High-Performance Liquid Chromatography (HPLC)

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Introduction

- HPLC is an advanced form of liquid chromatography that allows high-resolution separation, quantitative analysis, and purity testing of complex mixtures.
- It is a widely used analytical tool in:
  - Pharmaceutical industries
  - Quality control laboratories
  - Research and development
  - Regulatory submissions (ICH, USP, BP, IP)
- **In pharmaceutical analysis, HPLC is primarily employed for:**
  - Assay of active pharmaceutical ingredients (APIs)
  - Impurity profiling
  - Dissolution testing
  - Stability-indicating methods

Theory of HPLC

- HPLC is based on the principle of partition chromatography, where analytes are separated based on differences in their affinity (distribution) between a stationary phase (column packing) and a mobile phase (liquid solvent).
- **As the sample is injected into a flowing mobile phase and passed through the column:**
  - Components interact differently with the stationary phase.
  - Molecules with higher affinity for the stationary phase move more slowly.
  - Those with higher affinity for the mobile phase elute faster.
- This results in the separation of components, each of which produces a distinct peak on the chromatogram.

Retention Time (tR)

- The time it takes for an analyte to elute from the column.
- Used to identify compounds.

Theoretical Plates (N)

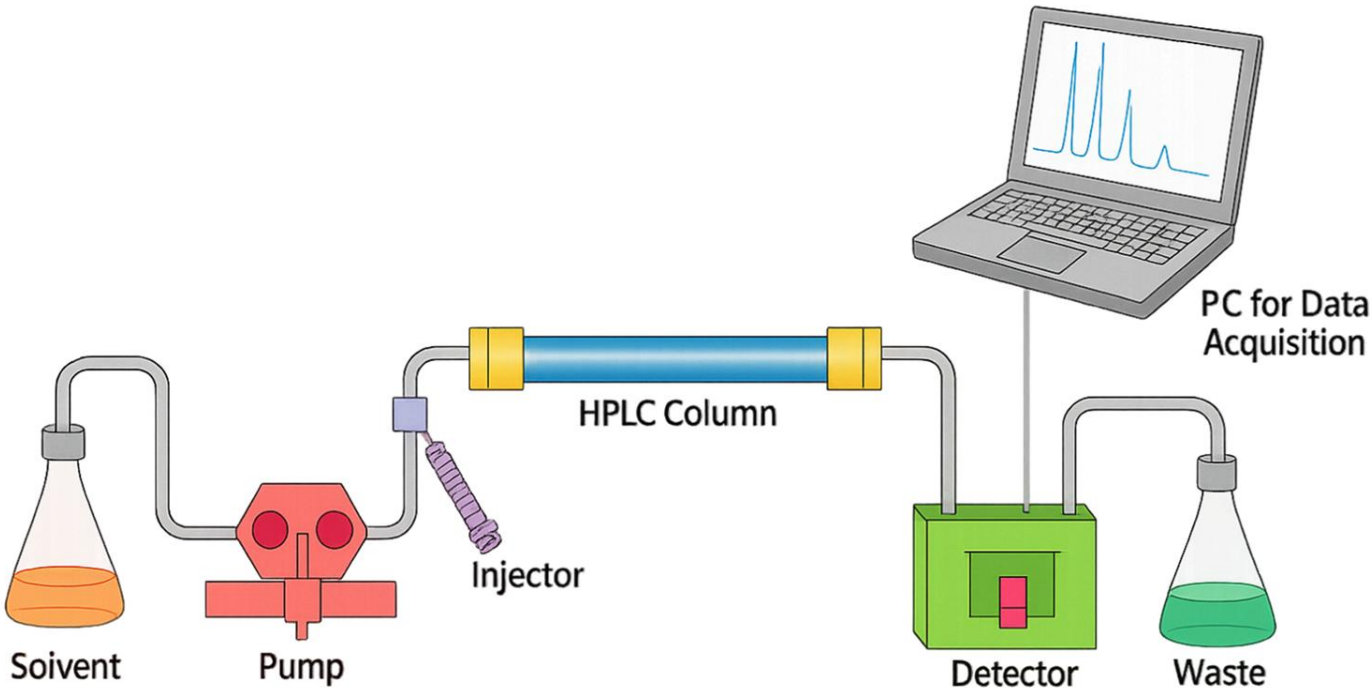
- Indicates column efficiency.
- Higher the number of plates, better the separation.

Selectivity (α) and Resolution (Rs)

- Describe how well two peaks are separated.
- Critical for distinguishing APIs from impurities and excipients.

Instrumentation of HPLC

The HPLC system consists of the following key components:



1. Solvent Reservoir (Mobile Phase Containers)

- Contains organic or aqueous solvents, or mixtures (e.g., water, acetonitrile, methanol).
- Must be degassed and filtered before use.
- Solvent choice depends on the polarity of analytes.

2. Pump

- Delivers the mobile phase at constant flow and pressure (typically 1–2 mL/min).
- Types:
  - **Isocratic pump** – constant mobile phase composition.
  - **Gradient pump** – mobile phase composition changes during the run (used for complex samples).

3. Injector

- Introduces the sample solution into the mobile phase stream.
- Manual injection (loop) or auto-samplers are used.
- Sample volume is usually 5–100 µL.

4. Column

- Packed with stationary phase particles (usually silica-based).
- Common dimensions: 150–250 mm length, 4.6 mm internal diameter.
- **Types of columns**:
  - **Reverse Phase (RP-HPLC)** – nonpolar stationary phase (e.g., C18) and polar mobile phase.
  - **Normal Phase** – polar stationary phase (e.g., silica) and nonpolar solvent.
  - **Ion-exchange** – for ionic compounds.
  - **Size exclusion (SEC)** – for large biomolecules.

5. Detector

- Detects compounds as they elute from the column. Common types:
- **UV-Visible Detector**: Most widely used in pharma for chromophore-containing drugs.
- **PDA (Photodiode Array)**: Detects across a range of wavelengths.
- **Fluorescence Detector**: High sensitivity for fluorescent compounds.
- **Refractive Index Detector**: Used for non-UV-absorbing substances like sugars.

6. Data Processor / Computer

- Records the chromatogram.
- Calculates retention time, area under the curve, and concentration.
- Software enables method development, peak integration, and report generation.

Applications of HPLC in Pharmaceutical Analysis

A. Assay of Active Pharmaceutical Ingredients (APIs)

- HPLC is used to determine the exact quantity of the drug in:
  - Raw materials
  - Finished dosage forms (tablets, injections, syrups)
  - **Example**: Assay of paracetamol, metformin, amoxicillin

B. Impurity Profiling

- Identifies and quantifies related substances, degradants, residual solvents, process impurities.
- Required by ICH guidelines for regulatory approval.
- **Example**: Impurity detection in valsartan, especially nitrosamines.

C. Drug-Excipient Compatibility

- Detects interactions that may affect drug stability or release.

- **Example**: Checking for degradation of aspirin in presence of moisture-retaining excipients.

D. Stability Studies

- Used in stability-indicating assay methods (SIAM).
- Detects and quantifies degradation products under stress (acid, base, heat, light).
- Ensures shelf life and storage conditions are appropriate.

E. Dissolution Testing

- Measures rate of drug release from solid dosage forms into the medium.
- HPLC is used to quantify drug dissolved over time points.
- Required for bioequivalence and quality assurance.

F. Bioanalytical Applications

- Estimation of drugs and metabolites in plasma, serum, urine.
- Used in pharmacokinetic studies and therapeutic drug monitoring.
- **Example**: Estimation of phenytoin levels in plasma.

G. Product Release and Regulatory Compliance

- Final product release testing according to USP/EP/BP/IP monographs.
- Batch release certificates require HPLC-based assays for content uniformity.

H. Herbal and Biopharmaceutical Analysis

- Used in standardization of herbal extracts.
- Separation and quantification of proteins, peptides, nucleotides in biologics.

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