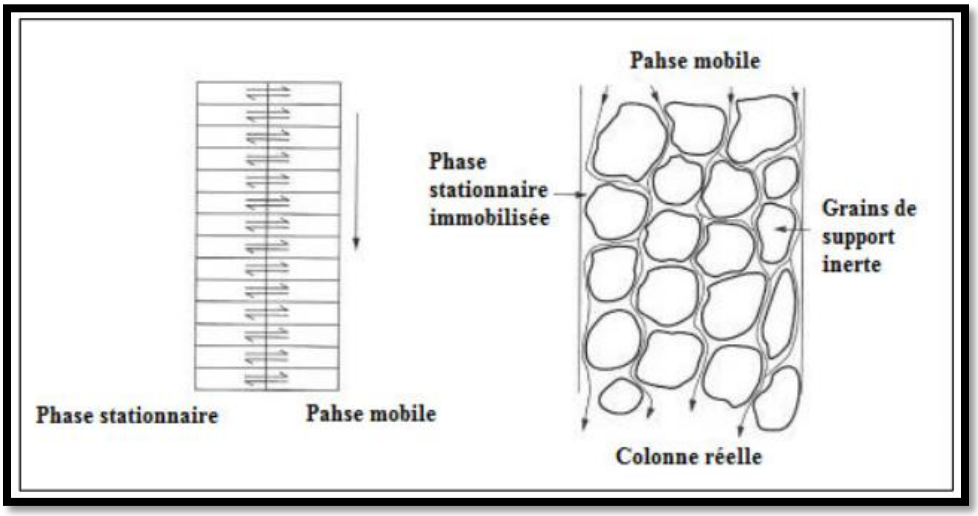


Fig 4. Plate’s theory

I.6.4.2. Theoretical Plate Height (HEPT or H)

The height equivalent to one theoretical plate (HEPT or H) is given by:

$$\mathbf{H} = \frac{\mathbf{L}}{\mathbf{N}}$$

The efficiency of the column increases when the number of theoretical plates increases or if **H** decreases at a constant length **L**. It can vary significantly depending on the type of column and the nature of the two phases.

I.6.4.3. Gaussian Elution Peaks

The efficiency of the column is calculated from the chromatographic peak, which can be modeled as a Gaussian curve (Figure 5). The dispersion of a peak is characterized by its standard deviation $\boldsymbol{\sigma}$ and its variance $\boldsymbol{\sigma}^2$.

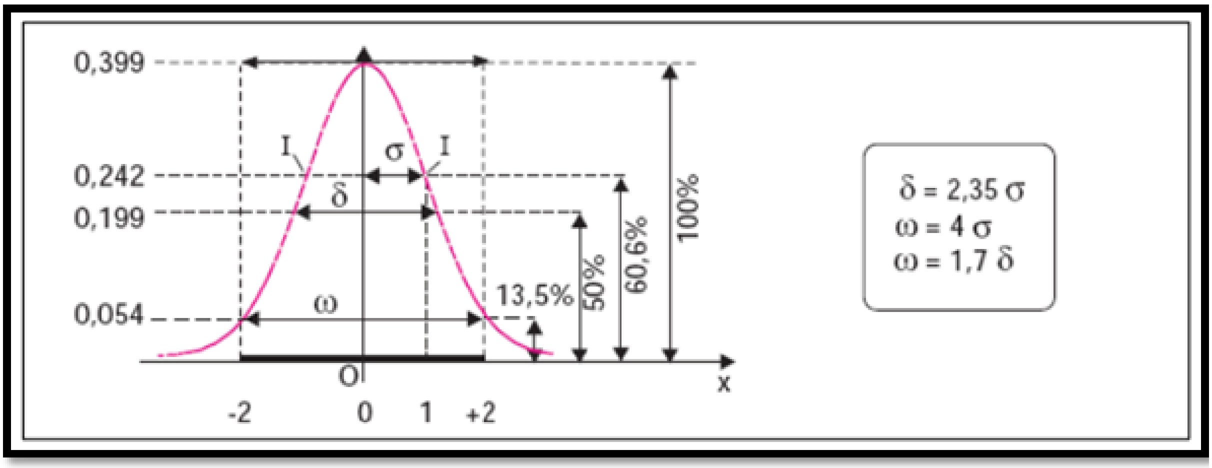


Fig 5. Gaussian Elution Peaks

- **δ**: the width at half height (measured at 50% of the total height).
- **σ**: the standard deviation of the peak (which is equal to half the width of the peak at 60.6% of its total height).
- Variance **v** = $\boldsymbol{\sigma^2}$
- **ω**: the width of the base of the peak measured at 13.5% of the total height.

The efficiency of a column is related to the width of the peaks and is defined as the variance per unit length of the column:

$$\mathbf{H} = \frac{\boldsymbol{\sigma}^2}{\mathbf{L}}$$

Since **H** is expressed in units of length, **σ** and **L** are also expressed in units of length.

The height **H** or the number of theoretical plates **N** can be determined graphically. The retention time $\mathbf{t_r}$ and **ω** (in time units) are measured graphically, allowing the determination of **N** and then **H**:

$$\mathbf{N} = \mathbf{16}\,\frac{\mathbf{t_r^2}}{\boldsymbol{\omega}^2}$$

This relationship can also be expressed in terms of the width at half-height δ:

$$N = 5.54\,\frac{t_r^2}{\delta^2}$$

This last equation is generally used because the peaks are often distorted at the base.

I.6.4.4. Actual Efficiency of a Column

N and **H** are two parameters used in the literature and by device manufacturers to evaluate column performance. They are provided for a defined solute under specified conditions.

When comparing the performances of two columns of different designs, it is preferable to replace $\mathbf{t_r}$ with the reduced time $\mathbf{t_R'}$, which does not take into account the dead time $\mathbf{t_m}$ spent by any compound in the mobile phase. The expressions become**:**

$$\mathbf{N}_{eff} = 16\,\frac{\mathbf{t_r^2}}{\boldsymbol{\omega}^2} \qquad ; \qquad N_{eff} = 5.54\,\frac{t_r^2}{\delta^2}$$

These corrected quantities are particularly useful to consider if the dead time is large compared to the retention time of the compound. This is notably the case in gas chromatography (**GC**) when comparing the performance of a capillary column to a packed column.

I.6.5. Quality of Separation

I.6.5.1. Selectivity of Column

The selectivity factor $\boldsymbol{\alpha}$ describes the position of two adjacent peaks (1 and 2) on a chromatogram. It corresponds to the ratio of the retention factors of the column for the two compounds (Figure 6). It defines whether the separation is chemically possible:

$$\alpha = \frac{t'_{r_2}}{t'_{r_1}}$$

where $\boldsymbol{\alpha}$: is always greater than 1**.**

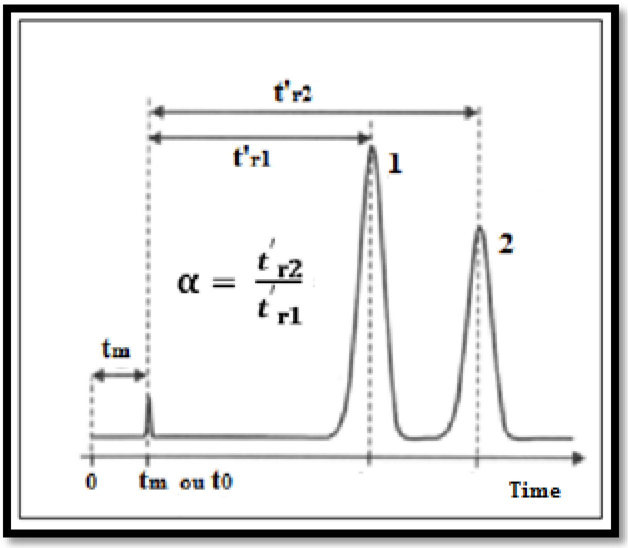


Fig 6. Selectivity factor

I.6.5.2. Resolution

The resolution **R** of a column provides a quantitative measure of its ability to separate two solutes. It is defined by:

$$\mathbf{R} = \frac{2(t_{r_2} - t_{r_1})}{\omega_1 + \omega_2}$$

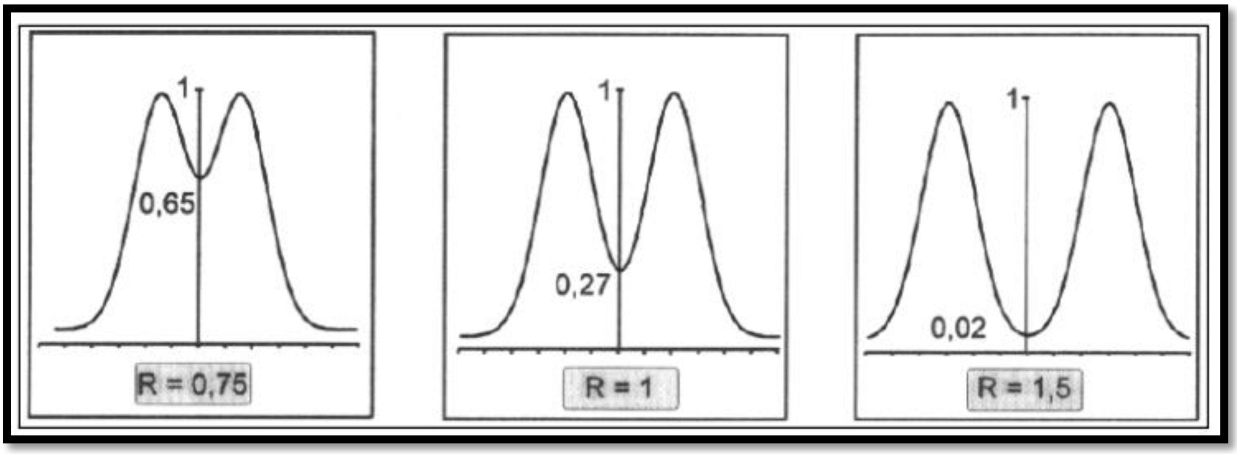


Fig 7. Resolution Factor

A resolution of **1.5** allows for almost complete separation of solutes **1** and **2**, which is not the case for a resolution of **0.75**.

For a resolution of **1**, peak **1** contains about **4%** of solute **2**, and peak **2** contains about **4%** of solute **1**.

For a resolution of **1.5**, the overlap is about **0.3%**.

The equation relating the resolution of a column to the number of theoretical plates, as well as the capacity and selectivity factors of two solutes on the column, is easily established:

$$\boldsymbol{R = \frac{\sqrt{N}}{4}\,(\frac{\alpha - 1}{\alpha})(\frac{K'_2}{1 + K'_2})}$$

Where $\mathbf{K_{2'}}$ is the capacity factor of the slowest species and **α** is the selectivity factor.

I.7. Optimization of Chromatographic Analysis

Resolution and elution time are the two most important dependent variables to consider. In any optimization, the goal is to achieve sufficient separation of the compounds of interest in the minimum amount of time. The parameters that condition **R** and $\mathbf{t_r}$ are the number of theoretical plates **N**, the capacity factor **α**, and the selectivity factor $\mathbf{K_{2'}}$:

$$\boldsymbol{R = \frac{\sqrt{N}}{4}\,(\frac{\alpha - 1}{\alpha})(\frac{K'_2}{1 + K'_2})}$$

I.7 – Optimization of Chromatographic Analysis

1. **Height Equivalent to a Theoretical Plate (H)**

- Resolution ↑ when **H** ↓.
- To reduce H:
  - Use smaller particle size.
  - Lower temperature (in GC).
  - Reduce film thickness (in HPLC).
  - Optimize mobile phase flow rate.

2. **Capacity Factor (k′)**

- Resolution improves when **k′** ↑ (optimal: 2–5).
- Drawback: longer elution time.
- Control:
  - In GC → change temperature.
  - In LC → adjust solvent composition.

3. **Selectivity Factor (α)**

- If α ≈ 1 → poor separation.
- To increase α (while keeping k′ between 1–10):
  - Modify mobile phase.
  - Adjust temperature.
  - Change stationary phase.

  - Use special chemical interactions.

I.8. Qualitative and Quantitative Analysis

I.8.1. Qualitative Analysis

- Identifies compounds by **retention time (t_r)** under fixed conditions.
- **Risk**: similar compound may share the same t_r.
- **Solution**: optimize experimental conditions to ensure each peak = single compound.

8.2. Quantitative Analysis

Once the solute(s) of interest have been identified, quantitative analysis is facilitated by the relationship: $\mathbf{m_i = K_i A_i}$

Where:

- **m_i:** mass of the injected solute **i**
- **A_i:** area of the peak representing this solute.
- **K_i :** proportionality constant.

I.8.2.1. Measurement of Peak Area A_i

The area is primarily measured using manual triangulation and automatic integration.

- **Triangulation:** The peak is approximated as a triangle, either by drawing tangents at the inflection points of the curve and calculating the area:

$$\boldsymbol{A_i = \frac{1}{2} H' \times \omega}$$

Or by measuring the width at half-height and calculating the area:

$$\boldsymbol{A_i = H \times \delta}$$

Or by measuring the widths at one-quarter β and three-quarters γ of the height:

$$\boldsymbol{A_i = \frac{1}{2} H \times (\beta + \gamma)}$$

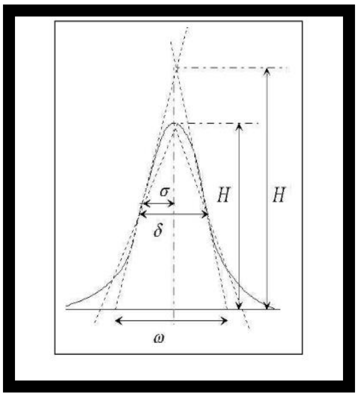


Fig 8. Triangulation

I.8.2.2. Determination of the Proportionality Constant

- In practice, it is difficult to measure **K** directly (because injection volumes are not exact).
- Therefore, **calibration methods** are used → quantitative chromatography becomes **relative** (compared to known substances).

Main methods:

1. **Internal Normalization**

- Assumes all **K_i are equal** (e.g., homologous series: alkanes, alcohols).
- Mass % of each solute:

$$\boldsymbol{m_i\% = \frac{A_i}{\sum_i A_i} \times 100}$$

2. **Internal Calibration (Internal Standard Method)**

- Add a known amount of a **reference compound (standard E)** to the sample.
- Requirement: the standard peak must not overlap with other peaks.
- Relations:

$$\boldsymbol{m_e = K_e \times A_e} \qquad ; \qquad \boldsymbol{\frac{m_i}{m_e} = \frac{k_i}{K_e} \times \frac{A_i}{A_e}}$$

We define:

$$\boldsymbol{K_{i/e} = \frac{K_i}{K_e}}$$

Thus, we will calculate the response of each solute relative to the standard. This method is general, precise, and reproducible.

$$\boldsymbol{K = \frac{m_e}{A_e}}$$

- External Calibration: This technique is only valid with excellent injection quality.

One injects a mass **m_e** of standard compound in solution (volume **V_e** injected).

$$\boldsymbol{m_e = K \times A_e}$$

Then, the same volume of solution containing the compound **m_i** is injected, and **m_i** is measured.

$$\boldsymbol{m_i = K \times A_i} \qquad ; \qquad \boldsymbol{m_i = \frac{m_e}{A_e} \times A_i}$$

II. Gas Phase Chromatography (GPC)

II.1. Introduction

Definition: Separation technique for volatile and thermally stable molecules (< 500 Da).

Inventors: A.J.P. Martin & R.L.M. Synge (Nobel Prize, 1952).

Applications: perfumery, wine analysis, petroleum industry, biology, fine chemistry, plastics.

Key point: GC is one of the most efficient separation techniques.

II.2. Principle

- Sample injected by syringe → vaporized in the injector.
- **Carrier gas** (He, N_2, sometimes H_2) transports analytes through the column.
- Compounds separate based on their **interactions with the stationary phase**.
- At the outlet, compounds are detected → often coupled with **Mass Spectrometry (GC–MS)** for identification

II.3. Apparatus

- **Injector** (sample vaporization).
- **Carrier gas** supply.
- **Column** (stationary phase where separation occurs).
- **Detector** (records peaks).
- **Data system** (chromatogram display).

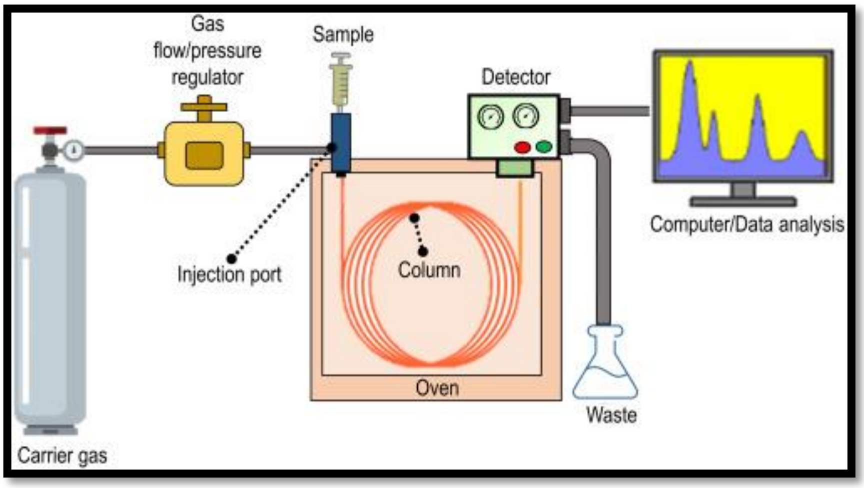


Fig 9. Simplified diagram of a gas chromatograph

II.3.1. Carrier Gas

- Role**:** Mobile phase → transports analytes from **injector → column → detector**.
- Types**:** Inert or unreactive gases (He, N_2, Ar, H_2).
- Choice**:** Depends on the **detector** used.
- Flow **rates:**
  - **Packed columns:** 30–40 mL/min
  - **Capillary columns:** 0.2–2 mL/min
- Effect **on resolution:**
  - Flow rate strongly affects separation quality.
  - Two opposing effects:
    - **Longitudinal diffusion** (better at high flow).
    - **Mass transfer resistance** (better at low flow).
- Optimization**:** Operator must choose the **best flow rate** for maximum resolution.

Fig 10. Carrier Gas supply in GC instrumentation

II.3.2. Injection System

The injection system introduces the sample (liquid, solid, or gas) into the chromatograph, where it is vaporized and carried by the mobile phase (carrier gas).

II.3.2.1. Direct Injection *(Septum Injector)*

- Most common system.
- **Structure**: Metal tube with glass insert, heated 20–30 °C above the least volatile component's boiling point.
- **Process**: Entire sample (1–10 µL) vaporized and transferred to the column.
- **Access**: Injection through silicone septum using micro-syringe (needle ~0.15 mm).

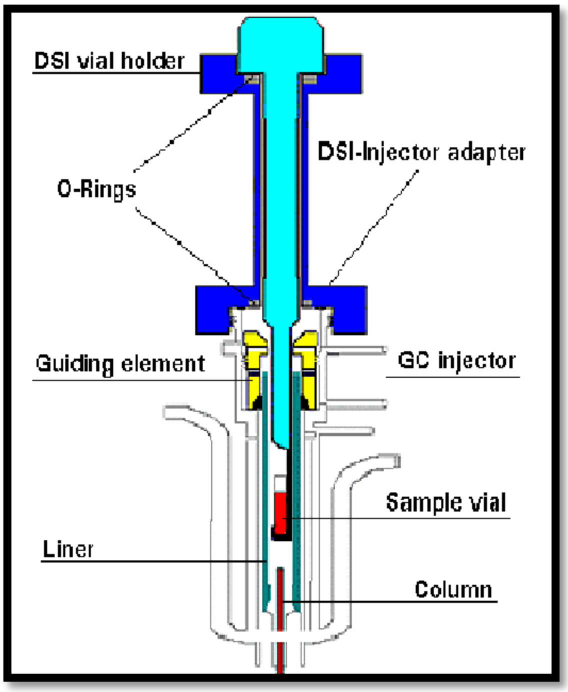


Fig 11. Direct Injection

II.3.2.2. Split/Splitless Injection

Split Mode:

- Carrier gas at high flow → sample stream split.
- Only a fraction enters the column (e.g., split ratio 1:100 → 1% enters).
- Suitable for capillary columns (low flow).

Splitless Mode:

- Entire sample vaporized and stays briefly in the liner.
- ~95% enters the column, ~5% vented.

- Used for very dilute or dirty samples (non-volatile residues).

Example: The flow rate of the capillary column is 1 mL/min. The split ratio is **100**. One-hundredth of the injected amount will pass through the capillary column.

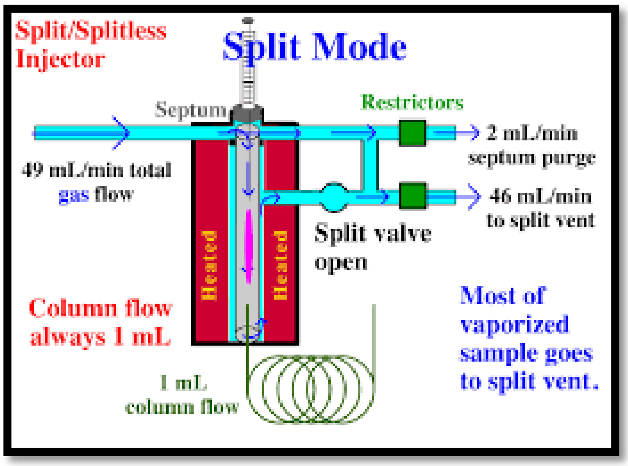


Fig 12. Split/Splitless Injection

II.3.2.3. On-Column Injection

- Sample deposited **directly inside the column** using a fine syringe.
- Now mostly automated → ensures reproducibility.
- **Post-analysis precaution:** Keep carrier gas flowing until injector cools below 100 °C (to protect column).

II.4. The Column (Stationary Phase)

There are two types of columns: **packed columns** and **capillary columns**.

II.4.1. Packed Columns

Packed columns are a few millimeters in diameter and typically about a meter in length. They are filled with inert support granules, usually silica, whose surface is impregnated or bonded with the stationary phase. Packed columns have been largely replaced by capillary columns, which offer significantly higher resolution power.

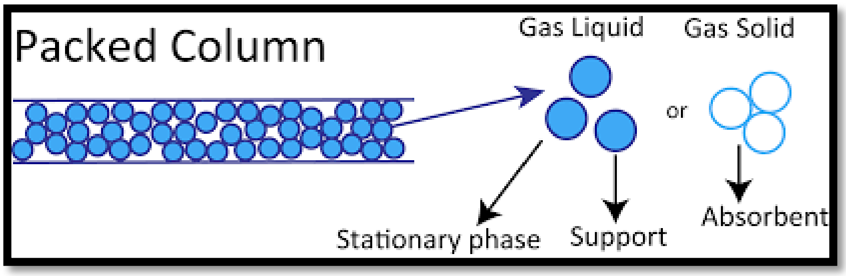


Fig 13. Packed Columns

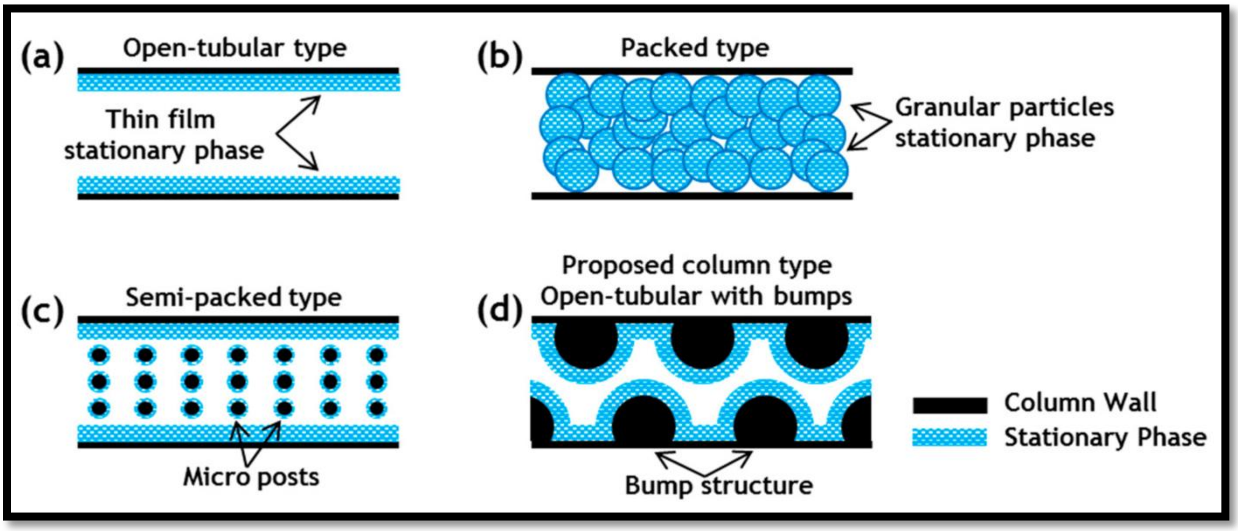


Fig 14. Packed Columns types

II.4.2. Capillary Columns

Capillary columns are simple tubes made of stainless steel, glass, or fused silica (inert materials regarding the stationary phase and samples). The inner diameter ranges from **0.1** to **0.5 mm**, and typical lengths can range from several tens of meters, up to **100** m.

Capillary columns are classified into three types: the wall-coated open tubular (WCOT) type, the support-coated open tubular (SCOT) type, and the porous-layer open tubular (PLOT) type (Figure 15).

To fit in the instrument, the column is coiled into spirals with diameters ranging from **10** to **30 cm**. The inner surface of the tube is coated with a stationary phase film, typically 0.1 to 5 μm thick. This film is applied through grafting or simple deposition, with grafting usually preferred for thermal stability. For example, Carbowax® is a capillary column with a polar polyethylene glycol film grafted onto the surface as the stationary phase. SE-30® is a non-polar capillary column with a film of polydimethylsiloxane as the stationary phase.

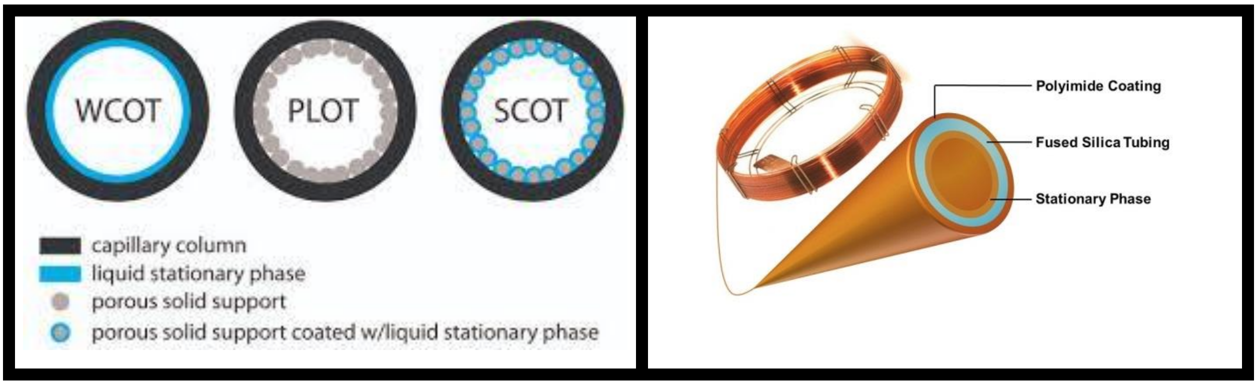


Fig 15. Different types of capillary columns

II.5. Oven

The main oven is a compartment placed between the injection chamber and the detector, containing the chromatographic column. It is equipped with heating elements that precisely control the oven temperature. Temperature control also allows for programmed temperature runs, which are useful for separating mixtures with very different retention capacities on the stationary phase.

Fig 16. Oven in GC

II.6. Detector

The detection system measures the signal emitted by different molecules and enables their identification. PC-based software now replaces traditional analog paper recorders for signal recording.

Several types of detectors exist, with two commonly used ones:

II.6.1. Thermal Conductivity Detector (TCD)

This was the most widely used detector in the early days of gas chromatography. Its principle is based on the fact that the resistance of a metal subjected to a constant current varies when exposed to a temperature gradient.

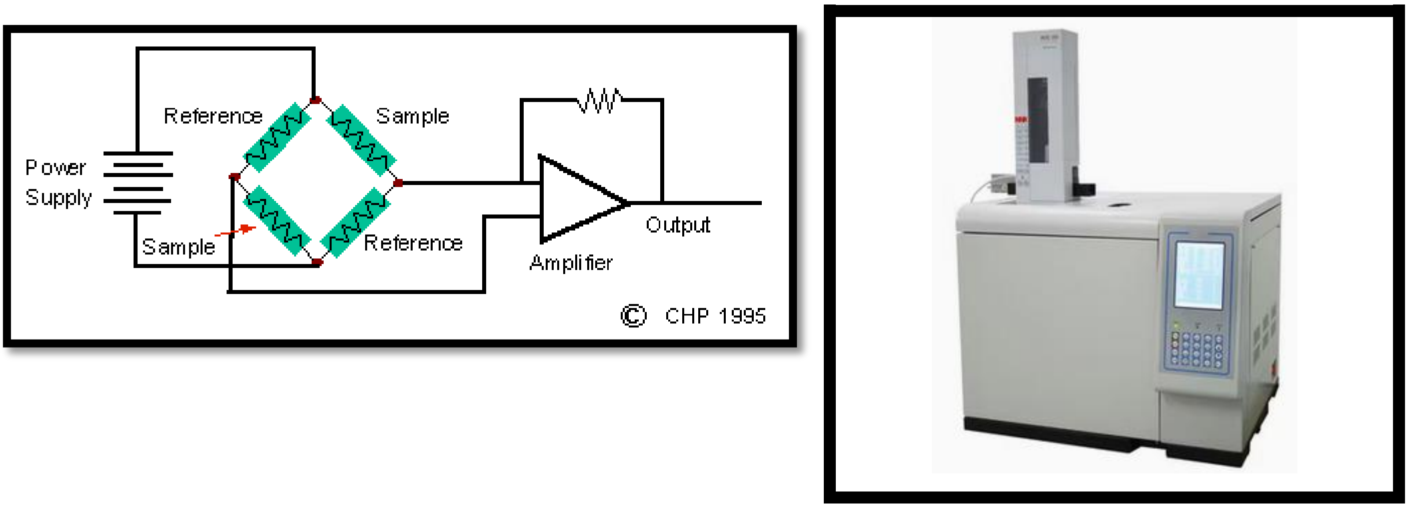


Fig 17. Detector used in GC

II.6.2. Flame Ionization Detector (FID)

Flame Ionization Detector (FID) is the most widely used detector in gas chromatography. The gas stream from the column enters a small burner, where the flame is fueled by a mixture of hydrogen and air. Combustion of the eluted organic compounds produces ions, which are collected using two electrodes. The resulting very weak current is converted into a voltage and recorded.

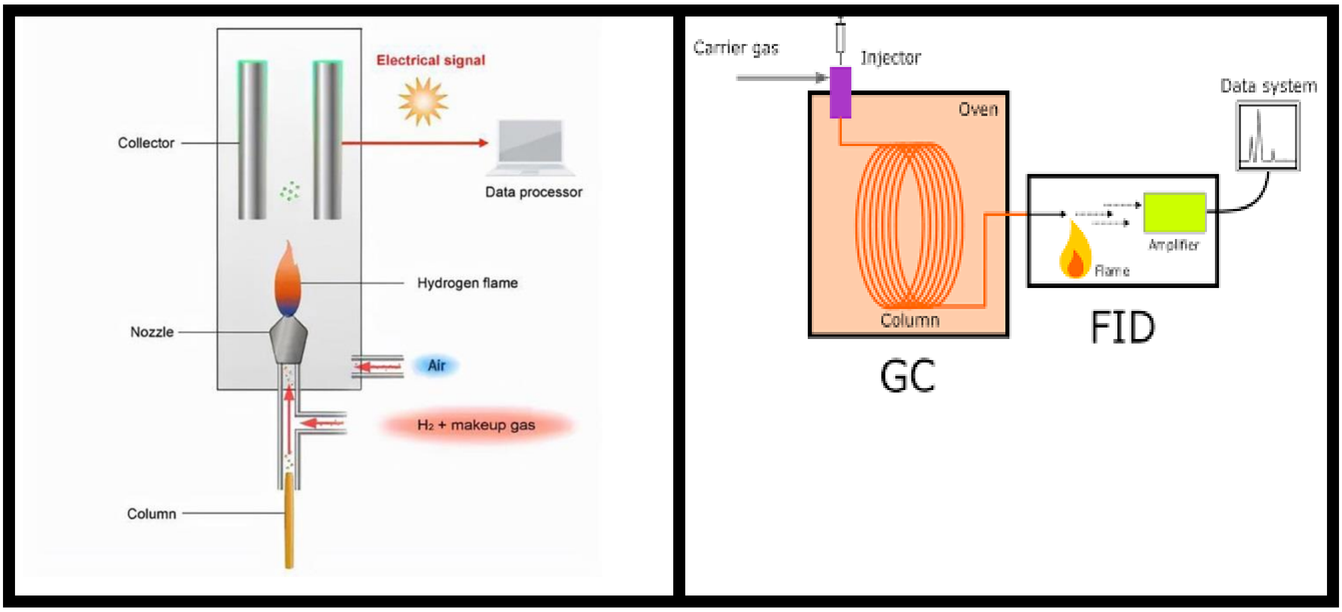


Fig 18. Flame Ionization Detector (FID)

II.6.3. Mass Spectrometry (MS)

The mass spectrometer is one of the most powerful detectors for gas chromatography. The combination of gas chromatography and mass spectrometry is referred to as **GC-MS** (Gas Chromatography-Mass Spectrometry). A mass spectrometer measures the mass-to-charge (m/z) ratio of ions produced from the sample via an ionization source. This source provides sufficient energy to break chemical bonds in the sample molecules, generating many fragments, which are helpful for identifying the molecular species entering the mass spectrometer based on the fragmentation spectrum or by comparing it with a spectral library.

Fig 19. Mass Spectrometry (MS) detector

II.6.4. Other Detectors

Other types of detectors include the nitrogen-phosphorus detector (NPD), which is specific for nitrogen and phosphorus compounds; the electron capture detector (ECD), highly sensitive to halogenated compounds; and the flame photometric detector, specific for sulfur and phosphorus-containing compounds. Typically, the detector temperature is set to be close to the injector temperature.

II.7. Factors Affecting Separation

II.7.1. Temperature

- **Low temperature** → slow exchange, long retention times, distorted peaks.
- **High temperature** → no equilibrium (component between the mobile and stationary phases does not have time to establish), poor separation (components appear simultaneously), simultaneous elution.
- **Best controlled** with programmed oven temperature (with electronic programmer).

II.7.2. Carrier Gas Flow Rate

The flow rate must:

- **Allow equilibrium between the mobile and stationary phases.**
- **If too fast → poor separation.**
- **If too slow → broad peaks due to diffusion.**
- **At the optimal flow rate, increasing the oven temperature improves peak sharpness.**

II.7.3. Column Length

- Longer column → better separation but broader peaks.
- Too long → requires high carrier gas pressure.

II.7.4. Nature of the Stationary Phase

- Types: non-polar (squalene, polyalkylsiloxane), polar (polyether, polyester).
- Must be inert and stable in its temperature range (too low → viscous, too high → evaporates).
- **Polarity rule**: like attracts like.

- Polar phase → retains polar compounds (elution by ↑ boiling point).
- Non-polar phase → retains non-polar compounds (elution by ↑ boiling point).

II.8. Practical Application

Operating Procedure for Performing a **GC** Analysis:

- Ensure that one (or more) column(s) is/are already installed in the chromatograph.
- Program the oven. If the experimental conditions are already known, use them. Otherwise:
  - A gradient method is often sufficient. Sweep a wide temperature range, for example from **50** to **250 °C** over **10** minutes.
  - If an isothermal method is desired, start with the same gradient, then tighten the extreme values through iteration to achieve the peak corresponding to the analyte of interest at the appropriate retention time.
- Prepare a sample solution at a concentration of approximately 1 mg/mL, in a volatile solvent (e.g., diethyl ether, cyclohexane, ethyl acetate).

- Select the column. By default, a non-polar column is sufficient. Polar columns are generally reserved for cases where the volatilities of the analytes to be separated are very close.
- Inject approximately 1 µL into the injector. By default, the injector temperature can be set to the highest temperature reached in the oven during the analysis.
- To clean the column, it can be left for a few minutes at the maximum operating temperature. This allows removal of the less volatile analytes that have not been eluted from the column at the end of the analysis and may otherwise elute during subsequent injections.

Example: GC Analysis of Geranium Oil. The chromatogram shows that geranium oil contains at least **16 compounds**. The conditions of the analysis are as follows:

- **Column**: **SUPELCOWAX 10**, **30m x 0.25mm ID**, **0.25µm film**
- **Oven**: **50°C** (**2 min**) to **280°C** at **2°C/min**, then **20 min**
- **Mobile Phase**: **Helium**, **25 cm/sec**
- **Detector**: **FID**
- **Injector**: **0.2µL**, split (**100:1**).

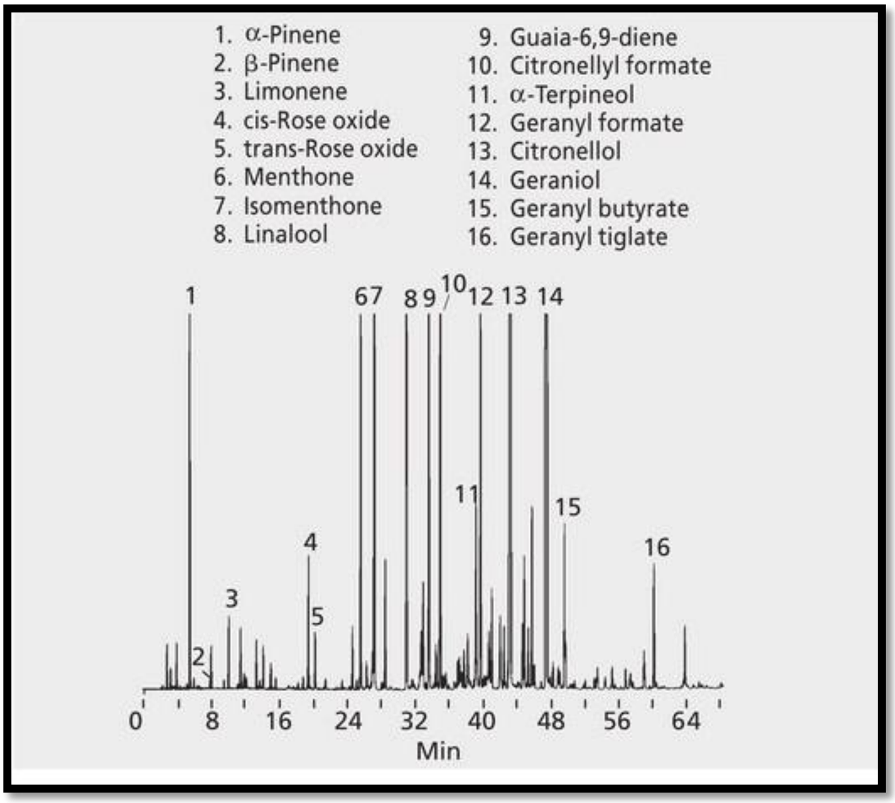


Fig 20. Spectrum **GC of Geranium Oil analysis**

II.9. Applications of Gas Chromatography

The use of this technique has become widespread across many industries, resulting in various analytical applications:

- **Pharmaceutical Industry Analytical Control:**
    - Raw material control / impurities, residual solvents,
    - In-process control of molecular integrity,
    - Finished product control / content,
    - Stability studies over time / degradation products.
- **Biological Medium Dosage (Extraction and Purification):**
    - Bioavailability studies,
    - Metabolic studies (identification/SM),
    - Therapeutic monitoring during treatment,
    - Biochemical dosage.
- **Toxicology Dosage:**
    - Search for toxins in acute poisoning (methanol, ethylene glycol...),
    - Drug dosage: amphetamines, opiates, cannabinoids,
    - Anti-doping control,
    - Substance abuse screening.
- **Agri-food Industry:**
    - Pesticide detection and dosage,
    - Detection and dosage of nitrosamines (nitrites).
- **Chemical Industry, Cosmetics:**
    - Analysis of essential oils, perfumes, flavors, hydrocarbons.

Gas chromatography is mainly used for analyzing thermally stable and sufficiently volatile liquids (boiling point **< 250 °C**) and for separating small molecules in amounts on the order of a few pictograms.

III. Column Liquid Chromatography

III. 1. General Overview

Column chromatography is a preparative method that allows for the separation of components of a mixture and their isolation, from samples that can weigh several grams (**50 mg** to about **20 g** in the laboratory, and up to **1 kg** in industry). However, it has several disadvantages:

- Large quantities of solvent are required for elution.
- The elution time is generally very long.
- Detection of compounds requires constant attention.

It is suitable for purifying small quantities of products when operating conditions are optimized. However, since the method is very empirical, its development often requires numerous trials.

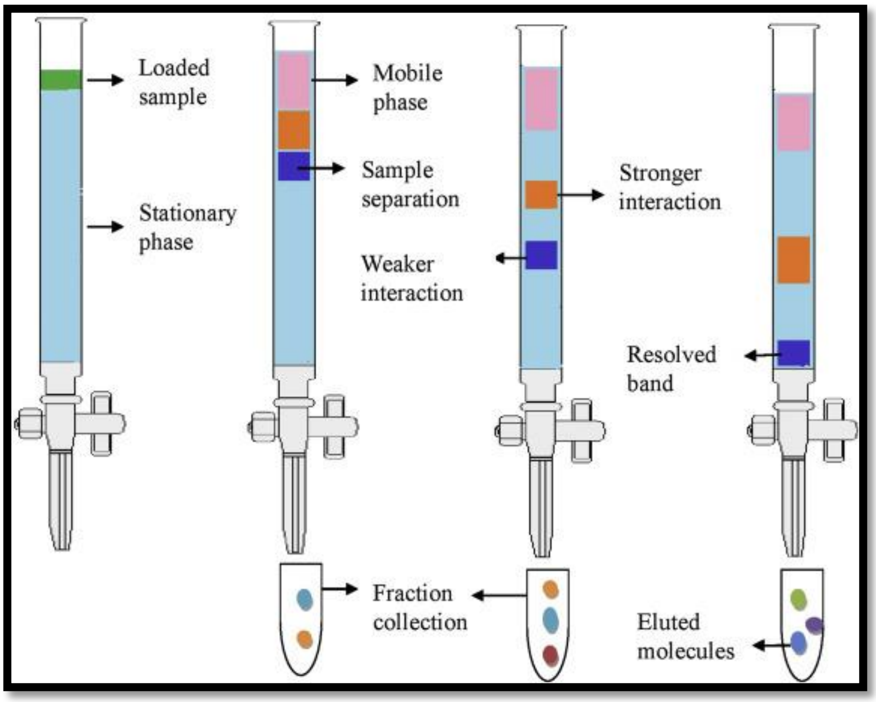


Fig 21. Column Liquid Chromatography Description

III.2. Column Liquid Chromatography

This is a liquid-solid chromatography. The stationary phase is a polar solid adsorbent. Reverse-phase adsorption chromatography: This is a liquid-solid chromatography where the stationary phase is non-polar.

Adsorption is a physicochemical phenomenon that involves the attachment of a substance in a liquid (or gas) state to a solid surface. This phenomenon involves complex forces between the solute and the adsorbent: electrostatic forces, inductive forces, London dispersion forces, hydrogen bonding, charge transfer forces, and others. In chromatography, adsorption must be reversible. The adsorbed substance is desorbed in solution by breaking the previous bonds.

III. 2.1. Description and Principle

It is a technique based on adsorption phenomena. The solid phase, often alumina or silica, fills a column of varying length and section. The concentrated sample in solution is deposited at the top of the column, and the separation of components results from the continuous flow of an eluent passing through the column by gravity or under slight pressure. One can use a single solvent as the eluent or progressively increase the polarity of the eluent to speed up the movement of the components.

The molecules are carried downward at varying speeds depending on their affinity for the adsorbent and their solubility in the eluent.

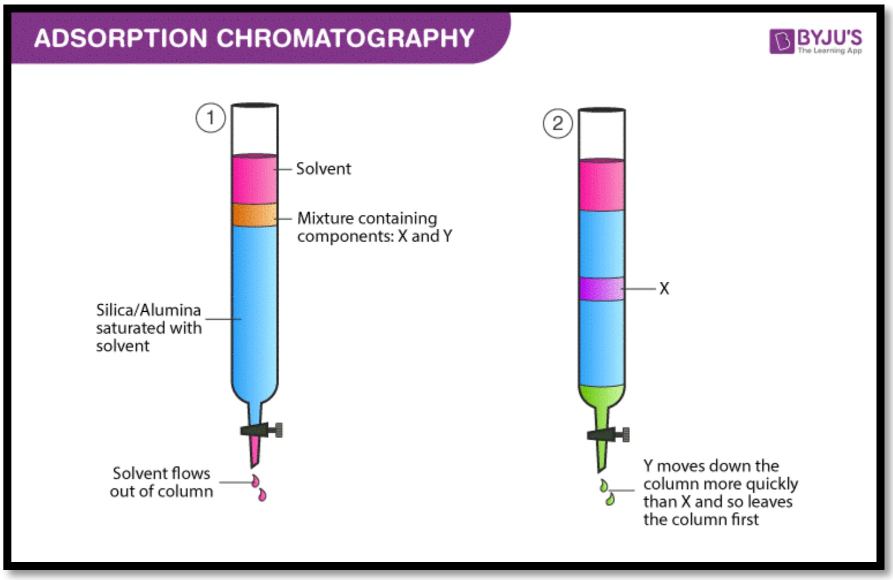


Fig 22. Principle of chromatography

III.2.2. Factors Affecting Separation Four factors come into play: the adsorbent, the eluent, the column size, and the elution speed.

III.2.2.1. Adsorbent

The most commonly used adsorbent is alumina (**Al_2O_3**), but it is limited to stable organic compounds because, in its basic form, it can cause the dehydration of esters, for example. Silica gel (**SiO_2**) is available as a white powder, used particularly for the separation of compounds that are not stable enough to be treated with alumina.

The particle size of the adsorbent should be larger than those used in Thin Layer Chromatography (**TLC**). The typical size range is between **50** and **200 µm**. The amount of adsorbent depends on the difficulty of separation and the sample mass. It can be considered that for each gram of sample, **30** to **50 g** of adsorbent is required if the polarity of the components to be separated is very different, and up to 200 g if the separation is difficult.

The adsorbents listed below are ranked in ascending order of their interaction strength with polar compounds:

- Cellulose, paper
- Kieselguhr, diatomaceous earth
- Starch
- Sugar
- Talc
- Sodium carbonate
- Magnesium oxide
- Silica gel
- Alumina
- Activated charcoal

In general, the more active the adsorbent, the more strongly it retains polar compounds.

III.2.2.2. Eluent

The eluent is usually a mixture of two solvents. At the beginning of the elution, the least polar solvent is used, which carries the least retained substances (the least polar) while targeting an **Rf ~ 0.3** for the least polar product. The composition of the eluent is gradually adjusted by adding the more polar solvent, allowing the more polar compounds retained on the adsorbent to migrate gradually downward. The polarity of the eluent may remain constant throughout the chromatography (isocratic elution).

The following liquids are ranked by increasing polarity (allotropic series):

- Petroleum ether
- Cyclohexane
- Tetra chloromethane
- Trichloroethylene
- Toluene

- Benzene
- Dichloromethane
- Diethyl ether
- Trichloromethane
- Ethyl acetate
- Pyridine
- Acetone
- Propan-1-ol
- Ethanol
- Methanol
- Water
- Acetic acid.

III.2.2.3. Column Size

The amount of adsorbent is such that it occupies a height approximately **10 times** the column diameter. An additional 10 cm space is usually provided above the adsorbent to place the solvent.

III.2.2.4. Elution Speed

It must be as constant as possible. It should be slow enough to allow the solute to be in equilibrium between the liquid and adsorbed phases. It should not be too slow, as substances will diffuse into the solvent, resulting in widening bands and poor separation.

III.2.3. Column Filling

This is the most delicate operation, as the filling must be as homogeneous as possible and free of air bubbles. The lower and upper surfaces of the adsorbent should be perfectly horizontal. Since the column is vertical, filling can be done by one of two methods:

- **Wet Filling**: Prepare a homogeneous mixture of the adsorbent and the least polar solvent used for development in a beaker by gradually adding the adsorbent to the solvent to create slurry that flows easily. Using a funnel, pour enough slurry so that the adsorbent gradually forms a layer about 2 cm thick. Tap the column walls to aid packing the adsorbent. Then open the valve so that the solvent flows slowly and continue adding the slurry in successive portions. Once all the adsorbent is added, let it settle until the liquid floating on top is clear. During the operation, the solvent level should always be above the adsorbent.

- **Dry Filling**: Fill the column two-thirds with the least polar solvent, and then add the adsorbent in successive portions using a funnel. Continuously tap the walls during addition to achieve maximum packing. Once the first portion forms a layer of about 2 cm, open the valve to allow the solvent to flow slowly. Finish as described earlier.

III.2.4. Depositing the Analyte

The Analyte is deposited with the valve closed, using a Pasteur pipette, as homogeneously as possible on the sides of the column, as low as possible. It should form a narrow cylindrical zone at the top of the column.

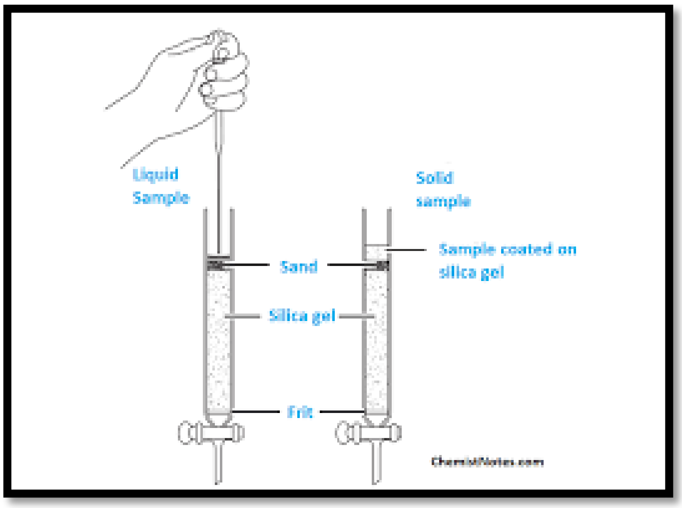


Fig 23. Deposit of the Analyte

III.2.5. Choosing the Solvent

If possible, use the smallest possible volume of the starting eluent. The deposit must then be fully absorbed before adding the elution solvent.

Two options are available if the raw product is solid:

- Dissolve it in the eluent and add as described earlier.
- Perform an impregnation on silica: mix the product in solution with **~5 times** its weight of silica. Evaporate to dryness and add this solid on top of the column before placing the sand. This technique is often used if the raw product is insoluble in the chosen eluent.

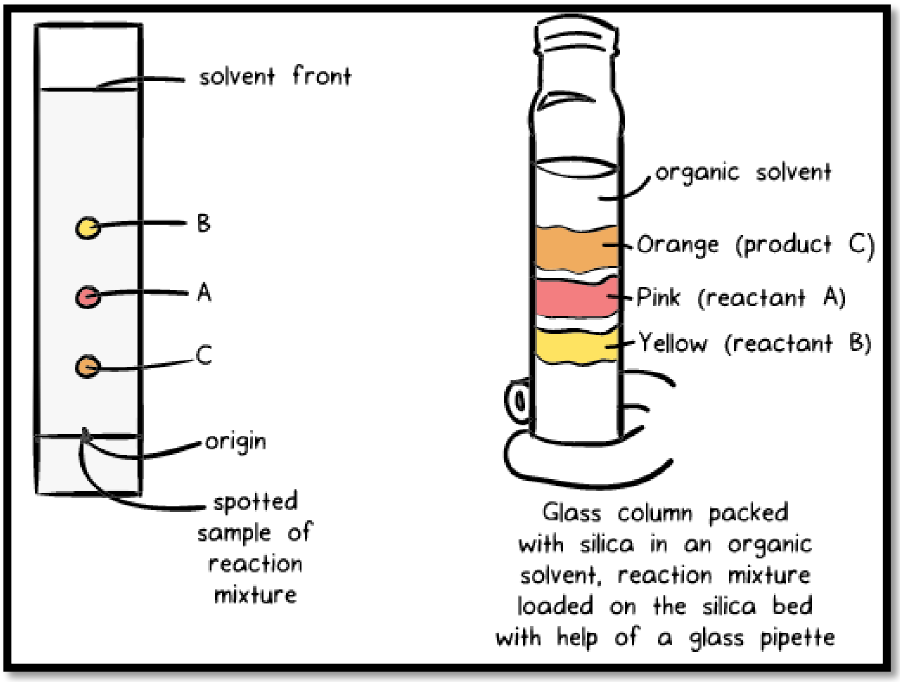


Fig 24. Choosing the Solvent

III.2.6. Elution

Elution must be continuous. The column can be fed continuously with a dropping funnel or the eluent can be added manually. Ensure that the surface of the adsorbent is never exposed to air, as a column left dry will deteriorate, leading to failure in separation due to cracks.

If a gradient eluent is used, allow the first solvent to flow before adding the second. For most operations, a flow rate of 5 to 50 drops per minute is appropriate (the lower limit corresponds to difficult separations). The volume of each fraction collected varies from 1 to 50 mL depending on the case. The fractions are collected and analyzed by TLC. A micro-drop from each fraction is placed on a **TLC** plate. Once the fraction analysis is completed, combine those that correspond to the same product, carefully discarding those that overlap.

The substances obtained are generally of very high purity. Automated systems are now available where the column is pre-packed, the gradient is programmed, and the device collects fractions. The interface directly gives the fractions containing a chromophore. Only TLC and evaporation are needed for further analysis.

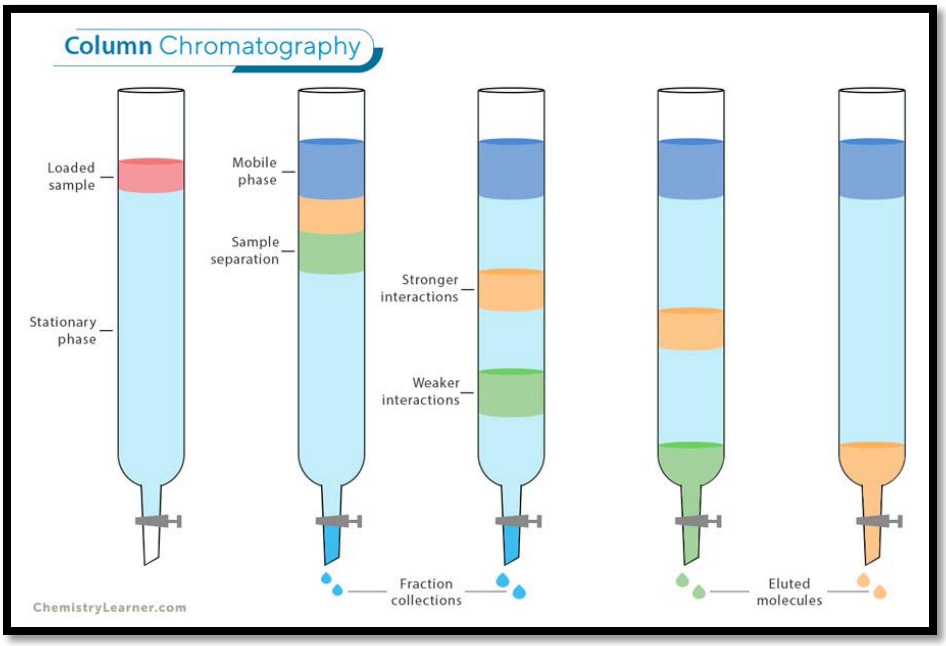


Fig 25. Elution description

III.2.7. Applications

The classic column chromatography is widely used for purifying organic synthesis products and also for separating components from plant extracts (phytochemistry).

However, it has several disadvantages:

- It requires large quantities of eluent.
- The elution time is generally very long (at least several hours).
- Detection of compounds requires constant attention.

Modern column chromatography has solved these problems, utilizing more sophisticated equipment.

IV. High-Performance Liquid Chromatography (HPLC)

IV.1. Introduction

- Separation technique for **quantitative & qualitative analysis**.
- Applications: analytical chemistry, toxicology, biochemistry.
- Advantages: analyzes **thermosensitive, large, or polar molecules** (unlike GC).
- Selectivity controlled by column + eluent composition.
- Efficiency lower than GC, but **chiral phases, ion pairing, hydrophobic interactions** extend possibilities.
- Separation mechanisms: partition, adsorption, ion exchange, exclusion.
- Operates under **high pressure (~200 bar)** for rapid elution.

IV.2. **Principle**

- Solutes dissolved in solvent → injected into **mobile phase**.
- Pump pushes eluent through column.
- Solutes interact differently with **stationary phase** → separation.
- Compounds exit column at different times → detected as **chromatographic peaks**.

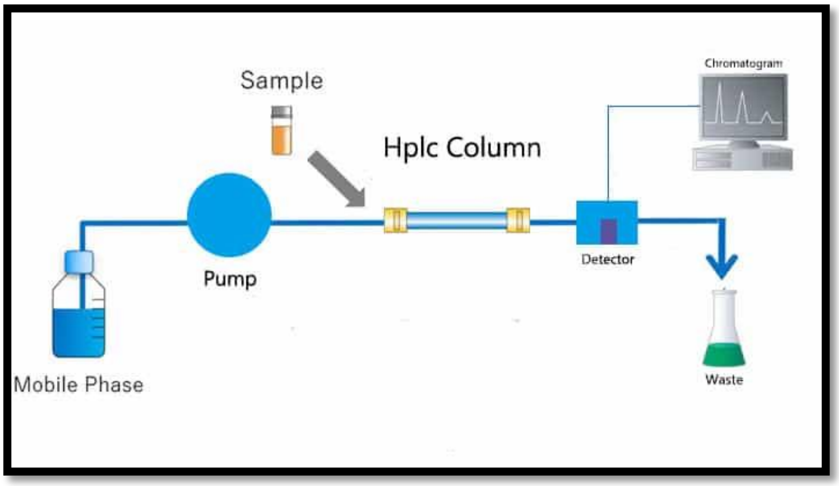


Fig 26. Principle of HPLC

IV.3. Equipment

All HPLC systems consist of several modules connected by narrow diameter tubes. One or more pumps control the flow rate of the eluting solvent. Downstream from the injector are the column(s) where separation occurs, followed by the detector. Below is a detailed overview of the essential components of an HPLC system.

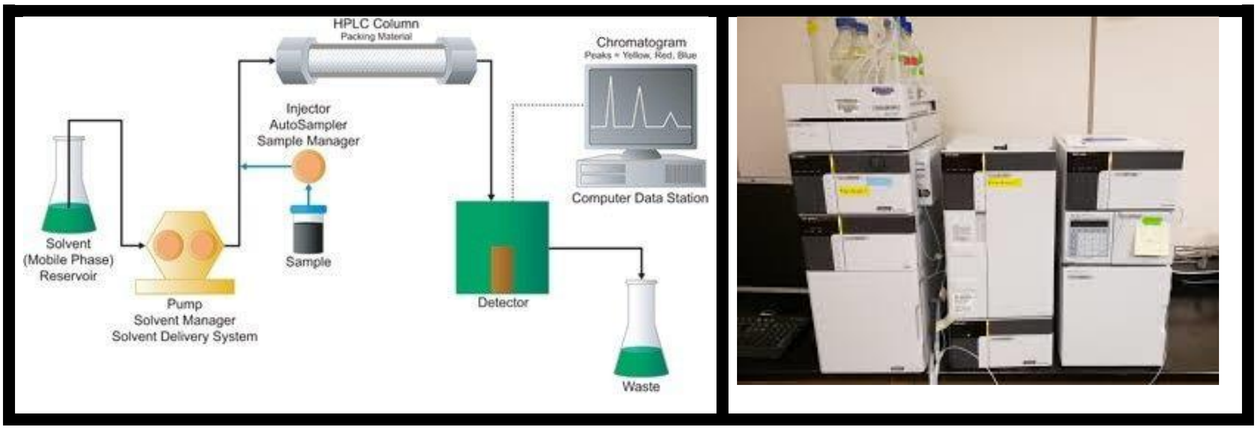


Fig 27. Equipment of HPLC

IV.3.1. Solvent Reservoir (eluent)

HPLC systems are equipped with one or more reservoirs containing a sufficient amount of the mobile phase. Different solvent reservoirs (with varying polarities) are available for gradient elution (mixing solvents at varying concentrations) using a dosing pump.

IV.3.2. Degasser

As the name suggests, this component removes gases (such as oxygen) dissolved in the solvent to prevent damage to samples or the stationary phase. There are two types of degassers:

- **Inert gas degasser**: A gas (usually helium) is bubbled through the mobile phase to remove dissolved gases.
- **Vacuum degasser**: A vacuum pump is used to reduce pressure inside a chamber containing the solvent, effectively separating dissolved gases, and is more effective than the inert gas method.

IV.3.3. Pump

The pump is responsible for storing the eluent and injecting it under pressure into the column. Pumps are the most delicate part of the apparatus and must meet strict criteria:

- Pressures up to 420 bars,
- Flow rates ranging from 0.1 to 10 mL/min,
- Resistance to corrosion from any solvent used.

The most common pumps have two pistons to regulate flow, and a gradient system allows the solvent composition to be varied during the analysis. They can operate in two modes:

- **Isocratic mode**: Using 100% of the same solvent throughout the analysis.
- **Gradient mode**: Varying the concentration of solvents in the mixture.

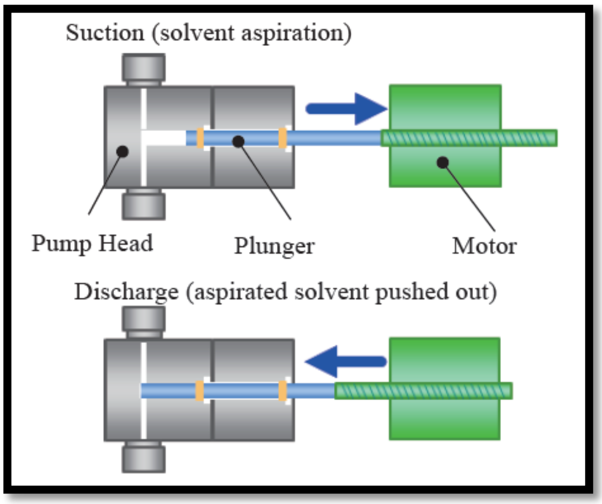


Fig 28. Pump of HPLC

IV.3.4. Injector

The most common injector in HPLC is the loop injection valve. The sample is introduced into a small loop using a syringe, then carried by the mobile phase into the column after rotating the valve. Injection must be fast to avoid flow disturbances. Typical sample volumes range from 5–500 µL, under pressures exceeding 10^4 kPa.

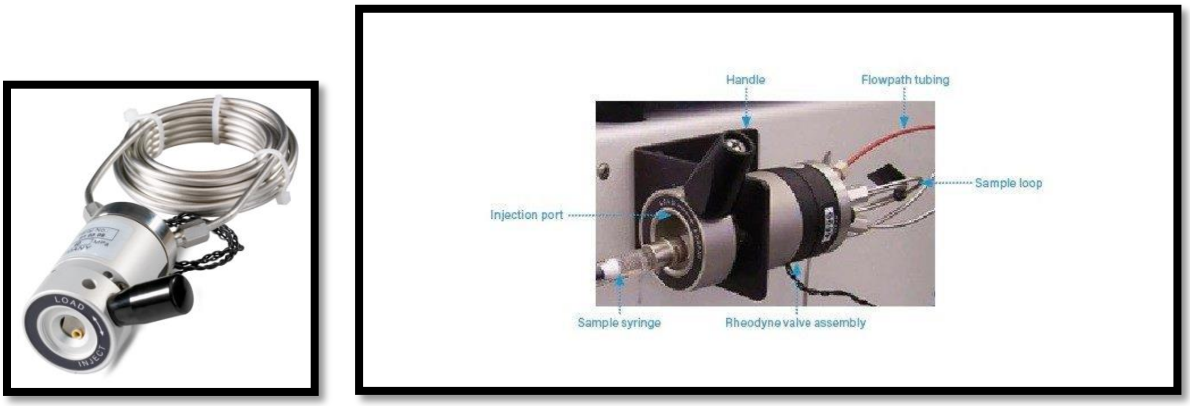


Fig 29. Injector of HPLC

IV.3.5. Column

The column is a tube constructed from a chemically inert material, typically stainless steel or glass. The column diameter ranges from 4 to 20 mm, and the length is typically between **15** and **30 cm**. The stationary phase is maintained between two porous discs at each end of the column. The mobile phase flow rate generally cannot exceed a few **mL/min**.

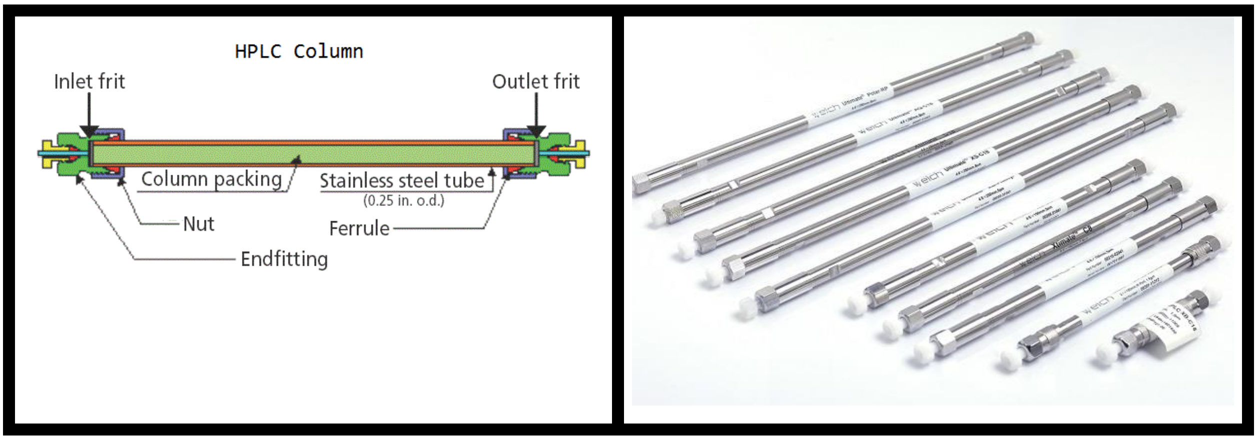


Fig 30. Column of HPLC

IV.3.6. Detectors

The detector continuously monitors the appearance of solutes, using various physical or chemical phenomena. The signal is recorded as a function of time, and it is often compared to the signal from the mobile phase alone. Common detectors include:

- Differential refractometer,
- Diode-array UV detector,
- Electrochemical,
- Fluor metric, among others.

Various coupling techniques are also used, such as:

- Infrared spectroscopy,
- Mass spectrometry,
- Nuclear magnetic resonance (NMR).

IV.4. Molecular Interactions between Mobile Phase and Solute

- Dielectric interactions,
- Hydrogen bonds,
- Van der Waals forces.

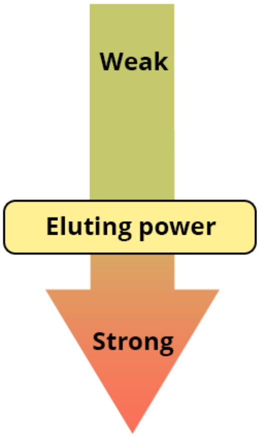
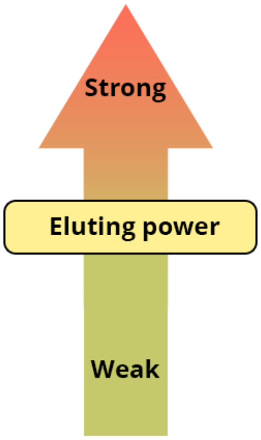
IV.4. 1. Elution strength and polarity

For polar compounds:

- The more polar the mobile phase is, the more it will carry the solutes.
- The less polar the mobile phase is, the less it will carry the solutes.

For less polar compound:

- The more polar the mobile phase is, the less it will carry the solutes, and vice versa. By mixing several solvents, the elution power of the mobile phase can be adjusted.

Polar stationary phase	Solvents ranked by ascending polarity	Non polar stationary phase
	Hexane Toluene Trichloroethane Dichloroethane Ether Ethyl acetate Acetonitrile Methanol Water	

IV.4.2. Snyder's Polarity

The distribution coefficient between the studied solvent and different stationary phases is measured.

Example: **<u>ethanol</u>**: proton donor, **<u>dioxane</u>**: proton acceptor, **<u>nitromethane</u>**: high dipole moment.

The overall polarity of a mixture of **3 solvents** will be **P' = log K_1 + log K_2 + log K_3**. By normalizing **$X_1 + X_2 + X_3 = 1$**,

Then

$$X_1 = \log\frac{K_1}{P'}$$

k': solute capacity factor.
P': overall polarity.
X: distribution coefficient.

IV.5. Mechanisms of Interaction with the Column

Chromatography can be classified into four types depending on the nature of the stationary phase:

- Adsorption chromatography,
- Partition chromatography,
- Size exclusion chromatography,
- Ion-exchange chromatography.

IV.5.1. Adsorption Chromatography

Adsorption Chromatography is the oldest method (Tswett, 1906), mainly used for organic compounds with molecular masses below 3000. Separation occurs by reversible adsorption of molecules on an adsorbent surface.

IV.5.1.1. Adsorbents: Must be insoluble, chemically inert, and have a large specific surface area for efficient separation. Common adsorbents are **silica gel** (polar, acidic, forms H-bonds; suitable for bases) and **alumina** (basic, suitable for acids). Other mineral salts and organic adsorbents may also be used. Polar molecules are generally retained more strongly.

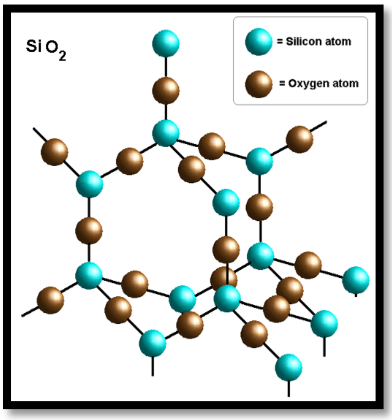


Fig 31. Silica (SiO_2) structure 3D

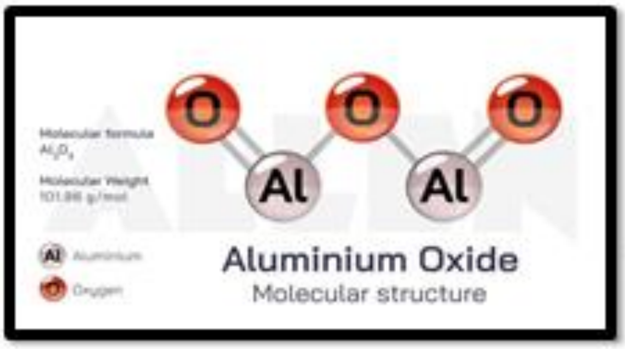


Fig 32. Alumina (Al_2O_3) structure 3D

IV.5.1.2. Mobile Phase: Solutes must be soluble in the eluent. Solvent mixtures are often used to adjust polarity. Less polar compounds move faster with the eluent, while more polar compounds are retained longer on the adsorbent.

IV.5.2. Partition Chromatography

- **Principle**: Partition chromatography is the most commonly used liquid chromatography technique. It operates by partitioning solutes between two immiscible phases: one stationary and one mobile.
- **Type**:
    - **Normal-phase partition chromatography**: The stationary phase is polar (e.g., amine or nitrile groups on silica), and the mobile phase is non-polar (e.g., hexane or isopropyl ether).
    - **Reversed-phase partition chromatography**: The stationary phase is non-polar (e.g., alkyl chains attached to silica), and the mobile phase is polar (e.g., water or methanol).

IV.5.3. Size Exclusion Chromatography

- **Principle**: Size exclusion chromatography (gel filtration/permeation) separates solutes based on their size. Larger molecules do not enter the pores of the stationary phase and elute first, while smaller molecules can enter the pores and elute later.

IV.5.4. Ion-Exchange Chromatography

- **Principle**: This technique isolates charged substances by using a stationary phase with ionized groups that exchange ions with the solute. Cation-exchange resins have negatively charged groups that attract positively charged ions, while anion-exchange resins attract negatively charged ions.

IV.6. Comparison between GC and HPLC

- **Advantages of HPLC over GC**:
    - The sample can be thermally sensitive, and HPLC allows the analysis of non-volatile compounds.
    - The choice of stationary and mobile phases is broader.

- **Disadvantages of HPLC compared to GC**
    - Diffusion coefficients in the mobile phase are higher.
    - Solute exchange with the mobile phase is slower compared to gas chromatography, leading to longer analysis times.
    - High pressure in the column in **HPLC** (viscosity of liquids is about **100** times greater than gases).

V. Thin Layer Chromatography (TLC)

V.1. Introduction

This technique is primarily based on adsorption phenomena and the principle of capillarity. When the plate on which the sample is deposited is placed in the chromatographic tank, the eluent moves up through the stationary phase (**PhS**).

However, depending on its density, solubility in the mobile phase, and the electrostatic forces holding the component on the stationary phase, each component of the sample moves at its own rate in the absorbent.

Example:

The figure below shows the separation of food coloring compounds by Thin Layer Chromatography.

Fig 33. TLC description

V.2. TLC technique description

The different components required to conduct the Thin Layer Chromatography experiment, along with the phases involved are:

V.2.1. The TLC container

The container will be a chromatographic tank, also known as Chamber is used to develop plates. It is usually made of glass, with a variable shape, sealed with a tight-fitting lid. It is responsible to keep a steady environment inside which will help in developing spots. Also, it prevents the solvent evaporation and keeps the entire process dust-free.

V.2.2. The Mobile Phase (PhM)

Also called the eluent: it is a solvent (or a mixture of solvents) that moves along a "stationary" phase, fixed on a support, carrying the sample components.

Depending on the type of analysis to be performed, the choice of eluent will vary:

- **For hydrocarbons**: hexane, petroleum ether, or benzene
- **For common functional groups**: hexane or petroleum ether, mixed in varying proportions with benzene or diethyl ether
- **For polar compounds**: ethyl acetate, acetone, or methanol

V.2.2. The Stationary Phase (PhS)

This is a layer of about ¼ of an adsorbent fixed onto a glass plate (or on a semi-rigid sheet of plastic, aluminum, or even paper) using a binder such as hydrated calcium sulfate (plaster), starch, or an organic polymer. After the sample is deposited on the stationary phase, the substances migrate at a speed depending on their nature and that of the solvent.

V.2.3. The Sample

It was dissolved (**2 to 5%)** in a volatile solvent, which is not necessarily the same as the eluent: the most commonly used solvents are chloroform (trichloromethane), acetone, or dichloromethane. The solution (max. 1 cm^3) is deposited on the plate, about 1 cm from the lower part.

V.3. Choice of Operating Conditions

V.3.1. Choice of Stationary Phase

There are three main types: silica, alumina, and cellulose. The supports are typically mounted on an aluminum plate. Some plates have a colorless dye in natural light, but it turns green under **UV** (ZnS, absorbing at **256 nm**). Here's how to guide your choice:

- **Silica**: This is the most common support. It's recommended to start with this one.
- **Alumina**: It is generally used for compounds with a basic character.
- **Cellulose**: It is used for strongly polar compounds like sugars or amino acids.

V.3.2. Choice of Eluent

Choosing the eluent is crucial. It is not always provided with the procedure, and it's important to know how to select it. The eluent is often a carefully balanced mixture of two or three solvents in specific proportions. Two factors influence the interaction between the eluent and the solute (mixture of compounds to be separated):

- **Solubility**: The solute must be dissolved in the eluent for migration to occur.

- **Polarity of the eluent**: It will determine the speed at which the compound migrates.

V.4. Performing TLC

TLC is carried out in three steps: preparation of the tank, preparation of the plate, and elution. Figure below summarizes the steps for performing Thin Layer Chromatography.

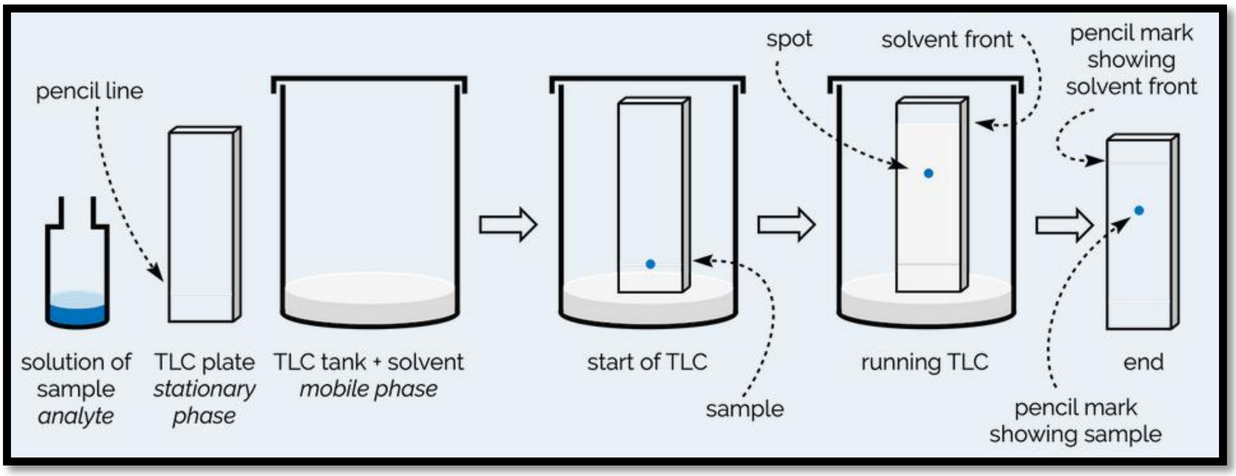


Fig .34. Performing TLC

V.5. Revelation

Some compounds are colored: no revelation is needed for them. Most are colorless. Here are a few methods used to reveal the plates.

V.5.1. UV Revelation

- If the plate is fluorescent, under a UV lamp, the whole plate appears green except where the spots are, which are circled with a pencil.
- Aromatic derivatives absorb UV light. Place the plate under a UV lamp and circle the colored spots.

V.5.2. Iodine Revelation

Many organic compounds form yellow-brown spots in the presence of iodine. In a flask, place the plate and a few iodine crystals, then seal it. The spots will appear.

V.5.3. Spray Revelation

This technique uses an atomizer containing the revelator in solution. Depending on the product to be revealed, the solution can be:

- **Ninhydrin** for amino acids (violet spots that turn brown and disappear after a few days)
- **50% sulfuric acid** for many organic compounds (appear as black spots).
- Complex mixtures of molybdenum oxide in the presence of cerium sulfate are also used.

V.6. Calculations and Interpretation

The final position of the spot is characteristic of the molecule. A value is assigned to it, the **R_f** (**Retention factor**), which has been cleverly translated as **Rapport frontal**. This **R_f** is the ratio of the distance traveled by the compound divided by the distance traveled by the eluent.

$$\mathbf{R_f = \frac{H}{h}}$$

Where :

H: height of elution of the product

h: height of elution of the solvent.

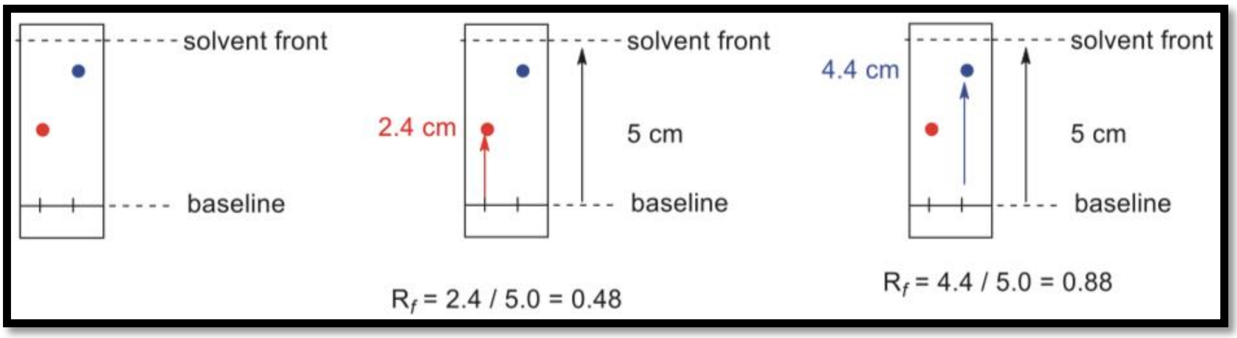


Fig 35. Calculation of R_f in TLC

The figure above shows how to determine the **R_f** in the case of upward liquid chromatography: Measure the distance **x** (H) traveled by the substance from the deposit line.

Also measure the distance **y** (h) traveled by the solvent from the deposit line to the solvent front. The ratio **x / y**, always less than 1, represents the $\mathbf{R_f}$ of the substance.

V.7. Bi-Dimensional Chromatography

When the mixture contains solutes with similar mobility, the separation power can be increased by performing two-dimensional chromatography: on the same support, a first chromatography is carried out using one solvent system, followed by a second chromatography using a different solvent system, perpendicular to the first one. As shown in the figure above, we start by performing chromatography in the usual way with a certain solvent system. This will result in the first separation. The plate is then rotated by 90°, and a new elution is performed using another solvent system (b).

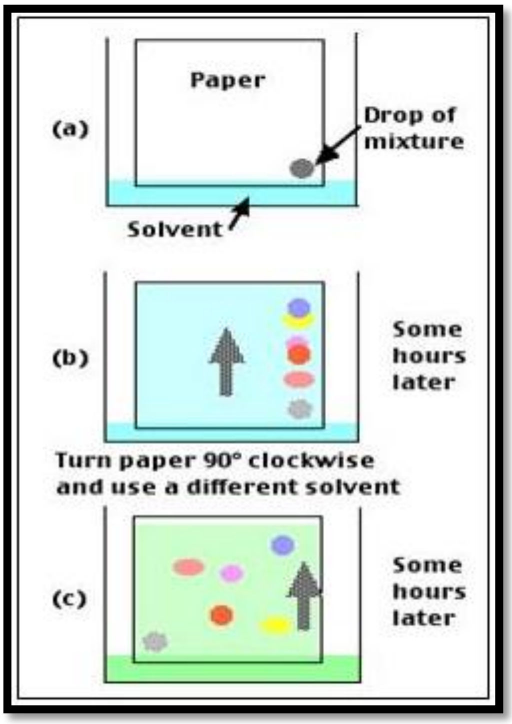


Fig 36. Bi-Dimensional Chromatography