

Chromatography

Presented By:-
Samarpan Mishra

Contents

1. Paper Chromatography (PC)
2. Thin-Layer Chromatography (TLC)
3. Column Chromatography (CU)
4. High-Performance **Thin-Layer** Chromatography (HPTLC)
5. High-Performance **Liquid** Chromatography (HPLC)
6. Gas Chromatography (GC)

Chromatography

Chromatography is a method used to **separate and identify** the different substances present in a mixture.

1. Paper Chromatography (PC)

Definition

Paper chromatography is a simple chromatographic technique. This method used to **separate and identify** the different substances present in a mixture.

Principle

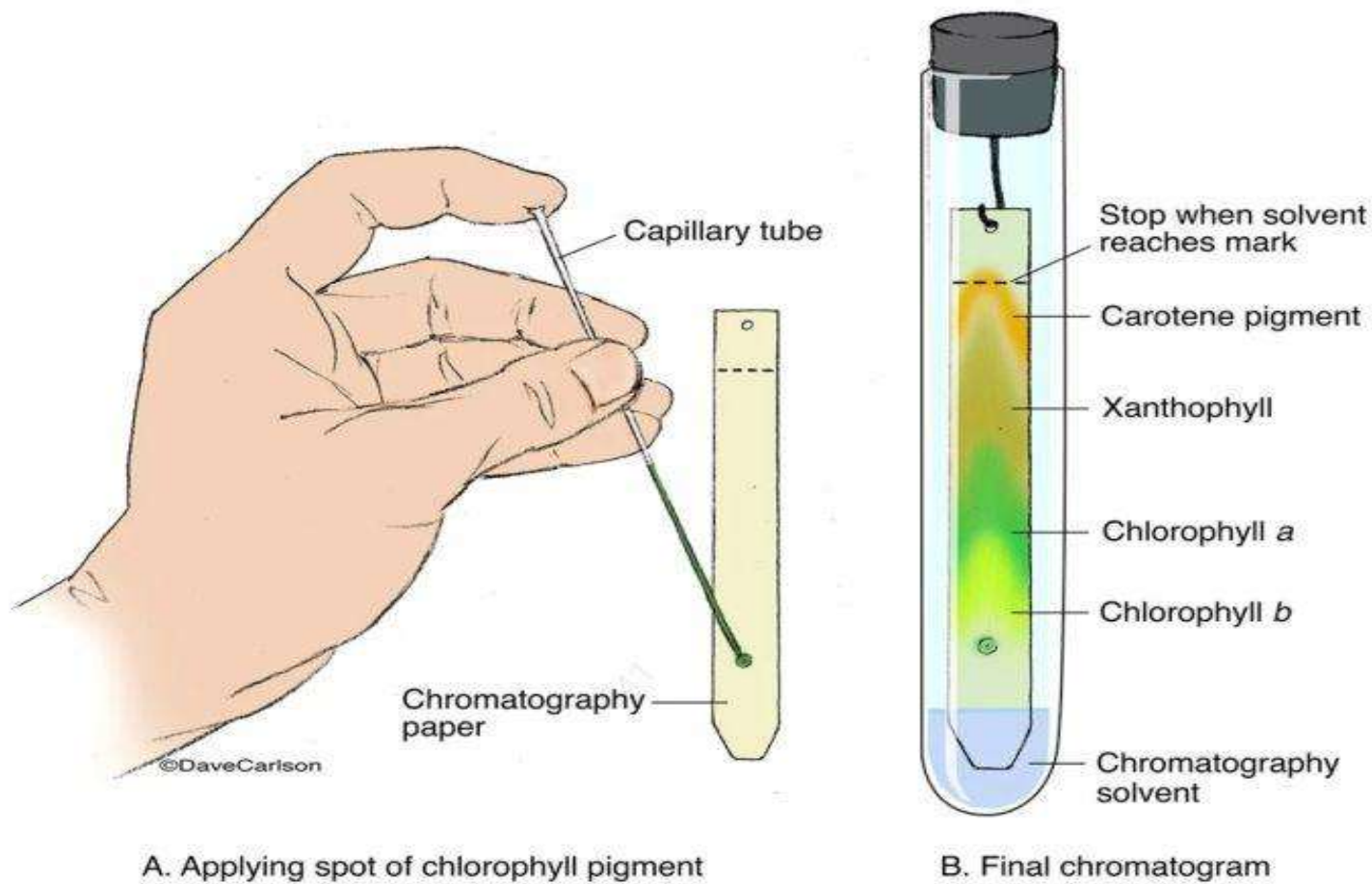
When the **solvent (mobile phase)** moves through the **paper (stationary phase)**, different components of the mixture move at **different speeds**. Therefore, they get **separated into different spots**.



Procedure

1. Take a suitable chromatography paper.
2. Draw a **baseline** with pencil near one end.
3. Apply a small spot of the sample/extract on the baseline.
4. Allow the spot to dry.
5. Place the paper in a developing chamber containing the mobile phase.
6. Ensure that the solvent level is **below the sample spot**.
7. Allow the solvent to move through the paper by capillary action.
8. Remove the paper when the solvent front reaches an appropriate distance.
9. Mark the solvent front immediately.
10. Dry the paper and detect separated spots using suitable reagents or UV light.
11. Calculate the **Rf values**.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$





Applications

1. Identification of **alkaloids, glycosides and flavonoids**
2. Separation of amino acids and sugars
3. Identification of plant constituents
4. Detection of adulteration in crude drugs
5. Preliminary phytochemical investigation

2. Thin-Layer Chromatography (TLC)

Definition

TLC is a chromatographic technique in which a **thin layer of adsorbent material**, such as silica gel, is coated on a plate to separate components of a mixture.

Principle

The **solvent (mobile phase)** moves up the **TLC plate (stationary phase)**. Different components move at **different speeds**, so they become separated into different spots.

- ❑ 🐢 **Slow-moving component:** Has **strong attraction to silica gel (stationary phase)** → stays more on the plate.
- ❑ 🏃 **Fast-moving component:** Has **weak attraction to silica gel** and greater attraction to the **solvent (mobile phase)** → moves farther up the plate.

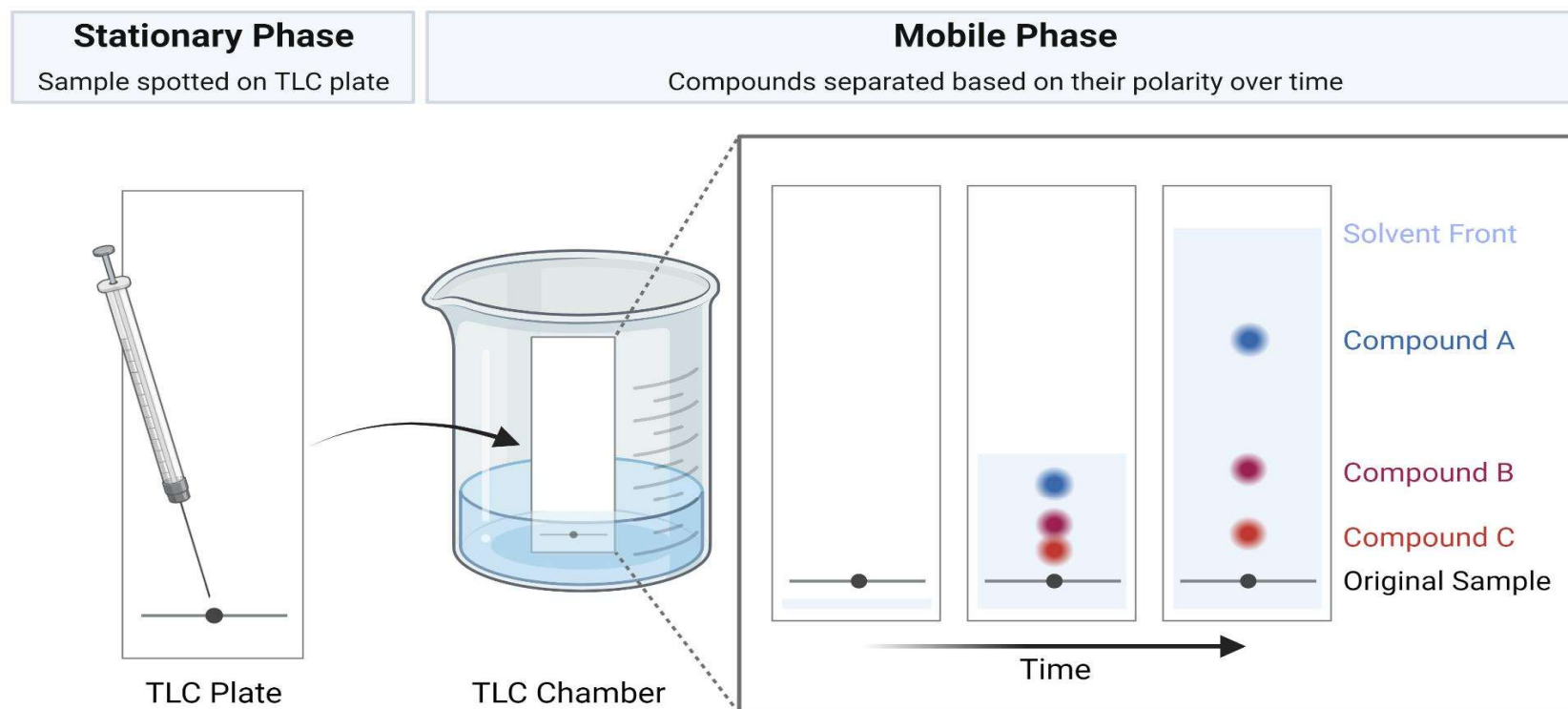


Procedure

1. Take a TLC plate coated with silica gel.
2. Activate the plate if required.
3. Draw a baseline using a pencil.
4. Apply the sample as a small spot on the baseline.
5. Place the plate in a saturated developing chamber.
6. Allow the mobile phase to move upward by capillary action.
7. Remove the plate when the solvent reaches the required height.
8. Mark the solvent front.
9. Dry the plate.
10. Visualize spots under **UV light** or by spraying suitable detecting reagents.
11. Calculate the R_f values.



Thin Layer Chromatography





Applications

1. Identification of constituents of crude drugs
2. Detection of **alkaloids, flavonoids, tannins, glycosides and terpenoids**
3. Detection of adulteration
4. Monitoring extraction and isolation
5. Purity testing
6. Fingerprinting of herbal drugs

3. Column Chromatography (CU)

Definition

Column chromatography separates the different components of a mixture using a **column packed with a stationary phase** (usually silica gel or alumina).

Principle

☞ When the mixture passes through the column, **different compounds move at different speeds** because they have different attraction toward the stationary phase.

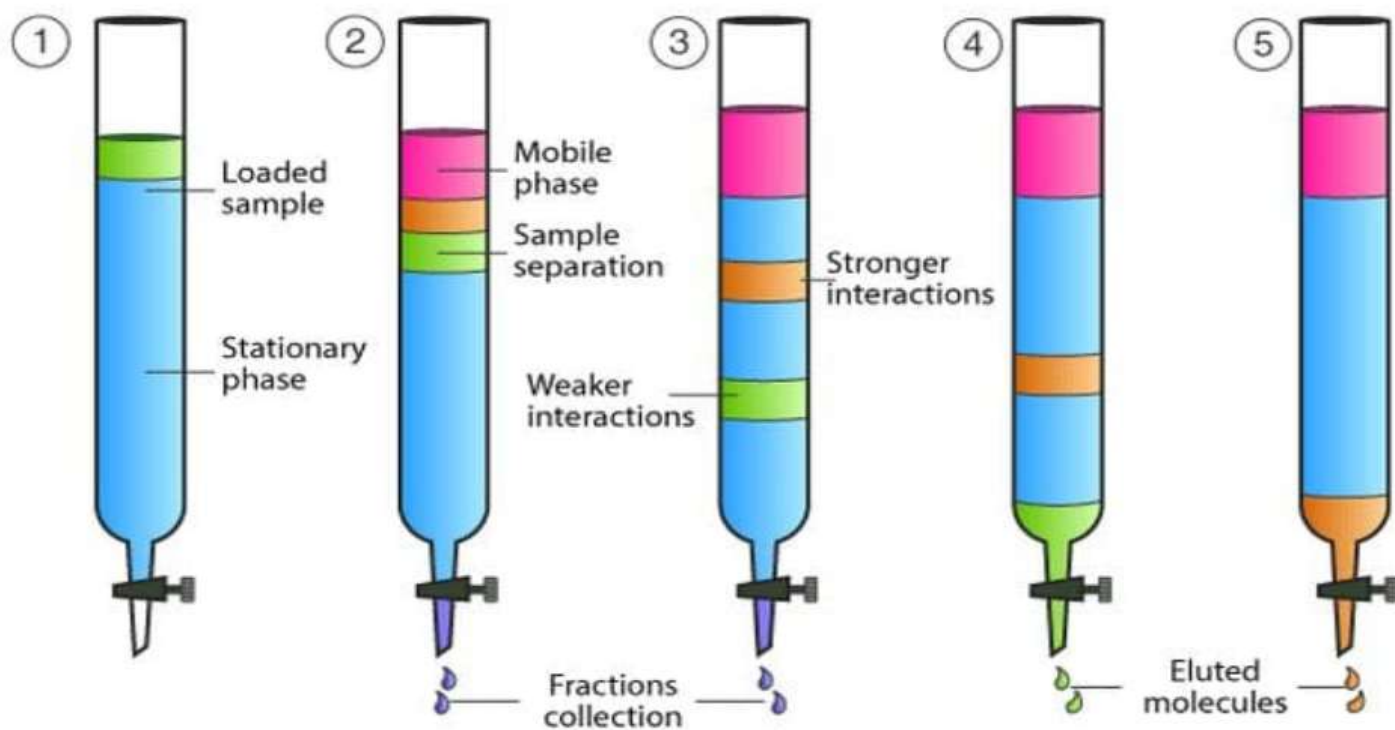
- ❑ **More attraction to stationary phase → moves slowly**
- ❑ **Less attraction to stationary phase → moves fast**
- ❑ **Therefore, the compounds come out of the column one by one, giving separation.**

Example:- Think of it like people walking through a crowded passage. Some move fast and some move slowly, so they become separated.



Procedure

1. Select a suitable chromatographic column.
2. Pack the column with the stationary phase.
3. Add the sample carefully on top of the stationary phase.
4. Add the mobile phase.
5. Allow the solvent to pass through the column.
6. Components move at different rates through the column.
7. Collect the eluate in separate fractions.
8. Analyze the fractions using TLC or another suitable method.
9. Combine fractions containing the desired compound.
10. Remove the solvent to obtain the purified compound.





Applications

1. Isolation of active constituents from crude drugs
2. Purification of alkaloids, glycosides and flavonoids
3. Separation of plant pigments
4. Isolation of essential-oil components
5. Purification of natural products
6. Preparation of samples for structural identification

4. High-Performance **Thin-Layer** Chromatography (HPTLC)

Definition

HPTLC is an **advanced form of TLC** that provides better separation, identification and quantitative analysis of substances.

Principle

In HPTLC, the sample is placed on a **special coated plate**. A **solvent (mobile phase)** moves up the plate and carries the chemicals with it.

- ▶ Chemicals that have **less attraction to the plate** → move **faster and farther**.
- ▶ Chemicals that have **more attraction to the plate** → move **slower and stay near the starting point**.



Procedure

1. Prepare the sample extract.
2. Use a precoated HPTLC plate, usually containing silica gel.
3. Apply samples accurately using an **automatic applicator**.
4. Develop the plate in a suitable mobile phase.
5. Remove the plate after development.
6. Dry the plate.
7. Detect separated compounds using UV/visible light or suitable derivatization.
8. Scan the plate using an HPTLC scanner.
9. Record peak areas and obtain a chromatographic fingerprint.
10. Compare results with reference standards.



Applications

1. Standardization of herbal medicines
2. Identification of marker compounds
3. Quantitative estimation of phytoconstituents
4. Quality control of crude drugs
5. Detection of adulteration/substitution
6. Herbal drug fingerprinting
7. Analysis of alkaloids, flavonoids, glycosides and essential-oil constituents

5. High-Performance **Liquid** Chromatography (HPLC)

Definition

HPLC is a technique used to separate, identify and measure different substances present in a mixture by using a liquid solvent under high pressure.

Principle

☞ In HPLC, a **liquid solvent (mobile phase)** is passed through a column containing a **stationary phase** under **high pressure**.

- ❑ Components that interact **less** with the stationary phase move **faster**.
- ❑ Components that interact **more** with the stationary phase move **slower**.
- ❑ Therefore, different components come out of the column at **different times**, and they are separated.

Example:- Think of the column as a **road**. Some substances travel quickly on the road, while others move slowly. This difference in speed separates them.

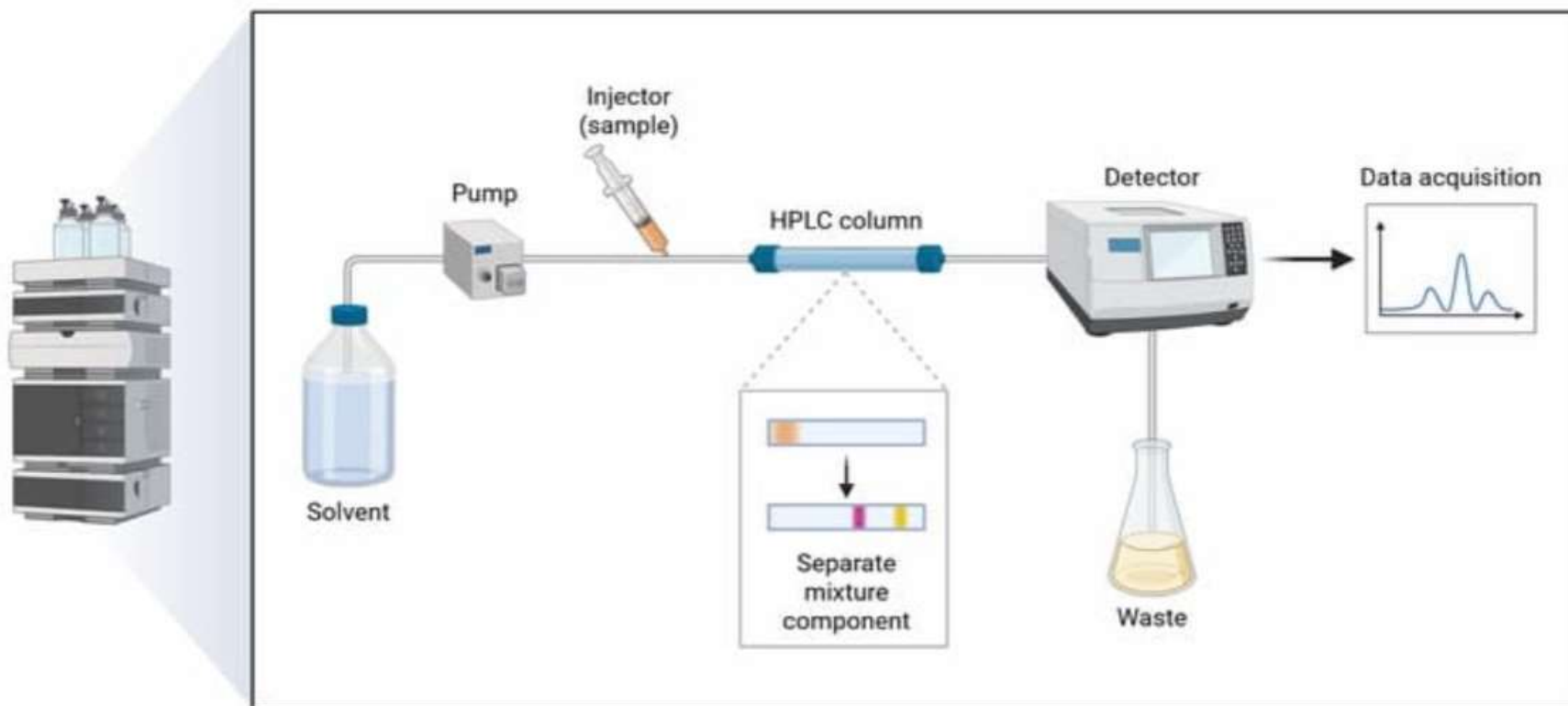


Procedure

1. Prepare the sample.
2. Prepare the mobile phase.
3. Degas the mobile phase if required.
4. Inject the sample into the HPLC system.
5. A high-pressure pump forces the mobile phase through the column.
6. Components separate inside the column.
7. Separated compounds pass through the detector.
8. The detector produces chromatographic peaks.
9. Identify compounds using retention time.
10. Quantify compounds using peak area or peak height.



High Performance Liquid Chromatography (HPLC)





Applications

1. Quantitative estimation of phytoconstituents
2. Analysis of alkaloids, flavonoids, glycosides and phenolic compounds
3. Standardization of herbal formulations
4. Quality control of crude drugs
5. Determination of marker compounds
6. Purity testing
7. Stability studies
8. Analysis of non-volatile compounds

6. Gas Chromatography (GC)

Definition

Gas chromatography is a technique used to separate and analyze **volatile substances** by using a **gas** as the mobile phase.

Principle

Gas Chromatography separates the different substances in a mixture **based on how fast they move through a column with a gas.**

Example:- Imagine 3 students running through a corridor:

- ❑ Student A 🏃 → Fast → reaches first
- ❑ Student B 🚶 → Medium → reaches second
- ❑ Student C 🐢 → Slow → reaches last

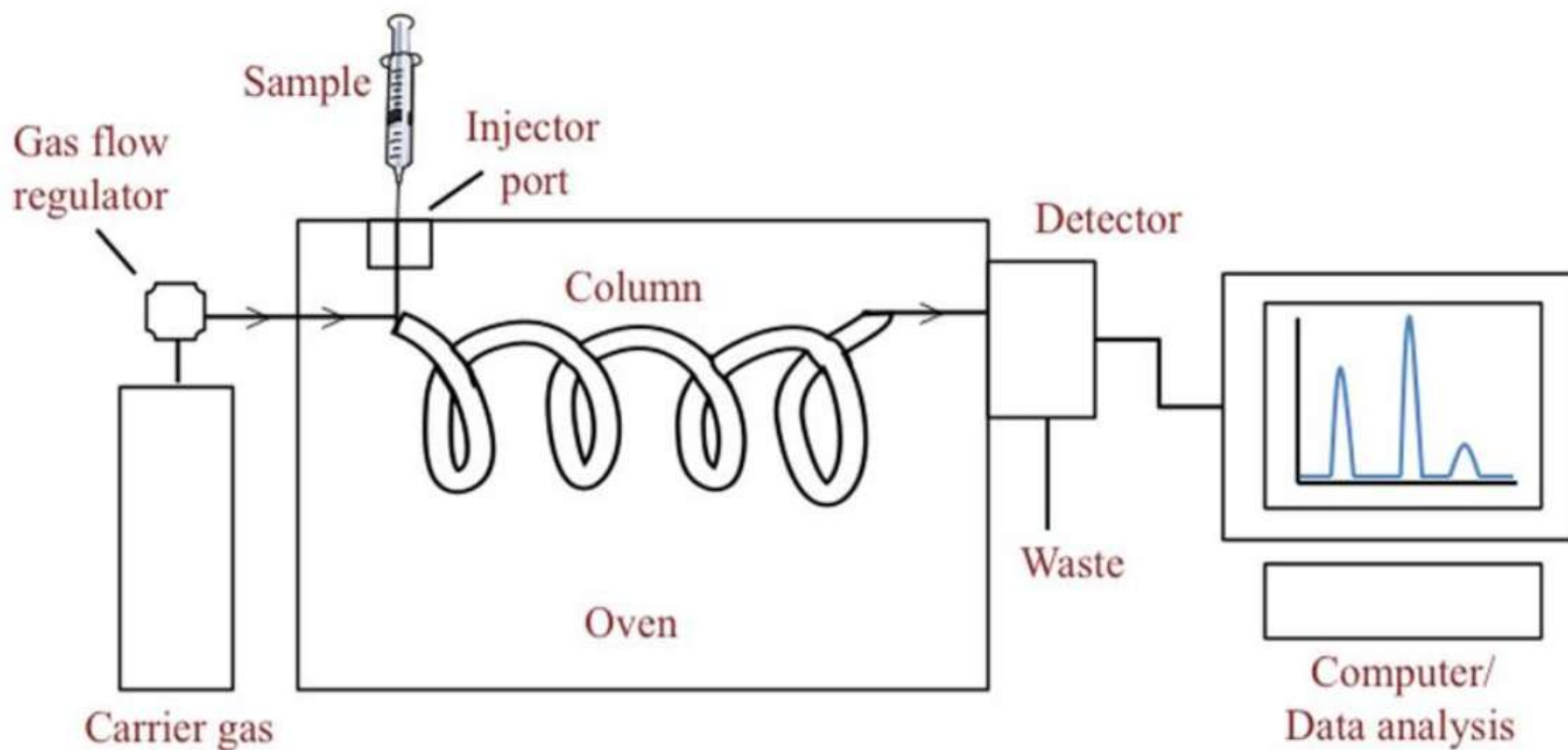


Procedure

1. Prepare a sample.
2. Inject the sample into the heated injector.
3. The sample is vaporized.
4. An inert carrier gas transports the vaporized sample into the column.
5. Components separate according to their volatility and interaction with the stationary phase.
6. Separated components reach the detector at different times.
7. The detector generates signals that appear as peaks on the chromatogram.
8. Identify compounds using their retention times and/or reference standards.
9. Quantify compounds from peak areas.



Gas Chromatography





Applications

1. Analysis of **volatile oils**
2. Identification of essential-oil constituents
3. Analysis of terpenes and hydrocarbons
4. Detection of volatile adulterants
5. Quality control of aromatic and volatile crude drugs
6. Analysis of residual solvents
7. Quantitative analysis of volatile phytoconstituents



Thank you

www.pharmrecord.com