

## DEPTH OF BIOLOGY - Level up your studies with DOB

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### UNIT –IV

**Gas chromatography** - Introduction, theory, instrumentation, derivatization, temperature programming, advantages, disadvantages and applications

**High performance liquid chromatography (HPLC)**-Introduction, theory, instrumentation, advantages and applications.

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## • High Performance Liquid <sup>1</sup> Chromatography. (HPLC).

→ To Separate, Identify & Quantify the Mixture of Component

→ HPLC is a type of Liquid Chromatography.

→ Liquid Chromatography because here Mobile Phase is Liquid

→ High Performance word is Used because resolution power of HPLC is too good.

→ Resolution power means ability to distinguish b/w 2 Components.

→ HPLC purify the Components on the basis of Polarity.

→ Polar  $\Rightarrow$  Like  $H_2O$  (Hydrophilic)

→ Non-Polar  $\Rightarrow$  Hydrophobic.

★ It gives High performance due to smaller particle size of the Stationary Phase (Avg. Particle Size is  $3.5-10\mu m$ ).

★ High pressure (1000-4000 psi) is applied for rapid separation of the Compounds.

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◦ Van - Deemter Equation → for HPLC 2.

$$(HETP = A + B/\mu + C\mu.)$$

HETP → Height Equivalent to Theoretical Plate.

(In HPLC → B = Negligible).

A = Eddy diffusion

B = Longitudinal diffusion

C = Resistance to Mass Transfer.

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[MOBILE PHASE].

★ The Solvent Used in HPLC Mobile Phase are Chosen on the basis of the type of HPLC selected for the purpose.

★ Commonly Used Solvents are

- Acetonitrile
- Methanol.
- Water.

★ Acidifier / Basifier / Buffer Solutions are added in small Amount for better Separation.

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### Stationary Phase in HPLC.

3.

- Always Solid Stationary Phase is Used.
- Particle Size range of Stationary Phase is  $3.5-10 \mu\text{m}$ .
- Two types of Stationary Phases are Used in HPLC.  $\rightarrow$ 
  - ① Normal Phase  $\rightarrow$  Silica Gel. (Polar in Nature).
  - ② Reversed Phase  $\rightarrow$  ODS-Silica Gel (Non-Polar in Nature)

### # Instrumentation & Working.

HPLC Instrument  $\Rightarrow$  (Contain different Parts).

- ① Solvent Reservoir  $\rightarrow$  Contains different Solvents.
- ② HPLC Pump  $\rightarrow$
- ③ Degasser attach with Vacuum pump  $\rightarrow$
- ④ Solvent Mixing Valve  $\rightarrow$
- ⑤ Solvent Injector  $\rightarrow$
- ⑥ Pre-Column or Guard Column  $\rightarrow$  Length of Pre-Column is 2-10 cm.  
 $\hookrightarrow$  It's Internal diameter is 4-5 mm.  $\nearrow$  Made up of stainless steel.

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g) Analytical Column → Length of Analytical Column is  $10-30$  cm.  
 Internal diameter 4-5 mm.  
 Made up of Stainless Steel.

\* These Columns Pre Column or Guard Column & Analytical Column are filled with Stationary Phase.

\* Commonly Used Stationary Phase is ODS Silica Gel.

h) Detector

i) Recorder

### Working

→ Solvent will be pump by the HPLC pump from the Solvent Reservoir to Degasser

& Degasser is Connected with Vacuum pump which Create High Vacuum (so, any gas or air dissolved in Solvent will be removed).

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→ Then after, this Solvent moves from Degasser to<sup>5.</sup> Solvent Mixing Valve (Mixing of Solvent takes place which will help to produce Uniform Mobile Phase).

⇒ Now, this Mobile Phase moves towards Pre-Column or Guard Column.

This Pre-Column or Guard Column removes impurities from the Mobile Phase.

⇒ Then Mobile Phase further moves towards Analytical Column then Detector.

★ Blank Mobile Phase will run for 10 Minutes which gives proper condition to HPLC Instruments.

⇒ Now, we introduce sample to sample injector with the help of Microsyringe.

→ After injecting the sample it will enter into Guard Column then Analytical Column<sup>\*\*</sup> (which contains stationary phase).

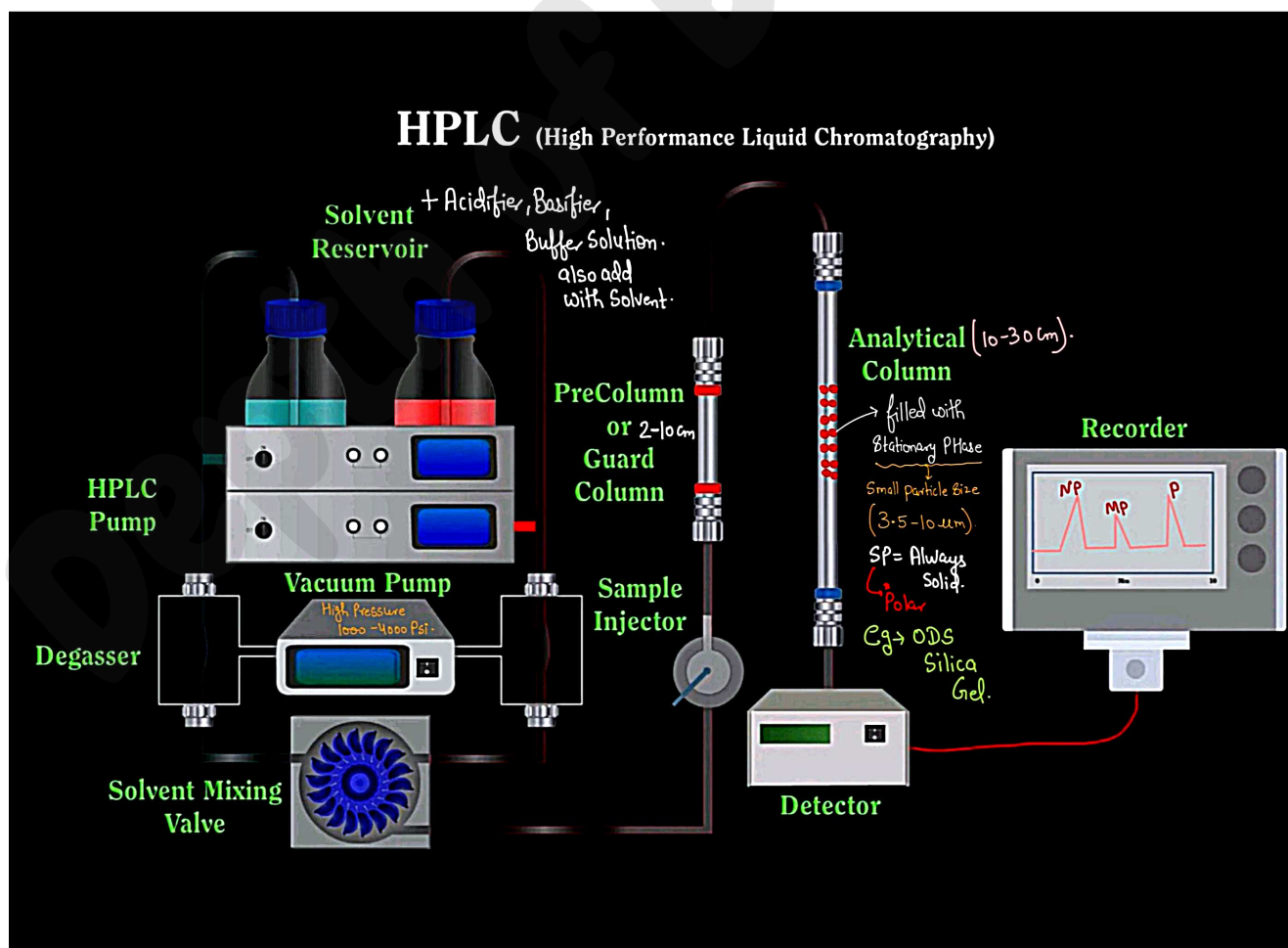
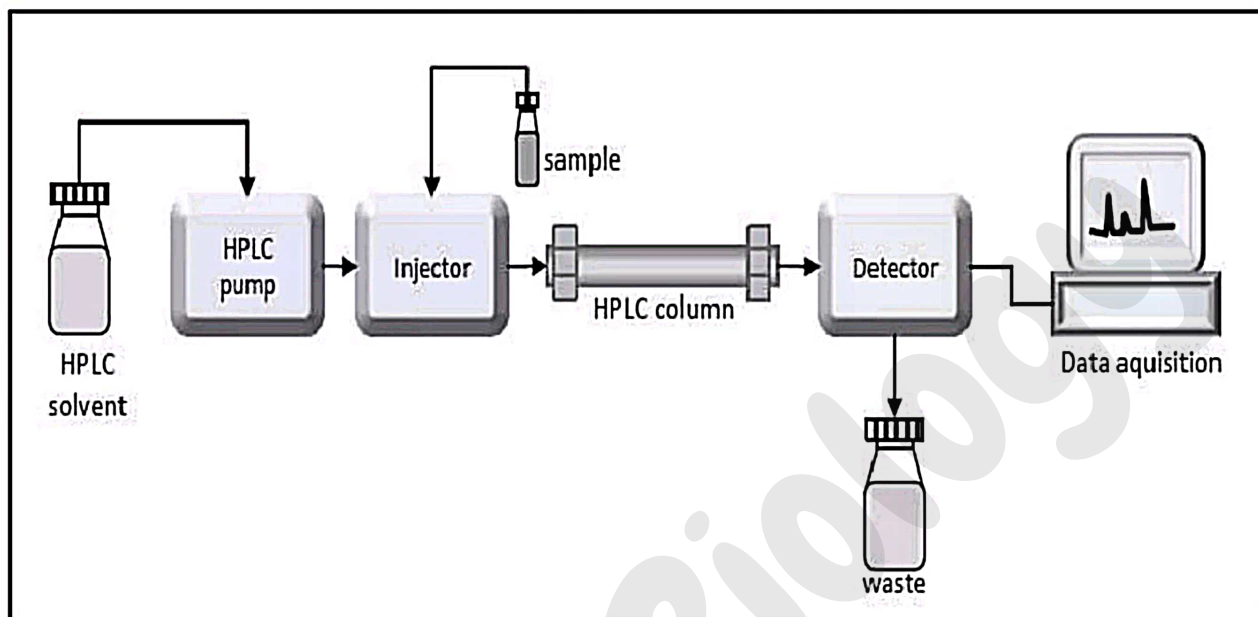
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⇒ Compounds (sample) which has less affinity towards <sup>6.</sup> Stationary Phase will reach to the Detector first.  
↓  
Then Moderate affinity Compound will reach.  
↓  
Then Highest affinity Compound will reach.

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### **The advantages of HPLC**

- **1- High speed**
- **2- High resolution**
- **3- High sensitivity**
- **4- Re-usable column**
- **5- No destruction of the components**
- **6- The instrumentation are automatic, computerized**
- **7- Sample is recovered completely**
- **8- Quantitative work is more easily and most sensitive**

### **Applications of HPLC (1)**

#### ☐ **Pharmaceutical Applications**

1. To control drug stability.
2. Tablet dissolution study of pharmaceutical dosages form.
3. Pharmaceutical quality control.

#### ☐ **Environmental Applications**

1. Detection of phenolic compounds in drinking water.
2. Bio-monitoring of pollutants.

#### ☐ **Applications in Forensics**

1. Quantification of drugs in biological samples.
2. Identification of steroids in blood, urine etc.
3. Forensic analysis of textile dyes.
4. Determination of cocaine and other drugs of abuse in blood, urine etc.

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### Applications of HPLC (2)

#### ☐ **Food and Flavour**

1. Measurement of Quality of soft drinks and water.
2. Sugar analysis in fruit juices.
3. Analysis of polycyclic compounds in vegetables.
4. Preservative analysis.

#### ☐ **Applications in Clinical Tests**

1. Urine analysis, antibiotics analysis in blood.
2. Analysis of bilirubin, biliverdin in hepatic disorders.
3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.