HPLC (High Performance Liquid Chromatography)

Principle of HPLC (High Performance Liquid Chromatography)

Basic Concept



- High Performance Liquid Chromatography (HPLC) is used to separate, identify, and quantify components in a mixture.
- **Separation** occurs because compounds distribute differently **between:**
  - **Mobile phase**: Liquid that moves
  - **Stationary phase**: Material packed inside the column

Core Principle

- When a sample mixture is injected into the mobile phase, it is carried through the column.
- Inside the column, different components interact differently with the stationary phase.
- **Some compounds:**
  - Stick strongly to the stationary phase → move slowly
  - Interact weakly → move quickly
- This difference in movement leads to separation

First Hope

Types of Interactions

- **Adsorption (Normal phase HPLC)**: Polar stationary phase, non-polar mobile phase
- **Partition (Reverse phase HPLC)**: Non-polar stationary phase (e.g., C18), polar mobile phase
- **Ion-exchange**: Separation based on charge
- **Size exclusion**: Separation based on molecular size
- **Affinity**: Based on specific binding interactions

Retention Time (tR)

- Time taken by a compound to reach the detector after injection
- Each compound has a unique retention time under fixed conditions

Modes of HPLC

- **Normal phase HPLC**
  - Polar stationary phase
  - Non-polar mobile phase

Copyright by Engacy Learning Private Limited. All rights reserved. Reproduction or distribution without written consent is strictly prohibited.

- **Reverse phase HPLC (most common)**
  - Non-polar stationary phase
  - Polar mobile phase
- **Ion exchange HPLC**
- **Size exclusion HPLC**

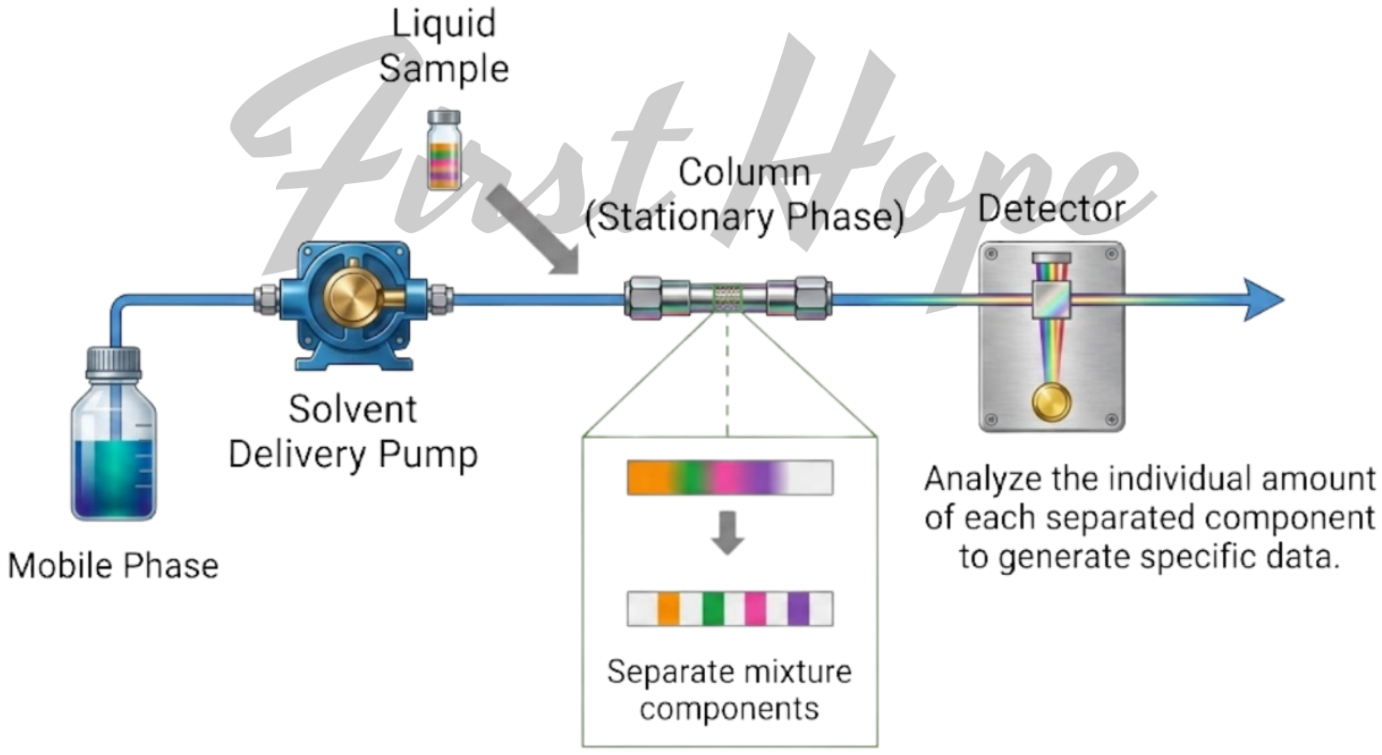
Need for High Pressure

- Small particle size in column packing increases resistance
- High pressure is required to push solvent through the column

Instrumentation of HPLC

- An **HPLC System** consists of several interconnected components, each with a specific function.

Main Components



A. Solvent Reservoir

- Stores mobile phase solvents
- Can contain one or more solvents
- Solvents must be filtered and degassed

B. Pump

- Pushes mobile phase through the system at high pressure

Copyright by Engacy Learning Private Limited. All rights reserved. Reproduction or distribution without written consent is strictly prohibited.

- Maintains constant flow rate
- **Types:**
  - Reciprocating pump
  - Syringe pump
  - Pneumatic pump

C. Degasser

- Removes dissolved gases from solvents
- Prevents bubble formation and noise in detector

D. Injector

- Introducing sample into the mobile phase
- **Types:**
  - Manual injector
  - Auto-injector
- Provides accurate and reproducible sample volume

E. Column

- Main part where separation occurs
- Packed with stationary phase particles
- **Types:**
  - Analytical column
  - Preparative column
- **Important parameters:**
  - Length
  - Diameter
  - Particle size

F. Column Oven

- Maintains constant temperature
- Improves reproducibility and peak quality

G. Detector

- Detects compounds as they come out of the column
- Converts signal into chromatogram

First Hope

Copyright by Engacy Learning Private Limited. All rights reserved. Reproduction or distribution without written consent is strictly prohibited.

- **Types:**
  - UV detector
  - PDA detector
  - Fluorescence detector
  - Refractive index detector
  - Mass spectrometer

H. Data System

- Records and processes data
- Gives chromatogram with peaks
- Calculates retention time and peak area

Flow Sequence

- Solvent reservoir → Pump → Injector → Column → Detector → Data system

Pharmaceutical Applications of HPLC

HPLC is one of the most important analytical techniques in the pharmaceutical industry due to its accuracy, sensitivity, and reproducibility.

1. Drug Analysis

- Identification and quantification of active pharmaceutical ingredients (APIs).
- Determination of drug purity.

2. Quality Control

- Ensures consistency in drug formulations.
- Detects impurities and degradation products.

3. Stability Studies

- **Used to study how drugs degrade over time under:**
  - Heat
  - Light
  - Humidity
- Helps in determining shelf life.

4. Dissolution Testing

- Measures the rate at which a drug dissolves in biological fluids.
- Important for bioavailability studies.

Copyright by Engacy Learning Private Limited. All rights reserved. Reproduction or distribution without written consent is strictly prohibited.

5. Bioanalysis

- **Determines drug concentration in:**
  - Blood
  - Plasma
  - Urine
- Used in pharmacokinetic studies.

6. Impurity Profiling

- Detects trace-level impurities.
- Essential for regulatory compliance (ICH guidelines).

7. Chiral Separation

- Separates enantiomers (optical isomers).
- Important because different enantiomers may have different biological effects.

8. Herbal and Natural Product Analysis

- Identification of active compounds in plant extracts.

9. Preparative Purification

- Isolation of pure compounds for research or production.

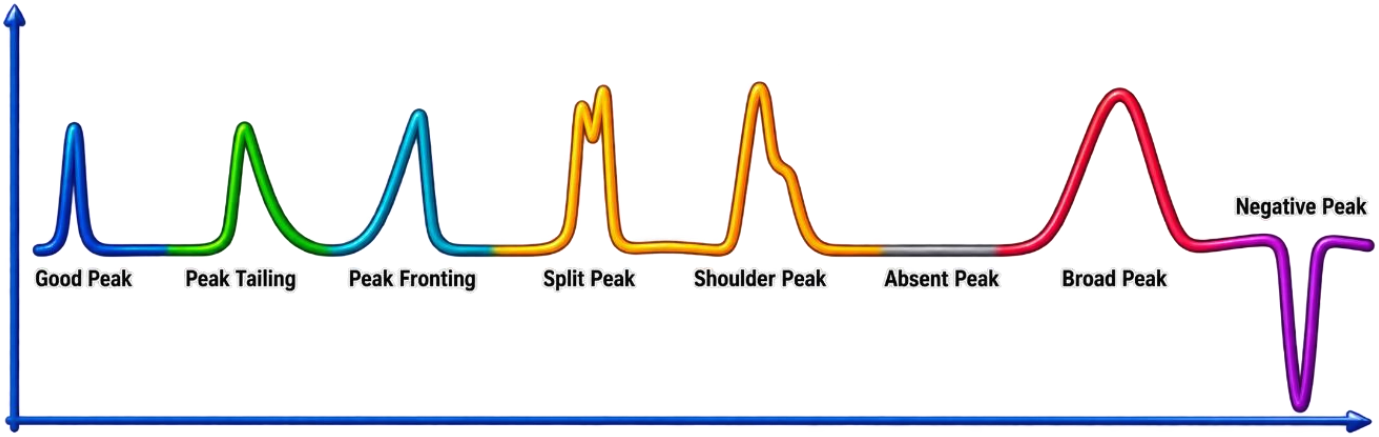
First Hope

Advantages in Pharmaceuticals

- High sensitivity and accuracy
- Suitable for non-volatile and thermally unstable compounds
- Wide applicability across drug types

Peak Shapes in HPLC

- In **HPLC**, the detector gives a signal in the form of peaks on a chromatogram.



Copyright by Engacy Learning Private Limited. All rights reserved. Reproduction or distribution without written consent is strictly prohibited.

First Hope

FREE PREVIEW

Click here to visit our website

PHARMACY PREMIUM NOTES

Complete subject and semesters are available on FirstHope app only.

Scan or click here

Click here to get the app >

Available on Google Play

JOIN THE FIRSTHOPE COMMUNITY

Join our Telegram channel

Click here to join Telegram >

Scan or click here

@FirstHopeNotes

Instagram

@firsthope.official

Click here to follow >

YouTube channel

FirstHope-Pharmacy / Live streams

Click here to watch >

WhatsApp channel

Follow FirstHope on WhatsApp

Click here to join >

FirstHope website

firsthope.co.in

Click here to visit >

B.Pharm / D.Pharm / M.Pharm / Pharm.D

Preview notes. Complete subjects and semesters in the FirstHope app.