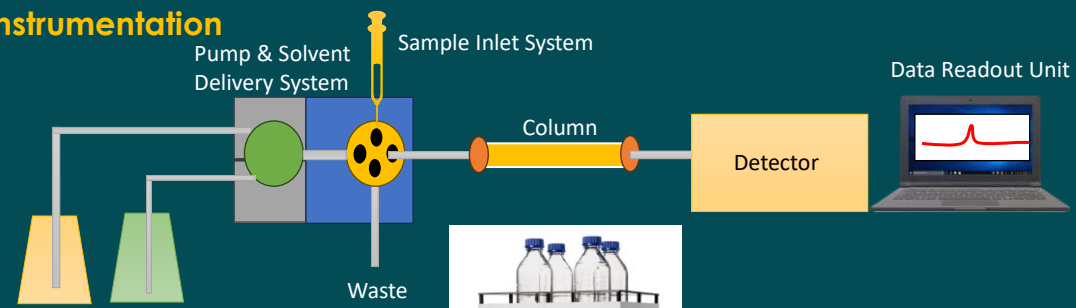
HPLC
Chromatography

Chromatography
Instrumental Analysis

HPLC

Instrumentation



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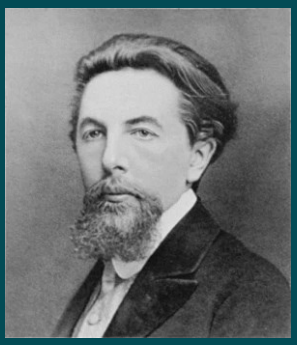
Introduction



- HPLC- High Performance Liquid Chromatography or High Pressure Liquid Chromatography
- It is a chromatographic technique used to separate, identify and quantify sample components in a liquid mixture, often characterized by high pressure
- It is a type of Liquid chromatography means all HPLC systems have one or more liquid/solvent as a mobile phase
- M. S. Tswett invented this method in around 1900s to study leaf pigments (mainly chlorophyll)
- It is a advance techniques of column chromatography

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Introduction

- Smaller particle size of column material enhance the surface are of separation and efficiency of column
- In 1960s – 40-60 um
- 1970s- 10-20 um
- 1980s- 5-10 um
- 1990s- 1-3 um

Parameter	Classical Column Chromatography	HPLC
Stationary Phase - Particle size	Large 60-200μ	Small 3-20μ
Column size length x int.diameter	Large 0.5-5m x 0.5-5cm i.d.	Small 5-50cm x 1-10mm i.d.
Column material	Glass	Mostly metal
Column packing pressure	Slurry packed at Low pressure - Often gravity	Slurry packed at High pressure > 5000 psi
Operating pressure	Low (< 20 psi)	High (500-3000 psi)
Flow rates	Low to very low	Medium - High (often > 3ml min^{-1})
Sample load	Low to Medium (g/mg)	Low to very low (μg)
Column efficiency i.e. Resolving power	(Low) < 500 theoretical plates per meter	(High) often > 1,00,000 plates per meter
Cost	Low - Few Hundreds	High - Few Lakhs
Detector flow cell volume	Large - 300 to 1000μl	Low 2 to 10μl
Types of Stationary phases available	Limited Range	Wide range
Scale of operation	Preparative Scale	Analytical and Preparative scale

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Types

1. **Normal Phase Mode:**
   - Stationary phase- Polar (Silica)
   - Mobile Phase- Non-polar
- In this techniques non-polar compound travel faster and eluted first that polar compound
2. **Reverse Phase Mode:**
   - Stationary phase- Non-Polar
   - Mobile Phase- Polar
- Most important techniques for pharmaceutical molecules (Polar in nature), In this techniques Polar compound travel faster and eluted first than non-polar compound
- Columns- ODS (Octadecyl Silane), C18, C8, C4, etc

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Principle: Based on type of packing material

1. **Adsorption-** distribution b/w liquid-solid
2. **Partition-** distribution b/w liquid-liquid
3. **Ion pair chromatography-** reverse phase column (non polar) converted into ion-exchange column temporary by using ion pair agent like pentane/hexane/heptane/octane sulfonic acid sodium salt, tetramethyl/tetra acetyl ammonium hydroxide.
4. **Ion-Exchange chromatography-** ion exchange resin is used to separate similar charged components **Affinity**
5. **Size exclusion/Gel permeation:** to separate different mol. size by using dextran, polyacrylamide, or agarose gel.
6. **Chiral phase:** to separate optical isomers by using chiral stationary phase
7. **Affinity:** for biological components

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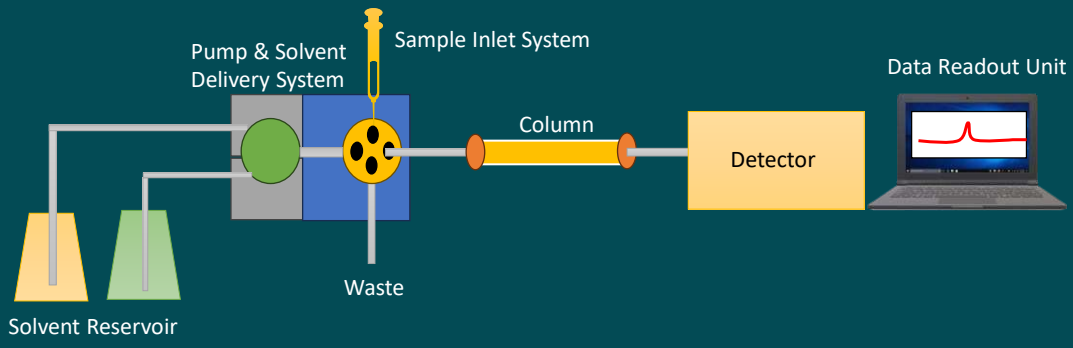

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INSTRUMENTATION

1. **Solvent/Mobile phase:** non-polar or polar depends on component nature (must filtered by 0.45 um filter)

- Isocratic separation – same or similar polar solvent used throughout the separation
- Gradient separation- combination of different solvent nonpolar: polar

2. **Pump/Solvent delivery system**

- Solvent or mobile phase used must be passed through column at high pressure (1000-3000 psi) because of particle size of column is very small (5-10 um).
- Mechanical pump- operate with constant flow rate and used a sapphire piston
- Pneumatic pumps- operate with constant pressure and high compressed gas

3. **Solvent Delivery system**

- Mix in specific ratio as per required

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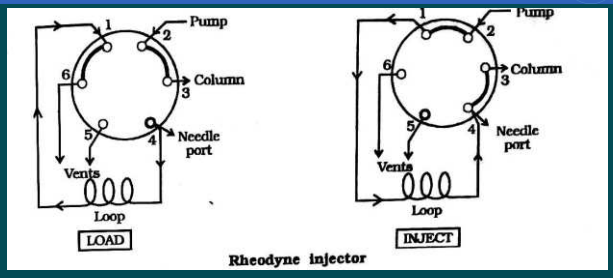
INSTRUMENTATION

4. Injection port: Automated sampling unit

a) Saptum injector

b) Stop flow

c) Rheodyne Injector (Loop valve type)



Rheodyne injector

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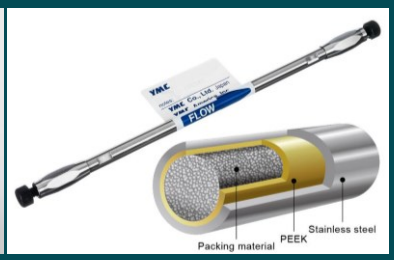
5. Column:

- Guard Column- It act as a prefilter for analytical column, having less quantity of adsorbent and improve the life of analytical column
- Analytical colum-
  - Made up of Stainless steel
  - 5-30 cm length
  - Diameter- 2-50 mm
  - Particle size- 1-20 um
  - Surface area per gm st phase- avg 400 sqmtr



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6. Detector:

UV detector	The light source is a D_2 lamp. This detector is used mainly to detect components having an absorption wavelength of 400 nm or less in the ultraviolet region.
UV-VIS detector	A D_2 lamp and a W lamp are used as the light source. This detector is effective in the detection of coloring components such as dyes and stains because of coverage of the visible light region.
Diode array detector (DAD)	Data on the spectrum from the ultraviolet to visible light range is also collected.
Fluorescence (FL) detector	Fluorescent substances can be detected specifically with high sensitivity.
Differential refractive index (RI) detector	Change in the refractive index is detected. Components absorbing no ultraviolet light can also be detected despite low sensitivity.
Conductivity detector	Mainly inorganic ions are detected by monitoring the conductivity.

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APPLICATION

The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy.

- Analysis of drugs
- Analysis of synthetic polymers
- Analysis of pollutants in environmental analytics
- Determination of drugs in biological matrices
- Isolation of valuable products
- Product purity and quality control of industrial products and fine chemicals
- Separation and purification of biopolymers such as enzymes or nucleic acids
- Water purification

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APPLICATION

The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy.

- Pre-concentration of trace components
- Ligand-exchange chromatography
- Ion-exchange chromatography of proteins
- High-pH anion-exchange chromatography of carbohydrates and oligosaccharides

PC
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