

High Performace Liquid Chromatography

A Summary

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1 Introduction

- HPLC stands for "High Performance Liquid Chromatography" or "High Pressure Liquid Chromatography"
- **Advantages**
 - Analysis of thermally unstable compounds
 - Analysis of nonvolatile compounds
- **Major requirement of LC**
 - Solute solubility in mobile phase
 - This is in contrast to GC which require solute volatility

2 Scope of HPLC

Adsorption chromatography (LSC)

Ion chromatography (IC)

Size-exclusion chromatography (SEC)

Partition chromatography separation of analytes by partitioning, most commonly to a stationary phase bonded to a solid support

Note that this replaces liquid-liquid chromatography with its problems of stripping of stationary phase

Hydrophobic interaction chromatography (HIC) for separation of proteins without denaturation

Hydrophilic interaction chromatography (HILIC) for separation of very polar analytes

Chiral chromatography

Affinity chromatography

3 Column Efficiency in HPLC

- Recall the van Deemter equation for GLC (commonly called GC) for packed columns:

$$H = A + \frac{B}{u} + Cu \quad (1)$$

- For longitudinal diffusion in LC, as the $D_l \approx 10^{-5}D_g$, the peak broadening due to longitudinal diffusion in mobile phase (liquid) phase in LC is *negligible*.
- i.e., $\frac{B}{u} = \frac{2\gamma D_m}{u}$

4 Pumps

5 Elution Techniques

6 Injectors

7 Columns

8 Detectors

9 Types of Chromatography in HPLC