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).

1. Definition of Chromatography

Chromatography is a method for separating the components present in various mixtures. It is used in analysis to **purify**, **identify**, and **quantify** compounds within different samples. The basic principle relies on concentration equilibria that occur when a compound is exposed to two immiscible phases: one, called the stationary phase, is trapped in a column or fixed on a support, and the other, called the mobile phase, moves in contact with the stationary phase. If multiple compounds are present, they are carried along at different rates, causing their separation.

Chromatography plays a crucial role in laboratory practice and is commonly used for:

- Monitoring the purity of reactants and reaction products.

- Tracking a reaction by analyzing the reaction medium.

- Isolating and purifying products from a reaction.

2. Historical Background

In **1906**, a Russian botanist named **Tswett** presented his first paper in **Warsaw** on a new type of adsorption phenomenon and its application to biochemical analysis. He described the formation of colored zones during the elution of plant pigments with petroleum ether in a column filled with calcium carbonate.

The term "chromatography" may have originated from this **separation of colored** compounds, as "**CHROMA**" (χρῶμα) in Greek means **color**. Tswett's work was largely forgotten for about twenty years, and it wasn’t until **1931**, with the publication by **Kuhn** and **Lederer** on the separation of carotene and xanthophyll isomers, that chromatography began to develop as an analytical tool.

From this point on, chromatography began to flourish:

- Around **1940**, **Martin and Synge** developed the practice and theory of chromatography and were awarded the **Nobel Prize** in **1952**.

- In **1952**, Gas Chromatography (GC) was developed.

- In **1968**, High-Performance Liquid Chromatography (**HPLC**) was developed.

- In **1979**, the first chiral separation was achieved by **HPLC**.

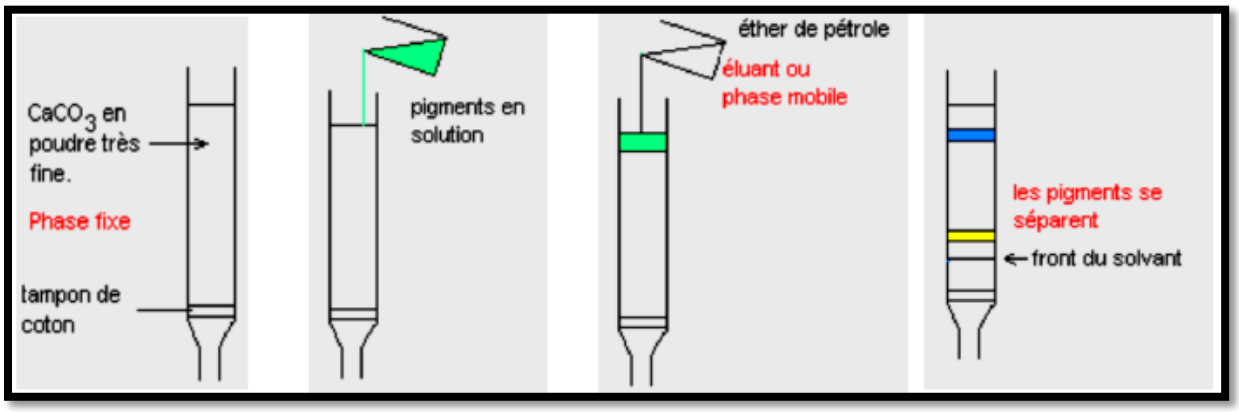


Fig 1. Representation of Tswett's experiment (1906)

3. Principle

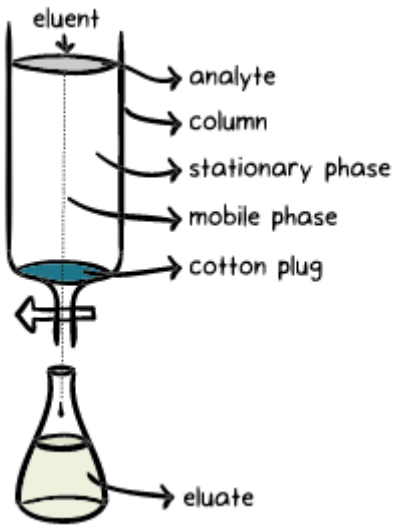
The principle of chromatography is based on the concentration equilibria of compounds present between two immiscible phases in contact:

- **Stationary Phase**: This can be solid or liquid and is contained within a column or fixed on a support.
- **Mobile Phase**: This can be a liquid, a gas, or a supercritical fluid, which moves over or through the stationary phase, carrying the solute with it. The process of carrying the solute is called elution. Each component migrates at a specific rate depending on its solubility in the mobile phase and its affinity for the stationary phase, which tends to retain it. Ultimately, this results in the separation of the components of the initial mixture.

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 thin-layer chromatography.
- **Eluate**: The mixture of solute and solvent that exits the column.

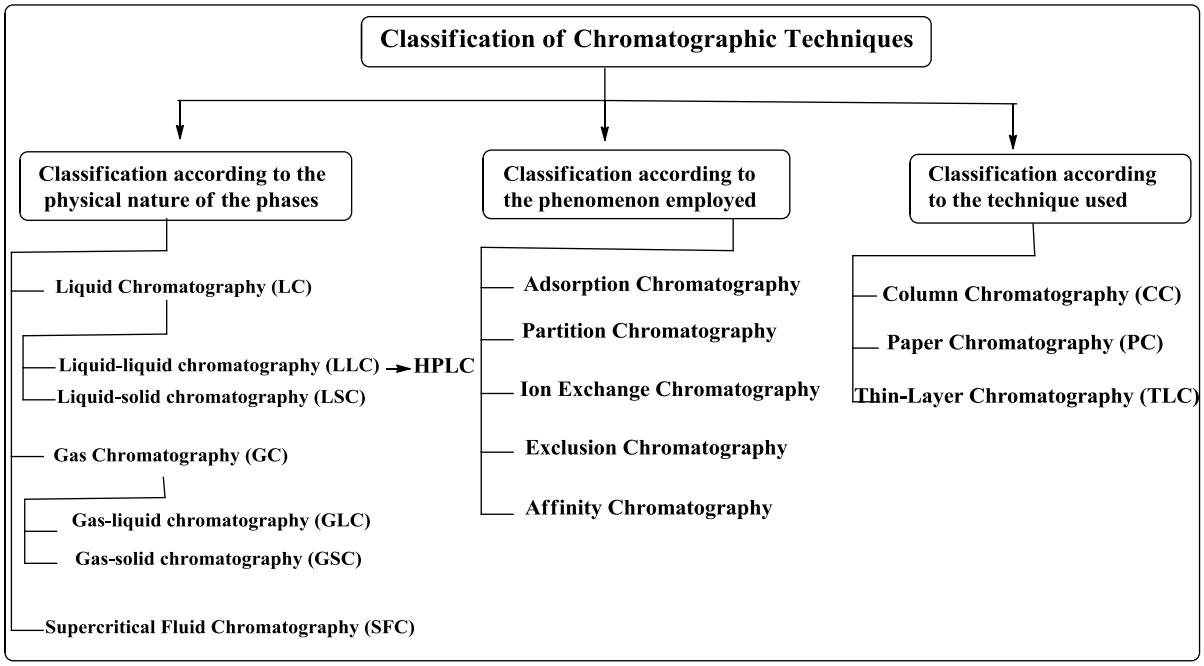


5. Classification of Chromatographic Techniques

The classification of chromatographies can be based on the mechanisms of separation. The factors involved in the distribution of molecules to be separated between the stationary and mobile phases include solubility in a liquid solvent, size (shape), polarity, electrical charge, and the presence of atomic groups forming specific sites. The various types of chromatography arise from the emphasis on one of these factors, but no mechanism is ever exclusively at play during a chromatographic separation.

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.



5.1. Classification According to the Physical Nature of the Phases

In this classification:

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).**

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).**

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.

5.2. Classification According to Chromatographic Phenomena

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

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.

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.

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.

5.2.4. Exclusion Chromatography, or Gel Permeation, or Molecular Sieve Chromatography: The stationary phase is a porous solid: large particles are excluded from the stationary phase, while small particles are included and diffuse into the pores of the gel, thus being delayed.

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, antibody-antigen).

5.3. Classification According to the Procedure Used

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

5.3.1. Column Chromatography (CC) The stationary phase is contained within a cylindrical column made of glass or metal.

5.3.2. Paper Chromatography (PC) : A cellulose surface, considered as a support, maintains a liquid stationary phase by imbibition.

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.

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: gas, volatile liquid, non-volatile liquid, solid, macromolecule, organic species, polar, ionic, etc.

The purpose of the analysis: identification of components in a mixture, whether or not to "couple" chromatography with a spectroscopic method or mass spectrometry (GC/MS), purity control, purification of products (preparative columns), continuous reaction monitoring to optimize parameters, quantification (dosage), etc.

6. Basic Theory - Fundamental Parameters

6.1. The 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.

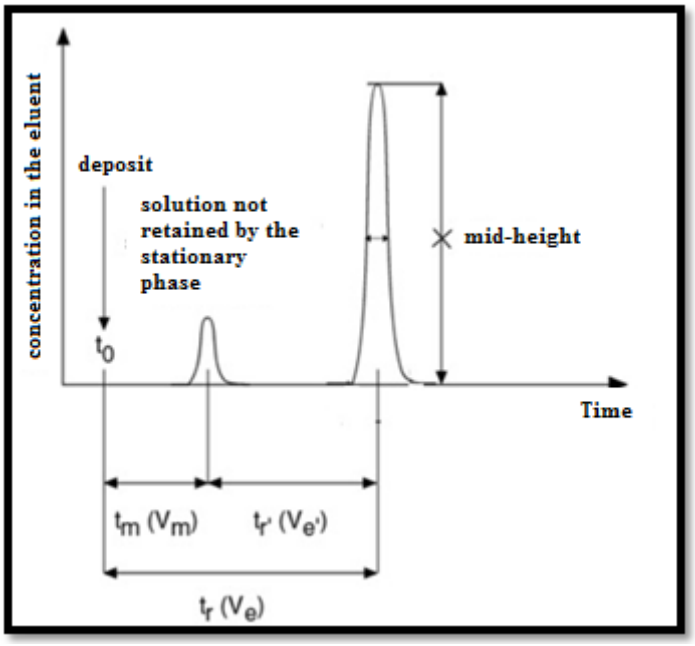


Diagram Showing the Characteristics of a Chromatogram

A chromatographic peak is characterized by:

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.

Retention Time The time taken by the molecules of a compound to be analyzed (solute) 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.

Corrected or Reduced Retention Time (**tr'**) This is the time taken by the solute molecules to traverse the distance from the entrance to the exit of the column, 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:

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

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:

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

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:

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

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 impregnated or not with a liquid) 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:

C_s: concentration of the solute in the stationary phase

C_m: concentration of the solute in the mobile phase.

6.3. Capacity Factor k' (or Retention Factor)

When a compound is introduced into the column, its total mass **m_T** is divided into two quantities: **m_M** in the mobile phase and mS in the stationary phase. These quantities remain constant during its migration in the column. They depend on **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 **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:

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

This relationship is also often encountered in the form:

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

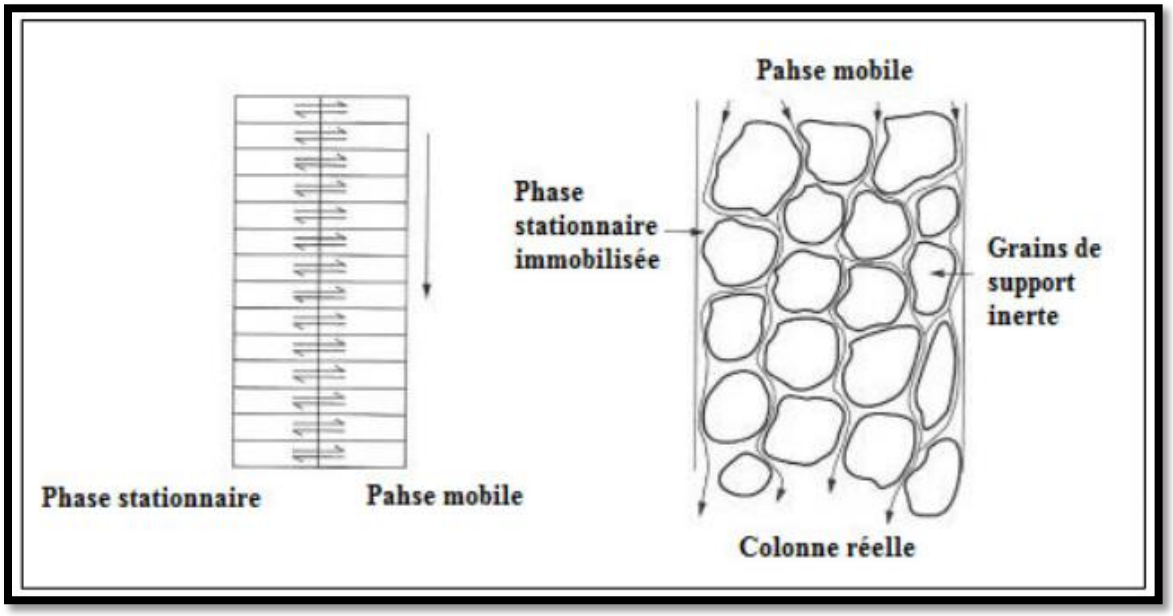
6.4. The Efficiency of a Column

a) 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 each new equilibrium, the solute has progressed through one more small disk in the column, called a theoretical plate (Figure 2).



Plates theory

Theoretical Plate Height (HEPT or H)

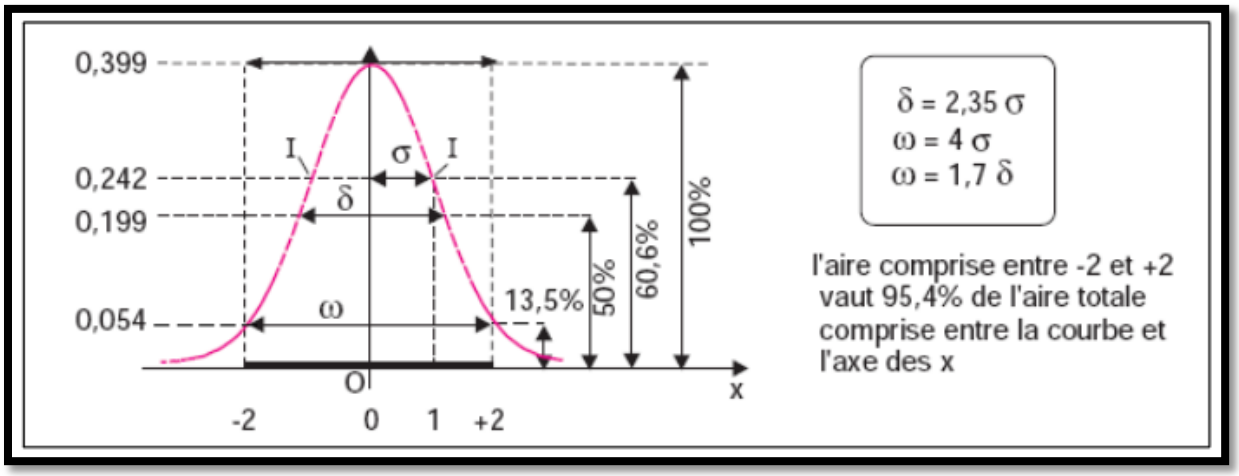
The height equivalent to one theoretical plate (HEPT or H) is given by:

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

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.

b) Gaussian Elution Peaks

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



- **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 $\mathbf{v = \sigma^2}$
- **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:

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

Since His 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 **tr** and **ω** (in time units) are measured graphically, allowing the determination of **N** and then **H**:

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

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.

c) 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 **tr** 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**:**

$$N_{eff} = 16 \frac{t_r^2}{\omega^2} \quad ; \quad N_{eff} = 5.54 \frac{t_r^2}{\delta^2}$$

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.

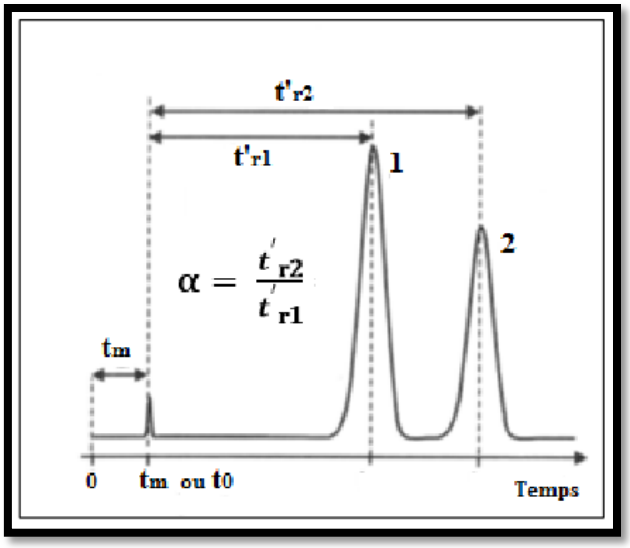
6.5. Quality of Separation

a) Selectivity of a Column

The selectivity factor **α** 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. It defines whether the separation is chemically possible:

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

where α : is always greater than 1**.**



Selectivity factor

b) Resolution

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

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

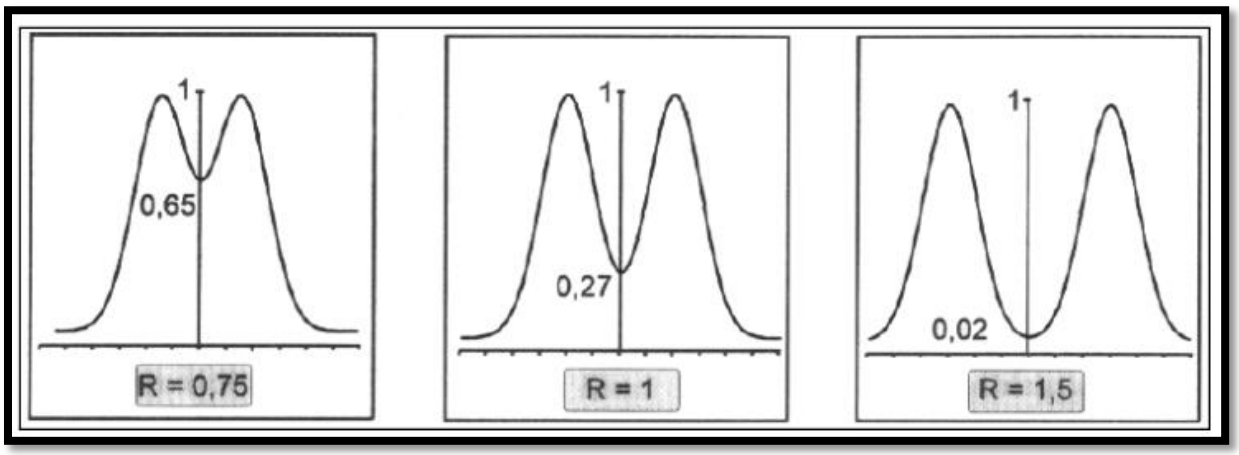


Figure 5: Resolution Factor

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:

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

where $K_{2'}$ is the capacity factor of the slowest species and α is the selectivity factor.

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 t_r are the number of theoretical plates **N**, the capacity factor **α**, and the selectivity factor $K_{2'}$:

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

7.1. Modification of the Height Equivalent to a Theoretical Plate

The resolution of a column is, by definition, proportional to the square root of the number of theoretical plates it contains. Increasing the number of plates results in a longer separation time unless this increase is achieved by reducing **H**.

Methods to minimize **H** include decreasing:

- The diameter of the support particles
- The temperature (in gas chromatography)
- The thickness of the liquid film (in HPLC)

The flow rate of the mobile phase can also be optimized.

7.2. Modification of the Capacity Factor

Separation can often be significantly improved by modifying the capacity factor $K_{2'}$. Increasing $K_{2'}$ improves the resolution factor at the expense of elution time. Optimal values of **k' are** generally between **2** and **5**.

The most common way to improve resolution is to optimize **k'**:

- For gaseous mobile phases, **k'** can be controlled by modifying the temperature.
- For liquid phases, changes in solvent composition often allow for adjustments to **k'** to achieve better separations.

7.3. Modification of the Selectivity Factor

Optimizing **k'** and **N** may not be sufficient for good separation of two solutes in a reasonable time if **α** is close to **1**. In this case, efforts should be made to increase **α** while keeping **k'** between **1** and **10**. Several options are possible, such as:

- Modifying the mobile phase
- Modifying the column temperature
- Modifying the composition of the stationary phase
- Using special chemical effects

An increase in temperature also leads to an increase in **k',** but it has little effect on the value of **α** in liquid-liquid or liquid-solid chromatography. However, temperature does influence ion exchange and will be a parameter to optimize.

8. Qualitative and Quantitative Analysis

8.1. Qualitative Analysis

This primarily serves to identify the components of a mixture. It involves identifying the solutes by their retention time, which, under given conditions (solvent, flow rate, column, etc.), is characteristic of the compound. It can happen that two different but very similar compounds have the same retention time. Therefore, it is essential to ensure that a given peak corresponds to a single solute. This is the aim of the entire phase of fine-tuning the operating conditions used. For the purposes of this discussion, we assume that each peak corresponds to a 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:

- $\mathbf{m_i}$**:** mass of the injected solute i
- $\mathbf{A_i}$**:** area of the peak representing this solute.
- $\mathbf{K_i}$ **:** proportionality constant.

a) 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:

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

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

$$A_i = H \times \delta$$

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

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

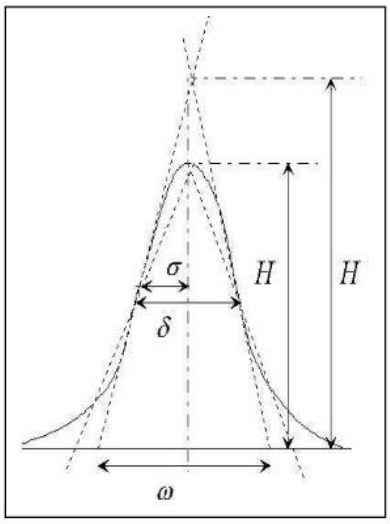


Figure 6: Triangulation

b) Determination of the Proportionality Constant

With standard chromatographs, it is impossible to directly calculate the proportionality constant by measuring the area of the recorded peak when a known, exact mass of a solute is injected. Syringes do not allow for precise sample volume measurement. Therefore, calibration methods must be employed, which, as in qualitative analysis, will make quantitative chromatography a relative procedure regarding known substances. Here are the main methods used:

- Internal Normalization: Assuming all $\mathbf{K_i}$ are equal (homologous series such as alkanes, alcohols, etc.), one obtains the mass percentages of each solute as follows:

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

- Internal Calibration: In this method, each peak to be evaluated is compared to the peak of a suitably chosen standard substance **E**, introduced in a known proportion into the mixture to be analyzed. It is essential that the standard peak does not overlap with any of the peaks in the chromatogram.

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

We define

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

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

- External Calibration: This technique is only valid with excellent injection quality.
One injects a mass $\mathbf{m_e}$ of standard compound in solution (volume $\mathbf{V_e}$ injected).

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

Then, the same volume of solution containing the compound $\mathbf{m_i}$ is injected, and $\mathbf{m_i}$ is measured.

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

Gas Chromatography

Gas chromatography (GC) is a powerful analytical technique used to separate and analyze volatile compounds in a sample. It's widely applied in various fields, from environmental monitoring to pharmaceutical analysis, due to its high sensitivity and resolution.

si

History and Principles of Gas Chromatography

1

Early Developments (1900s)

The foundational principles of chromatography were established early in the 20th century. Scientists began exploring methods for separating complex mixtures using various stationary and mobile phases.

2

Martin and Synge's Contribution (1941)

Archer Martin and Richard Synge's pioneering work on partition chromatography laid the theoretical groundwork for gas chromatography, earning them the Nobel Prize in Chemistry.

3

First Gas Chromatograph (1952)

A.J.P. Martin and A.T. James developed the first practical gas chromatograph, revolutionizing analytical chemistry and paving the way for diverse applications.

4

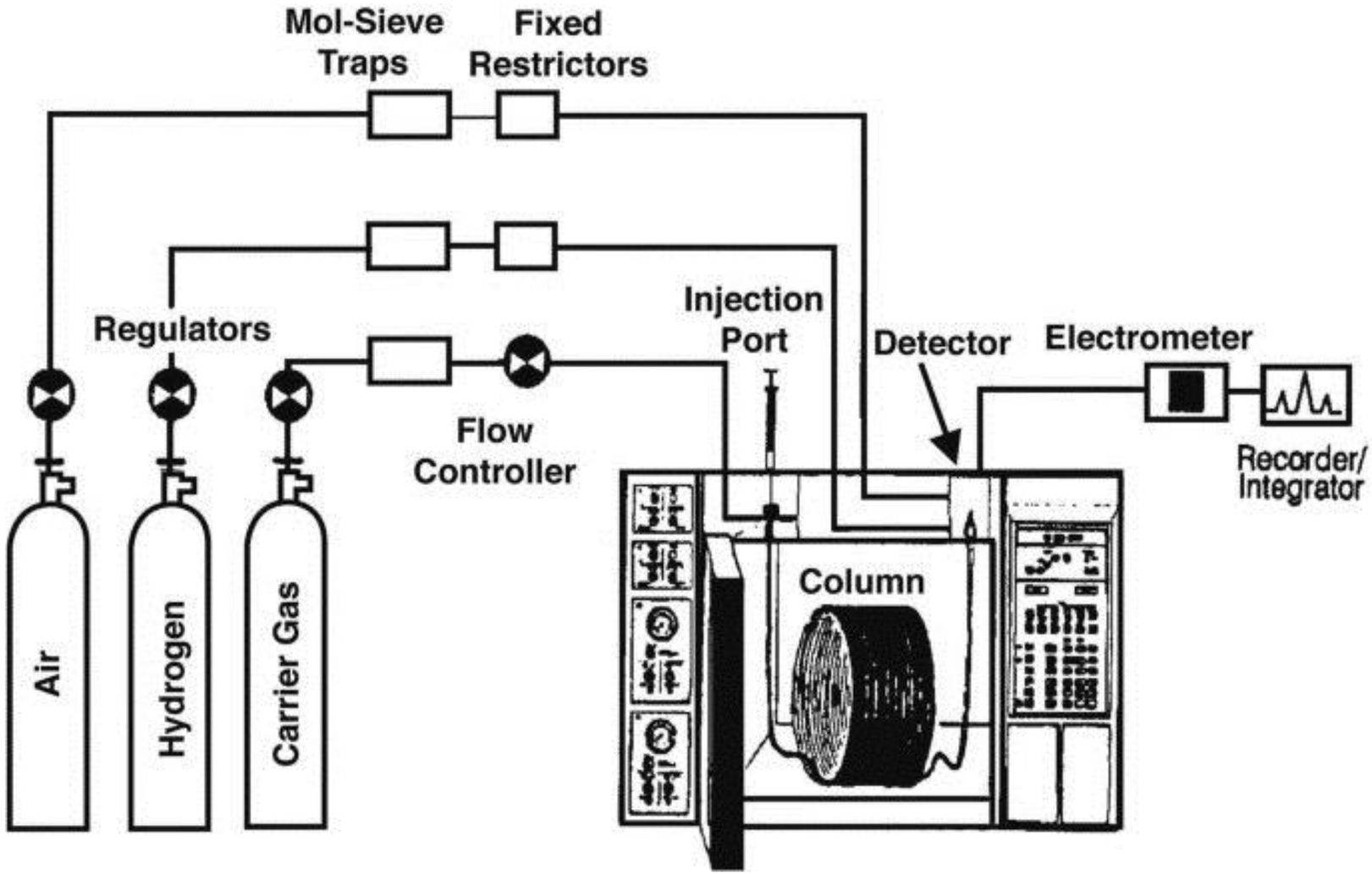
Modern GC (Present)

Modern gas chromatography has advanced significantly, with improvements in instrumentation, detectors, and separation techniques leading to higher sensitivity and resolution.

Instrumentation and Components of a Gas Chromatograph

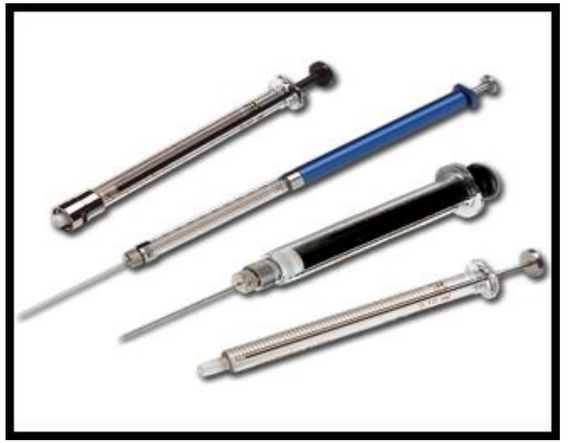
Carrier Gas System

Provides the mobile phase, typically inert gases like **helium** or **nitrogen**, ensuring consistent flow and pressure.



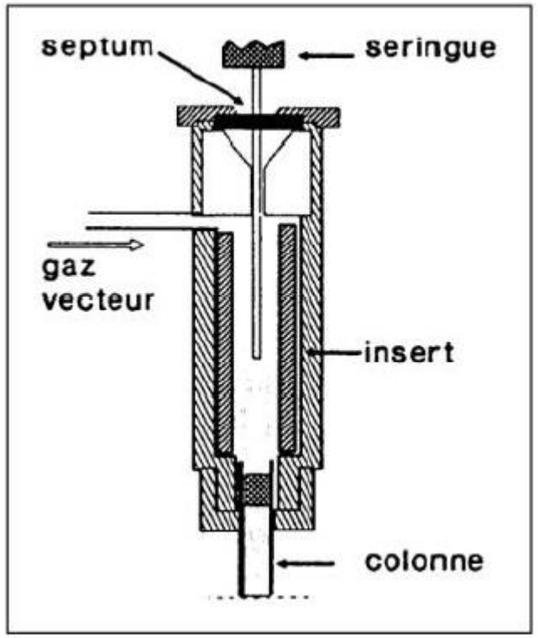
Injection System

Introduces the sample into the GC column, requiring precise control of injection volume and temperature to maintain sample integrity.



Sample injection

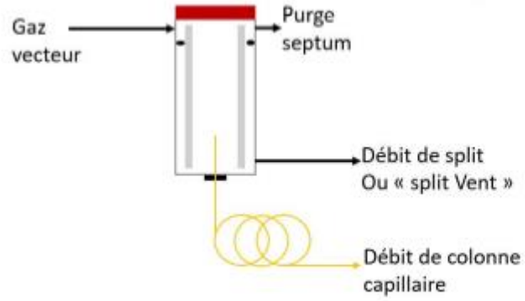
The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash port at the top of the column. The temperature of the sample port is usually about 50°C.



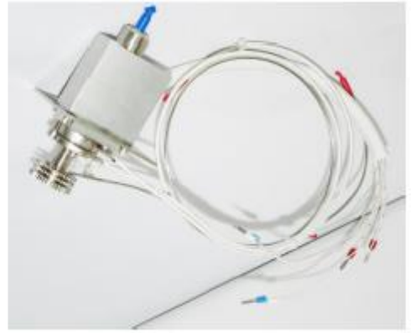
Injection split/splitless

Split: introducing a portion of the injected material into the column and driving the remaining portion outside the system.

Splitless injection is a variation of split injection and was designed particularly for trace analyses in highly diluted samples. Features of splitless injection. Suitable for trace analysis.

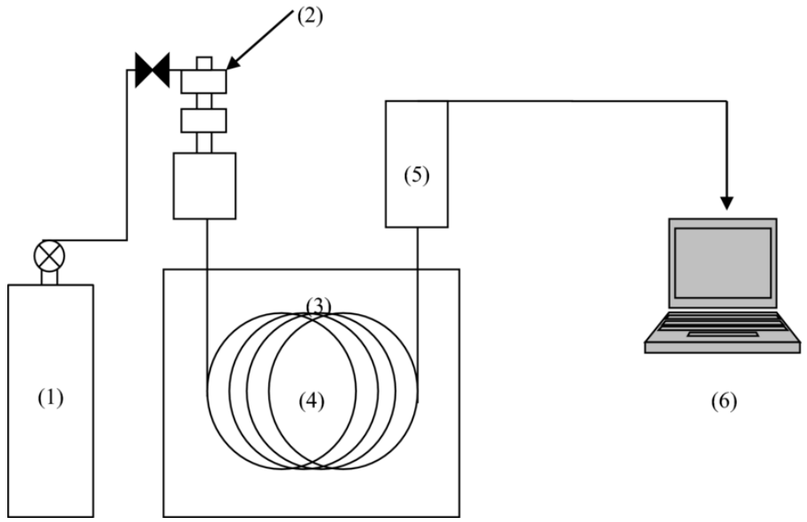


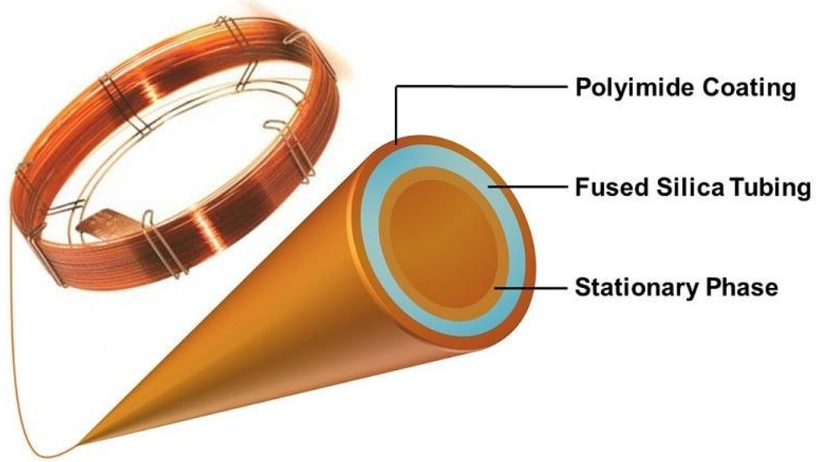
The split / splitless injector

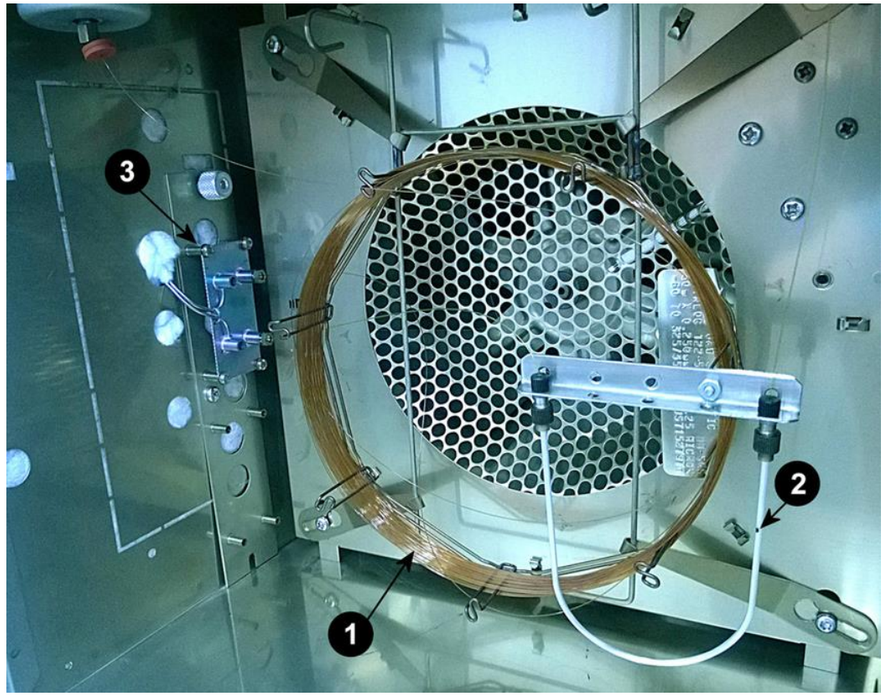


Column and Oven

The heart of the GC, the column (packed or capillary) separates components based on their interaction with the stationary phase. The oven regulates temperature for optimal separation.

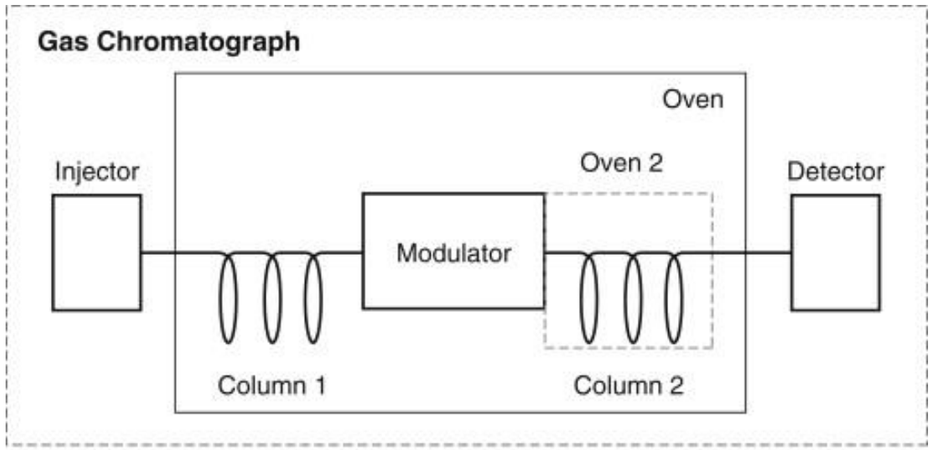


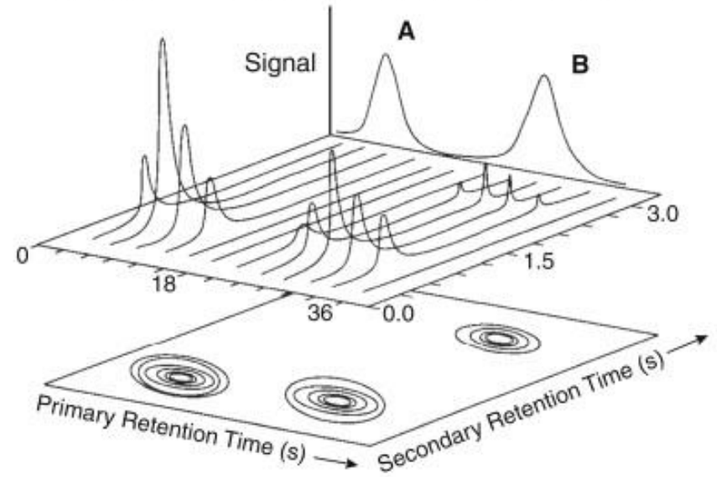


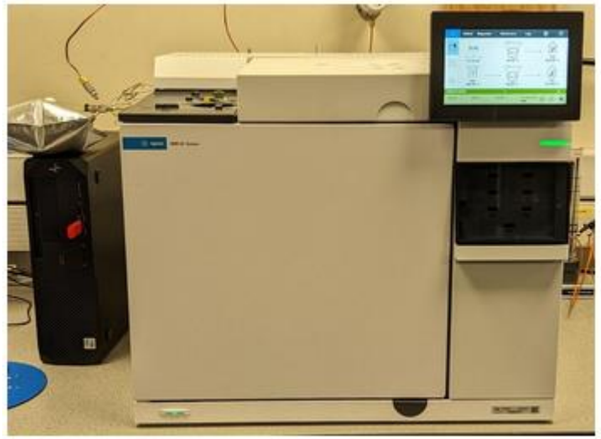


Detector System

Detects the separated components as they elute from the column, producing signals proportional to their concentration. Common detectors include FID, TCD, and MS.







Sample Preparation and Injection Techniques

1 Liquid Injection

Common technique for liquid samples, using microsyringes for precise injection volume control. Requires careful technique to avoid overloading the column.

2 Gas Injection

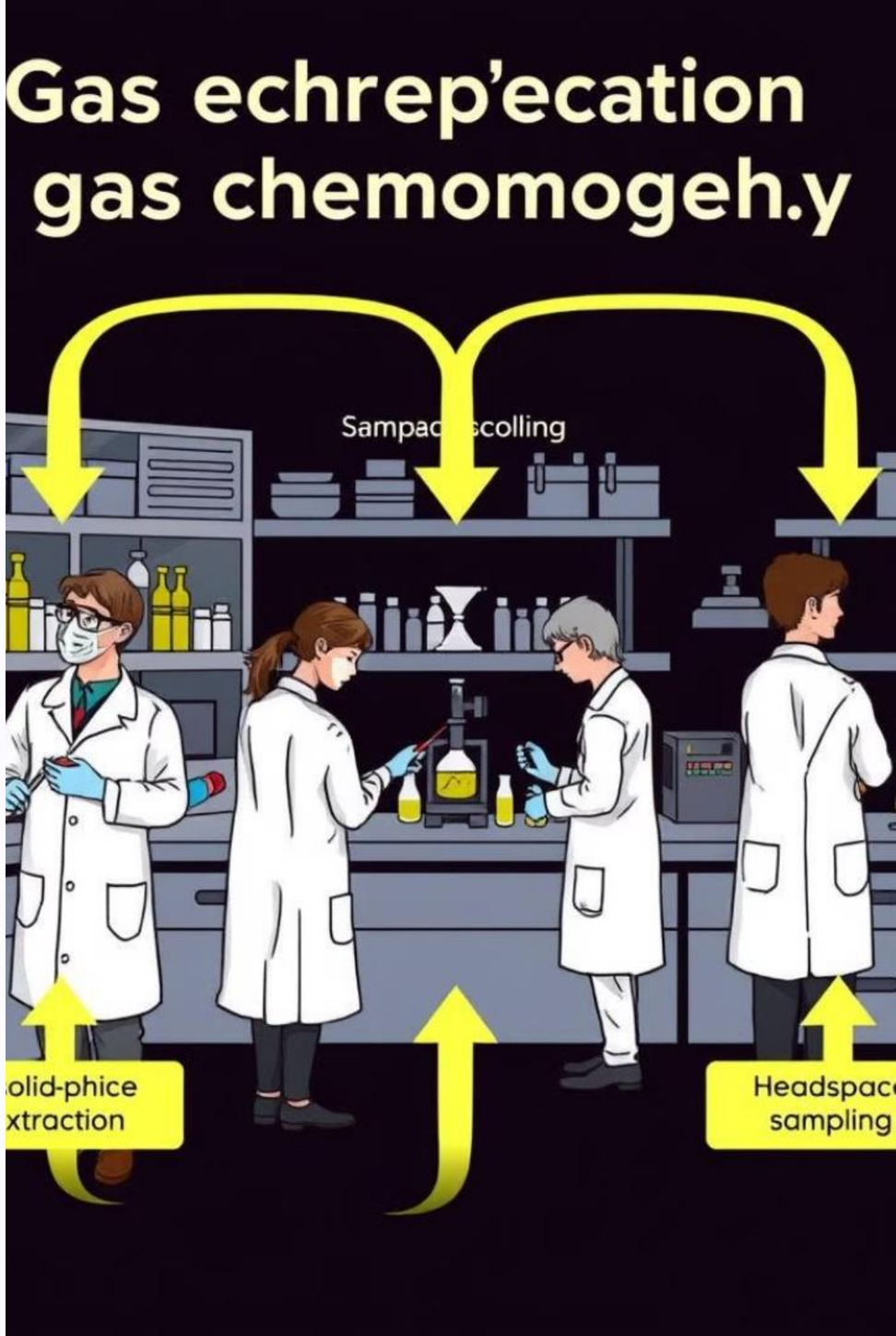
Suitable for gaseous samples, using gas-tight syringes or valves. Requires precise pressure control and volume measurement.

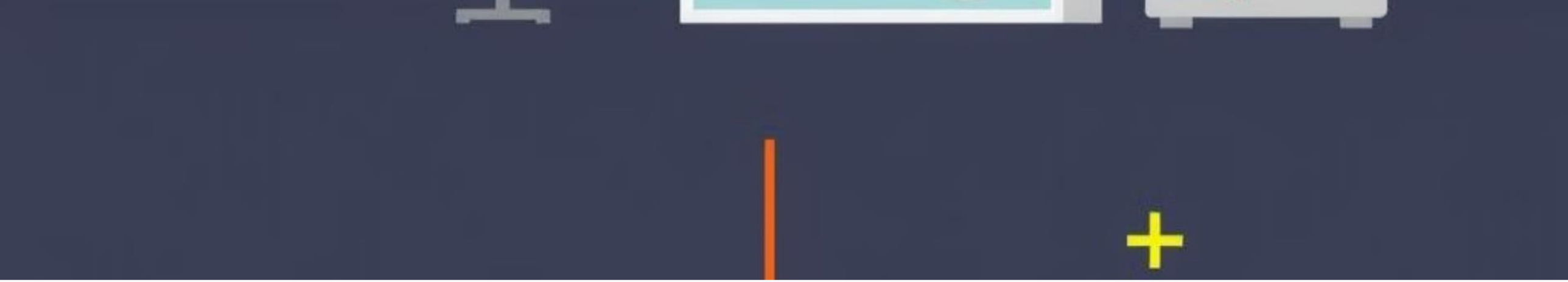
3 Headspace Analysis

Analyzes volatile compounds in a sample's headspace, avoiding direct injection of the sample matrix. Useful for analyzing volatile components in liquids or solids.

4 Solid-Phase Microextraction (SPME)

A solvent-free extraction technique where analytes are absorbed onto a fiber, then thermally desorbed into the GC. Useful for trace analysis.





Separation Mechanisms in Gas Chromatography

Partition Chromatography

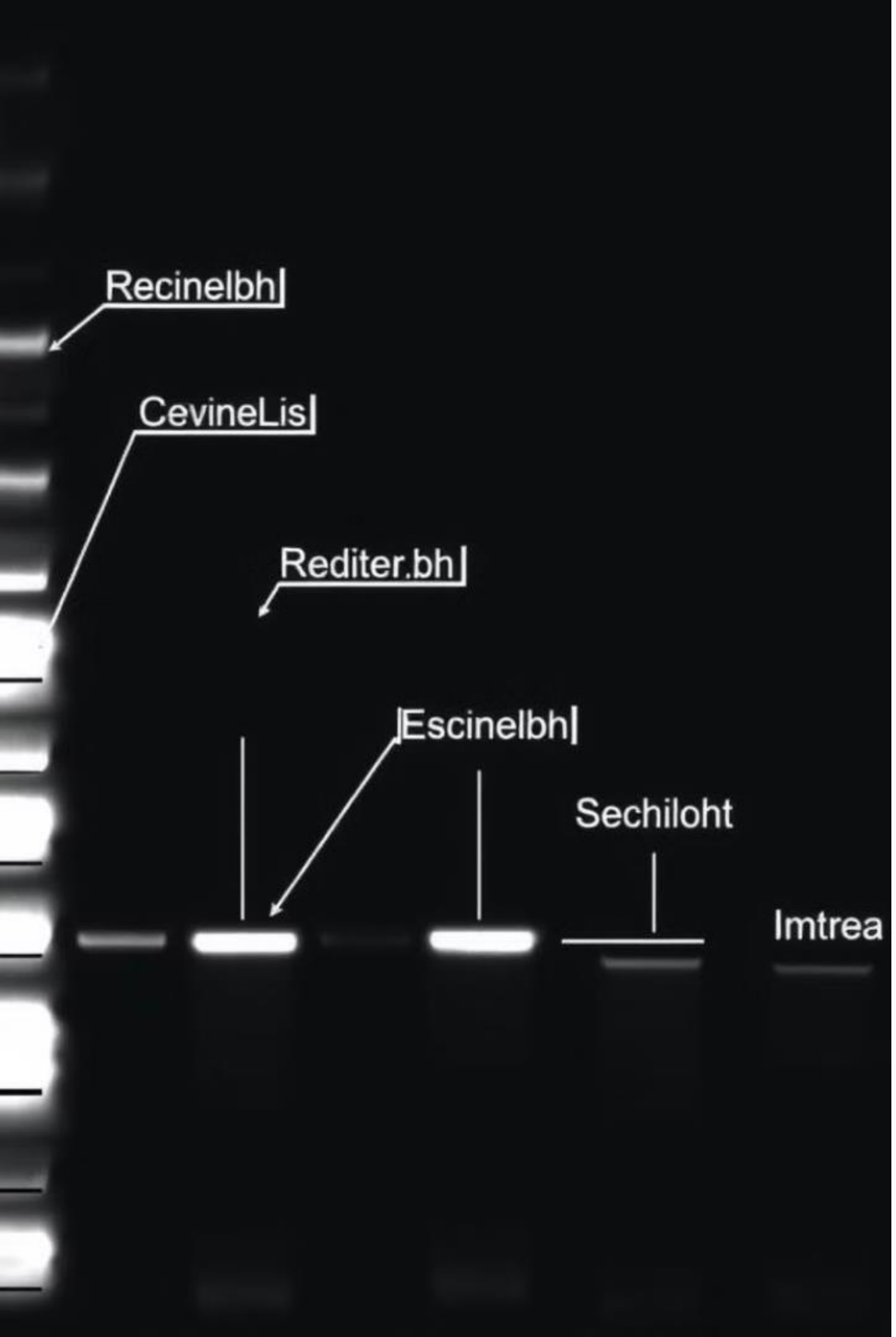
Separation based on the differential partitioning of analytes between the stationary and mobile phases. The most common mechanism in GC.

Adsorption Chromatography

Separation based on the differential adsorption of analytes onto the surface of the stationary phase. Often used for separating non-polar compounds.

Size Exclusion Chromatography

Separation based on the size and shape of the analyte molecules. Less common in GC than partition chromatography.



Qualitative and Quantitative Analysis

1

Peak Identification

Retention times are compared to standards to identify components. Spectral data (from MS detectors) is often used for confirmation.

2

Peak Area Measurement

Peak areas are proportional to the concentration of each component in the sample.

3

Calibration Curve

A calibration curve is constructed using standards of known concentrations to relate peak area to concentration.

4

Quantitative Results

The concentration of each component is calculated from the peak area and the calibration curve.

Applications of Gas Chromatography

Environmental Monitoring

Detecting pollutants in air and water samples.

Food Safety

Analyzing food for contaminants and adulterants.

Pharmaceutical Analysis

Assessing drug purity and stability.

Forensic Science

Analyzing evidence in criminal investigations.

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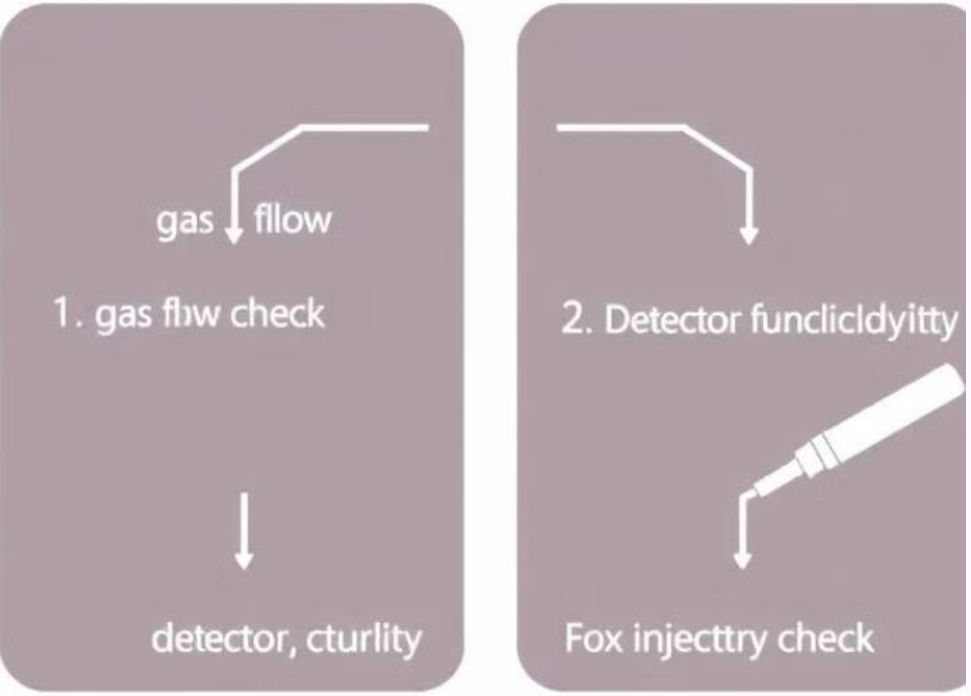

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Troubleshooting and Maintenance

1 Regular Calibration

Maintaining accurate calibrations to ensure consistent and reliable results.

2 Column Maintenance

Regularly checking column performance and replacing columns as needed.

3 Detector Cleaning

Periodically cleaning or replacing detectors to maintain sensitivity and reduce noise.

4 Leak Detection

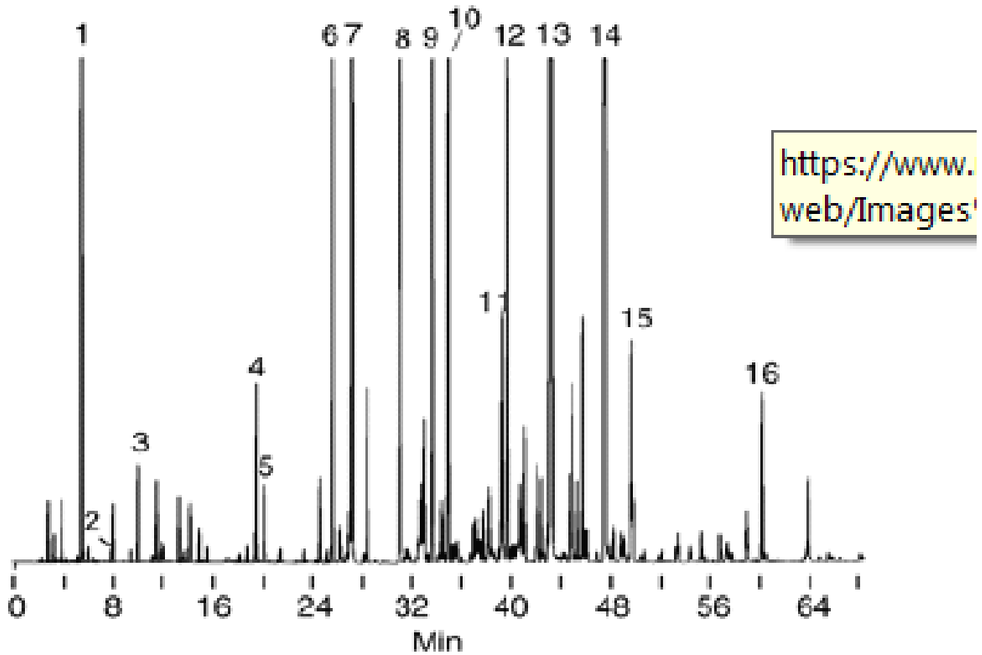
Regularly checking for leaks in the system to ensure proper gas flow and prevent contamination.

Example

GC analysis of geranium oil.

The chromatogram obtained in Figure below, indicates that geranium oil contains at least 16 compounds.

1. α-Pinene
2. β-Pinene
3. Limonene
4. cis-Rose oxide
5. trans-Rose oxide
6. Menthone
7. Isomenthone
8. Linalool
9. Guaia-6,9-diene
10. Citronellyl formate
11. α-Terpineol
12. Geranyl formate
13. Citronellol
14. Geraniol
15. Geranyl butyrate
16. Geranyl tiglate



Conditions of the analyte :
- Column: SUPELCOWAX 10, 30m x 0.25mm ID, film de 0.25µm
- Four: 50°C (2 min) to 280°C at 2°C/min, puis 20 min
- Phase mobile : helium, 25cm/sec
- Detector.: FID
- Injector: 0.2µL, split (100:1).

Example

GC analysis of geranium oil.

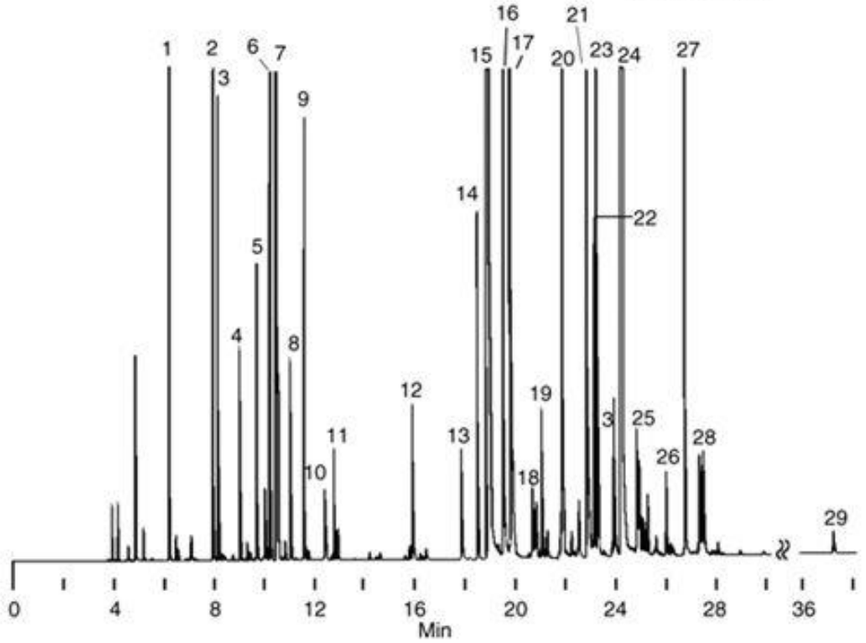
The chromatogram obtained in this

Figure, indicates that menthe oil contains

at least **29 compounds**.

ANALYSE D'HUILES ESSENTIELLES

1. α-Pinene
2. β-Pinene
3. Sabinene
4. Myrcene
5. α-Terpinene
6. L-Limonene
7. 1,8-Cineole
9. γ-Terpinene
10. para-Cymene
11. Terpinolene
12. 3-Octanol
13. 1-Octen-3-ol
14. trans-Sabinenehydrate
15. L-Menthone
16. Menthofuran
17. D-Isomenthone
18. Beta-Bourbonene
19. Linalool
20. Methyl acetate
21. Neo-menthol
22. Terpinene-4-ol
23. β-Caryophyllene
24. L-Menthol
25. Pulegone
26. α-Terpineol
27. Gemacrene-D
28. Piperitone
29. Viridiflorol



Mr. William Faas of A.M. Todd Company provided the sample and assisted with peak identification.

Chromatogramme de l'huile essentielle de menthe

*Colonne: **SUPELCOWAX 10, 60m x 0.25mm, film de 0,25µm***
Four : 4 min (75°C) à 200°C puis 4°C/min, enfin 5 min à 200°C.
Phase mobile: hélium, 25cm/sec (mesuré à 155°C)
Det.: FID
Inj.: 0.2µL, split (100:1)

Future Trends and Developments in Gas Chromatography

Future trends in gas chromatography include miniaturization, increased automation, higher sensitivity detectors, and advanced data analysis techniques. The integration of artificial intelligence and machine learning is expected to further enhance the capabilities of GC in various applications.

Ongoing research focuses on developing new stationary phases with improved selectivity, robustness, and efficiency. The use of micro-GC systems for portable and rapid analysis is also gaining traction. Hyphenated techniques, combining GC with other analytical methods like mass spectrometry (MS), continue to provide valuable insights into complex sample matrices.

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High Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is a powerful analytical technique used to separate, identify, and quantify components in a mixture. It's widely applied in various fields, including pharmaceuticals, environmental science, and food analysis. This presentation will delve into the fundamental principles, techniques, instrumentation, and applications of HPLC, providing a comprehensive overview for both beginners and experienced users.

Principles of HPLC

1 Partitioning

HPLC relies on the differential partitioning of analytes between a stationary phase and a mobile phase. Molecules with higher affinity for the stationary phase move slower, while those with greater affinity for the mobile phase move faster.

2 Retention Time

The time taken for a particular analyte to elute from the column is called its retention time. This is characteristic for each compound under specific chromatographic conditions and is crucial for identification.

3 Resolution

Resolution refers to the ability of the HPLC system to separate two closely related compounds. High resolution is essential for accurate quantitative analysis of complex mixtures.

4 Peak Area

The area under the peak in a chromatogram is directly proportional to the concentration of the analyte. This relationship is exploited for quantitative analysis.

Types of HPLC Techniques

Reverse Phase HPLC

The most common type, employing a **nonpolar** stationary phase and a **polar** mobile phase.

Normal Phase HPLC

Uses a **polar** stationary phase and a **nonpolar** mobile phase, less commonly used than reverse phase.

Ion Exchange HPLC

Separates molecules based on their charge using ion exchange resins.

Stationary Phases and Mobile Phases

Stationary Phases

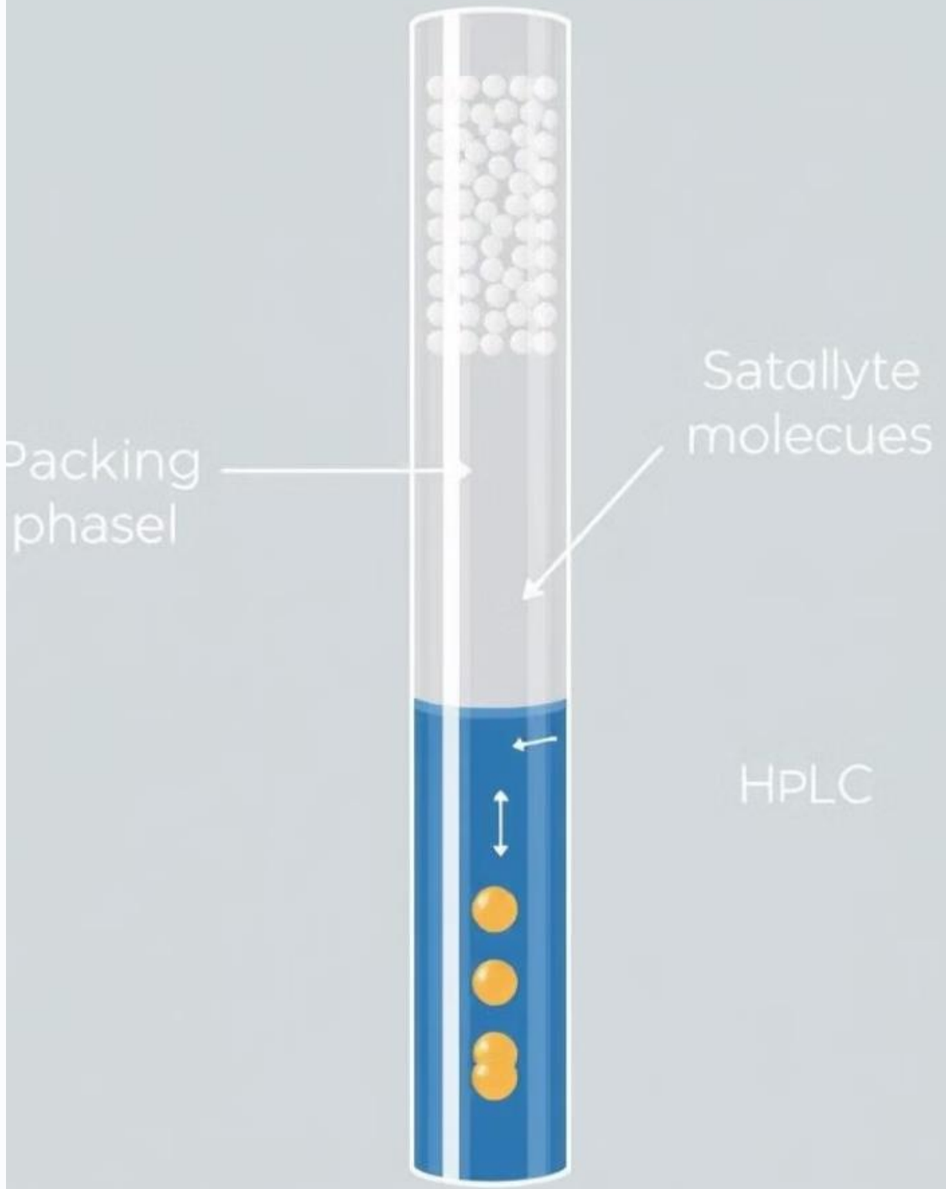
A wide range of stationary phases are available, including **silica-based materials**, **polymeric resins**, and specialized bonded phases tailored for specific separations. The choice of stationary phase depends on the properties of the analytes.

Mobile Phases

Mobile phases can be **single solvents** or **mixtures of solvents**, selected to optimize separation based on the analyte properties and stationary phase. Factors like solvent strength and viscosity are crucial.

Gradient Elution

This technique involves changing the composition of the mobile phase during the separation, improving resolution and reducing analysis time.



Instrumentation and Components of an HPLC System

1

Solvent Delivery System

Delivers the mobile phase at a constant flow rate and pressure.

2

Sample Injector

Introduces the sample into the flowing mobile phase.

3

Column

The heart of the system, where separation occurs.

4

Detector

Detects the eluted components based on their physical properties.

5

Data System

Processes and displays the chromatographic data.

Sample Preparation and Injection

1

Sample Dissolution

The sample is dissolved in a suitable solvent compatible with the mobile phase.

2

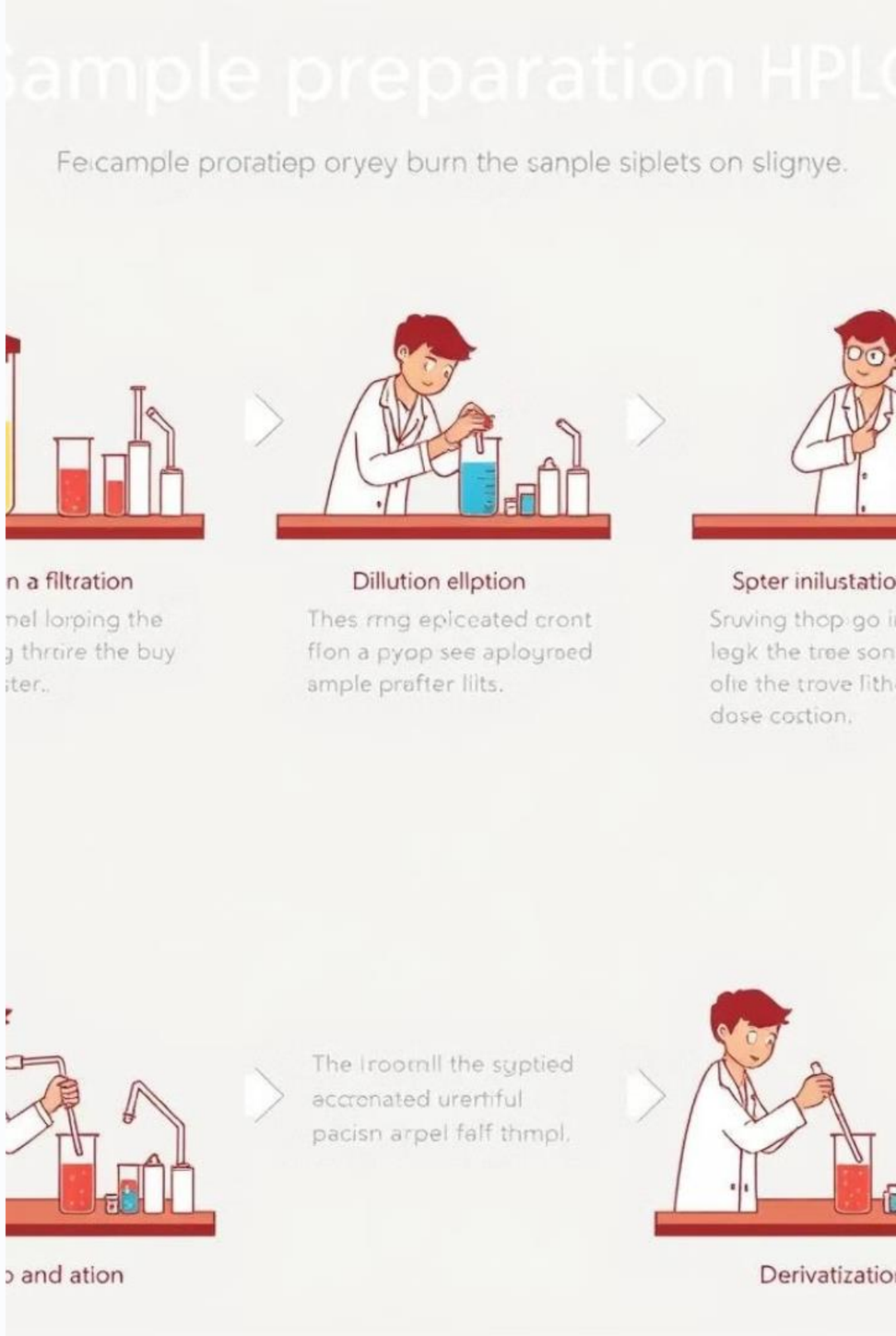
Filtration

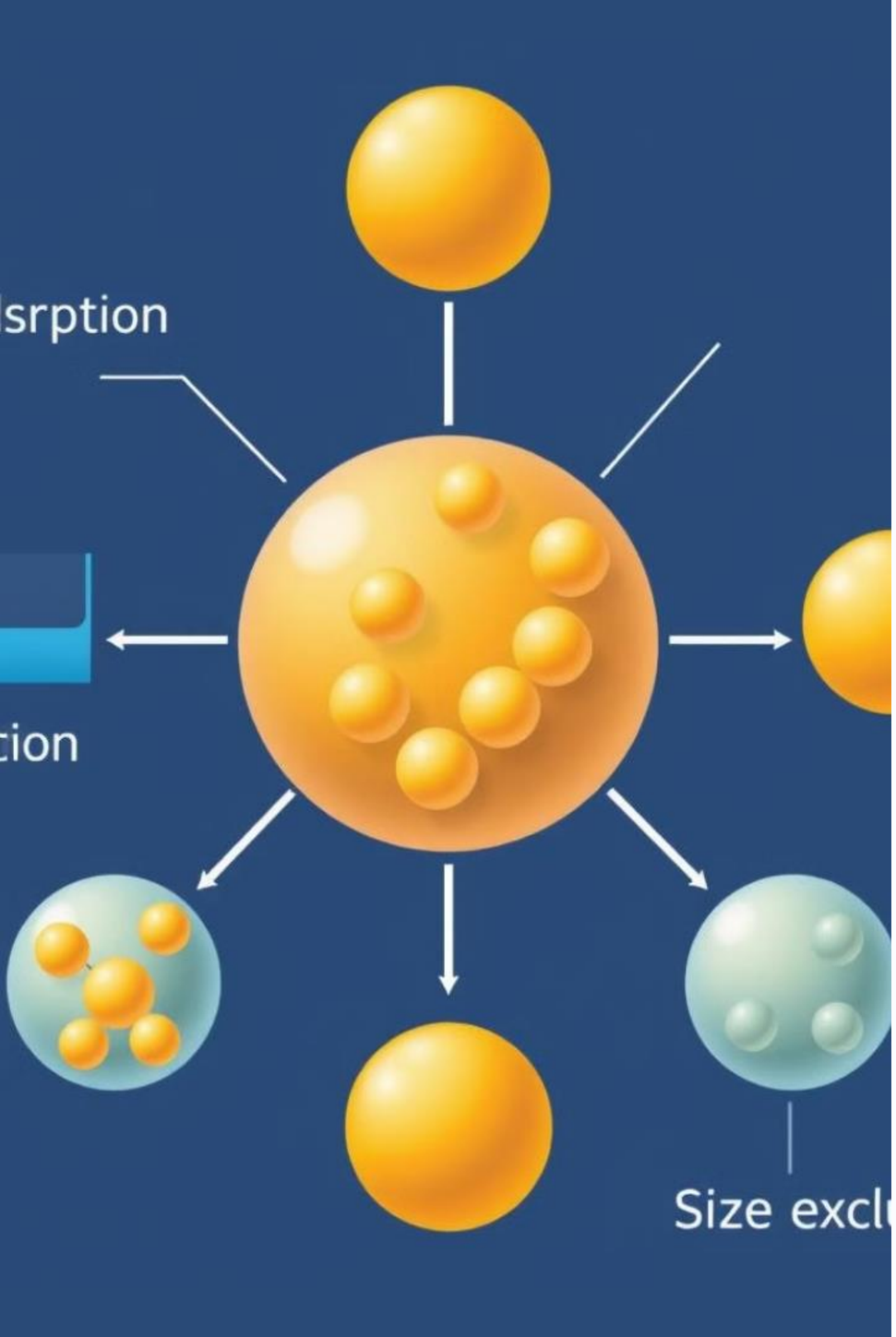
Removes particulate matter to prevent clogging of the column.

3

Injection

The prepared sample is injected into the HPLC system using an autosampler or manual injection.





Separation Mechanisms in HPLC

Adsorption

Analyte interacts with the surface of the stationary phase.

Partition

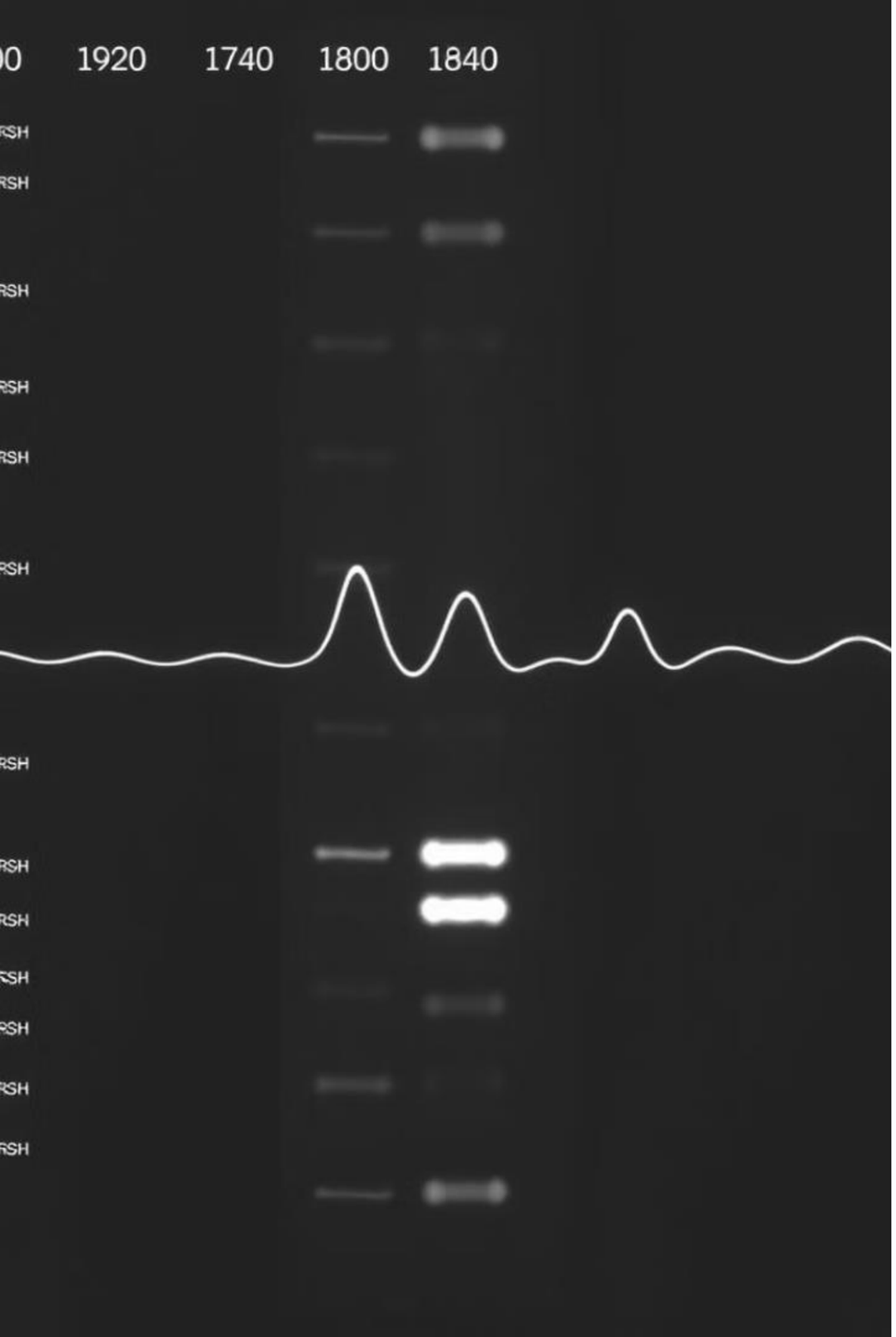
Analyte distributes between the stationary and mobile phases.

Ion Exchange

Analyte is separated based on its charge.

Size Exclusion

Analyte is separated based on its molecular size.



Data Analysis and Quantification

Peak Identification	Retention Time Matching
Peak Area Integration	Quantitative Analysis
Calibration Curves	Concentration Determination

Applications of HPLC

1 Pharmaceutical Analysis

Purity testing, quantification of active ingredients, and impurity profiling.

2 Environmental Monitoring

Detection and quantification of pollutants in water and soil samples.

3 Food Safety

Analysis of pesticide residues, toxins, and contaminants in food products.

Advantages, Limitations, and Future Trends in HPLC

Advantages

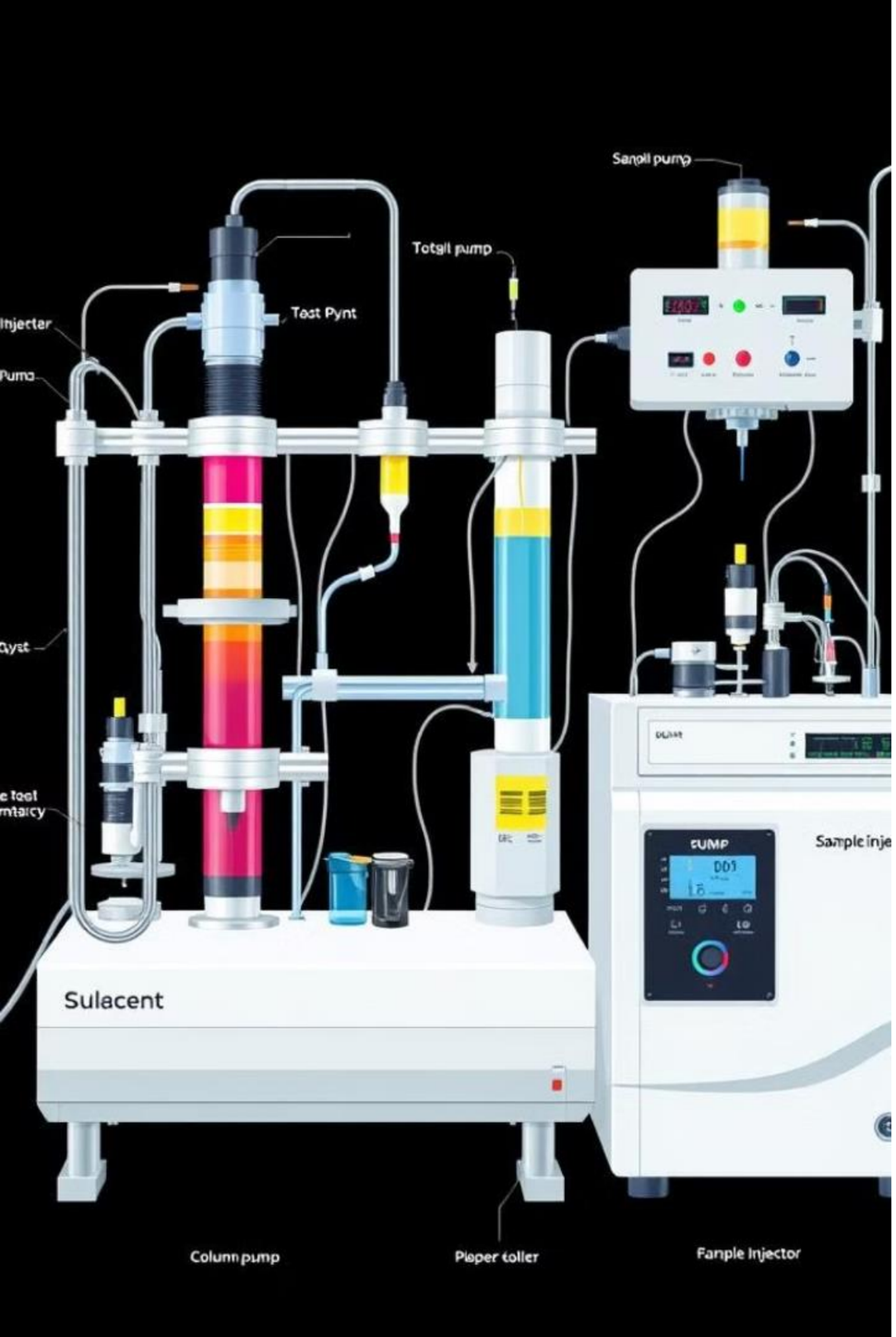
High sensitivity, excellent resolution, versatile, widely applicable.

Limitations

Can be expensive, requires specialized training, sample preparation may be complex.

Future Trends

Miniaturization, hyphenated techniques (HPLC-MS, HPLC-NMR), increased automation, improved sensitivity.



Liquid Column Chromatography: Principles and Applications

Liquid column chromatography (LCC) is a powerful analytical technique used to separate, identify, and quantify individual components within a mixture. This versatile method finds applications in diverse fields, from pharmaceuticals and environmental science to food analysis and materials research. Its ability to resolve complex mixtures makes it an indispensable tool for both qualitative and quantitative analysis.

Throughout this presentation, we'll explore the fundamental principles of LCC, delve into practical aspects of its application, and examine various types of detectors and data analysis techniques. By the end, you'll have a comprehensive understanding of this essential separation technique.

Introduction to Chromatography

1 Separation Technique

Chromatography is a powerful technique that separates components of a mixture based on their differential affinities for a stationary and a mobile phase.

2 Diverse Applications

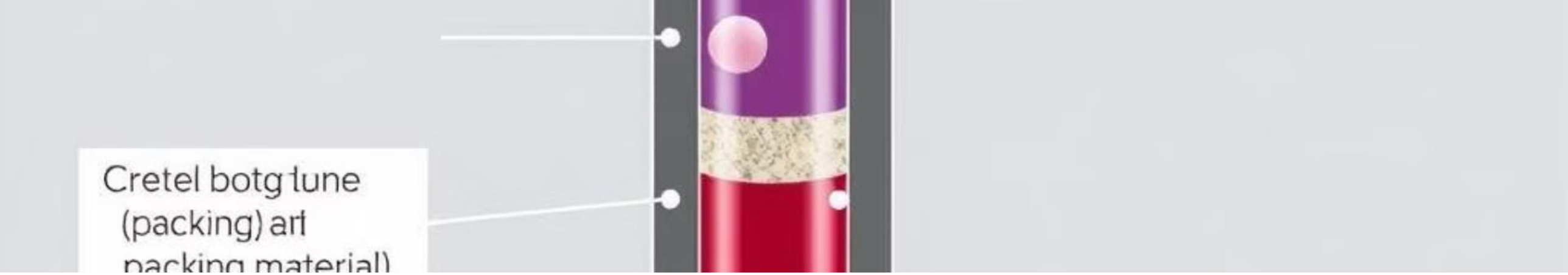
It's used in various fields, including chemistry, biology, and medicine, for identifying and quantifying substances.

3 Underlying Principles

The separation is achieved through a combination of physical and chemical interactions between the components and the phases.

4 Types of Chromatography

Different types exist, including gas, liquid, and thin-layer chromatography, each suited for specific applications.



Fundamentals of Liquid Column Chromatography

Stationary Phase

The stationary phase is a solid or a liquid supported on a solid, and it interacts with the components of the sample to achieve separation.

Mobile Phase

The mobile phase is a liquid that carries the sample through the column, enabling differential migration of components.

Separation Mechanisms

Separation occurs based on differences in the affinity of the components for the stationary and mobile phases.

Retention Time

Retention time is the time it takes for a component to travel through the column and is crucial for identification.

Column Selection and Packing

Column Dimensions

Column length and diameter influence separation efficiency and resolution. Longer columns generally provide better separation.

Packing Materials

Various materials are available, each with different properties and selectivity for specific separations. Silica is common.

Particle Size

Smaller particles lead to higher efficiency but require higher pressure. The choice depends on the instrument's capabilities.

Mobile Phase Preparation and Selection

1

Solvent Selection

The choice depends on the sample's polarity and the stationary phase. The mobile phase should be compatible with the detector.

2

Solvent Purity

High-purity solvents are essential to avoid contamination and ensure accurate results. HPLC-grade solvents are often used.

3

Solvent Mixing

Often, a mixture of solvents is used to achieve optimal separation by adjusting the polarity of the mobile phase.

4

Degassing

Dissolved gases in the mobile phase can cause bubbles and disrupt the flow, leading to poor separation. Degassing is crucial.

Sample Preparation and Injection

1

Dissolution

The sample needs to be dissolved in a suitable solvent compatible with both the sample and the mobile phase.

2

Filtration

Filtration removes particulate matter that could clog the column and damage the instrument.

3

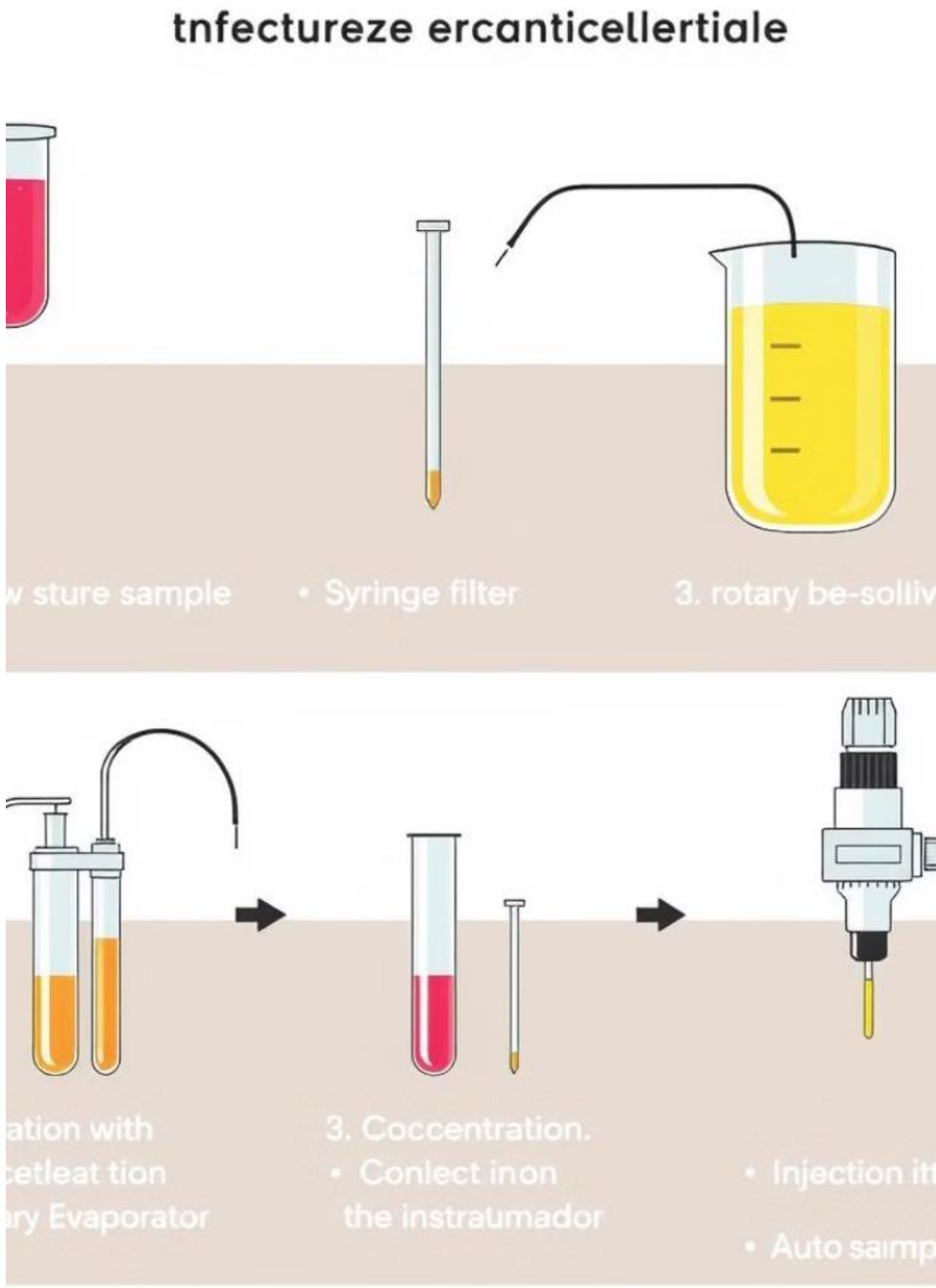
Dilution

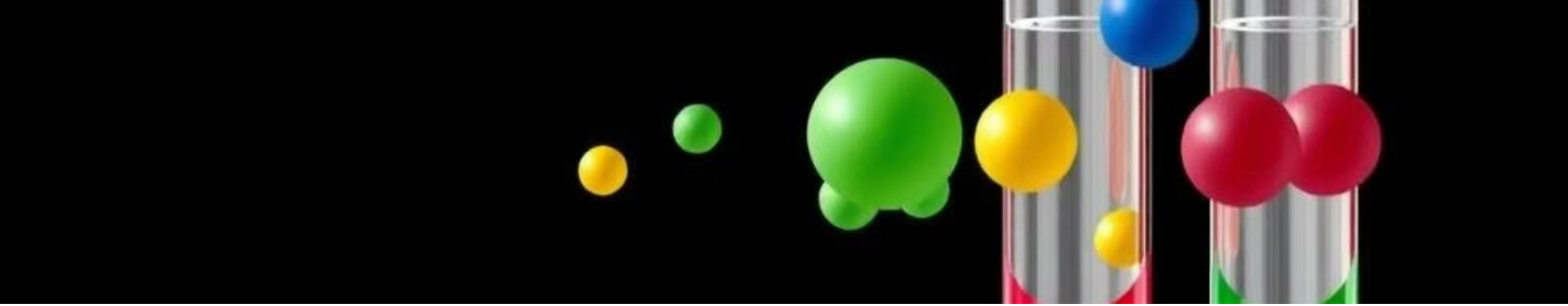
If the sample is too concentrated, dilution is necessary to prevent column overloading and to improve separation.

4

Injection

Precise injection of a known volume of the prepared sample into the instrument is crucial for quantitative analysis.





Separation Mechanisms and Optimization

Adsorption

Separation based on differences in the adsorption of components onto the stationary phase.

Partition

Separation based on the differential distribution of components between the stationary and mobile phases.

Ion Exchange

Separation based on the electrostatic interactions between charged components and the stationary phase.

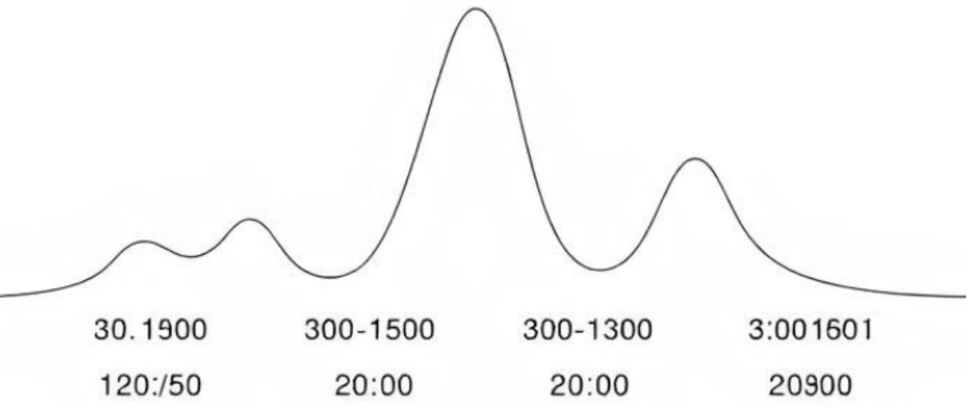
Size Exclusion

Separation based on the size and shape of the components. Larger molecules elute first.

Detection Techniques and Instrumentation

Detector Type	Principle	Applications
UV-Vis	Absorption of ultraviolet or visible light	Widely applicable
Fluorescence	Emission of light after excitation	Sensitive for fluorescent compounds
Refractive Index	Changes in refractive index of the mobile phase	Universal but less sensitive
Mass Spectrometry	Measurement of mass-to-charge ratio	Provides structural information

Data Analysis and Interpretation



1 Peak Identification

Retention time is compared to standards or databases to identify components.

2 Peak Area Measurement

The peak area is proportional to the amount of each component.

3 Calibration Curves

Calibration curves are created to determine the relationship between peak area and concentration for quantitative analysis.

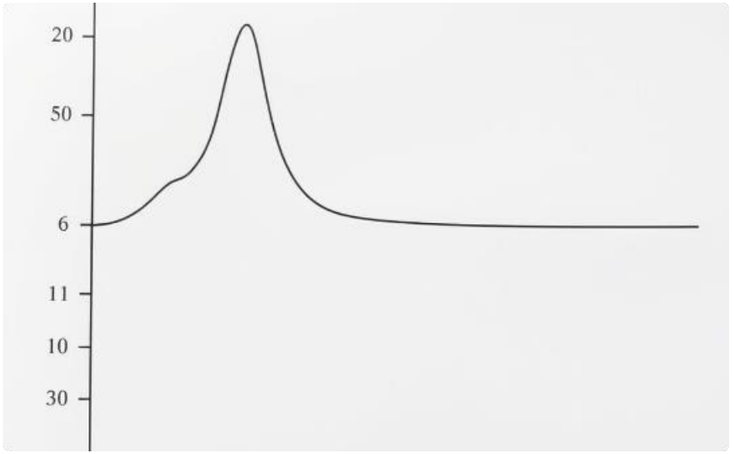
4 Software Tools

Specialized software aids in data processing, integration, and reporting.

Practical Considerations and Troubleshooting

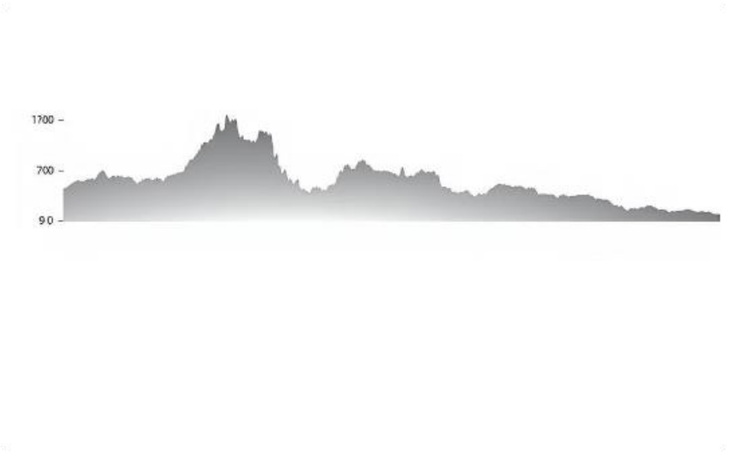
Column Clogging

Caused by particulate matter; requires filtration and column replacement.



Peak Tailing

Indicates poor column efficiency; caused by several factors such as improper sample preparation or column degradation.



Baseline Noise

Poor signal-to-noise ratio can result from instrument issues or environmental factors; requires careful system setup and diagnostics.

Thin Layer Chromatography (TLC): Principles and Applications

Thin-layer chromatography (TLC) is a widely used analytical technique in chemistry and biochemistry for separating and identifying components of a mixture. It's a simple, rapid, and inexpensive method that provides valuable information about the composition of a sample. This presentation will explore the fundamental principles of TLC, its various applications, and the steps involved in performing a TLC experiment.

Introduction to Thin Layer Chromatography (TLC)

1 **Separation Technique**

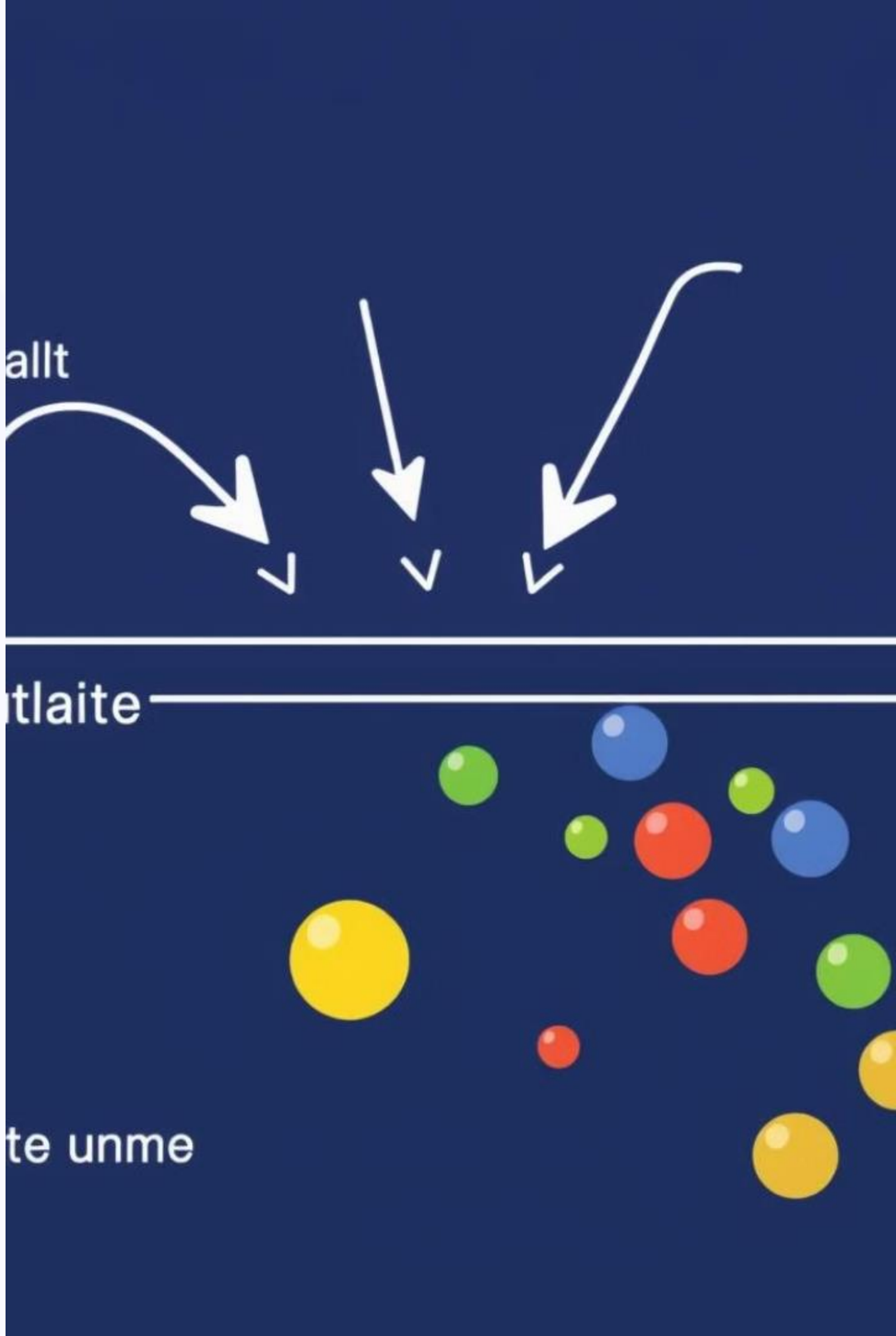
TLC is a powerful technique for separating mixtures based on their differential adsorption to a stationary phase.

2 **Simple and Rapid**

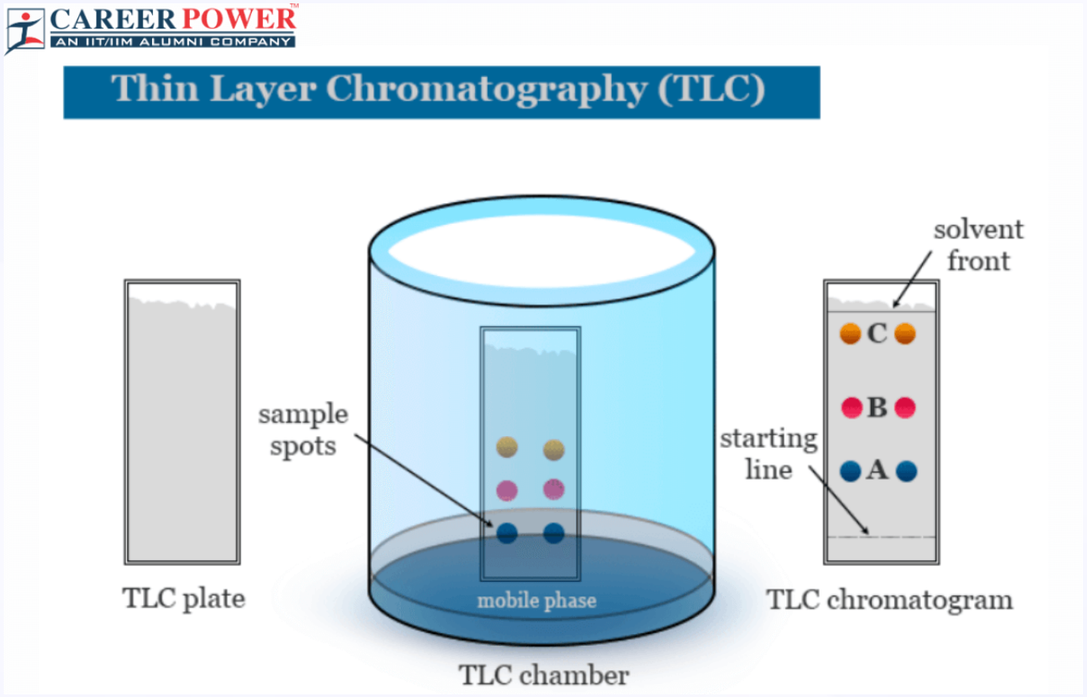
It offers a quick and easy way to analyze complex samples, providing results in a short time.

3 **Qualitative and Quantitative Analysis**

TLC can be used for both identifying components (qualitative) and determining their amounts (quantitative).



Basic Components of a TLC System



TLC Plate

A glass or plastic plate coated with a thin layer of absorbent material (stationary phase).

Developing Chamber or TLC chamber

A closed container where the TLC plate is developed, maintaining a saturated atmosphere.

Mobile Phase

A solvent or solvent mixture that moves through the stationary phase, carrying the sample components.

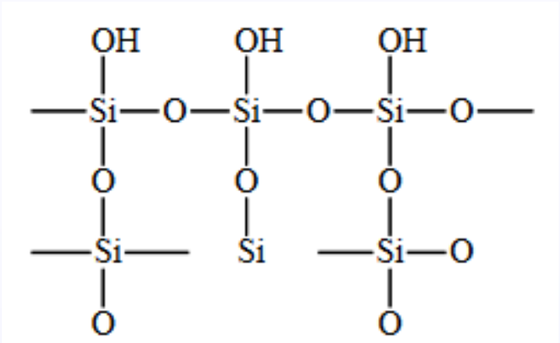
Sample Application

Micropipettes or capillaries are used to spot the sample onto the plate.

Choosing the Appropriate Stationary and Mobile Phases

Stationary Phase

Silica gel is the most common stationary phase, offering high surface area and polarity.



Mobile Phase

The choice depends on the sample's polarity. Nonpolar solvents for nonpolar compounds, and polar solvents for polar compounds.

Table 1. Polarity Indices for Common Liquid Chromatography Solvents

Solvent	Polarity Index (P')
Alkanes and Cyclohexane	0.1
Toluene	2.4
Dichloromethane	3.1
Isopropanol	3.9
Tetrahydrofuran	4
Ethanol	4.3
Ethyl Acetate	4.4
Acetone	5.1
Methanol	5.1
Acetonitrile	5.8
Water	10.2

Optimization

Experimentation is key to finding the optimal combination for efficient separation of the specific compounds in the sample.

Sample Preparation and Spotting Techniques

1

Sample Dissolution

Dissolve the sample in a suitable solvent, ensuring a homogenous solution.

2

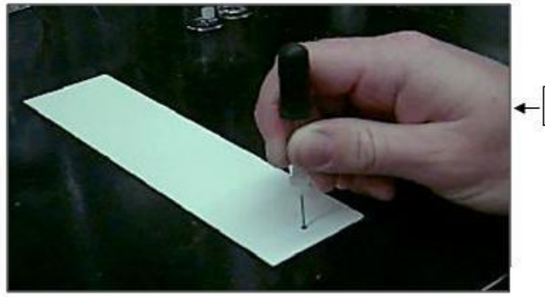
Spotting

Apply small, concentrated spots of the sample onto the TLC plate using a capillary tube or micropipette. Allow the spots to dry completely before developing.

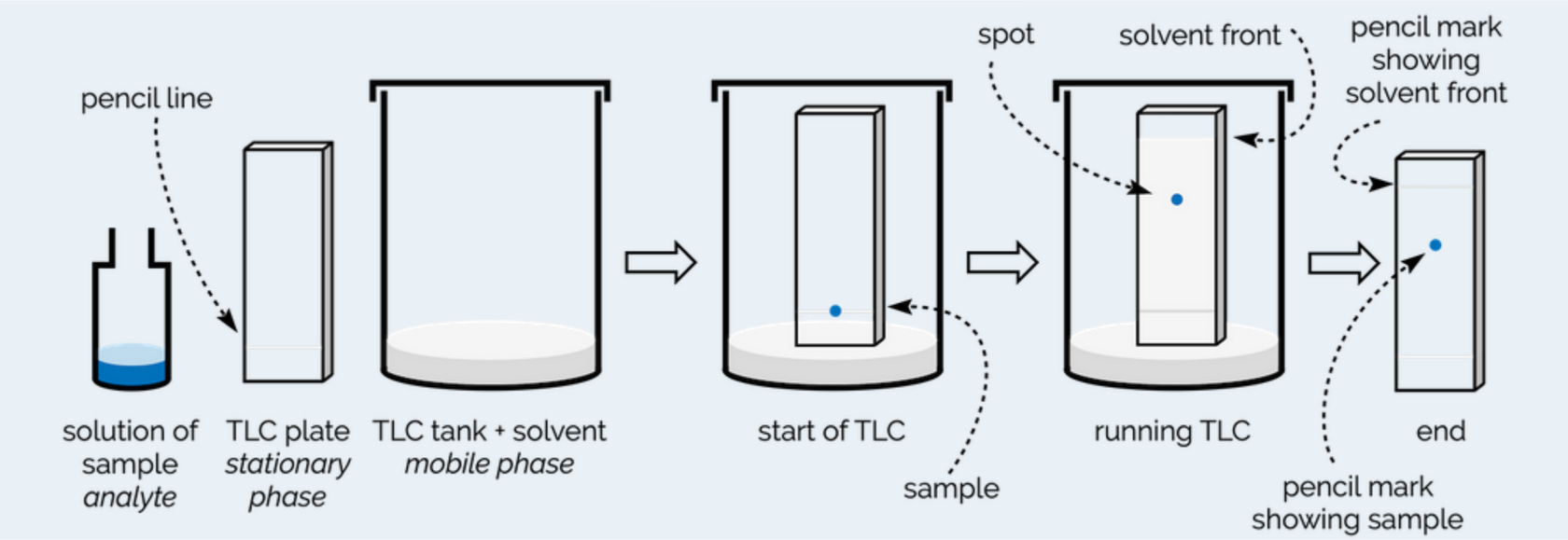
3

Spotting Technique

Spotting is a crucial step, as it influences the separation quality.
Avoid overloading the spots and ensure they are small and evenly spaced.



Developing the TLC Plate



1 Plate Placement

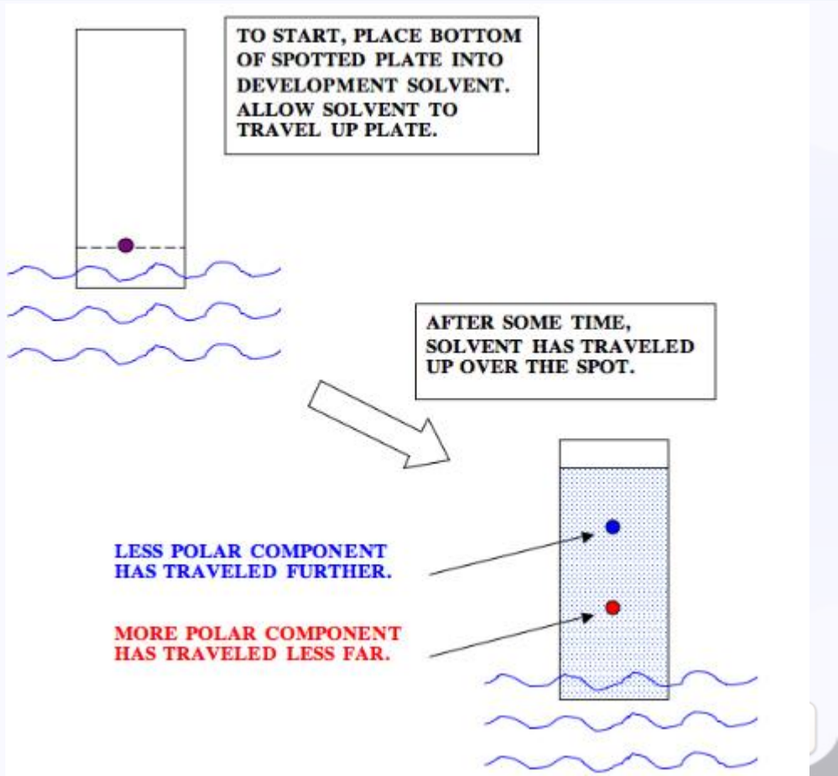
Carefully place the spotted TLC plate into the developing chamber, ensuring the solvent level is below the sample spots.

2 Development

Allow the solvent to ascend the plate until it reaches a predetermined level. This process separates the components based on their interaction with the stationary and mobile phases.

3 Removal and Drying

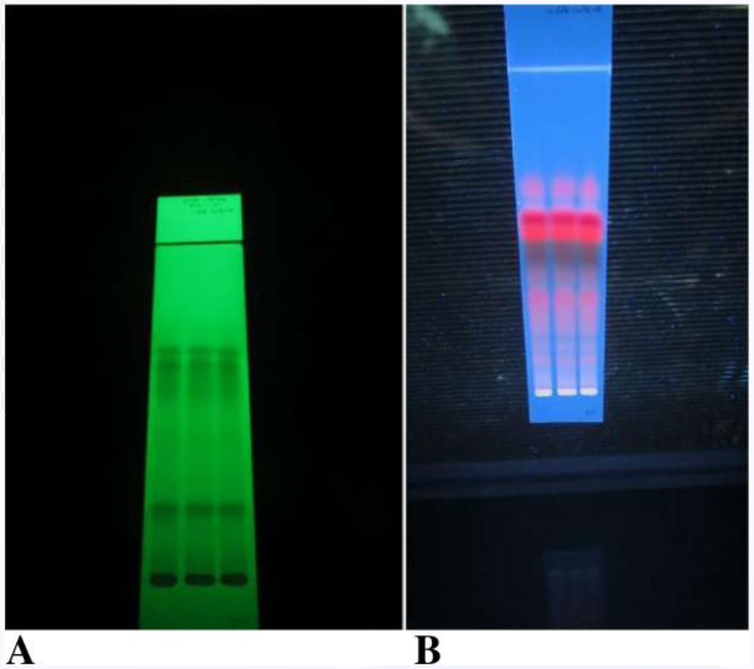
Remove the plate from the chamber once the solvent front has reached the desired height, and let it air dry completely. This step prepares the plate for visualization.



Visualization and Documentation of TLC Results

UV Light

Many compounds fluoresce under UV light, making them visible on the plate.



Iodine Staining

Iodine vapor stains most organic compounds, revealing their positions.

Advantages and Limitations of TLC

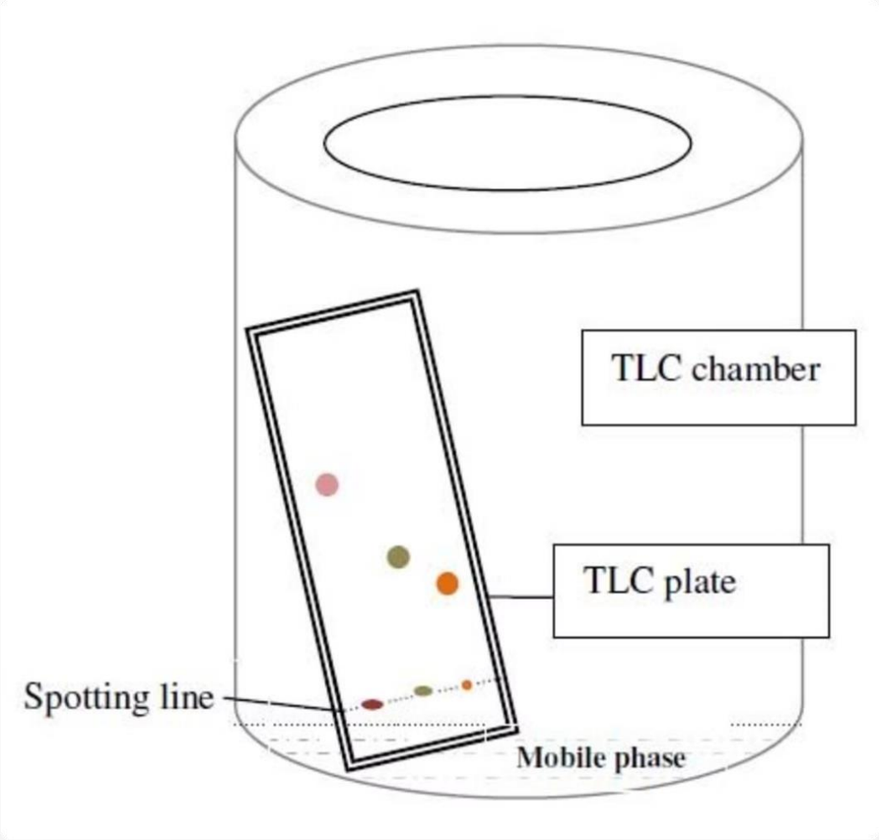
Advantages

- Simple and inexpensive
- Rapid analysis
- Requires minimal sample
- Versatile

Limitations

- Semi-quantitative
- Not suitable for volatile compounds
- Resolution can be limited

Case Studies and Real-World Applications of TLC



Pharmaceutical Analysis

Purity testing of drugs and identifying impurities.

Forensic Science

Analyzing evidence such as drugs, inks, and dyes.

Environmental Monitoring

Detecting pollutants in water and soil samples.