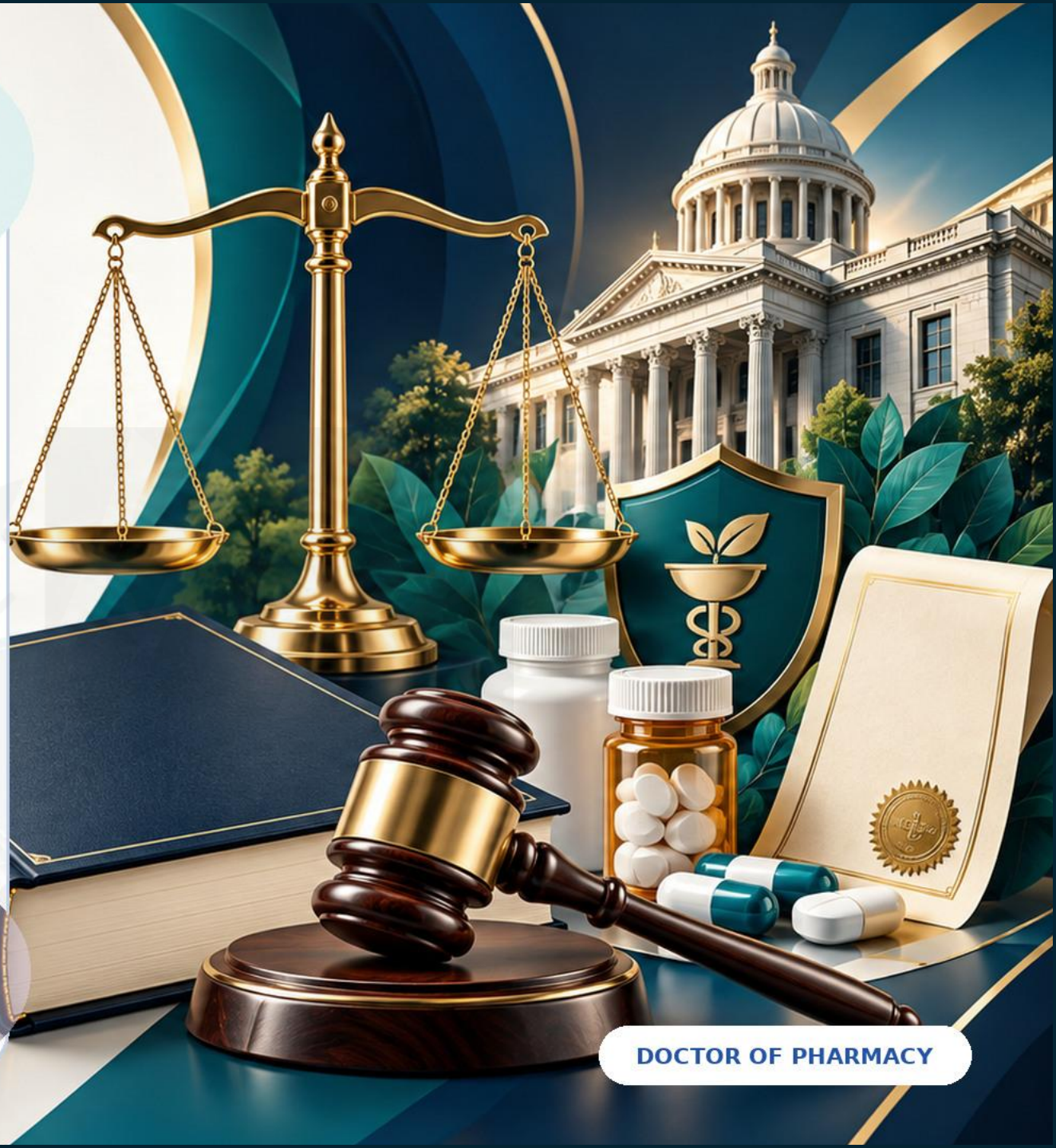
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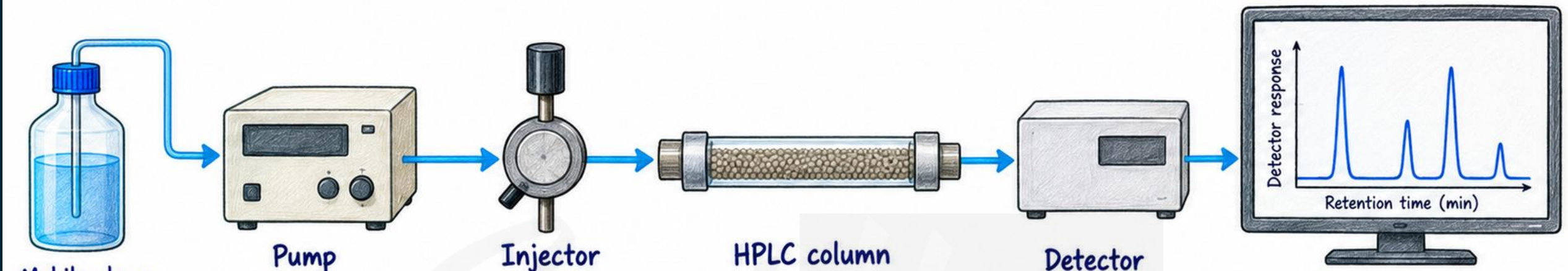
CHAPTER 2.5

# High Performance Liquid Chromatography (HPLC)

PPT CHAPTER COVER



# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY




**Mobile phase**
(solvent reservoir)

**Pump**
Moves the mobile phase through the system at a controlled flow rate.

**Injector**
Introduces a small volume of sample into the mobile phase.

**HPLC column**
Contains small stationary-phase particles.

**Detector**
Measures components as they elute from the column.

**Chromatogram**
Each component gives a peak at a characteristic retention time.

## Principle and operation

- HPLC uses controlled liquid flow through an efficient column to separate dissolved components.
- A pump moves the mobile phase through small stationary-phase particles.
- Different interactions give different retention times.
- HPLC can use reversed-phase, normal-phase, ion-exchange or size-exclusion mechanisms.

## Applications

- It supports API assays, impurity analysis, dissolution measurements and stability studies.

## Performance requirements

- The separation and detector must provide suitable performance for the intended measurement.

# RETENTION, EFFICIENCY AND RESOLUTION

## Retention time

- Retention time is the interval from injection to a peak's elution. Compare it under controlled conditions, rather than treating it as unique proof of identity.

## Theoretical plate number

- Theoretical plate number describes column efficiency.

## Selectivity

- Selectivity describes relative retention between components.

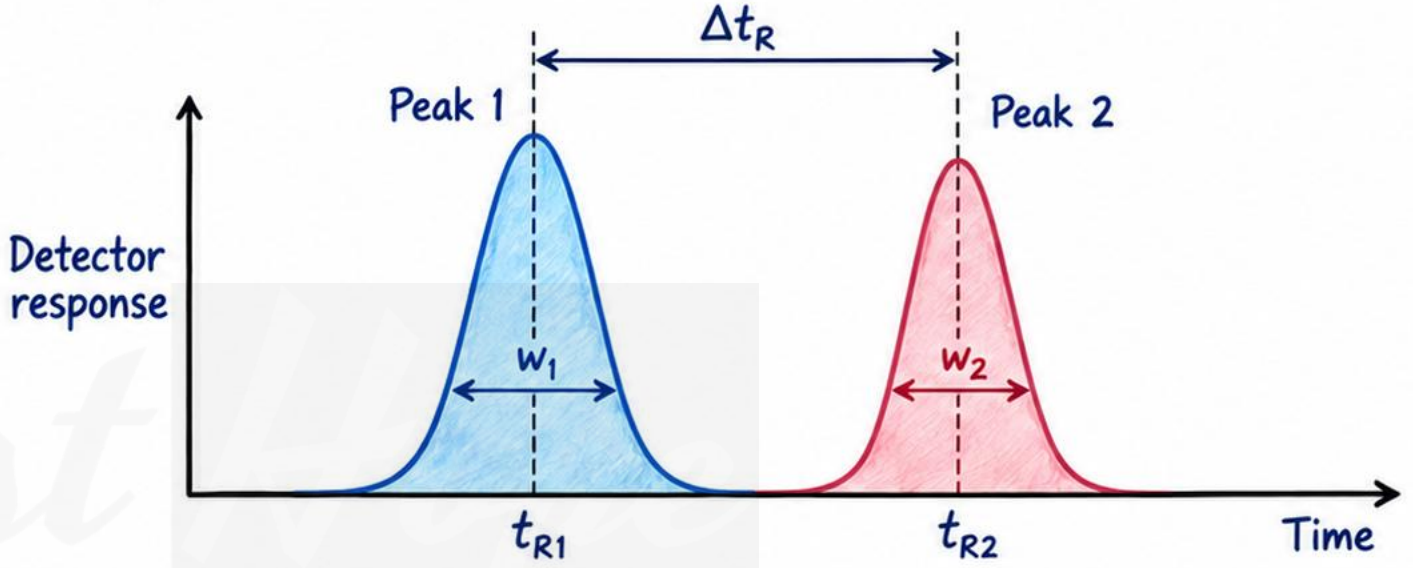
## Resolution

- Resolution describes how well adjacent peaks separate and also depends on peak width. High plate number alone does not guarantee adequate resolution.

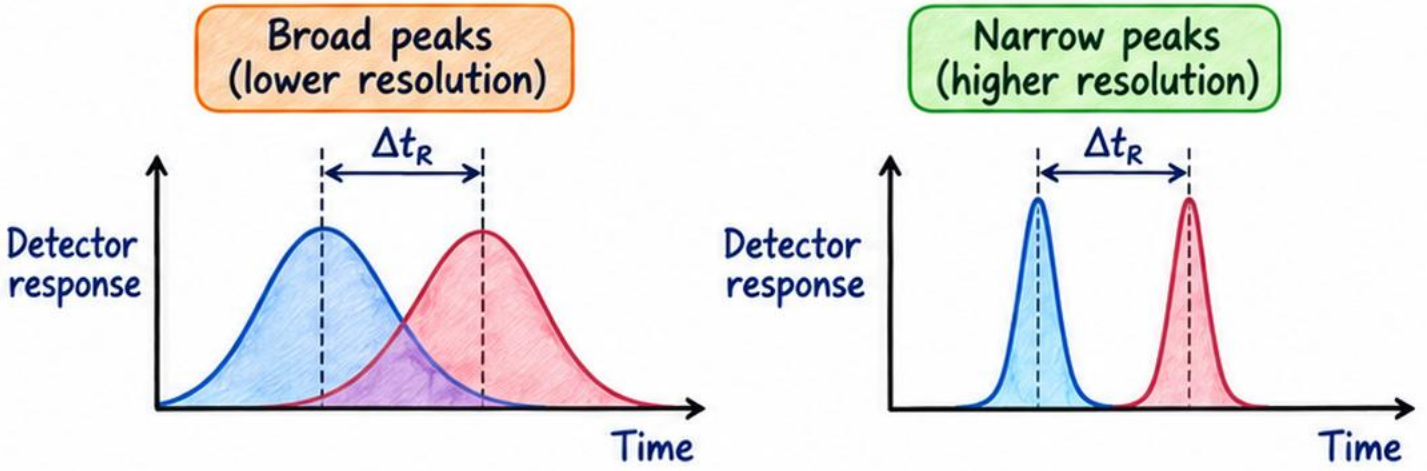
## Peak area

- Peak area can support quantitation after appropriate calibration and integration.

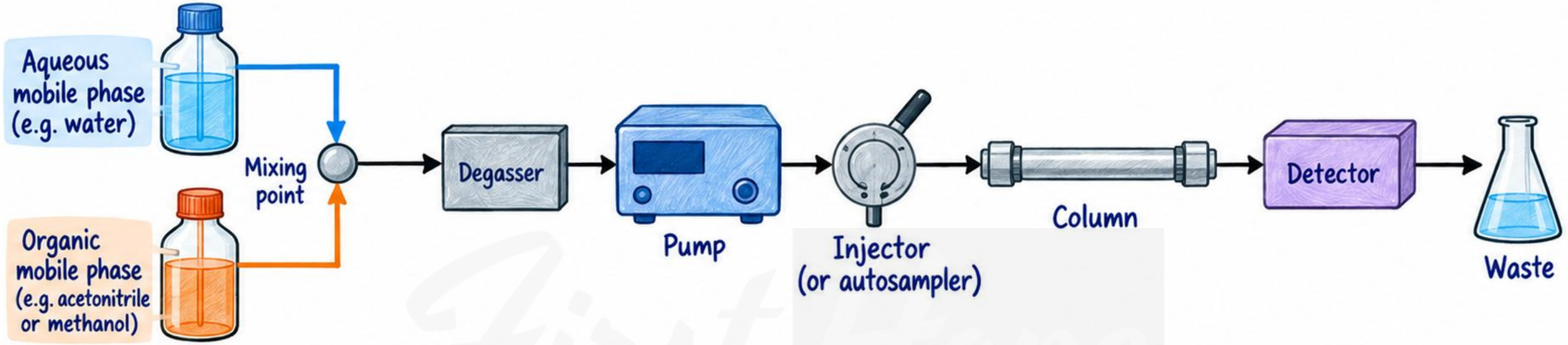
### Chromatogram with retention times, peak widths and resolution




### Effect of peak width on resolution (same retention-time gap, different Rs)



# MOBILE PHASE, PUMP AND INJECTION




## Mobile phases and reservoirs

- Reservoirs hold suitable aqueous and organic mobile phases, often including water, acetonitrile or methanol.
- Filter and degas as appropriate for the method.

## Pump operation

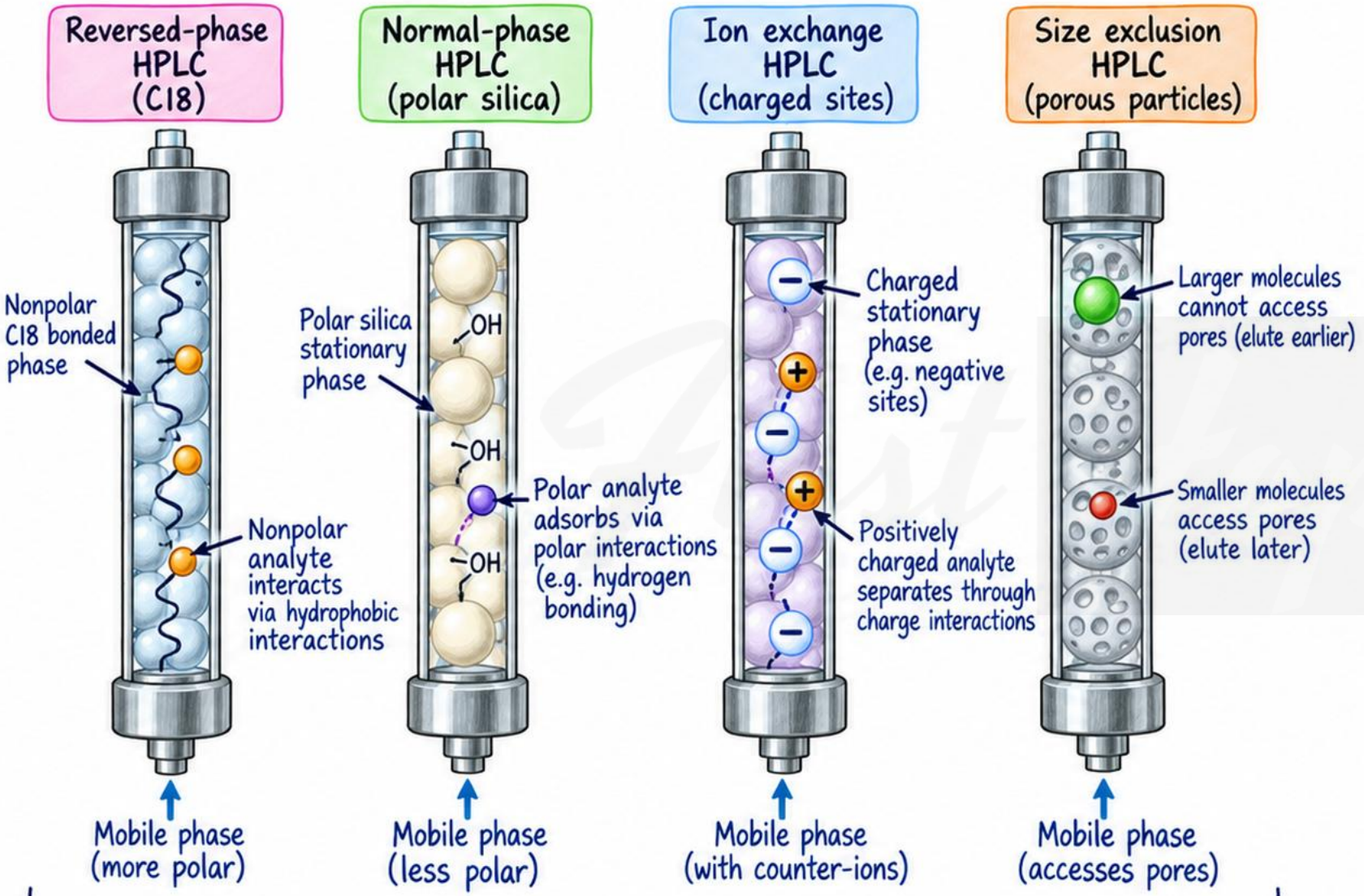
- The pump delivers controlled flow against column resistance.
- Isocratic operation keeps composition constant; gradient operation changes it during the run.

## Injection

- An injector or autosampler introduces a defined sample volume.

**Example conditions (from PDF)**
1–2 mL/min and 5–100 µL as conventional examples, with actual values depending on the system.

# HPLC COLUMNS AND SEPARATION MODES




Conventional examples: 150 – 250 mm length, 4.6 mm internal diameter

Modern dimensions and particle sizes vary with the method.

## Reversed-phase HPLC

- Reversed-phase HPLC commonly uses a relatively nonpolar bonded phase such as C18 and a more polar mobile phase.

## Normal-phase HPLC

- Normal-phase HPLC uses a polar stationary phase and less polar solvents.

## Ion exchange

- Ion exchange separates through charge interactions.

## Size exclusion

- Size exclusion separates by access to pores and hydrodynamic size.

## Column dimensions

- The PDF describes conventional columns about 150–250 mm long with 4.6 mm internal diameter.
- Modern dimensions and particle sizes vary with the method.

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