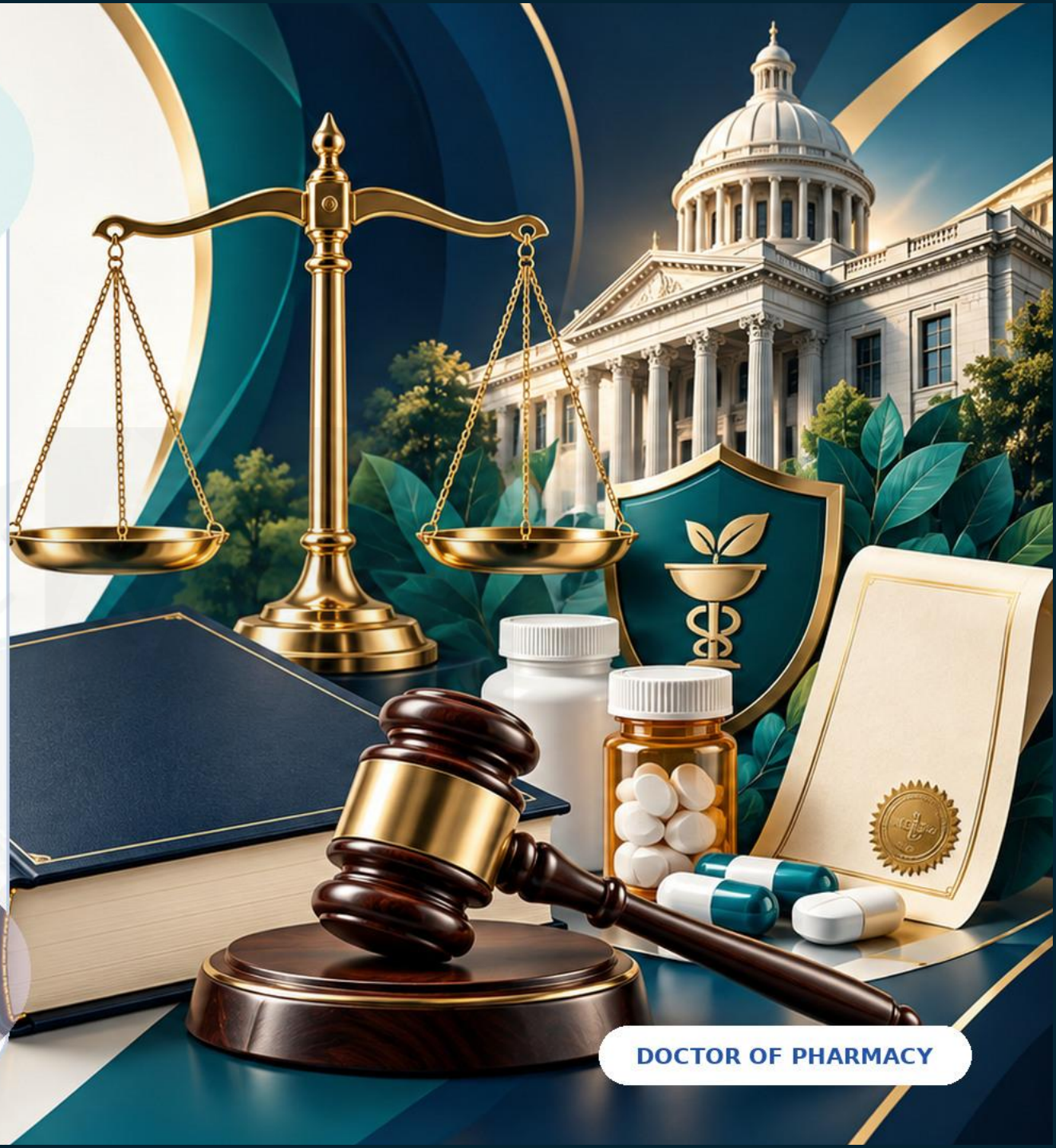
PHARM.D • YEAR 3

Pharmaceutical Analysis

CHAPTER 2

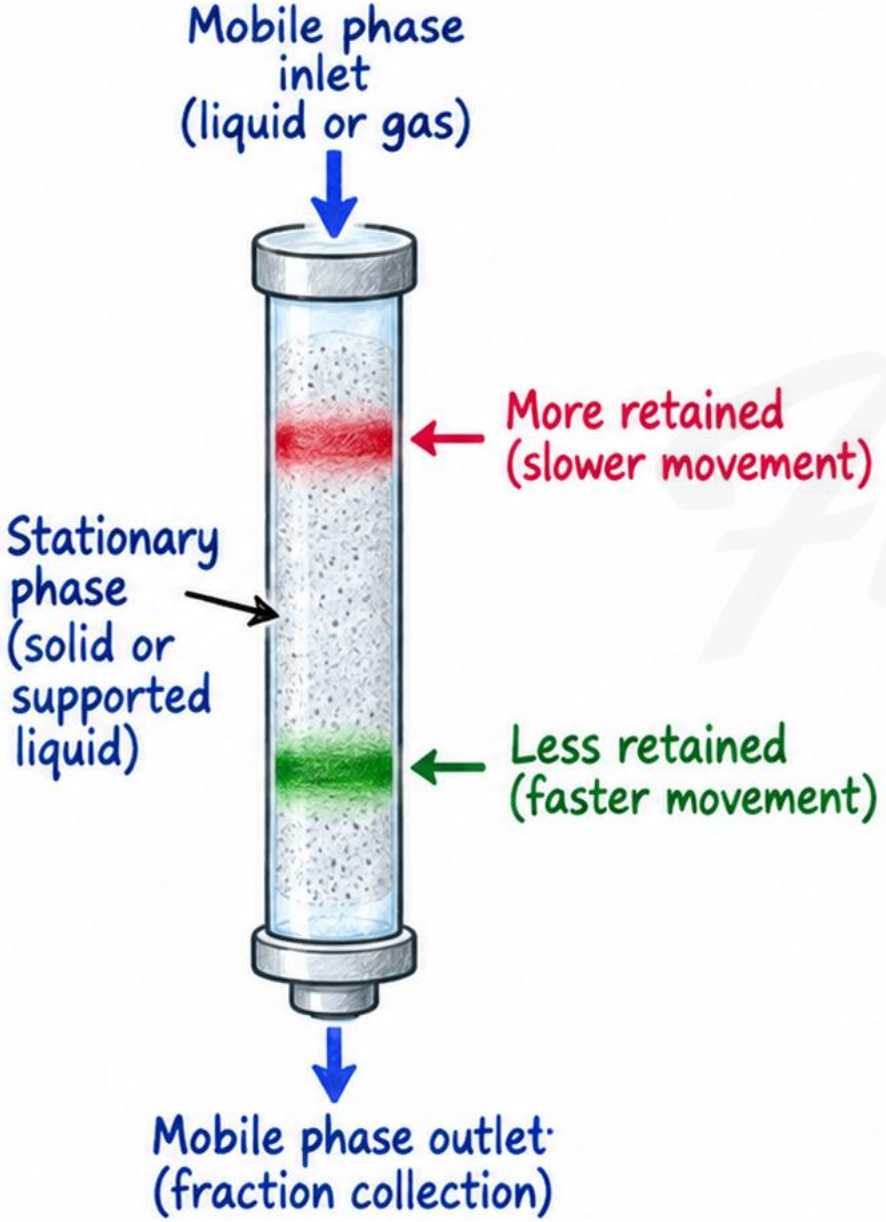
# Chromatography

PPT CHAPTER COVER



DOCTOR OF PHARMACY

# CHROMATOGRAPHY: BASIC PRINCIPLE




## Principle

- Chromatography separates components because they interact differently with a stationary phase and a moving mobile phase.
- A component retained more strongly spends longer in the stationary phase and migrates more slowly.
- A component favored by the mobile phase moves faster.

## Phases

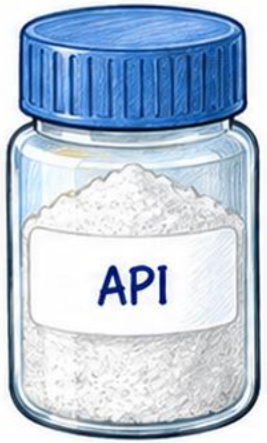
- The stationary phase may be a solid or a supported liquid.
- The mobile phase may be a liquid or gas.

## Applications

- Separation can support identification, purification and quantitative measurement.

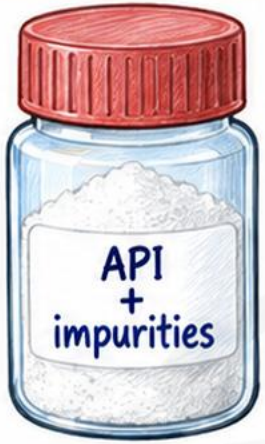
# PHARMACEUTICAL USES OF CHROMATOGRAPHY

## API assay

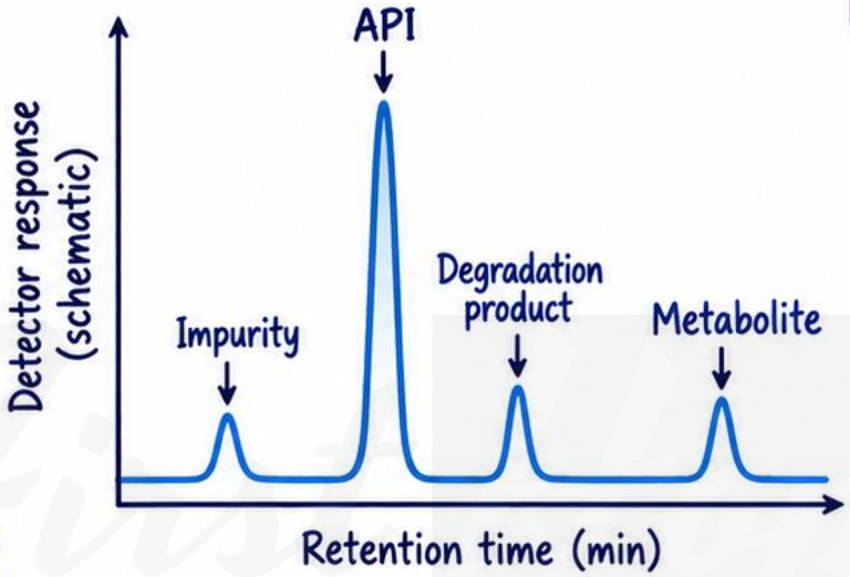



Drug substance assay

## Impurity test




Detection of impurities or degradation products




## Stability sample




Assessment of degradation products

## Biological sample




Measurement of drugs and metabolites in pharmacokinetic or bioavailability studies

## ROLE AND APPLICATIONS

- Chromatographic methods support API assays, purity assessment and detection of impurities or degradation products.
- They also help measure drugs and metabolites in pharmacokinetic or bioavailability studies.

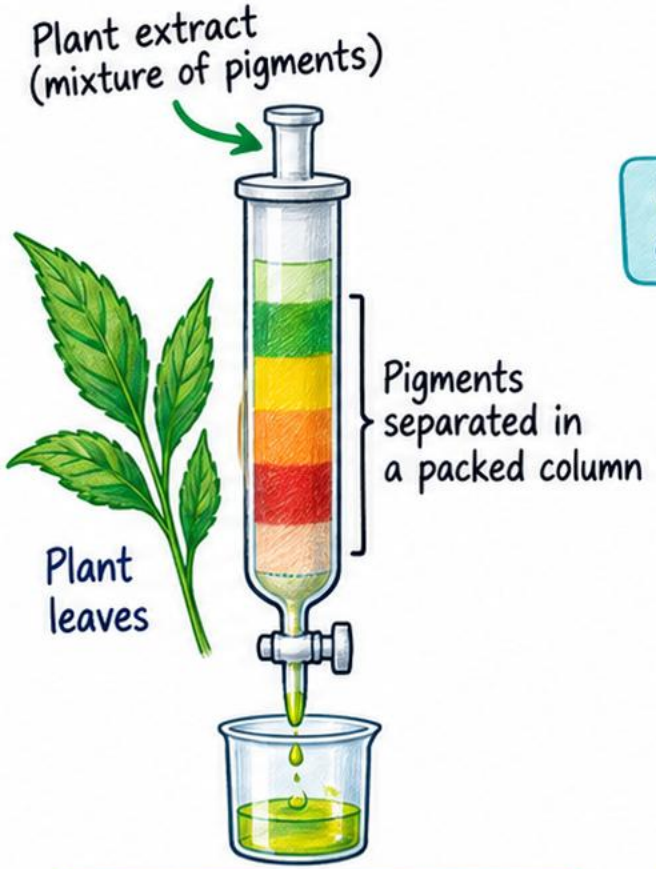
## QUANTITATION AND SAMPLE PREPARATION

- A suitable detector and calibrated response enable quantitation.
- Sample preparation must recover the analyte and control matrix interference.

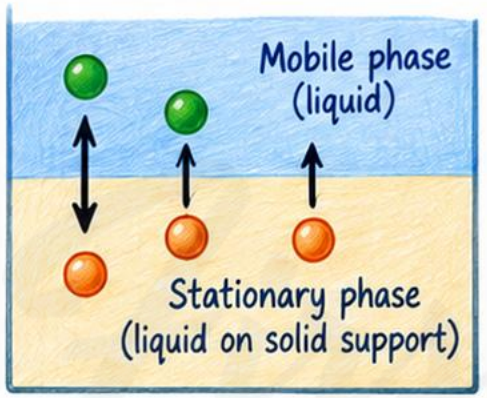
## METHOD CONSIDERATIONS AND IDENTIFICATION

- The method should match its purpose and provide sufficient selectivity.
- A separated peak or spot gives analytical evidence, but identification may require comparison with a reference and additional information.

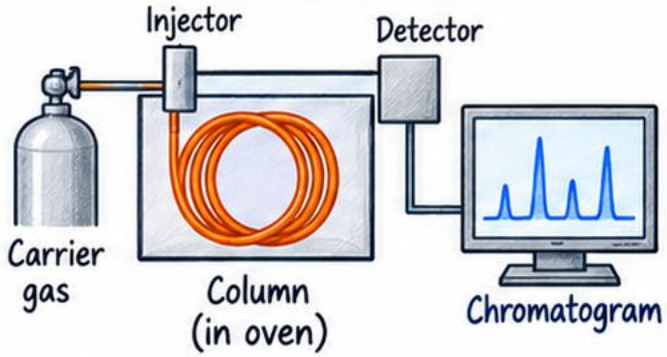
# DEVELOPMENT OF CHROMATOGRAPHY



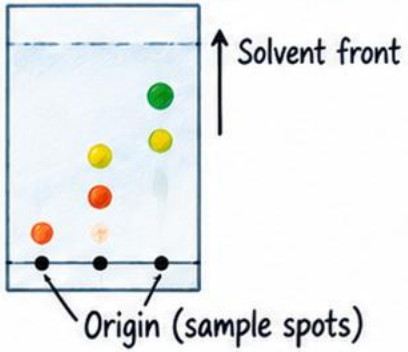

**Partition chromatography concept (Martin and Synge)**




**Gas chromatography (GC)**




**Thin-layer chromatography (TLC)**




## 1. Mikhail Tsvet's early work

- Mikhail Tsvet's early work separated plant pigments using a packed column and helped establish chromatography.

## 2. Martin and Synge

- Martin and Synge developed partition chromatography, which expanded the theoretical basis of separation.

## 3. Gas chromatography and thin-layer chromatography

- Gas chromatography and thin-layer chromatography later broadened practical applications.

## 4. High-performance liquid chromatography (HPLC)

- High-performance liquid chromatography increased the capability for reproducible pharmaceutical analysis.

## 5. Capillary electrophoresis

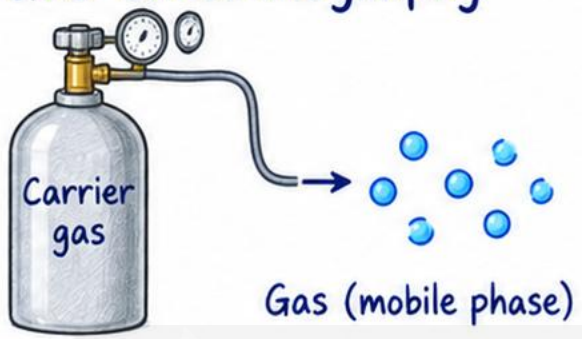
- Capillary electrophoresis is a related separation technique based on an electric field rather than ordinary chromatographic partitioning.

# CLASSIFICATION BY PHASE AND FORMAT

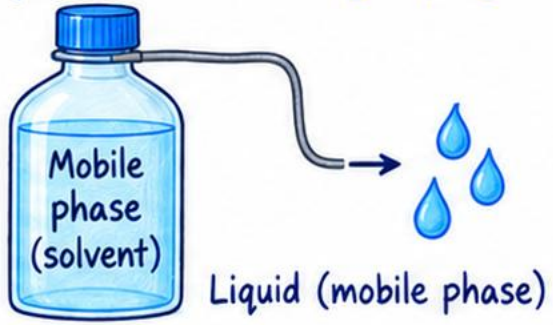
## Mobile phase:

- Gas chromatography uses a gas, while liquid chromatography uses a liquid.

Gas chromatography



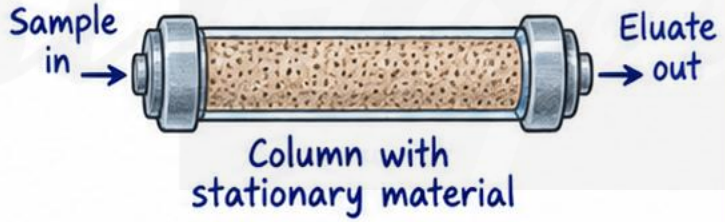

Liquid chromatography



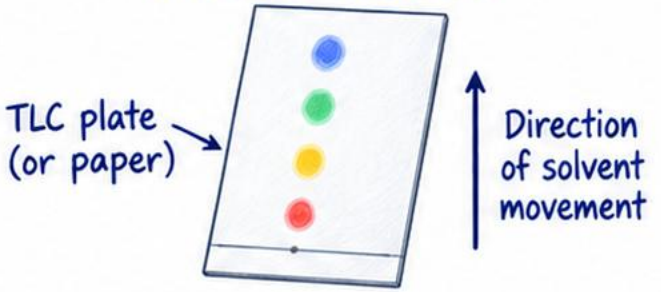

## Physical format:

- Column methods contain stationary material in a tube, while planar methods use a flat support such as a TLC plate or paper.

Column method



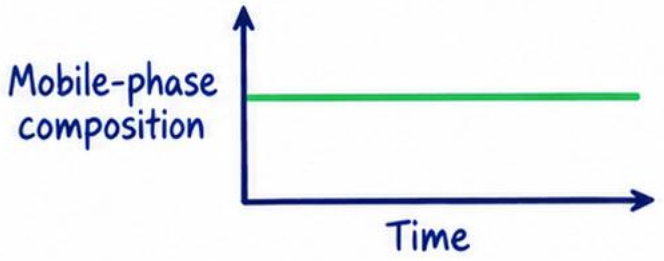

Planar method



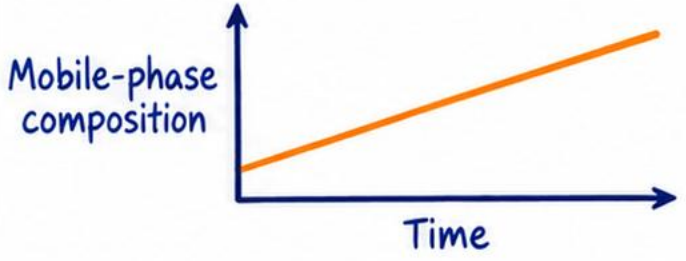

## Elution mode:

- Isocratic elution keeps the mobile-phase composition constant.
- Gradient elution changes composition during the separation.

Isocratic elution




Gradient elution




These are different classification dimensions, so a single method can fit several categories at once.

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