**Q1. What are the main differences between High Performance Liquid Chromatography and Gas Chromatography?**

•In HPLC the mobile phase is a liquid whereas in GC the mobile phase or carrier is a gas.
•HPLC is useful for analysis of samples which are liable to decompose at higher temperatures. GC involves high temperatures so compounds are stable at such temperatures.
•GC is applied for analysis of volatile compounds whereas non volatile compounds can be easily analyzed on HPLC
•GC cannot be used for analysis of high molecular weight molecules whereas HPLC has applications for separation and identification of very high molecular weight compounds
•HPLC requires higher operating pressures than GC because liquids require higher pressures than gases for transport through the system
•HPLC columns are short and wide in comparison to GC columns

**Q 2. Which type of High Performance Liquid Chromatography technique is most widely used?**
A. Reverse phase Chromatography has the widest range of applications. The stationary phase comprises non polar organic chains bound to inert silica surface and mobile phase comprises of aqueous or aqueous-organic mixtures comprising of polar solvents of varying degrees of polarity. The elution sequence is polar followed by less polar and least polar or non polar compounds eluting last through the column.

**Q3. What is the separation principle in Size Exclusion Chromatography?**
A. In size exclusion chromatography the separation does not involve chemical interactions between eluting molecules and stationary phase. The separation takes place on the basis of molecular size with larger molecules eluting first and small molecules in the end. Small molecules are retained longer in the pores of the stationary phase therefore they get eluted last.

**Q4. Why is it necessary to degass the mobile phase?**
A. Mobile phases entrap air from the atmosphere and this trapped air gets released as small bubbles under high pressures encountered during the HPLC analysis. Such bubbles can lead to noise in detector response or hinder flow of mobile phase through columns. In order to overcome such problems degassing of mobile phase becomes essential

**Q5. Which is the most commonly used detector in High Performance Liquid Chromatography and why?**
A. The most commonly used detector in HPLC is the UV-VIS detector. The reason for its predominant use is that it gives specific response to a particular compound or class of compounds. Most of the organic compounds absorb at specific wavelengths covered in the available wavelength range of the detector.

**Q6. What do you understand by a bulk property detector and a specific property detector?**
A. A bulk property detector responds to some property of mobile phase and sample combination

passing through it at any point of time such a Refractive index or Electrochemical detector whereas a specific property detector is responsive only to the characteristic property of the eluting molecule and is independent of changes in mobile phase composition such as UV-Vis and Fluorescence detectors.

**Q7. What do you understand by Isocratic and Gradient elution?**

A. When the composition of the mobile phase is not changed through the chromatographic run the operation is termed as isocratic. It can involve a single solvent or a mixture of two or more solvents mixed in a fixed proportion. In gradient operation the composition at start of run is programmed to change at a predetermined rate and the composition at the end of run is different from the composition at the start.

**Q8. What are the desirable features of a High Performance Liquid Chromatography detector?**

A. The desirable features of a detector are

- Sensitivity towards solute over mobile phase.
- Low dead volume to eliminate memory effects
- Low noise
- Low detection limits
- Large dynamic linear range

**Q9. What do you understand by theoretical plate concept and how HETP affects the separation of HPLC column?**

A. Plate theory concept was introduced to explain efficiency of columns. The concept assumes that a state of instantaneous equilibrium exists between the concentration of solute in stationary phase and the mobile phase and further the column is imagined to be divided into a number of theoretical plates. Any analyte spends a finite time in each plate and this is the equilibrium time. Smaller the plate height the greater is the number of plates in a given length (HETP) and better is the column resolution.

**Q10. What are the benefits of Fast LC or UHPLC?**

A. Fast or UHPLC technique makes use of small particles below 2 µ size Use of such particle sizes result in high resolution and as small columns can be used it results in completion of analysis in much less time thereby reducing consumption of expensive solvents.

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