HPLC

- **What is chromatography ?**
- Chromatography is a separation technique that uses the size, shape, chemical properties or charge of molecules in a sample to separate the sample into its constituent components.
  Chromatography is a physical method of separation in which the components to be separated are, distributed two phases, one of which is stationary phase while the other is mobile phase, moves in a definite direction.

BACK TO BASIC

- **Chromatography Principle**
- Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. □e factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights. Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster

BACK TO BASIC

- **Why HPLC?**
- HPLC came about because not all compounds can be vaporized and analyzed on a GC
- Separation of a wider range of compounds -- high MW, polar, and ionic compounds
- Highly efficient separations achieved in HPLC due to interactions of both m.p. and s.p. with the components of a mixture.
- Improved separation within a much shorter time

WHY ALL THIS

- What is High-performance liquid chromatography (HPLC)?
- What parameters parameters are used as a standard for a particular compound
  Principle of High-Performance Liquid Chromatography (HPLC)
  Instrumentation of High-Performance Liquid Chromatography (HPLC)
  Types of High-Performance Liquid Chromatography (HPLC)

WHAT MORE

What is (HPLC)?

- **High-performance liquid chromatography** represents an automated system for the separation of compoundsin mixture using a liquid mobile phase, which is passed across the stationary phase under high pressure in order to speed up the operation.
- -The effluent of the column is monitored by special detectors andthe signals for the eluted components are recorded in a special recorder which amplifies such signals and record them as peaks similar to those obtained in gas chromatography.

TYPES OF HPLC TECHNIQUES:

- A. Based on modes of chromatography
- B. Based on principle of separation
- C. Based on elution technique
- D. Based on the scale of operation
- E. Based on the type of analysis

- 1. Normal phase mode
  2.Reverse phase mode

TYPE Based on modes of chromatography

- 1. Adsorption chromatography
  2. Ion exchange chromatography
  3. Ion pair chromatography
  4.Size exclusion(or)Gel permeation chromatography
  5. Affinity chromatography
  6. Chiral phase chromatography

B. Based on principle of separation

- 1. Isocratic separation
  2. Gradient separation

C. Based on elution technique

- 1. Analytical HPLC
  2. Preparative HPLC

D. Based on the scale of operation

- 1. Qualitative analysis
  2. Quantitative analysis

E. Based on the type of analysis

HPLC INSTRUMENTATON

- **HPLC INSTRUMENTATON BASIC INFORMATION:**
- 1. Solvent delivery system
  2. Pumps
  3. Sample injection system
  4. Column
  5. Detectors
  6. Recorders and Integrators

- **Applications of High-Performance Liquid Chromatography (HPLC**

Applications of HPLC

Applications of HPLC

- Pharmaceutical applications of HPLC are Tablet dissolution study of pharmaceutical dosages form, Shelf-life determinations of pharmaceutical products, Identification of active ingredients of dosage forms, Pharmaceutical quality control applications, Detection of phenolic compounds in Drinking Water, Identification of compounds in sediment samples, Bio-monitoring of pollutant, Quantification of the drug in biological samples. • Identification of anabolic steroids in serum, urine, sweat, and hair,Determination of cocaine and metabolites in blood Clinical Quantification of ions in human urine Analysis of antibiotics in blood plasma, Estimation of bilirubin and biliverdin in blood plasma in case of hepatic disorders,Detection of endogenous neuropeptides in extracellular fluids of brain.

Applications of HPLC

- Other applications include testing the quality of soft drink and drinking water, Analysis of beer, Sugar analysis in fruit juices, Analysis of polycyclic compounds in vegetables,Trace analysis of military high explosives in agricultural crops.

- Chemical Separations
- Purification

Chemical Separations
Purification

Applications of HPLC

- **HPLC USES**

HPLC USES

- 1. Separations fast and efficient (high resolution power)
  2. Continuous monitoring of the column effluent
  3. It can be applied to the separation and analysis of very complex mixtures
  4. Accurate quantitative measurements.
  5. Repetitive and reproducible analysis using the same column.
  6. Adsorption, partition, ion exchange and exclusion column separations are excellently made
  7. HPLC is more versatile than GLC in some respects, because it has the advantage of not being restricted to volatile and thermally stable solute and the choice of mobile and stationary phases is much wider in HPLC
  8. Both aqueous and non aqueous samples can be analyzed with little or no sample pretreatment
  9. A variety of solvents and column packings are available, providing a high degree of selectivity for specific analyses.
  10. It provides a means for determination of multiple components in a single analysis.

USES

Advantages HPLC

- **Advantages of High-Performance Liquid Chromatography (HPLC)**
- By using this High-Performance Liquid Chromatography (HPLC) technique it is possible to perform structural, and functional analysis, and purification of many molecules within a short time.
- This technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules
- In HPLC, mobile phase passes throuıgh columns under 10–400 atmospheric pressure, and with a high (0.1–5 cm//sec) flow rate.
- In this technique, use of small particles,and application of high pressure on the rate of solvent flow increases separation power, of HPLC and the analysis is completed within a short time

- **PARAMETERS USED IN HPLC:**
- 1.Retention time
  2.Retention volume
  3.Separation factor
  4. Resolution
  5. Height Equivalent to a Theoretical Plate (HETP)
  6. Efficiency
  7. Asymmetry factor

PARAMETERS USED

- **What are hplc detectors**
- The work of detector is to detect and give the information to the recorder which shows it in a form of a chromatogram. Every compounds has its own properties which is not completely the same with one another, thus this arises a need to have different detectors for different compounds. Before beginning the separation by HPLC it is thus very important to study about the nature of the compound and select the detector accordingly. The selection of wrong detector misguides our journey of separation and quantification.

What are hplc detectors

Types of hplc detectors

- **Types of hplc detectors**
- 1. Refractive index detectors
  2. U.V detectors
  3. Fluorescence detectors
  4. Electro chemical detectors
  5. Evaporative light scattering detectors
  6. IR detectors
  7. Photo diode array detector:

- **what are most common hplc detector**
- Detectors used depends upon the property of the compounds to be separated. Detectors are elemental detectors (atomic absorption/emission, inductively coupled plasma–mass spectrometry and microwave-induced plasma); optical detectors (UV/visible, IR/Raman, optical activity, evaporative light scattering and refractive index); luminescent detectors (fluorescence/phosphorescence, chemiluminescence/bioluminescence); electrochemical detectors (potentiometry, novel material/modified electrodes, array electrodes and pulsed and oscillometric techniques); mass spectrometric detectors (time-of-flight/MALDI, Fourier transform ion cyclotron resonance mass spectrometry, electrospray/thermospray, atmospheric pressure ionization and particle beam); and other detection systems (nuclear magnetic resonance, radioactivity detectors, surface plasmon resonance)

common hplc detector

MODES OF HPLC

- HPLC Modes
  - Normal-phase (NPC)
  – Separation based on adsorption of the analyte onto a polar surface (silica)
  - Reversed-phase (RPC)
  – Separation based on analytes' partition coefficients between the mobile phase and the bonded stationary phase
  - Ion-exchange (IEC)
  – Separation based on ion-exchanging with the counter-ions and ionic interaction with the bonded ionic group
  - Size-exclusion (SEC orGFC)
  – Separation based on analyte's molecular size and sieving action of the column packing

Limitations of HPLC

- **Limitations of HPLC**
- Lack of a Universal DetectorThe lack of a universal detector is often mentioned, although the UV–vis detec-tor comes close to one for chromophoric compounds. Refractive index detection fits the bill, but suffers from low sensi-tivity and incompatibility with gradient elution. Evaporative light scattering detection (ELSD) was a contender, but was surpassed by charged aerosol detec-tion (CAD). CAD uses a nebulizer with corona discharge detection and has bet-ter sensitivity (low ng) and ease-of-use than ELSD
- Less Separation Efficiency than Capillary Gas Chromatography Conventional HPLC has a practi-cal peak capacity (Pc) of ~200 using columns with ~20,000 plates under gradient conditions — not particularly effective for very complex samples
- Relatively More Difficult for Novices The bewildering number of HPLC modules, columns, mobile phases, and operating parameters renders HPLC difficult for the novice.
- Still Arduous, Particularly for Regulated Testing HPLC is versatile, quantitative, sensi-tive, and extremely precise. It can also be time-consuming and arduous, particularly for regulated analysis under good manufacturing practices (GMP).

- **Conclusion**
- HPLC is a complex technique because of its myriad combinations of modules, columns or mobile phases, and operating parameters. Initially chromatographic techniques were used to separate substances based on their color as was the case with herbal pigments. With time its application area was extended considerably. Nowadays, chromatography is accepted as an extremely sensitive, and effective separation method.
  HPLC technique which has many superior features including especially its higher sensitivity, rapid turnover rate, its use as a quantitative method, can purify amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, drugs, antibiotics, and steroids

Conclusion

- LETS JUST DISCUSS

Questions on HPLC