APPLICATION OF HPLC in PHARMACEUTICAL ANALYSIS

Lecture by

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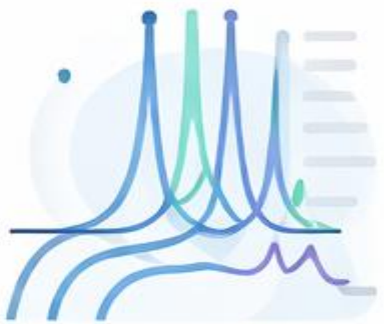
A comprehensive guide to high-performance liquid chromatography



Principles

Understand the fundamental concepts of HPLC.

Components

Explore the key instruments and their functions.

Modes of operation

Learn how different separation modes are achieved.

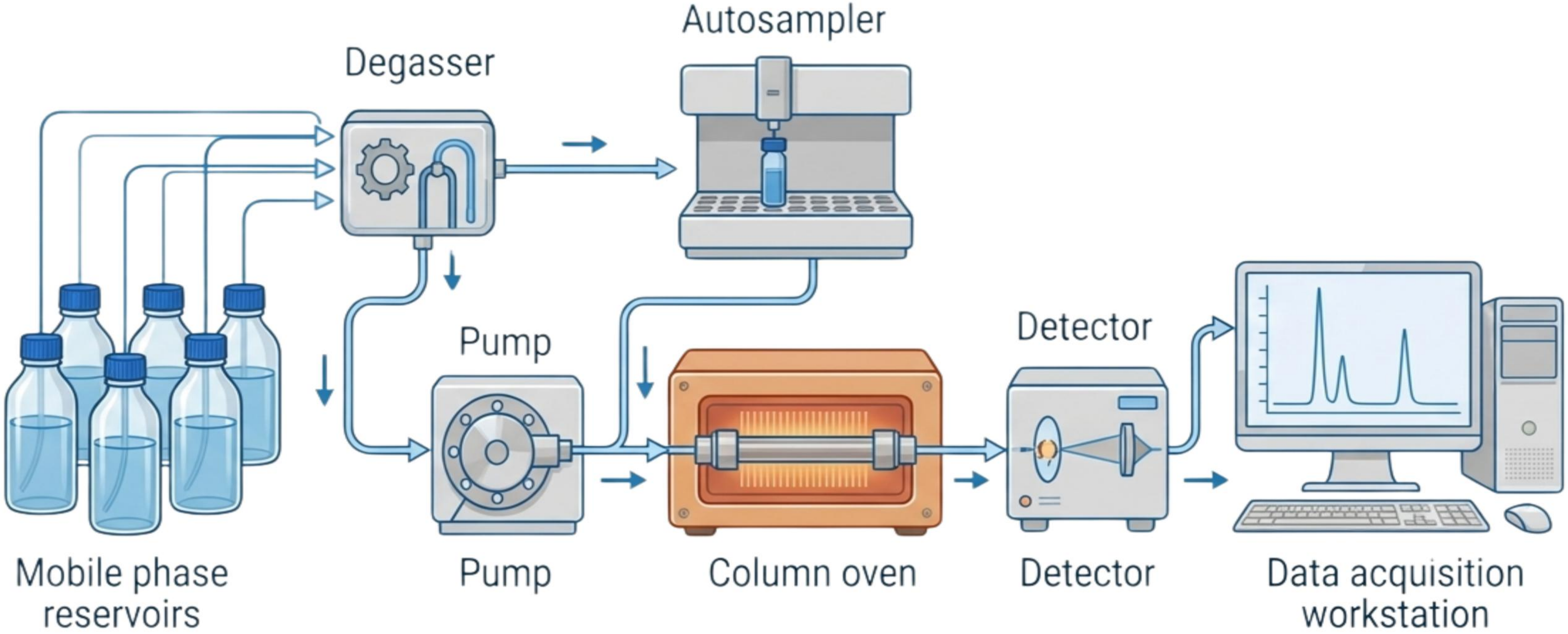
Critical applications in pharmaceutical science.

See how HPLC supports drug development, quality control, and regulatory compliance.

PHARMACEUTICAL ANALYSIS

ANALYTICAL CHEMISTRY

A MODERN HPLC SYSTEM AT A GLANCE



All working in concert to deliver accurate, reproducible separations.

Introduction to

High-Performance Liquid Chromatography

A powerful analytical technique for separating, identifying, and quantifying complex mixtures with precision.

CORE ANALYTICAL CAPABILITIES

A powerful tool for separating, identifying, and quantifying complex mixtures dissolved in liquid.

EXCEPTIONAL SENSITIVITY

Detects and analyzes compounds at parts per million (ppm) concentrations.

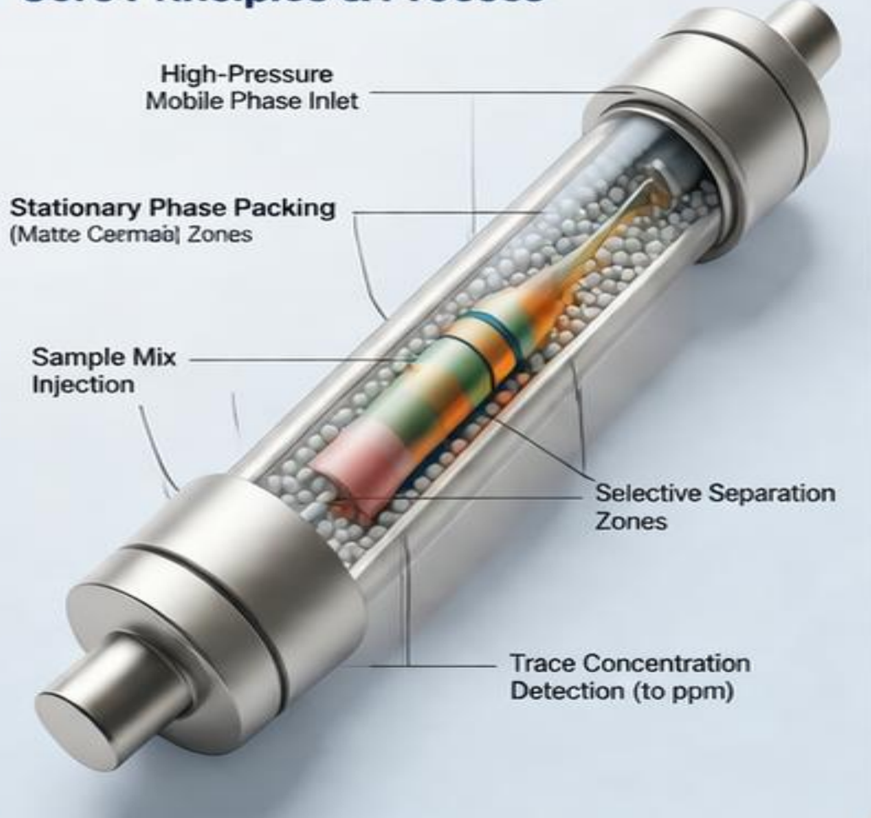
SYSTEM EVOLUTION

Tracing the advancement from basic high-pressure liquid chromatorgaphy to modern, automated HPLC instruments.

DIVERSE APPLICATION SPECTRUM

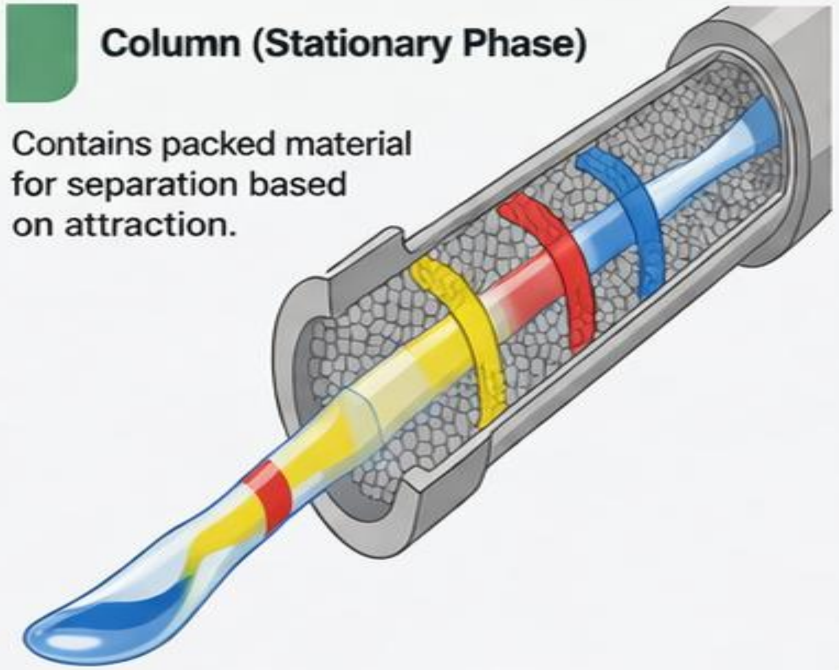
Critical across pharmacenticals, food, cosmetics, environmental science, forensics, and industrial testing.

Core Principles & Process

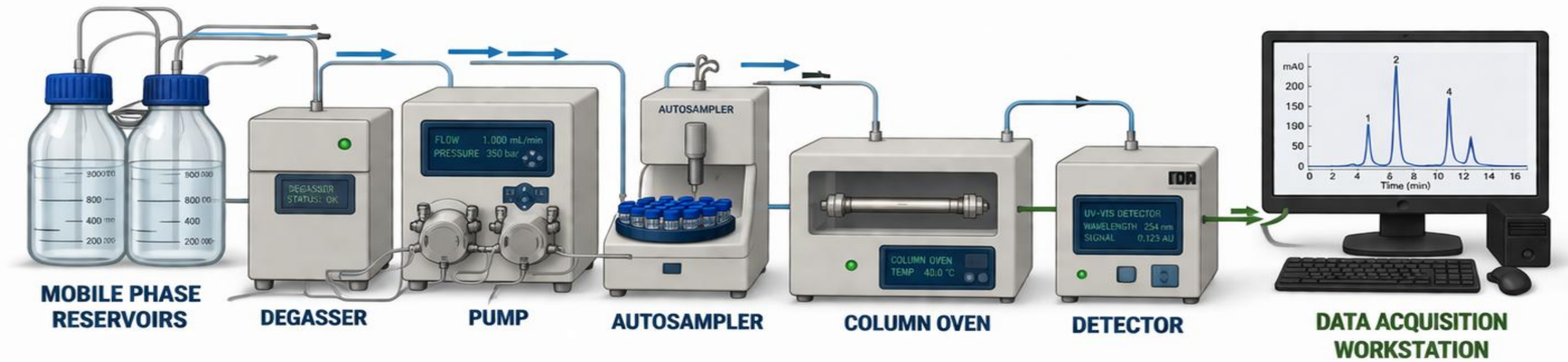


Column (Stationary Phase)

Contains packed material for separation based on attraction.



HPLC Components: The Flow Path



Mobile Phase Reservoirs

Bottle-like containers that hold the solvent (or mixture of solvents) used to carry the sample through the system.

Degasser

A critical component that removes dissolved gasses from the mobile phase to prevent bubbles, ensuring pump stability and consistent flow rates.

Pump

This module is responsible for delivering the mobile phase at a constant, high pressure and flow rate throughout the entire system.

Autosampler

An automated device that precisely injects a specified volume of the sample solutions, into tha high-pressure mobile phase stream.

Column Oven

This component houses the chromatgraphy column, the heart of the separation process, maintaning it at a stable and precise temperature.

Detector

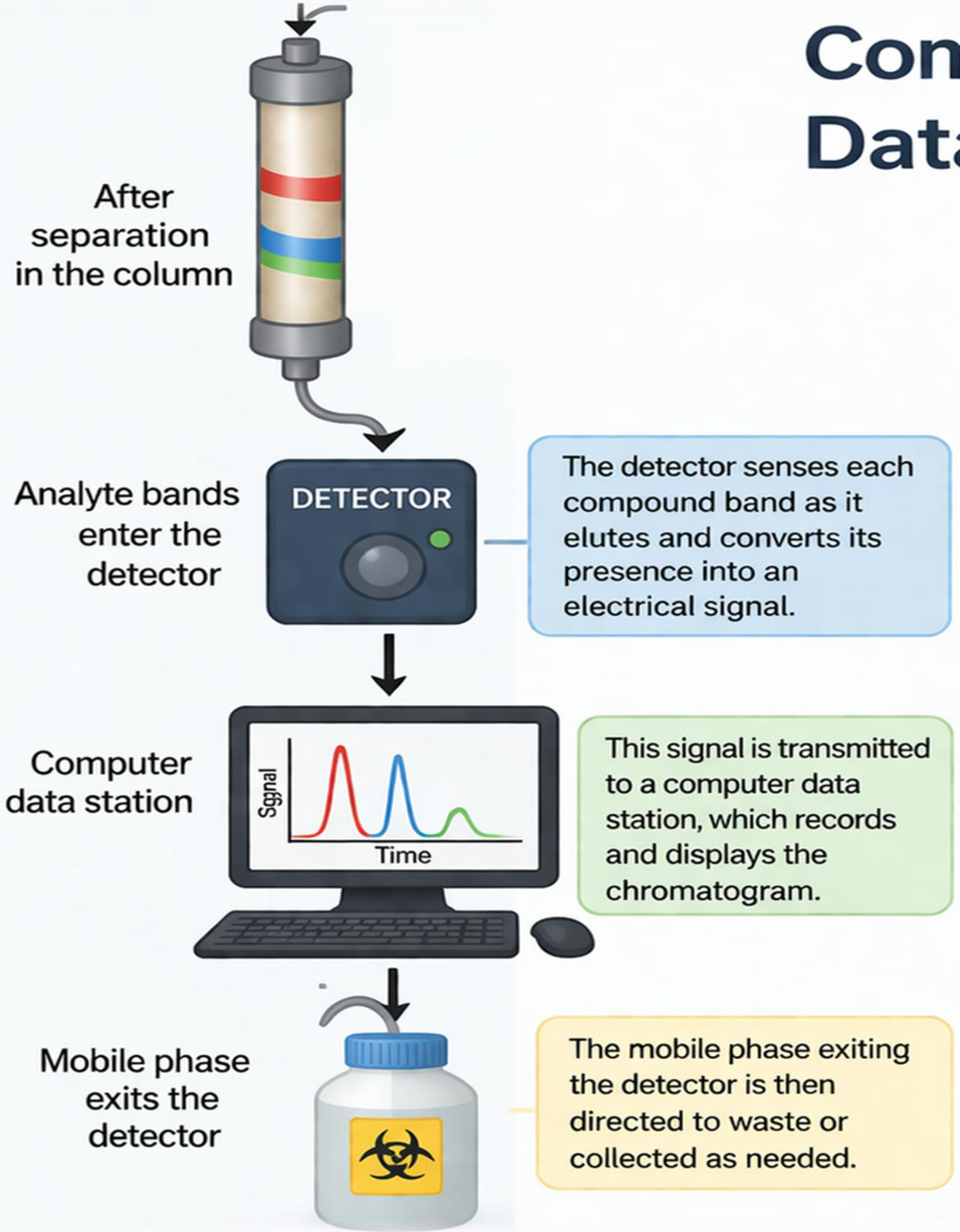
As the separated components leave the column, the detector measures the moncentration or mass of each component. Common detectors include UV-Vis or mass spectrometers.

Data Acquisition Workstation

A computer with specialized software that controls all the system's modules, recording the signal from the detector and processing it to generate a chromatogram, which is then used to quantify the sample components.

The mobile phase flows from the reservoirs through the degasser, pump, autosampler, column oven (containing the column), and detector. The data acquisition workstation controls the system and converts the detector signal into a chromatogram for analysis and quantification.

Components: Detection & Data Acquisition



After separation in the column, the mobile phase carries analyte bands into the **detector**. Since most compounds are colorless and invisible to the naked eye, the detector senses each compound band as it elutes and converts its presence into an electrical signal.

This signal is transmitted to a **computer data station**, which records and displays the chromatogram.

The mobile phase exiting the detector is then directed to waste or collected as needed.

Types of HPLC Detectors

UV-Absorbance Detector

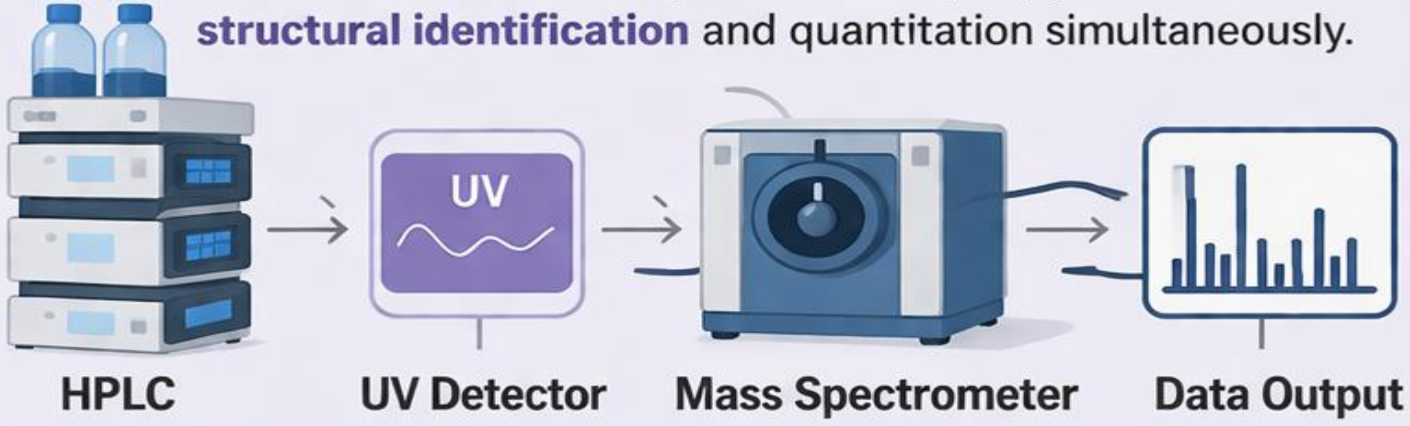
The most common detector. Used when the analyte absorbs **ultraviolet or visible light.** Highly sensitive and suitable for a broad range of pharmaceutical compounds.

Fluorescence Detector

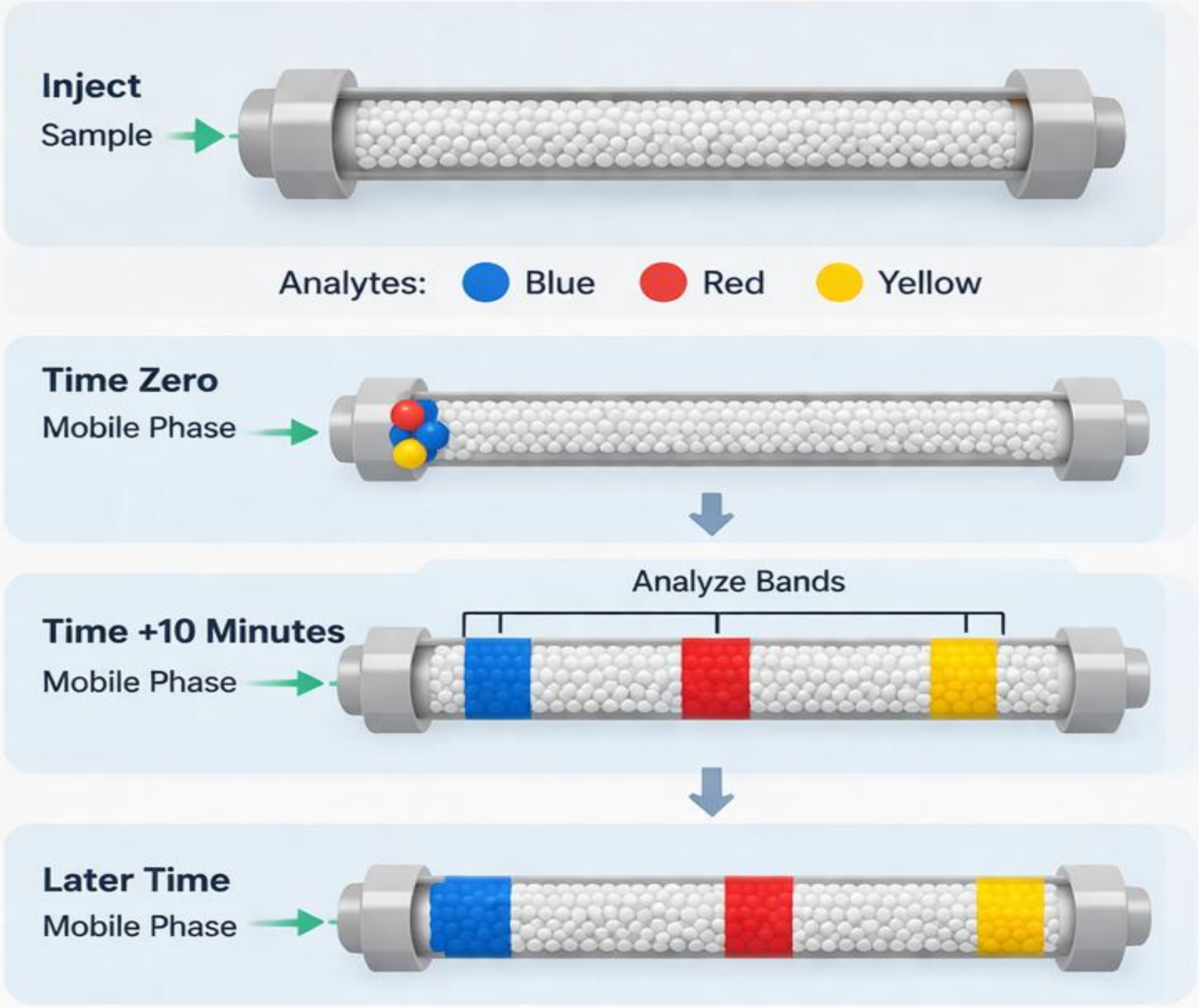
Used for compounds that naturally **fluoresce** or can be derivatized to do so. Offers exceptional sensitivity and selectivity for trace-level analysis.

LC/MS (Mass Spectrometer)

The most powerful approach — coupling UV detection **in series** with a mass spectrometer (MS) provides both **structural identification** and quantitation simultaneously.



How Does HPLC Work?



Compounds migrate at different speeds based on their **relative affinities** for the mobile and stationary phases.

Separation occurs because of a **competition** between the mobile phase and stationary phase for each analyte. As the mobile phase flows through the packed column, individual compounds migrate at different speeds based on their relative affinities:

Yellow Dye — Fastest

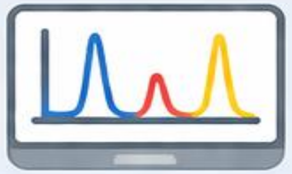
Preferentially attracted to the **mobile phase**; moves rapidly and elutes first.

Red Dye — Intermediate

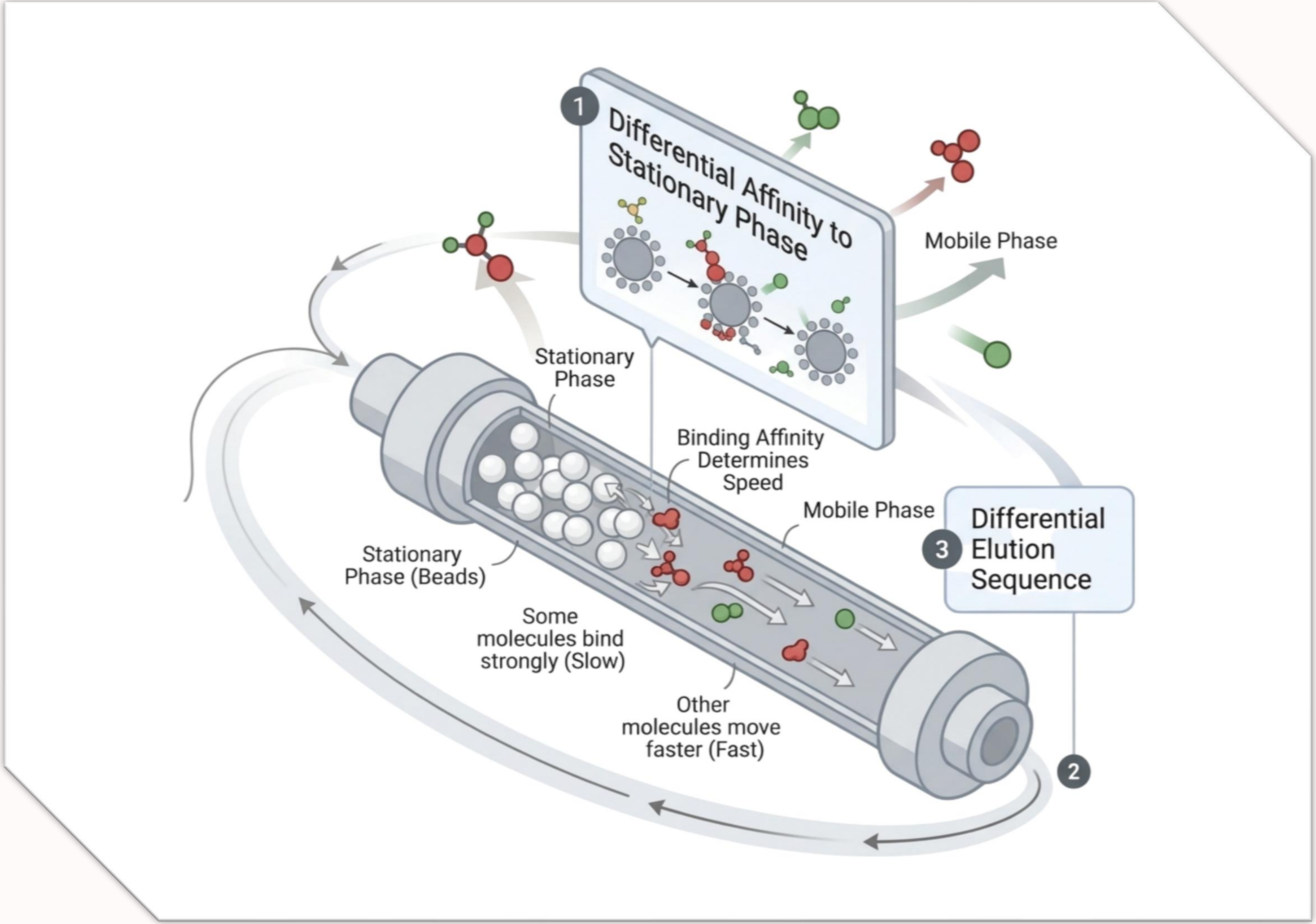
Balanced attraction to both phases; travels at an **intermediate speed** through the column.

Blue Dye — Slowest

Strongly attracted to the **stationary phase**; most retained compound and elutes last.



The detector records the eluted compounds as peaks in a **chromatogram** in order of their elution times.


1
Differential Affinity to Stationary Phase
Mobile Phase
Stationary Phase
Binding Affinity Determines Speed
Mobile Phase
Stationary Phase (Beads)
3
Differential Elution Sequence
Some molecules bind strongly (Slow)
Other molecules move faster (Fast)
2

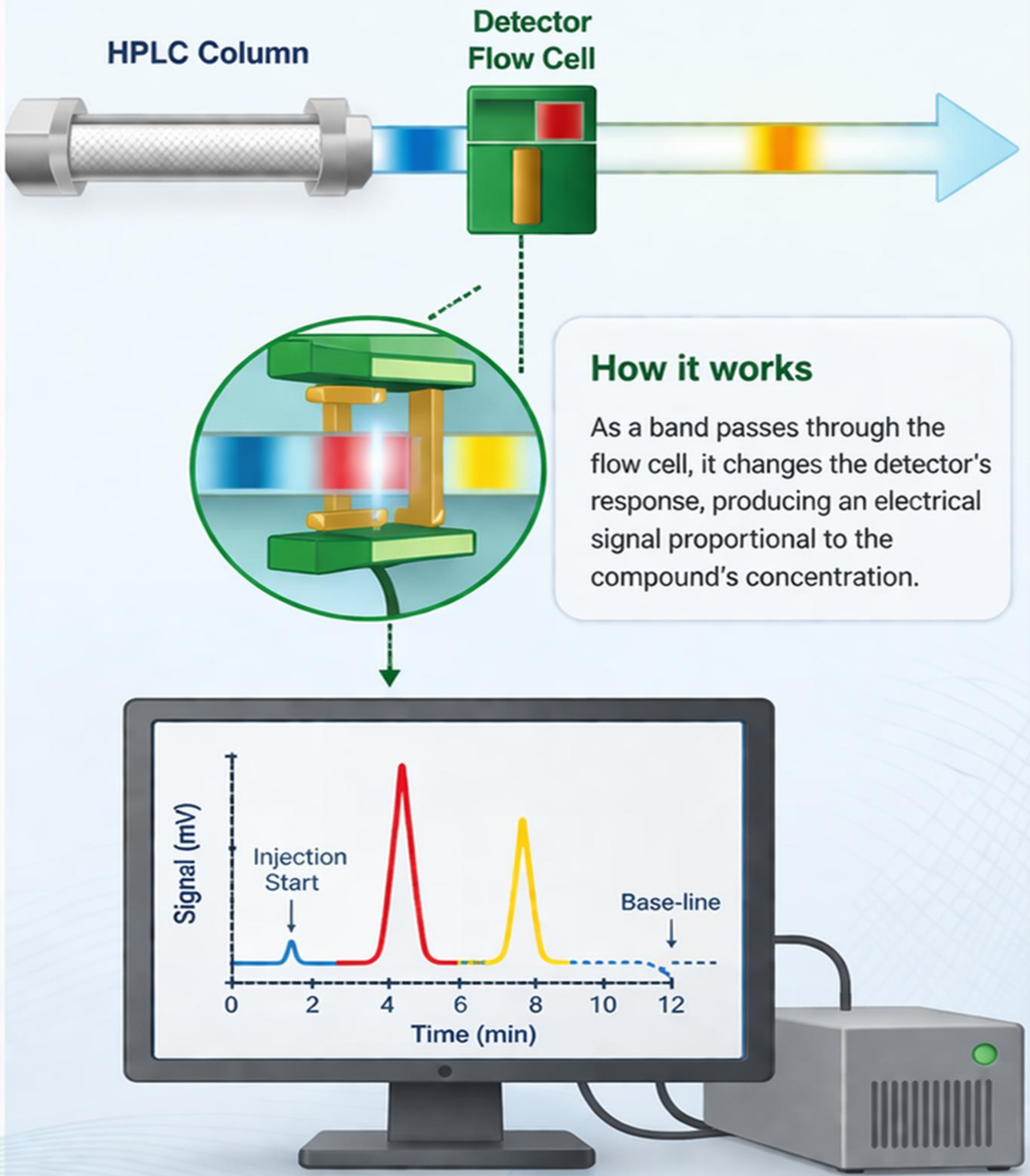
From Column to Chromatogram: The Detector Flow Cell

As each separated compound band exits the column, it flows immediately into the **detector's flow cell**.

The detector senses the presence and concentration of each band in real time, generating a **proportional electrical signal**.

This signal is relayed to the computer data station, where it is plotted as a **chromatographic peak** — ultimately producing the chromatogram that serves as the analytical record of the separation.

Each peak in the chromatogram represents a separated compound and its concentration.

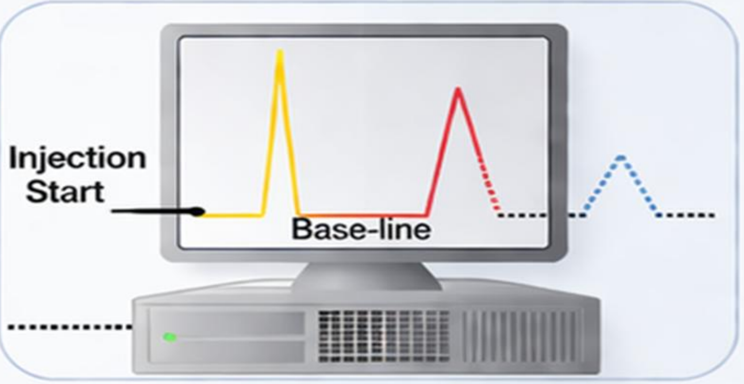


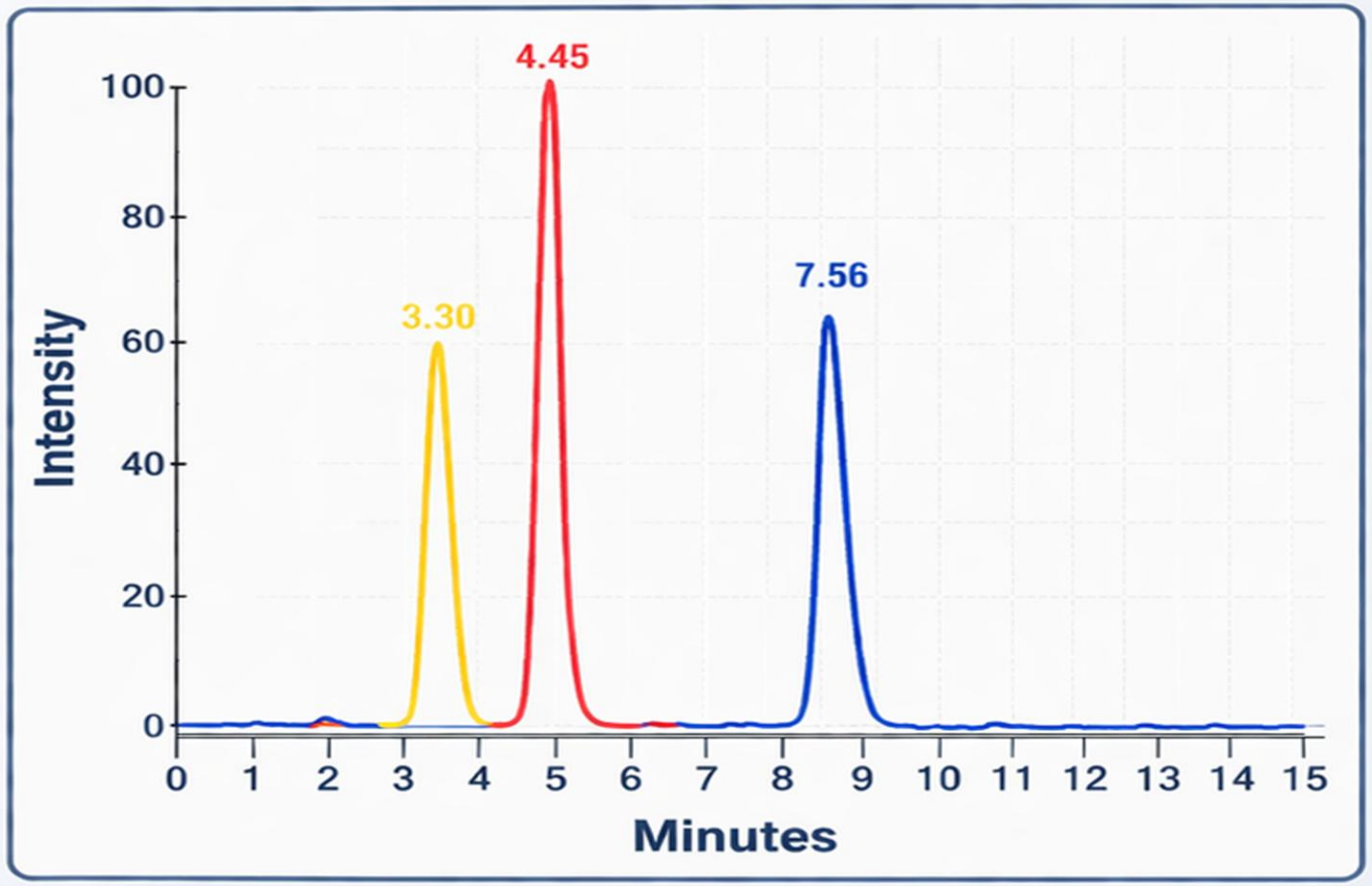
Reading the Chromatogram

How Peaks Are Generated

The **baseline** represents pure mobile phase passing through the flow cell. When an analyte band enters the cell, the detector signal rises proportionally to concentration, creating a peak. The peak height and area are used for **quantitation**, while the retention time identifies the compound.

- **Yellow band** ⟶ first, sharpest peak
- **Red band** ⟶ second peak
- **Blue band** ⟶ last, most retained peak





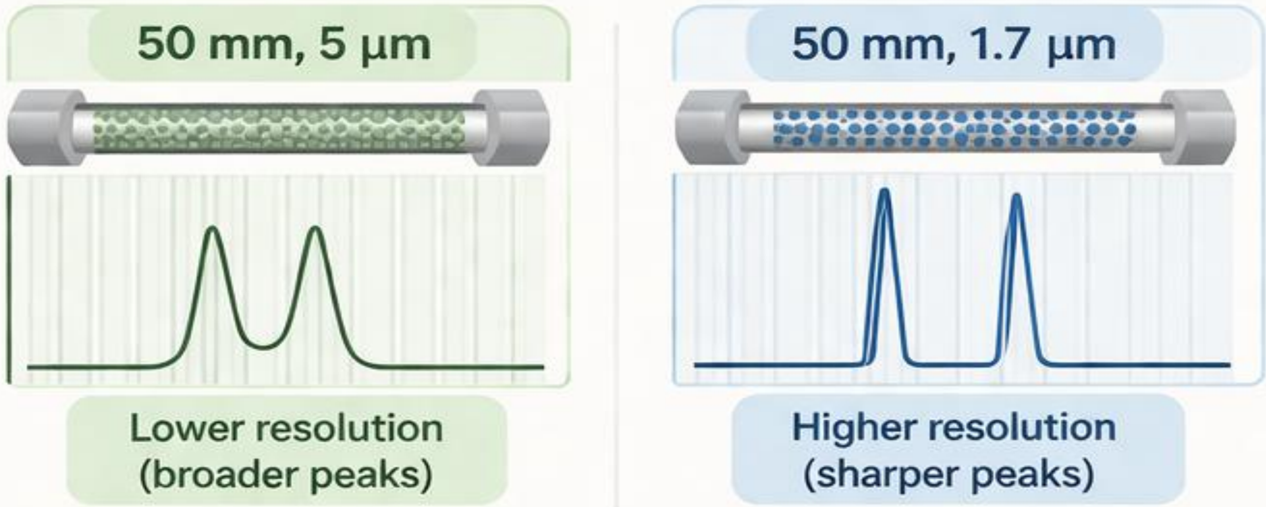
Separation Performance: Resolution

Chromatographic resolution (RS) is the degree to which two compounds are separated. Two principal factors govern the overall resolution achievable by an HPLC column:

1 Mechanical separation power

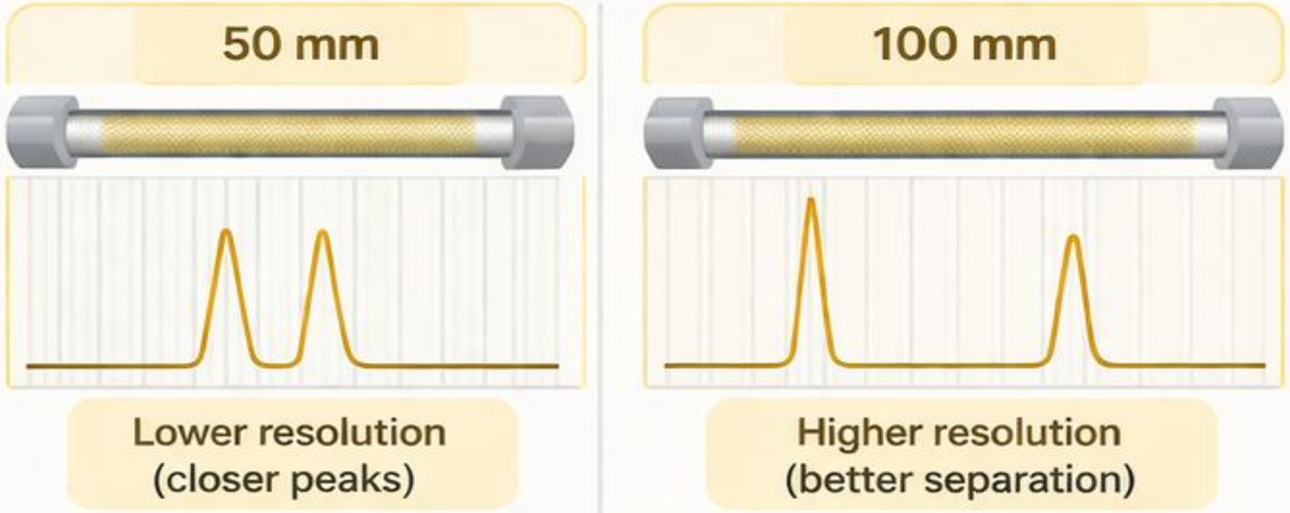
A. Particle Size

Smaller particles create **higher efficiency** (sharper peaks, better resolution) but generate **higher backpressure**. Reducing particle size from 5 µm to 1.7 µm dramatically improves peak separation on the same column length.



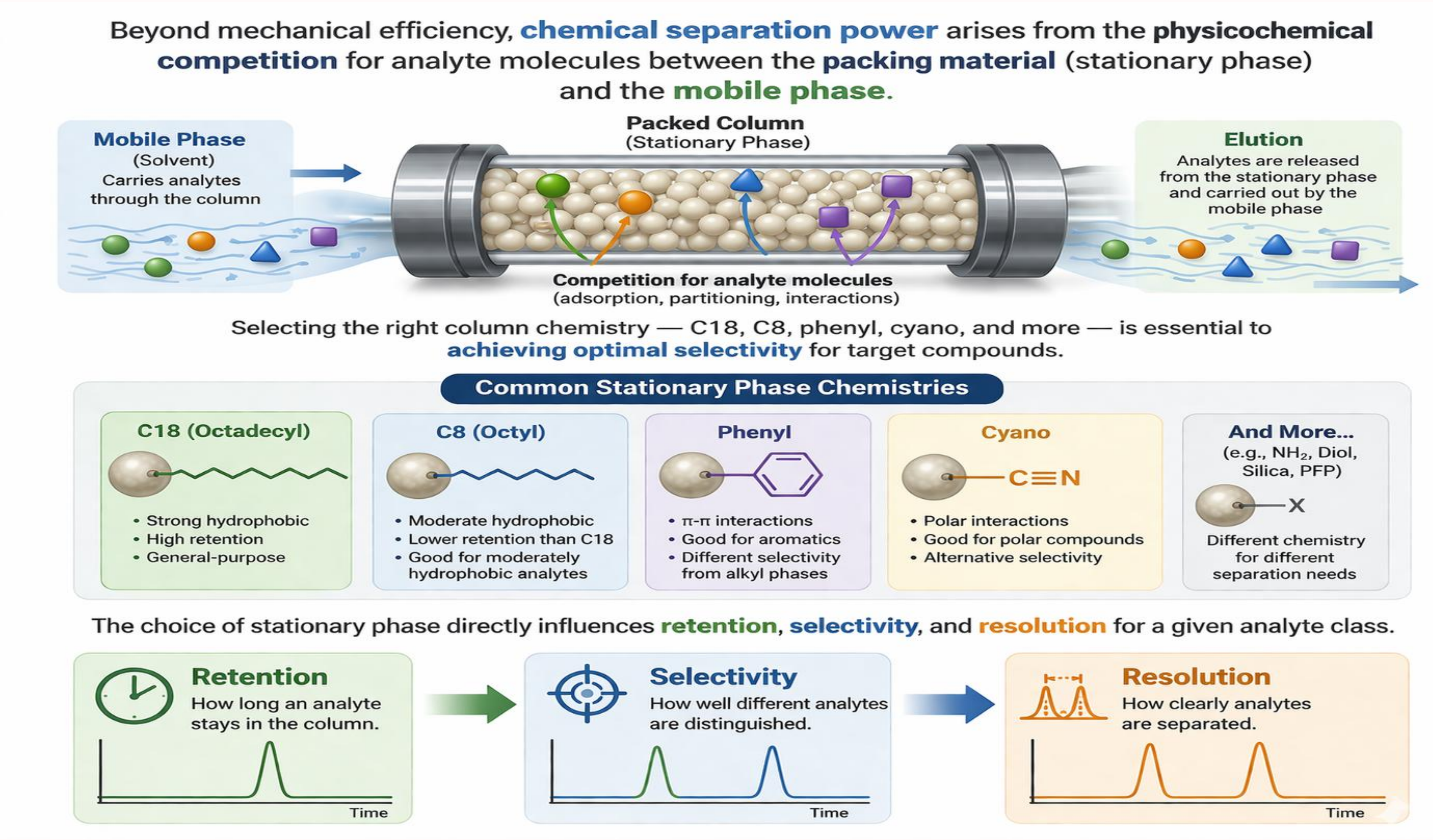
B. Column Length

For a given particle size, **increasing column length** provides more mechanical separation power by extending the path the analytes travel through the stationary phase.

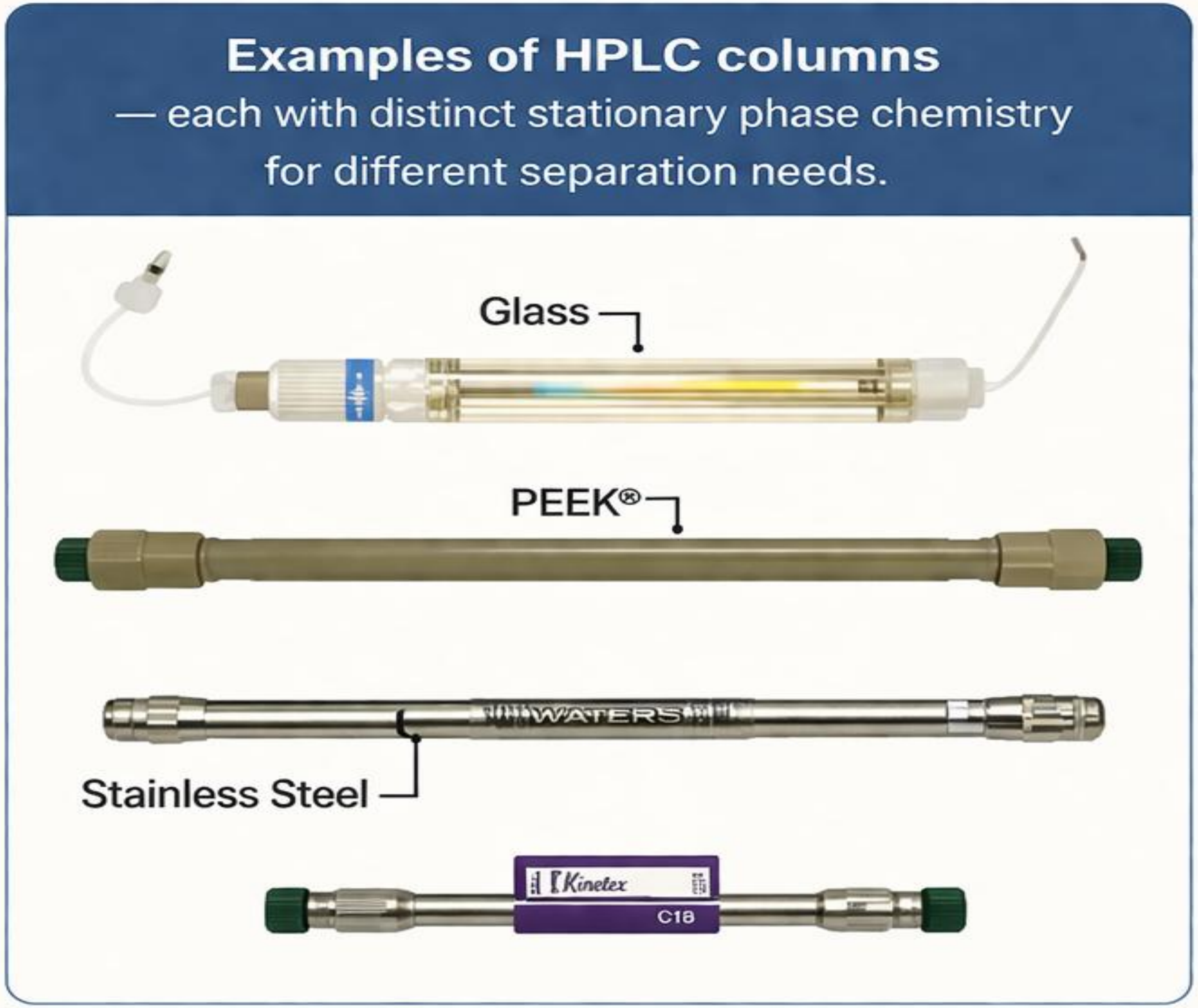


Smaller particles and longer columns both increase mechanical separation power, leading to better resolution.

2. Chemical Separation Power: Stationary Phase Chemistry



Chemical Separation Power: Stationary Phase Chemistry



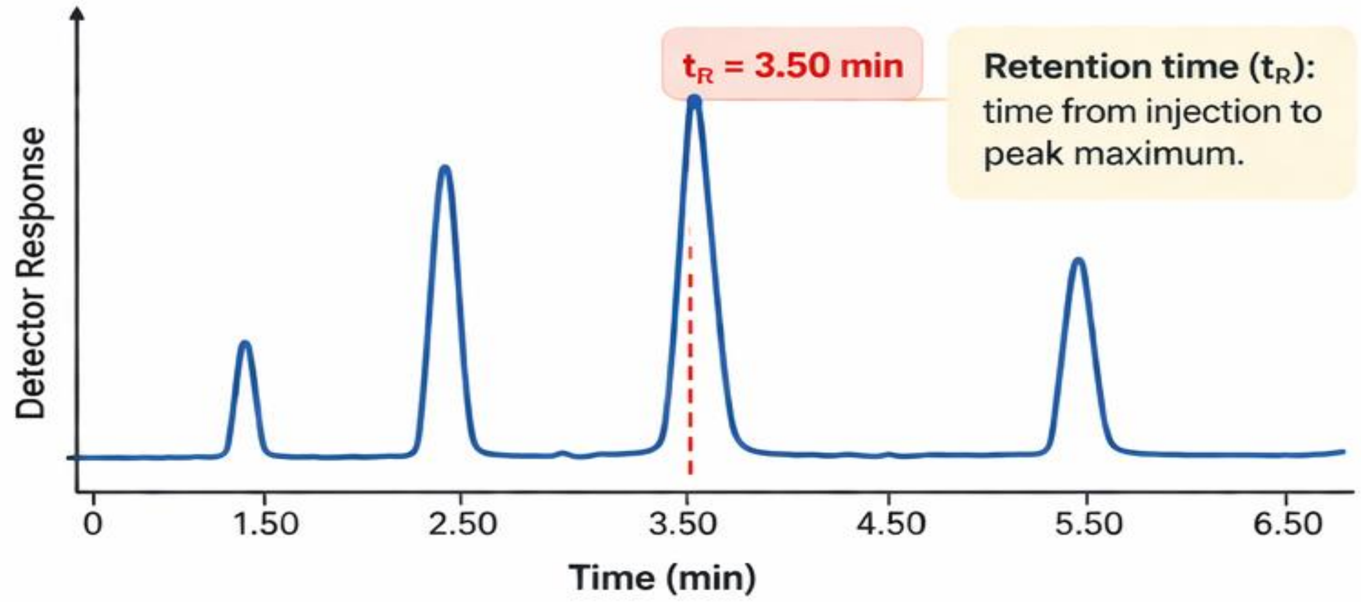
HPLC Use: Identifying Compounds

Retention Time as an Identity Marker

Compound identification in HPLC relies on **retention time** — the time elapsed from injection to peak maximum.

By comparing each peak's retention time with that of injected **reference standards** run under identical chromatographic conditions (same mobile phase, stationary phase, flow rate, and temperature), an unknown compound can be **positively identified.**

This approach is widely used in **pharmaceutical quality control** to confirm the identity of active **pharmaceutical** ingredients (**APIs**) and **excipients.**



Matching retention time under identical conditions = **positive identification**

1 Inject Sample
Introduce the unknown sample into the HPLC.

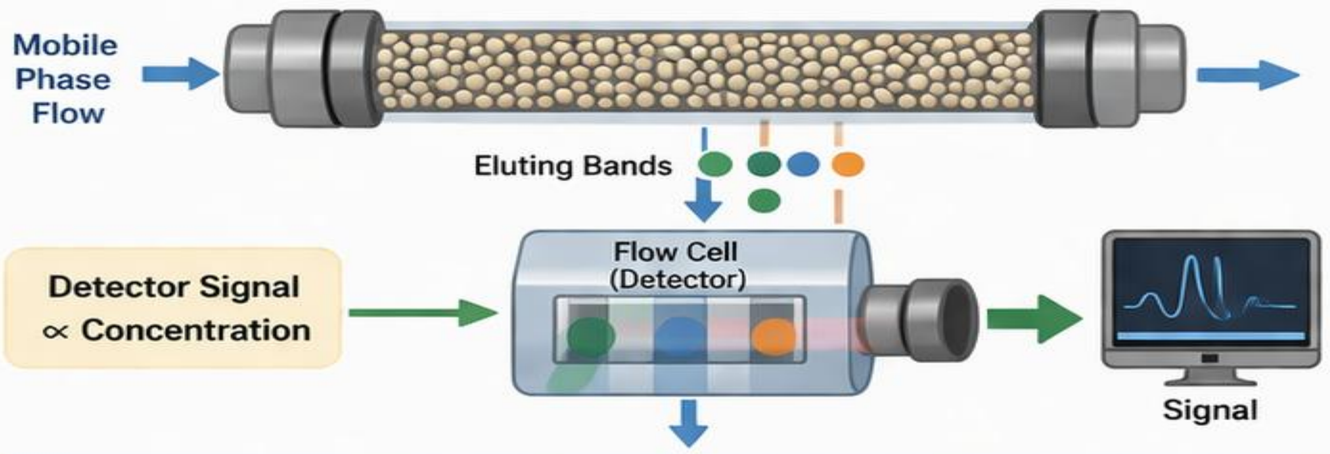
2 Separation
Compounds separate based on interactions with the stationary and mobile phases.

3 Detect Peaks
The detector records peaks at specific retention times.

4 Compare & Identify
Compare retention times with reference standards to identify the unknown.

Match = Identified!

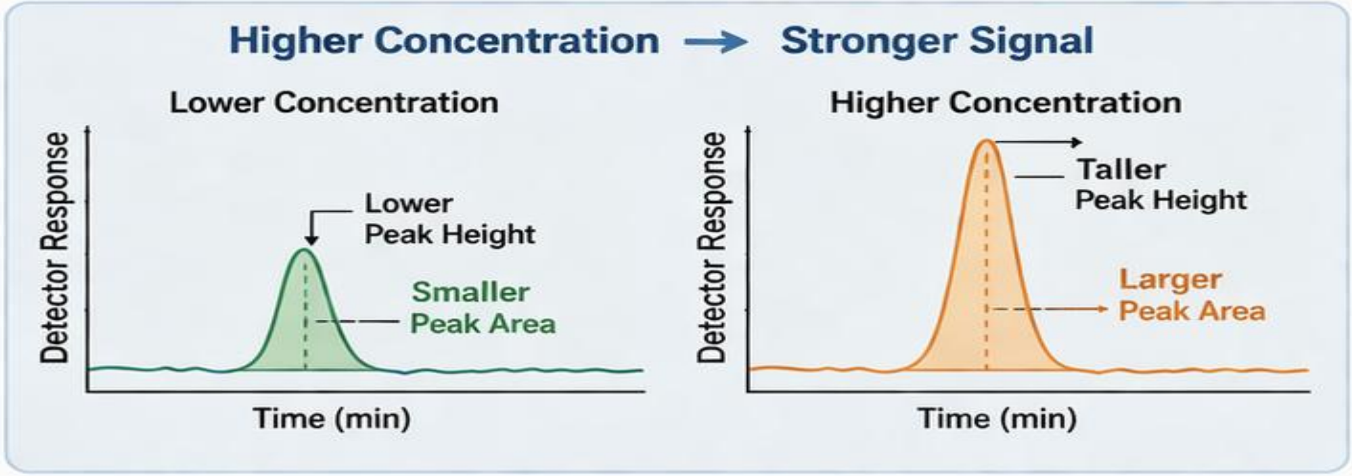
HPLC Use: Quantitating Compounds

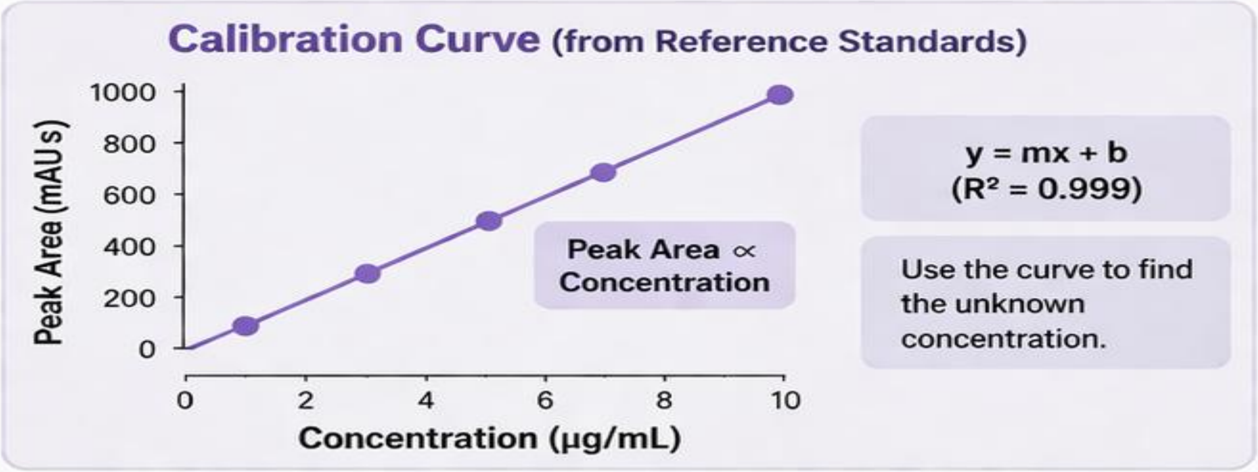


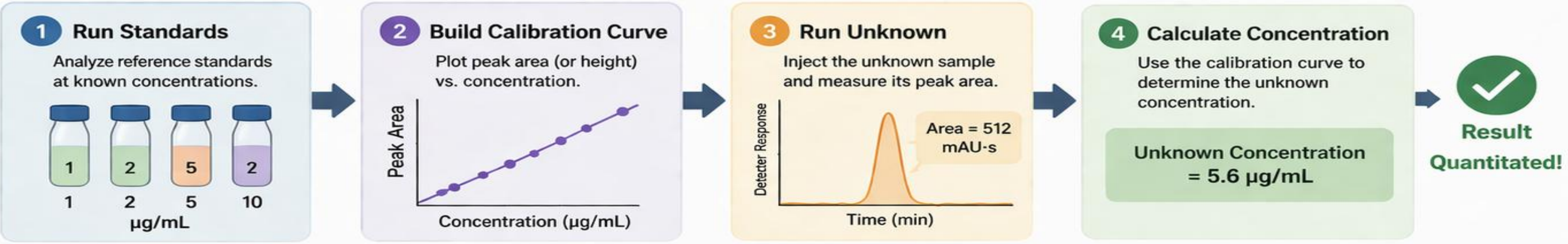
Concentration from Peak Response

The detector responds proportionally to the **concentration** of each compound band as it passes through the flow cell. A higher concentration produces a **stronger signal**, resulting in a **taller peak** above the baseline and a **greater peak area**.

Quantitation is typically performed by constructing a **calibration curve** from reference standards at known concentrations. Peak area or peak height is then used to calculate the concentration of the analyte in the unknown sample — critical for **assay and content uniformity testing** in pharmaceuticals.



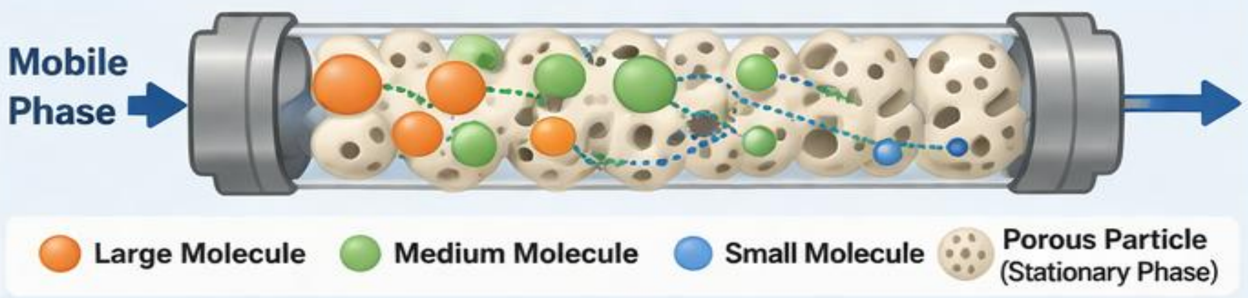


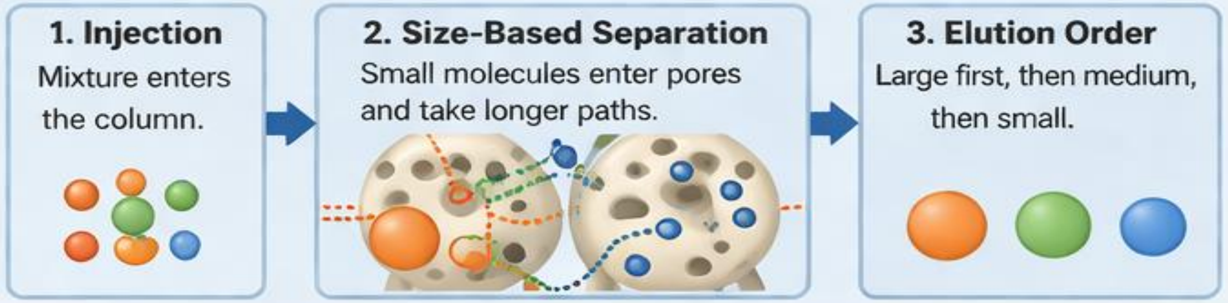


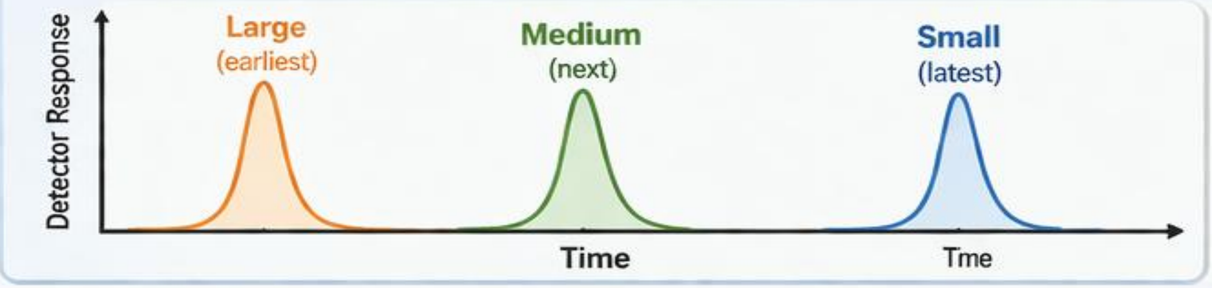
HPLC Use: Separation Mechanisms

1. Separation Based on Size

Achieved using **controlled-porosity silica** packings (size-exclusion chromatograhy).
Smaller molecules penetrate more pores and spend more in:he stationary bed, **eluting later**.
Larger molecules are excluded from smaller pores and **elute earlier**. Ideal for **polymers** and **biopharmaceuticals**.

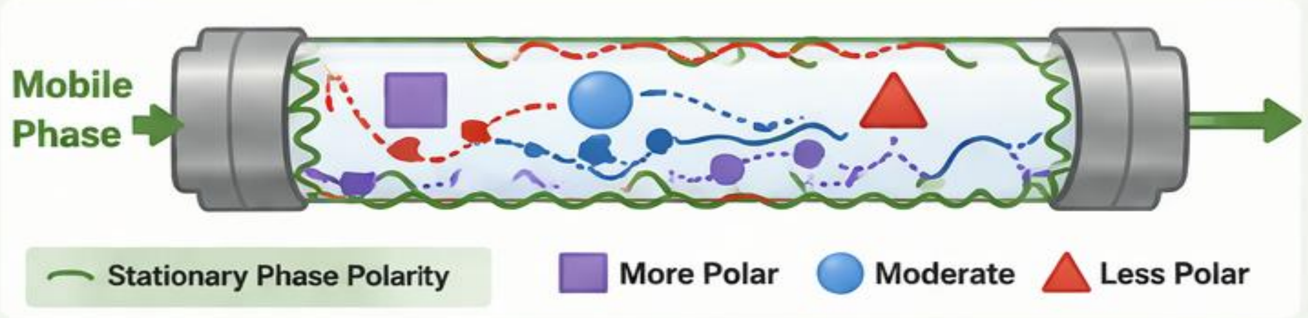


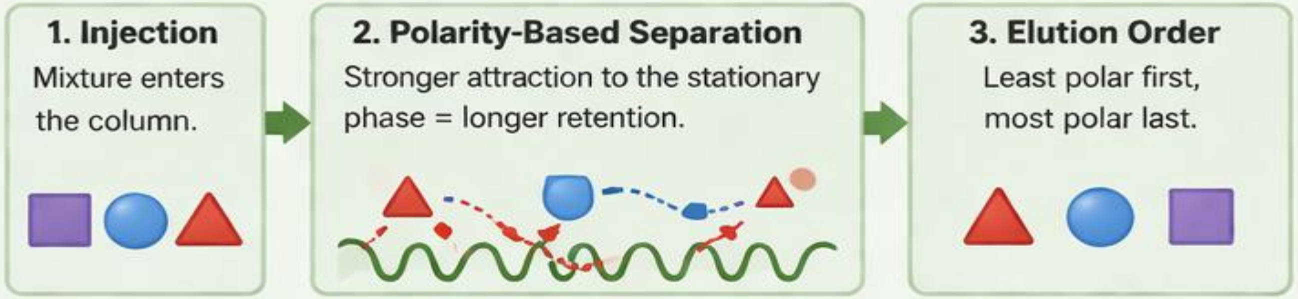


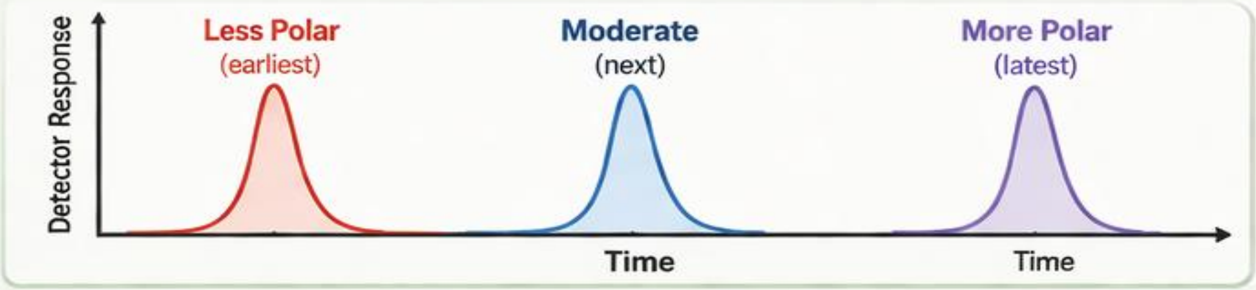


2. Separation Based on Polarity

"Like attracts like" — the mobile and stationary phases are chosen with **different polarities** to create competition.
Compounds similar in polarity to the stationary phase are **more strongly retained**; those similar to the mobile phase **elute faster**.
The foundation of both **normal**- and **reversed-phase** HPLC.







Different mechanisms, same goal: **separate compounds** so they elute at different times.

Normal-Phase vs. Reversed-Phase HPLC

Normal-Phase HPLC

Uses a **polar stationary phase** (e.g., bare silica)
with a **nonpolar mobile phase** (e.g., hexane).
Polar compounds are **retained longer**.
Historically the classical mode of liquid chromatography.

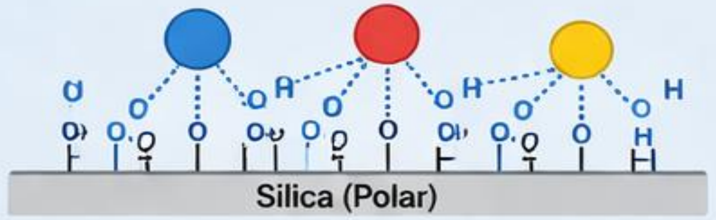
Stationary Phase Is **Polar** (Silica)

Sample

Mobile Phase Is **Non-Polar** (Hexane)

WHY Do They Separate?

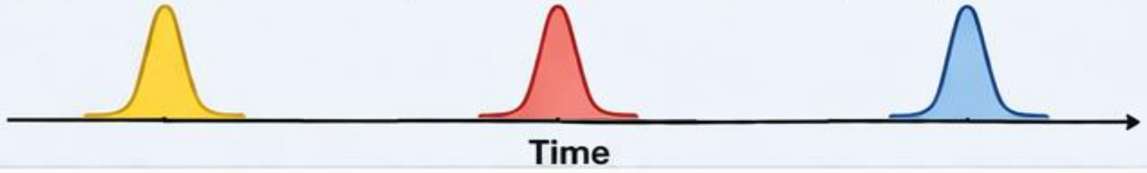
Polar stationary phase attracts polar analytes.
Stronger interactions = **longer retention**.



- Polar Compound
- Medium Polarity
- Nonpolar Compound

Elution Order (earliest → latest)

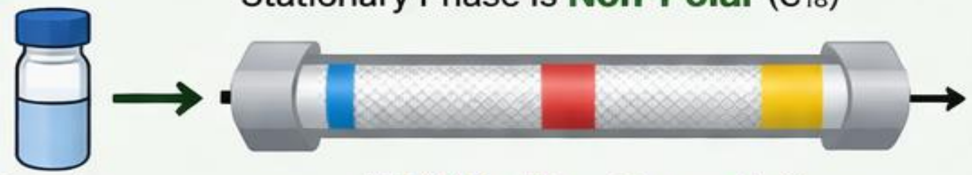
Nonpolar (weak interaction) → **Medium Polarity** (moderate interaction) → **Polar** (strong interaction)



Reversed-Phase HPLC

The opposite of normal phase — uses a **nonpolar stationary phase** (e.g., C18) with a **polar mobile phase** (e.g., water/acetonitrile).
This is by far the most commonly used mode in pharmaceutical analysis today, offering broad applicability and robust method development.

Stationary Phase Is **Non-Polar** (C_{18})

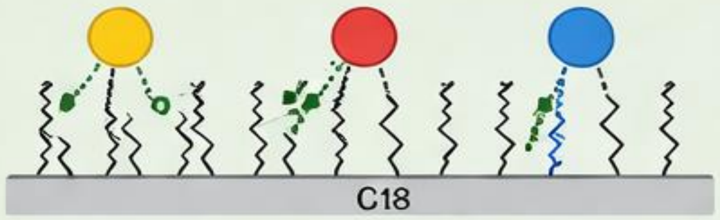


Sample

Mobile Phase Is **Polar** (Aqueous)

WHY Do They Separate?

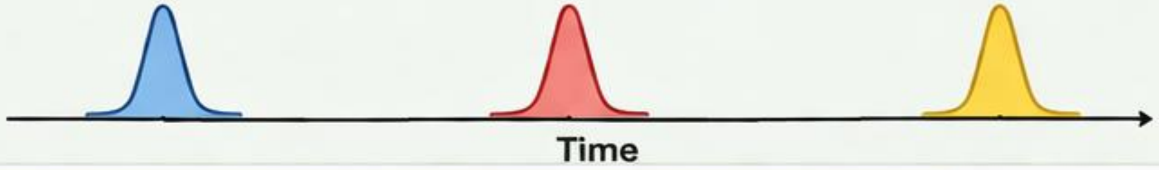
Nonpolar stationary phase retains **nonpolar** analytes.
Stronger hydrophohic interactions = **longer retention**.



- Nonpolar Compound
- Medium Polarity
- Polar Compound

Eluder Order (earliest → latest)

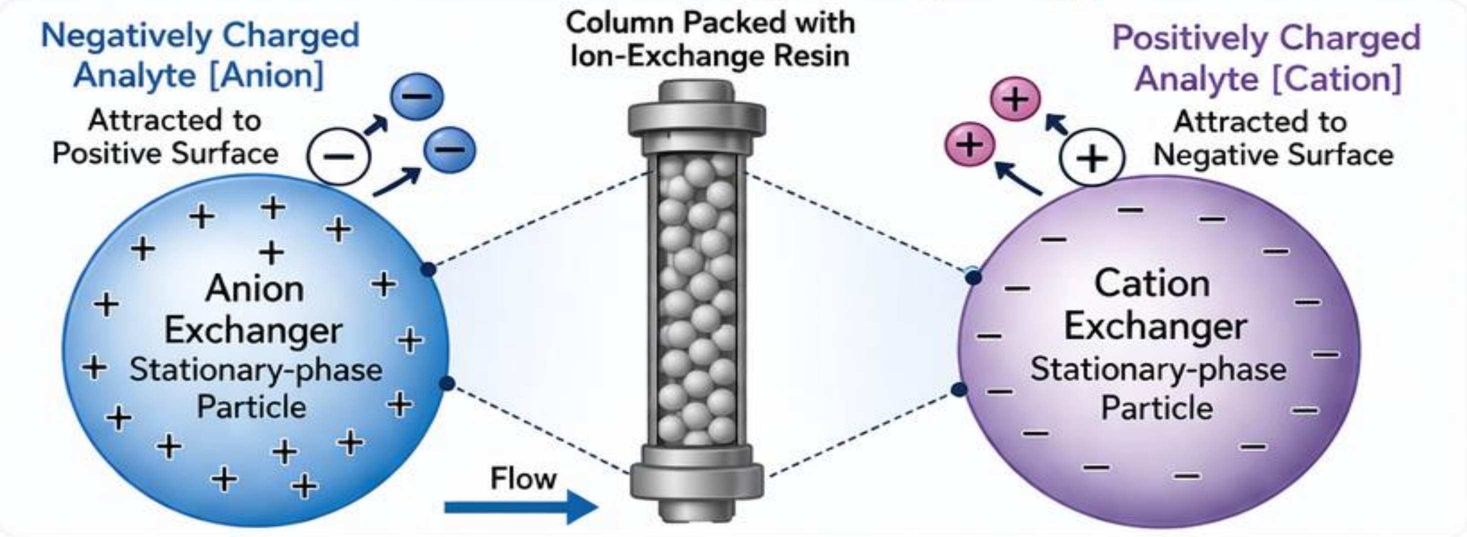
Polar (weak interaction) → **Medium Polarity** (moderate interaction) → **Nonpolar** (strong interaction)



Different stationary/mobile phase polarity = different interactions = different elution order

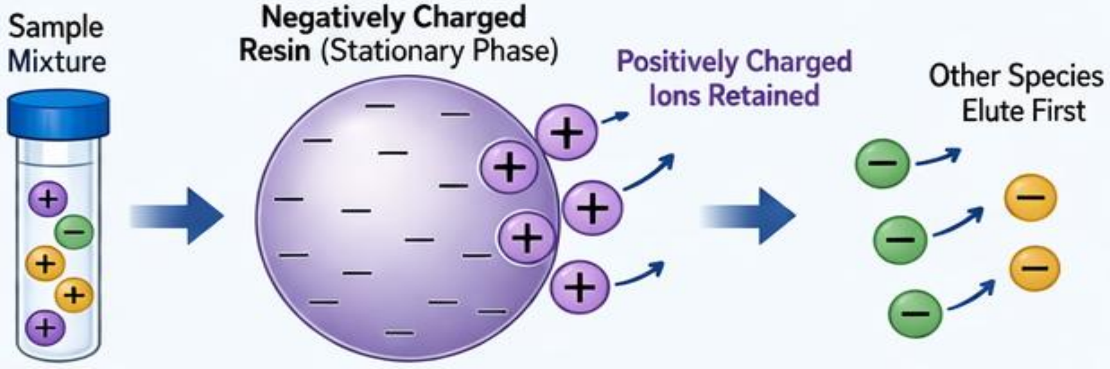
3. Separation Based on Charge: Ion-Exchange Chromatography

Ion-exchange chromatography (IEC) exploits **electrostatic interactions** between charged analytes and oppositely charged stationary phase functional groups. The governing principle: ***likes repel, opposites attract.***



Cation Exchange

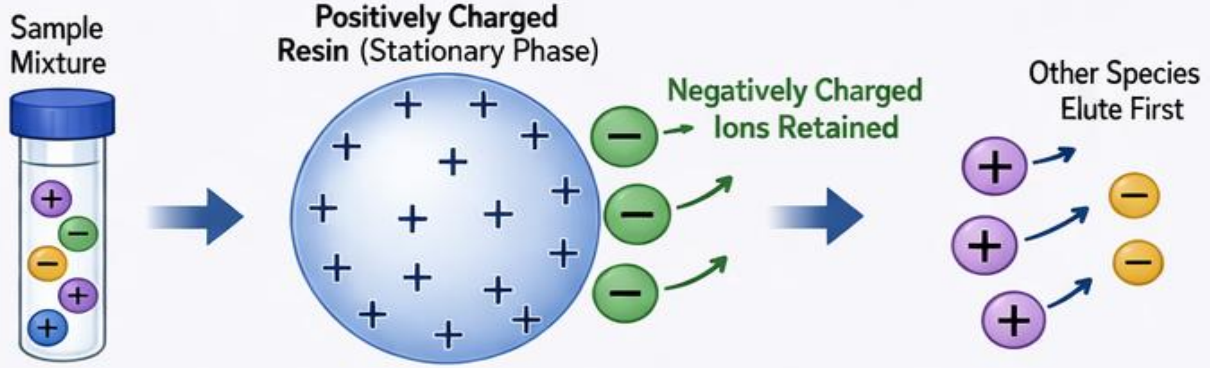
Retains and separates **positively charged ions** on a **negatively charged** stationary surface. Used for basic drugs and amino acids.



Best for basic drugs, amino acids, and other positively charged molecules at the working pH.

Anion Exchange

Retains and separates **negatively charged ions** on a **positively charged** stationary surface. Used for acidic compounds, nucleotides, and proteins.



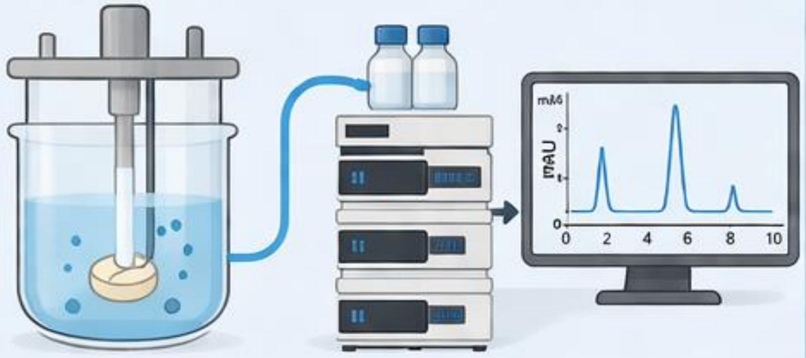
Best for acidic compounds, nucleotides, proteins, and other negatively charged molecules at the working pH.

Pharmaceutical Applications of HPLC

HPLC is indispensable throughout the pharmaceutical life cycle — from development through manufacturing and post-market surveillance. **Key applications include:**

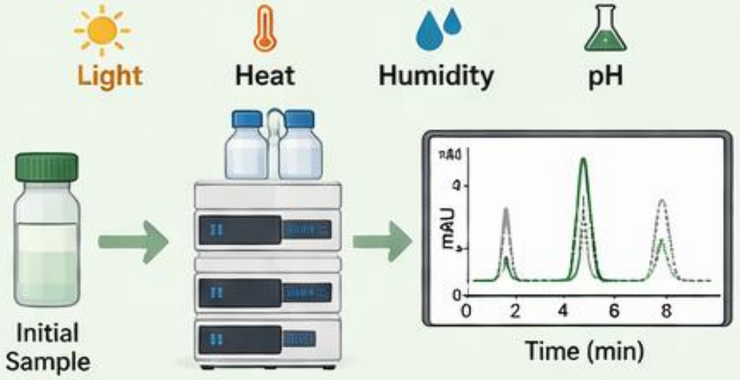
Tablet Dissolution Testing

Measures the rate and extent to which an API is released from a dosage form, ensuring **bioavailability** meets specification.



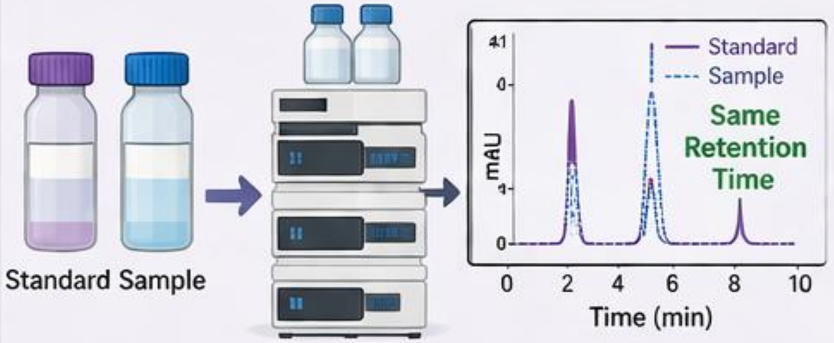
Drug Stability & Shelf-Life

Monitors **degradation** of APIs over time under various stress conditions to establish expiry dates and storage requirements.



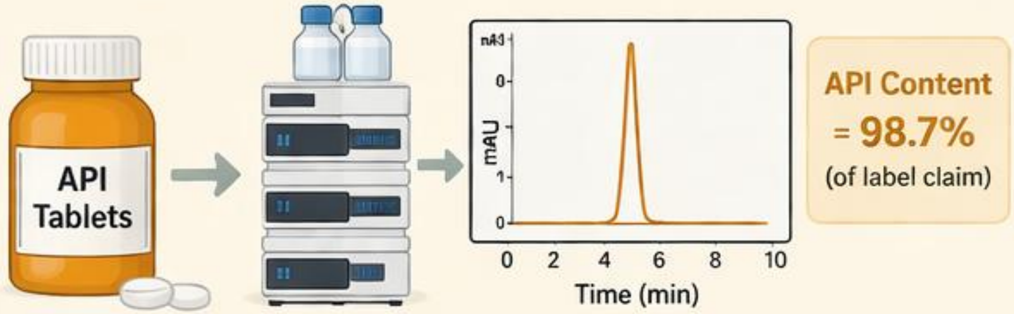
Identification of Active Ingredients

Confirms the identity of APIs and excipients by comparing **retention times** against certified reference standards.



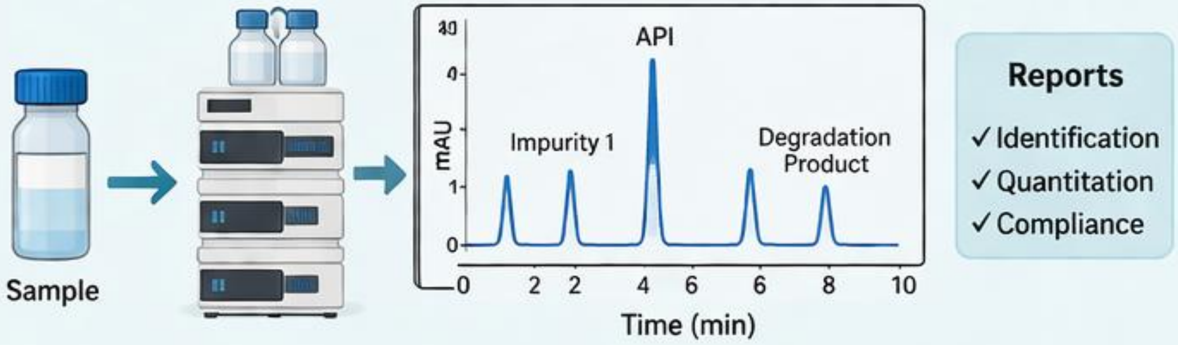
Quality Control & Assay

Quantifies API content in finished products to confirm **potency** and compliance with pharmacopoeial specifications.



Impurity Profiling

Detects and quantifies related substances, degradation products, and process impurities at **trace levels** per ICH guidelines.

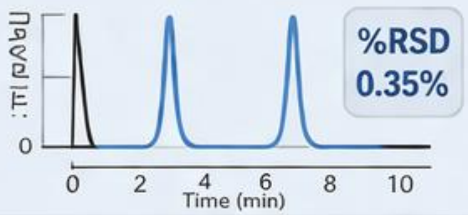


Advantages & Disadvantages of HPLC

✓ Advantages

Rapid & automated — high throughput with minimal analyst intervention

Highly precise — reproducible quantitative results with low %RSD



Sensitive — detects compounds at ppm and ppb levels

Versatile — applicable across a wide range of compound classes and matrices

Validated methods widely accepted by regulatory authorities (FDA, EMA, USP)

✕ Limitations

Sample preparation required — samples must be extracted and clarified prior to injection to protect the column and system

No imaging capability — HPLC provides chemical information only; structural elucidation requires coupling with MS or NMR

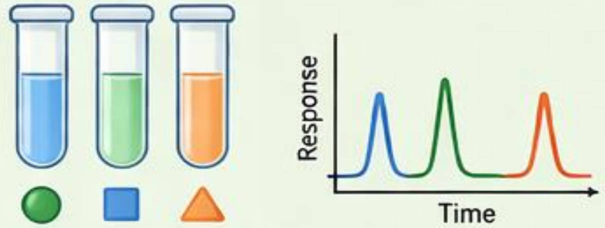
Method development time — optimizing conditions for new analytes can be resource-intensive

Solvent consumption — generates chemical waste that must be disposed of appropriately

Isocratic HPLC

In **isocratic elution**, the composition of the mobile phase remains constant throughout the entire chromatographic run. The ratio of organic to aqueous solvent does not change from injection to the end of the run.

Best Suited For



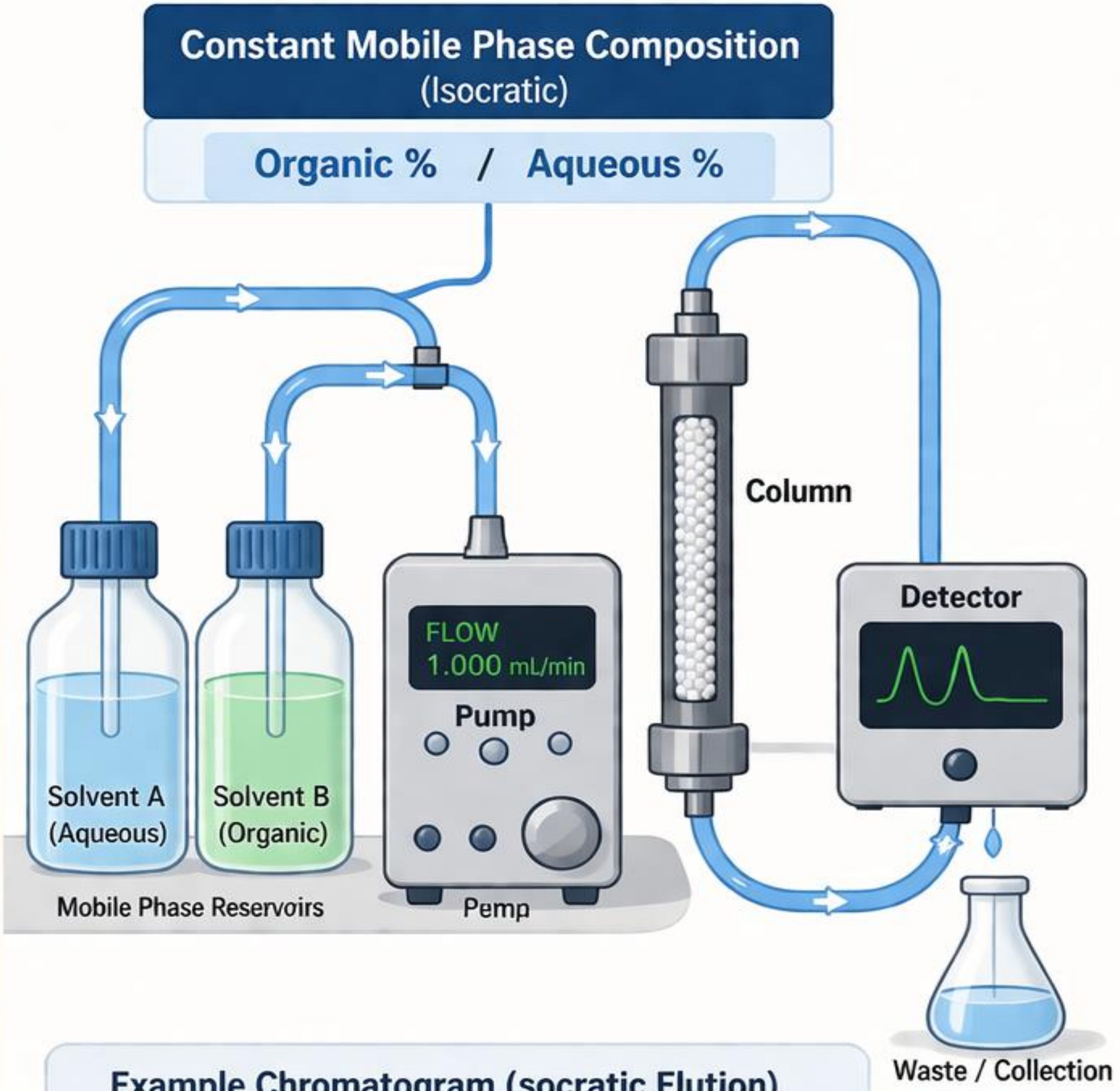
Separating compounds with **similar polarities** or **closely matched retention times.** Ideal for routine quality control analyses where analyte behavior is well-characterized and predictable.

Advantages

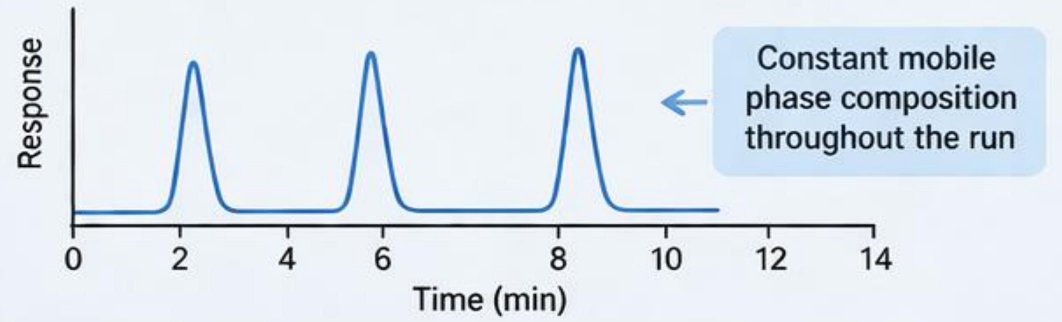
- Simple and straightforward to implement
- Requires less complex instrumentation
- Excellent for routine, validated methods

Limitations

- Poor resolution for mixtures with a **wide range of polarities**
- **Longer run times** when analyzing complex, multi-component samples
- Strongly retained compounds **may not elute** in a reasonable timeframe

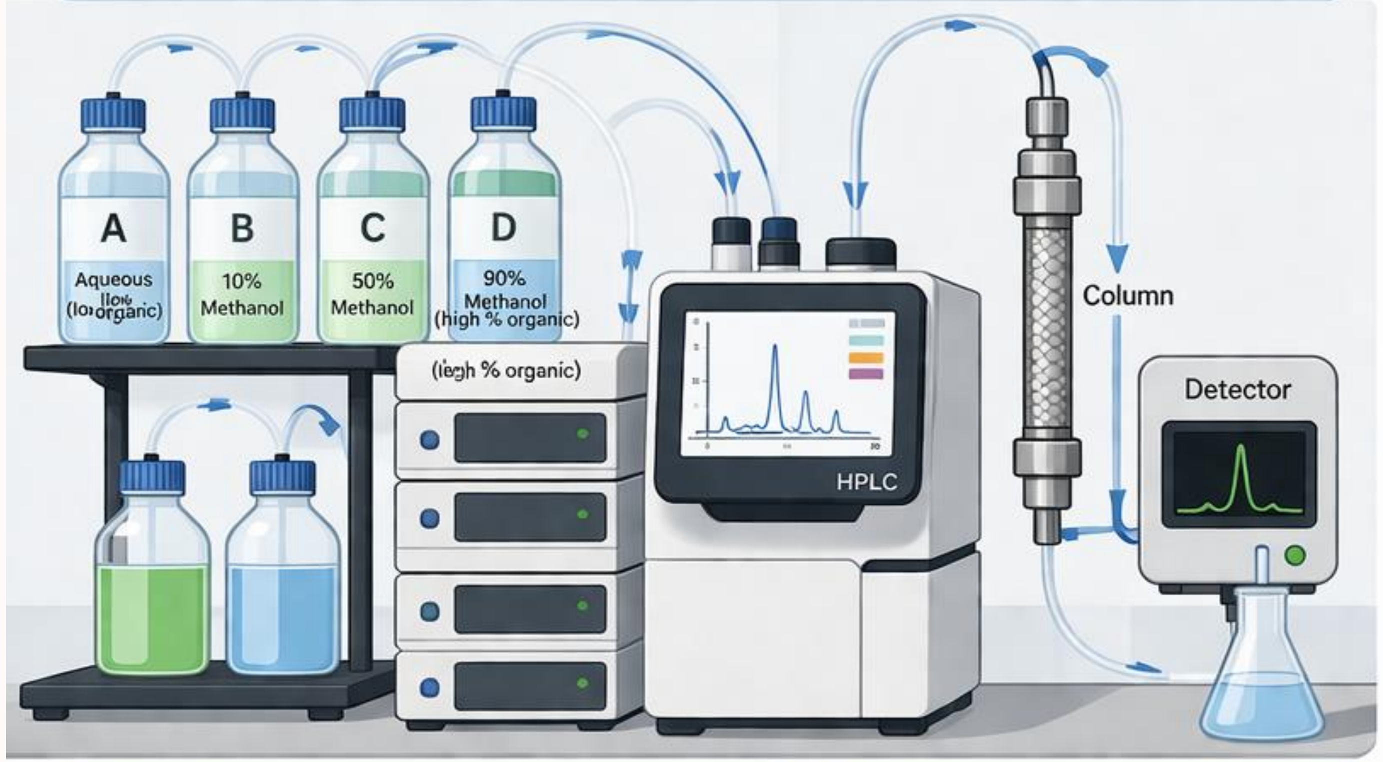


Example Chromatogram (socratic Elution)

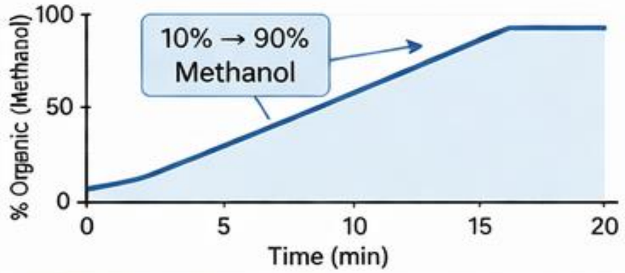


Gradient HPLC

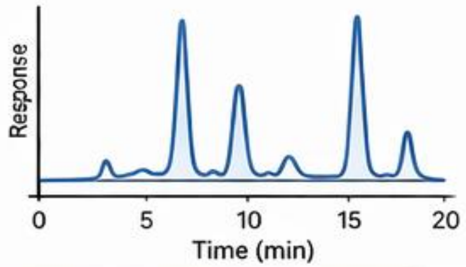
In **gradient elution**, the mobile phase composition is **varied progressively** during the run — for example, starting at 10% methanol and increasing to 90% over the course of the analysis. This programmed change in solvent strength enables efficient separation of analytes with widely differing polarities.



Example Gradient Profile (Mobile Phase)



Resulting Chromatogram



The gradient allows early elution of weakly retained compounds and later elution of strongly retained compounds.

Best Suited For

Complex mixtures containing compounds of **widely varying polarities** — common in pharmaceutical impurity profiling, natural product analysis, and biologics characterization.

- Impurity Profiling
- Natural Product Analysis
- Biologics Characterization

Limitations

- Requires more sophisticated instrumentation (gradient pump, mixer)
- More complex method development and validation
- Longer equilibration time required between injections

Advantages

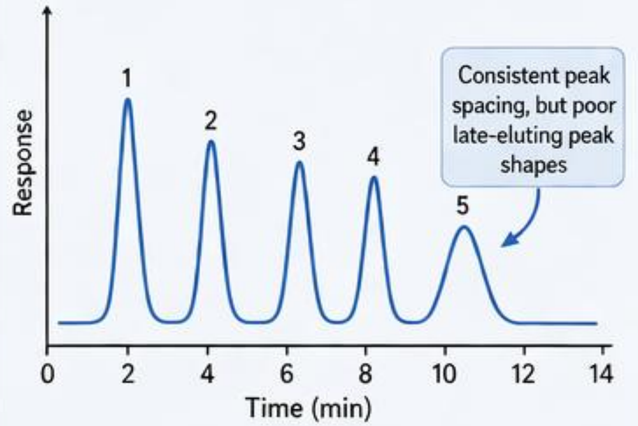
- **Sharper peaks and better resolution across a wide polarity range**
- **Reduced total analysis time**
- **Column regeneration through high-organic wash step**

Isocratic vs. Gradient HPLC

Basis of comparison	Isocratic elution method	Gradient elution method
Definition	Is an HPLC mode in which the concentration of the mobile phase remains **constant** over the full analysis time. (A, B → Constant composition)	Is an HPLC mode in which the concentration of the mobile phase is **changed** throughout the analysis. (A, B → Changing composition)
Retention of components	Retention of the components is **not affected** because of the unchanging concentration	Retention of the components is **affected** because of the varying concentration
Polarity of the mobile phase	Polarity of the mobile phase remains the **same** throughout (Constant polarity)	Polarity of the mobile phase may **gradually increase or decrease** throughout the separation process (Changing polarity)

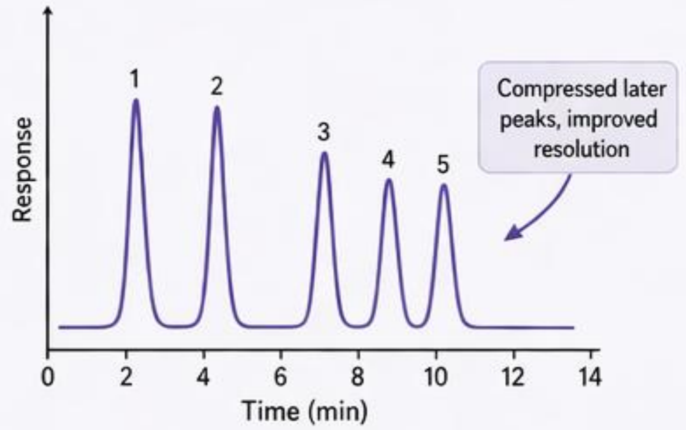
The chromatogram comparison illustrates the key difference: **isocratic elution** produces consistent spacing between peaks but can result in poor late-eluting peak shapes, while **gradient elution** compresses later peaks and improves overall resolution for complex mixtures — at the cost of added method complexity.

Isocratic Elution
(Constant Mobile Phase)



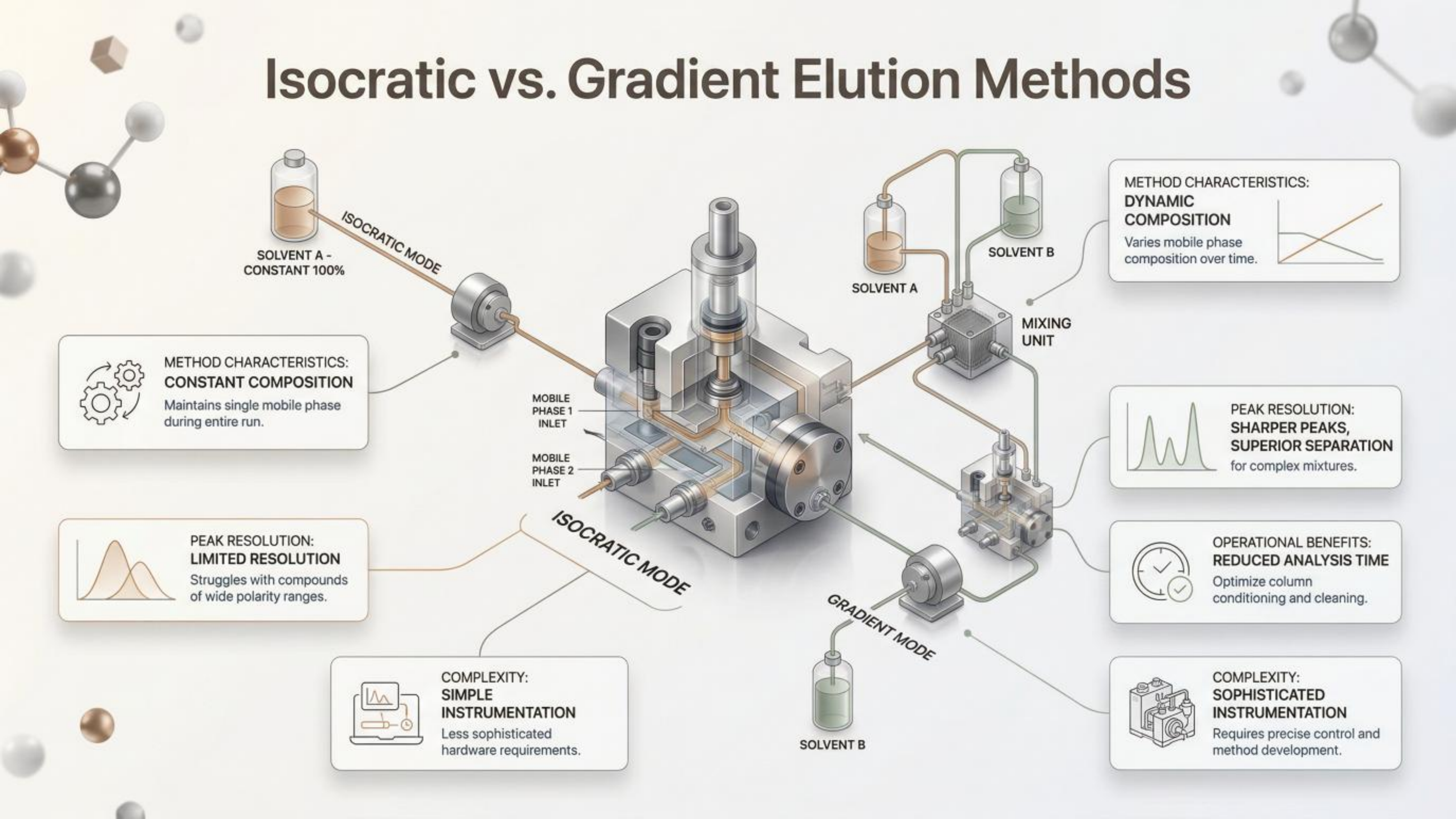
- Constant mobile phase composition
- Peaks are evenly spaced
- Late peaks may be broad/tail
- Suitable for simpler mixtures

Gradient Elution
(Changing Mobile Phase)



- Mobile phase composition changes over time
- Later peaks elute sooner and are sharper
- Better resolution for complex mixtures
- Requires more method development

Key Takeaway:
Isocratic = Simple & Consistent | **Gradient** = Powerful & Efficient (for complex samples)


Isocratic vs. Gradient Elution Methods
SOLVENT A - CONSTANT 100%
ISOCRATIC MODE
METHOD CHARACTERISTICS:
CONSTANT COMPOSITION
Maintains single mobile phase during entire run.
MOBILE PHASE 1 INLET
MOBILE PHASE 2 INLET
PEAK RESOLUTION:
LIMITED RESOLUTION
Struggles with compounds of wide polarity ranges.
ISOCRATIC MODE
COMPLEXITY:
SIMPLE INSTRUMENTATION
Less sophisticated hardware requirements.
SOLVENT A
SOLVENT B
MIXING UNIT
METHOD CHARACTERISTICS:
DYNAMIC COMPOSITION
Varies mobile phase composition over time.
PEAK RESOLUTION:
SHARPER PEAKS, SUPERIOR SEPARATION
for complex mixtures.
OPERATIONAL BENEFITS:
REDUCED ANALYSIS TIME
Optimize column conditioning and cleaning.
GRADIENT MODE
SOLVENT B
COMPLEXITY:
SOPHISTICATED INSTRUMENTATION
Requires precise control and method development.

Effect of Organic/Aqueous Phase Ratio on Elution

In **reversed-phase HPLC (RP-HPLC)**, the ratio of organic solvent (e.g., acetonitrile, methanol) to aqueous phase (e.g, water, buffer) is one of the mos most critical variables contordling retention and selectivity.

1. Retention time and Elution order

↑ Higher Organic Content

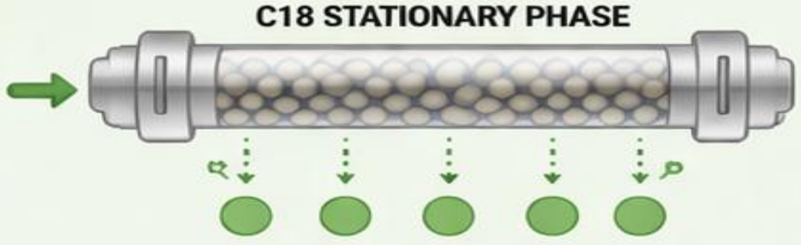
Mobile phase becomes **less polar**. Interaction between nonpolar analytes and the **C18** stationary phase decreases.

Result: **faster elution** (shorter retention times) for hydrophobic compounds. Peaks sharpen; tailing is reduced.

MOBILE PHASE

High % Organic (e.g., acetonitrile, methanol)

Low % Aqueous (water, buffer)



Weaker interaction → faster elution

↑ Higher Aqueous Content

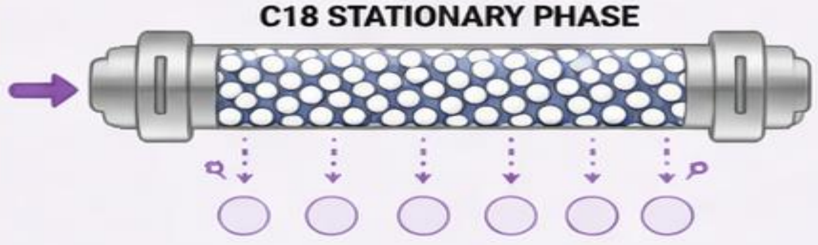
Mobile phase becomes **more polar**. Nonpolar compounds are pushed toward the stationary phase by the aqueous environment.

Result: **longer retention times** for hydrophobic analytes. Polar compounds may elute early or remain unretained.

MOBILE PHASE

High % Aqueous (water, buffer)

Low % Organic (acetonitrile, methanol)



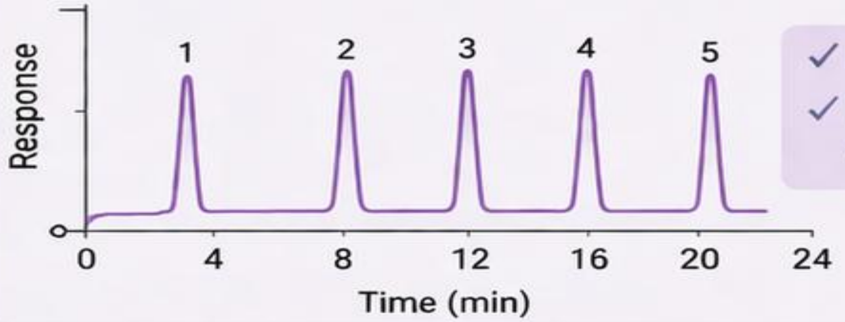
Stronger interaction → longer retention

Optimizing the organic-to-aqueous ratio is the **primary lever** for adjusting retention times and resolving closely eluting peaks during RP-HPLC method development.

Higher Organic Content (faster elution)

- ✓ Shorter retention times
- ✓ Sharper peaks
- ✓ Less tailing

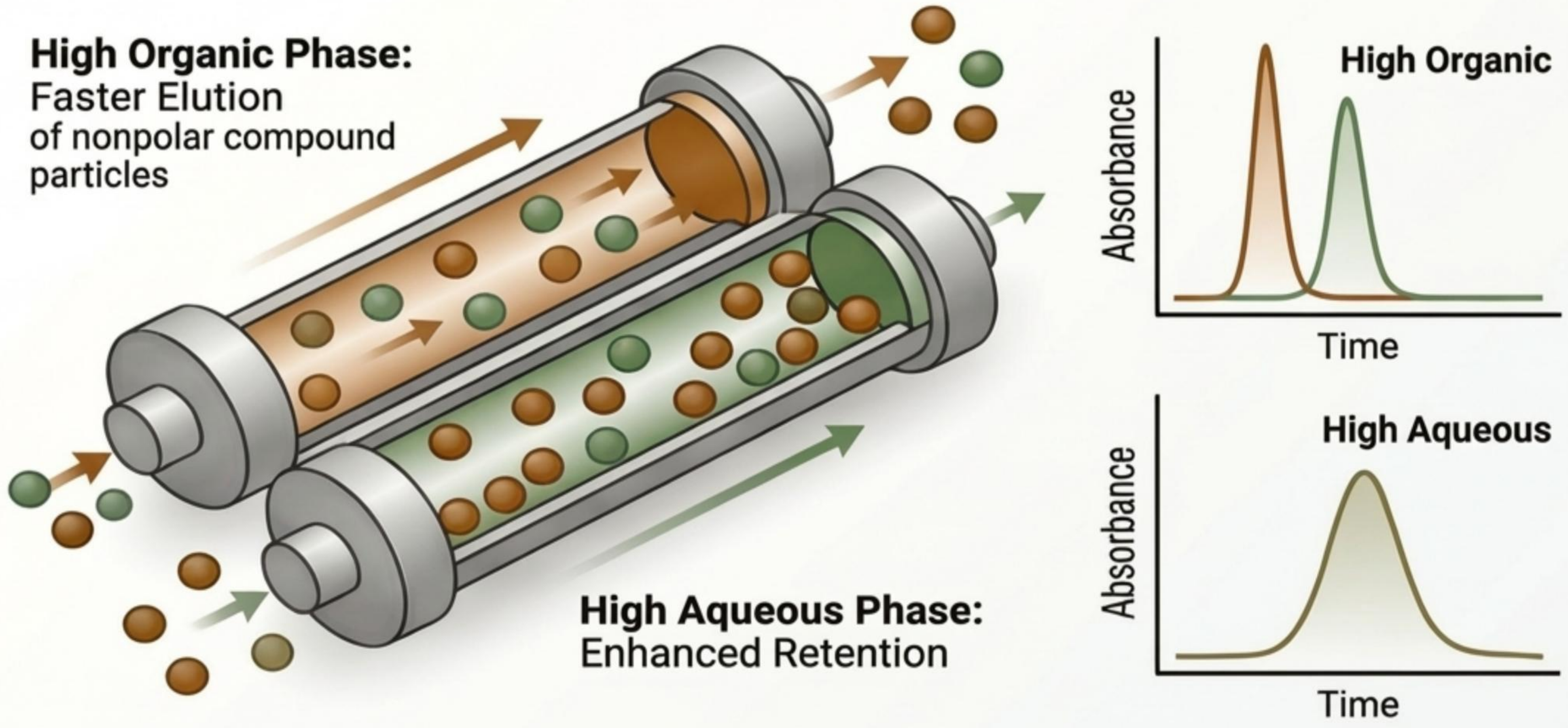
Higher Aqueous Content (longer retention)



- ✓ Longer retention times
- ✓ Polar compounds elute early or not retained

Key Takeaway: **Higher organic** = less retention, sharper peaks, faster elution | **Higher aqueous** = more retention, later elution

Organic-to-Aqueous Phase Ratio
(e.g., Acetonitrile in Water)

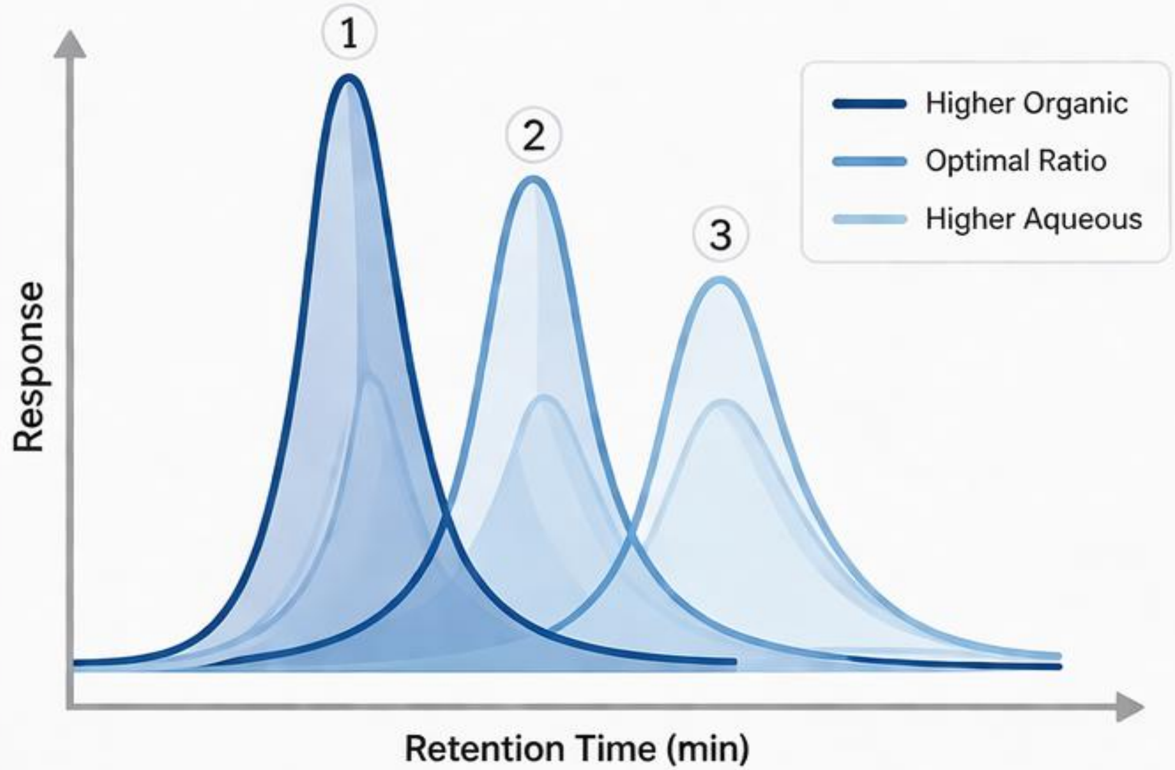


- Reversed-Phase HPLC context
- Organic content decreases nonpolar retention (faster elution)
- Aqueous content enhances retention
- Adjusts separation speed

- Faster flow elution
- Organic content decreases nonpolar retention
- Minimalist comparative area-curve chromatograms

Organic/Aqueous Ratio:
2. Peak Shape & Resolution

Changing the organic/aqueous ratio changes peak shape and resolution.



More Organic (Stronger Elution) → **More Aqueous** (Weaker Elution)

SHARPER PEAKS
Less tailing
Faster elution

BETTER RESOLUTION
Improved separation of close peaks

BETTER QUANTITATION
More accurate results
Lower detection limits

Higher Organic Content → Sharper Peaks

Increasing organic phase content generally **sharpens peaks and reduces peak tailing** for hydrophodic compounds. This improves quantitation accuracy and lowers detection limits.

Higher Aqueous Content → Improved Polar Separation

A more polar mobile phase can improve the separation of **highly polar compounds**, but risks **broad or tailing peaks** for nonpolar analytes due to extended column interaction.

Optimal Ratio = Best Resolution

Careful optimization of the organic-to-aqueous ratio is critical for **resolving closely eluting peaks** — a key step in pharmaceutical impurity method development.

KEY TAKEAWAY: Adjusting the organic/aqueous ratio directly affects peak shape, retention time, and resolution. Optimize to achieve the best separation for your analytes.

Effect of Mobile Phase pH on Peak Separation

The pH of the mobile phase is a **critical method parameter** in RP-HPLC and ion-exchange HPLC. It directly governs the ionization state of analytes, which in turn influences retention time, peak shape, selectivity, and resolution — particularly for **acidic and basic drug molecules**.

1. Effect of Ionization of Analytes

Because ionization fundamentally changes how a molecule interacts with both the aqueous mobile phase and the nonpolar stationary phase, even small pH shifts (±0.5 units) can produce dramatic changes in chromatographic behavior — making **precise pH control** essential in validated pharmaceutical methods.

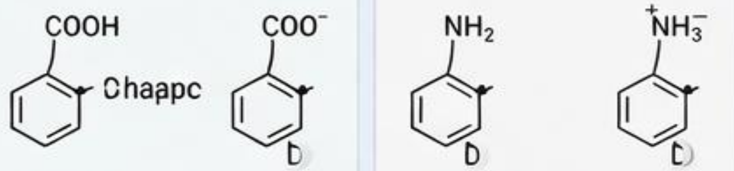
Small pH shifts (±0.5 units) can lead to **big changes** in separation!

HOW pH AFFECTS IONIZATION

pH determines the ionization state of acidic and basic analytes, altering their polarity.

Acidic analyte ($HA \rightleftharpoons A^- + H^+$)

Basic analyte ($B + H^+ \rightleftharpoons BH^+$)



Acidic analyte: **Low pH** (protonated) → **High pH** (ionized)

Basic analyte: **Low pH** (ionized) → **High pH** (neutral)

Increasing pH

IMPACT ON CHROMATOGRAPHIC BEHAVIOR

Ionization changes affect retention and peak shape.

Aqueous Mobile Phase (polar)

Nonpolar Stationary Phase

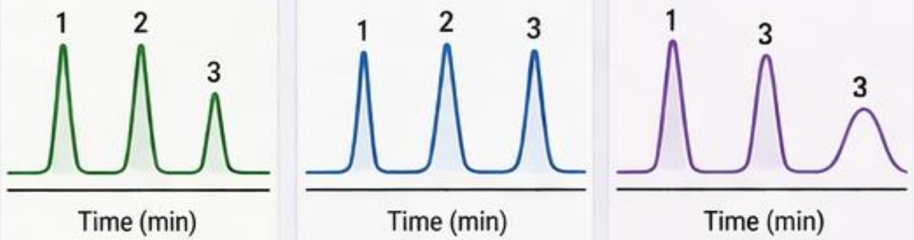
More Ionized (More Polar)
- → Spends more time in aqueous phase
- → **Shorter retention**

Less Ionized (More Nonpolar)
- → Spends more time on stationary phase
- → **Longer retention**

Ionization also affects peak shape and selectivity.

EFFECT OF pH ON CHROMATOGRAMS

Low pH (More ionized) | **Optimal pH** (Balanced ionization) | **High pH** (Less ionized)



Low pH:
- Shorter retention
- Earlier elution
- May improve peak shape for basic compounds

Optimal pH:
- Best resolution
- Good peak shape
- Balanced retention

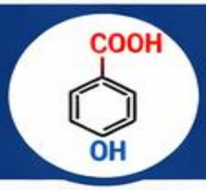
High pH:
- Longer retention
- Later elution
- Risk of peak broadening

Increasing pH

KEY TAKEAWAY: pH controls ionization, which drives retention, peak shape, and selectivity.
Careful pH optimization = better separation, sharper peaks, and reliable results.

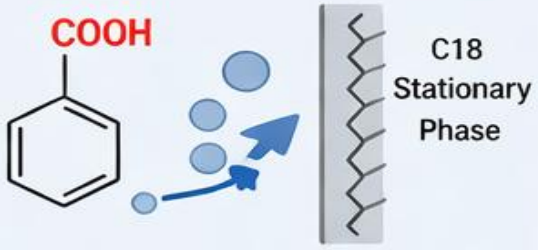
pH Effects: Acidic vs. Basic Compounds

In RP-HPLC, the pH of the mobile phase controls the ionization state of analytes, which directly affects **retention time, peak shape, selectivity, and resolution.**

Acidic Compounds


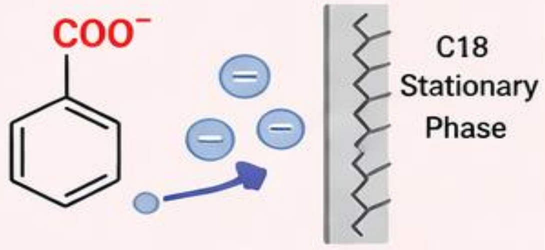
(e.g., Carboxylic Acids, Phenols)

Low pH (below pKa)
Neutral (non-ionized) form

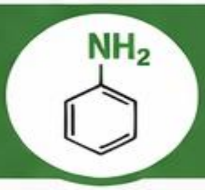


- Stronger hydrophobic interaction with C18 stationary phase
→ **Longer retention time**

High pH (above pKa)
Ionized form (A^-)



- More soluble in aqueous mobile phase
→ **Shorter retention time**

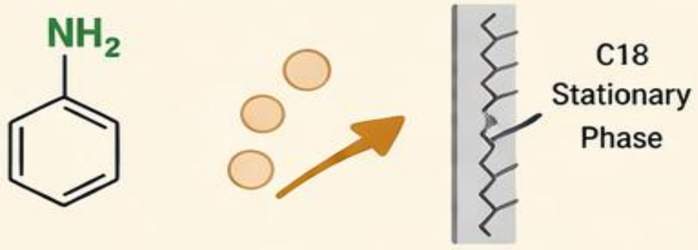
Basic Compounds


(e.g., Amines, Alkaloids)

Low pH (below pKa)
Protonated (ionized) form (BH^+)

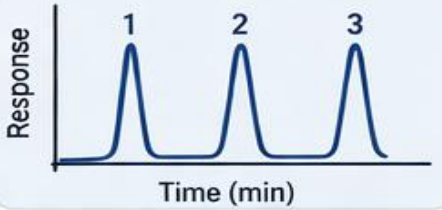
- More polar
→ **Elutes faster**

High pH (above pKa)
Neutral (non-ionized) form



- Increased hydrophobic character
→ Stronger stationary phase interaction → **Longer retention time**

KEY TAKEAWAY: The relationship between pH and pKa is the key — analyte retention in RP-HPLC is **maximized for neutral species and minimized for ionized species.**

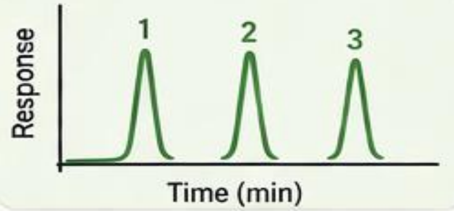


Stronger retention
= Peaks elute later

Neutral species
(non-ionized)
= **Max retention**

Ionized species
= **Min retention**

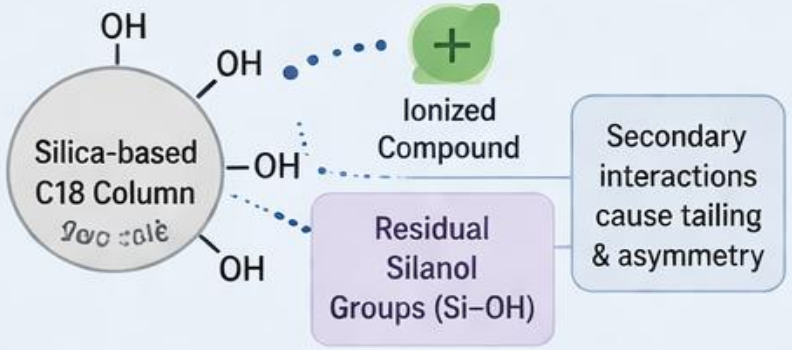
Weaker retention
= Peaks elute earlier



pH Effects: Peak Shape, Selectivity & Resolution

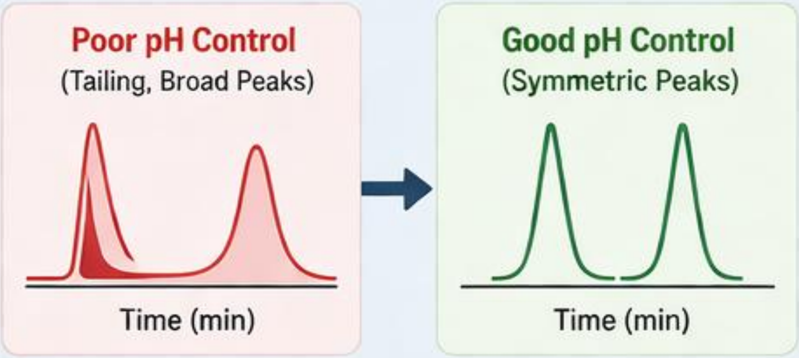
2. Peak Shape

Poorly controlled pH leads to peak tailing and broadening.



Ionized compounds interact with residual silanol groups on silica, causing asymmetric peaks.

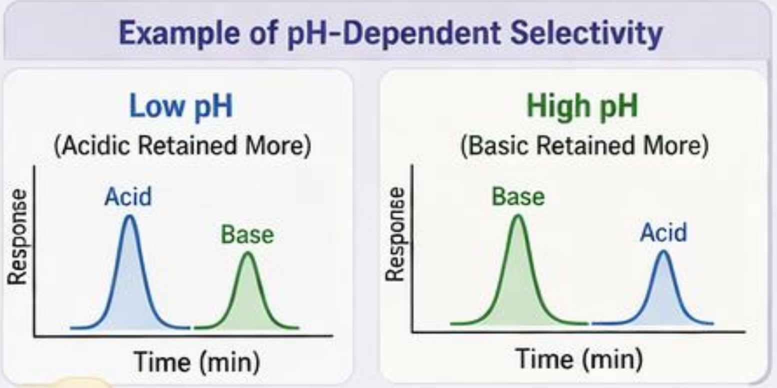
Buffers (phosphate, acetate, formate) stabilize pH and dramatically improve peak symmetry.



3. Selectivity & Resolution

Changing pH alters the relative retention **order** of acidic and basic analytes, providing a powerful tool for resolving closely eluting peaks.

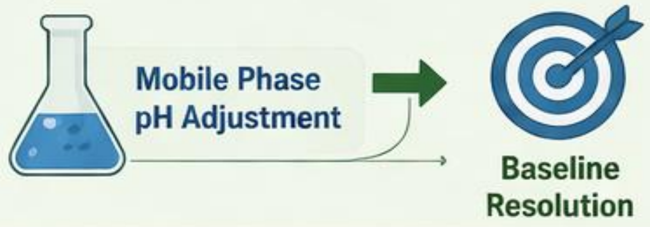
In mixtures of acidic and basic drugs, pH adjustment can **selectively retain one compound** while allowing another to elute earlier.

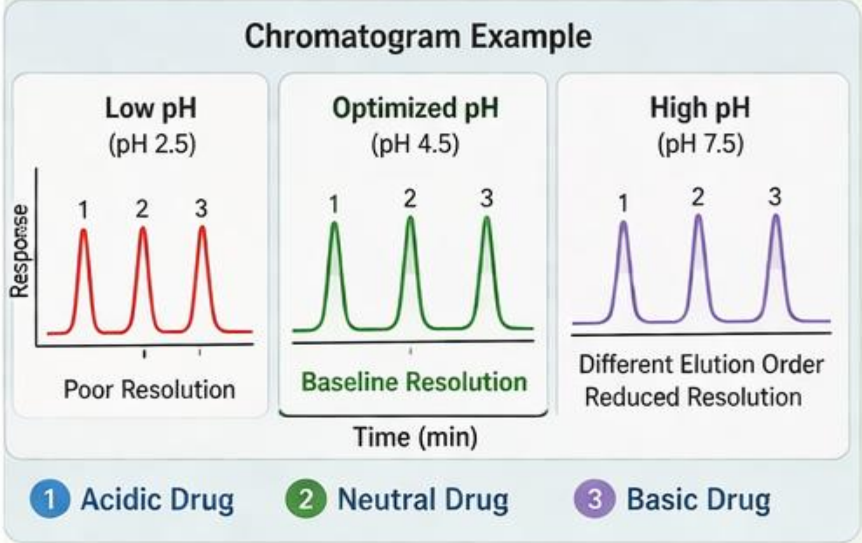


pH adjustment changes who stays longer and who comes out first.

Practical Example

In a pharmaceutical formulation containing both acidic and basic active ingredients, fine-tuning the mobile phase pH is often the decisive step in achieving **baseline resolution** of all components within an acceptable run time.

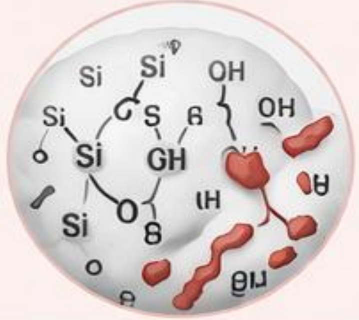




The right pH = the right separation

3. pH Stability of HPLC Columns

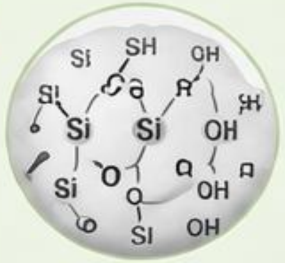
Silica-based HPLC columns — the most widely used in pharmaceutical analysis — have a defined **pH operating range** that must be respected to preserve column performance and longevity.



1. pH < 2 — Danger Zone

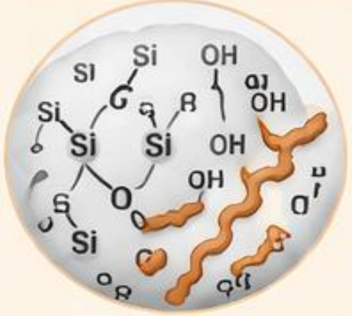
Silica begins to **dissolve** at extremely low pH, leading to column bed collapse, void formation, and shortened column life.

2. pH 2–8 — Safe Operating Range



Silica-based columns are **stable** within this range. Most pharmaceutical RP-HPLC methods are developed within **pH 2.5–7.5** for optimal stability and reproducibility.

3. pH > 8 — Degradation Zone



At high pH, **silanol groups degrade**, leading to increased peak tailing, loss of retention, and irreversible damage to column selectivity.

For analyses requiring extreme pH conditions, **hybrid silica or polymer-based columns** with extended pH stability **(pH 1–12)** should be considered.


Thank You
for Exploring the Power of
HPLC
in Pharmaeccutical Analysis
Enabling Precision. Ensuring Quality.
Advancing Patient Safety.
BUFFER
pH 4.0
ACETONITRILE
WATER
HPLC SYSTEM
READY
CHROMATOGRAM
Time (min)
Peak RT (min) Area % Area Compound
1 2.35 15423 15.21 Impurity A
2 4.87 25631 25.24 API
3 7.12 18953 18.67 Impurity G
4 9.43 31424 30.88 Impurity C
5 11.35 10322 10.20 Impurity D
Drug Substance Assay & Purity
Drug Product Quality Control
Impurity Identification & Quantification
Method Development & Validation
Stability Testing & Degradation Studies
Regulatory Compliance & Patient Safety