HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Introduction

High performance liquid chromatography technology has a wide range of applications and is widely used in the fields of chemistry, food, life sciences, medicine and environment. HPLC has wide applicability to samples and is not restricted by the volatility and thermal stability of the analytical object. Almost all compounds including high boiling point, polar, ionic compounds and macromolecular substances can be analyzed and determined by HPLC.

Basic principles of HPLC:
The liquid to be tested is injected into the chromatographic column and moves in the stationary phase by pressure. Due to the different species tested, the interaction between the substance and the stationary phase is different, and the order of different substances leaving the chromatographic column is different. The detector obtains different peak signals, and then analyzes and compares these signals to determine the substance contained in the analyte.

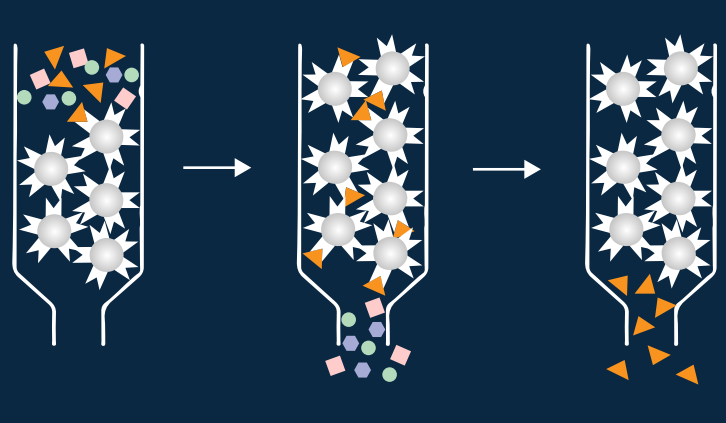
There are many different ways to separate target compounds using liquid chromatography, and the choice of technique usually depends on the physicochemical properties of the target molecule.

Commonly used modes of separation are based on:

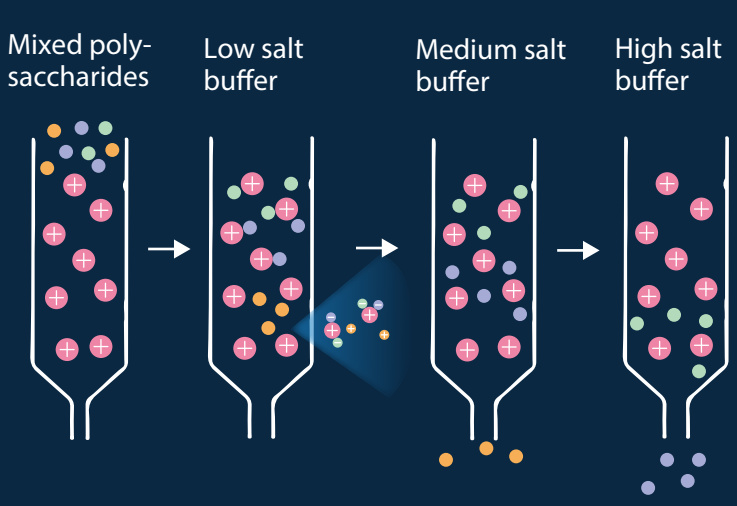
- Specific binding interactions (affinity chromatography)
- Charge (ion exchange chromatography)
- Size (size exclusion chromatography/gel filtration chromatography)
- Hydrophobic surface area (hydrophobic interaction chromatography and reverse phase chromatography)
- Multiple properties (multimodal or mixed-mode chromatography)

Affinity Chromatography

Affinity Chromatography uses the binding characteristics of the stationary phase to adsorb the target product and achieve separation and purification.



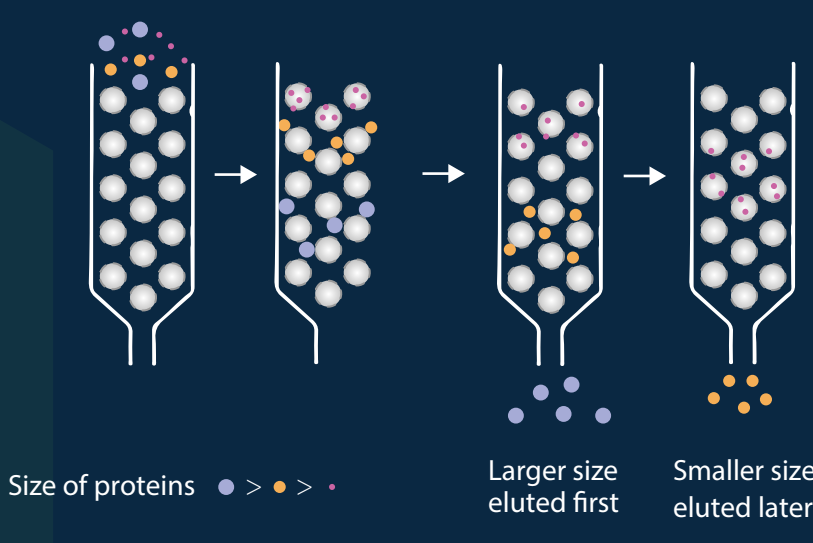
Ion Exchange Chromatography



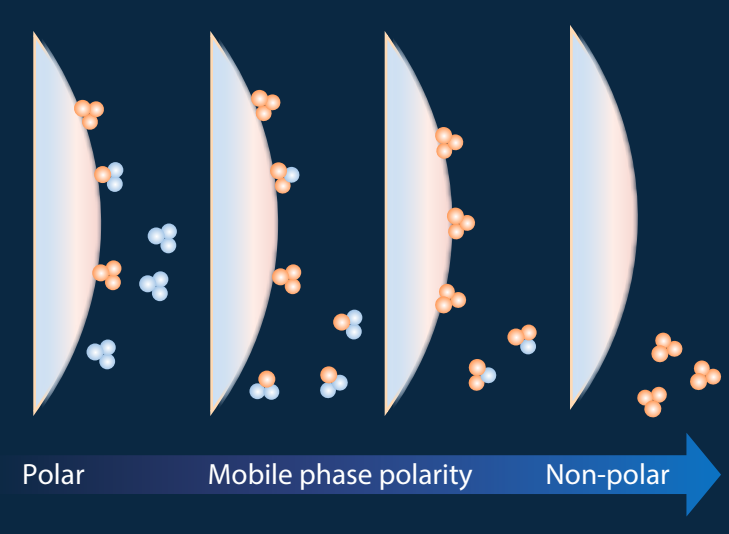
Ion exchange chromatography is based on the difference in protein charge for separation. The positively charged basic protein binds to the negatively charged matrix ion. The negatively charged acidic protein binds to the positively charged matrix ion. The sample is added to the chromatographic column containing ion exchange resin. The elution of proteins from the column depends on gradually increasing the salt concentration of the mobile phase, which weakens the ionic interaction and promotes its downward movement. Therefore, proteins that interact weakly with the resin are eluted first, and the elution order and resolution of the separated proteins depend on their charges.

Size Exclusion Chromatography

Size exclusion spectroscopy (also called gel filtration chromatography) separates proteins based on their molecular weight. The column packing with fine, porous beads allows for separation of small and big molecules, since the smaller molecules can enter the pores. Thus, they move slower than bigger molecules that cannot interact and enter the pores.



Reversed-Phase Chromatography



Most commonly used HPLC type with a non-polar stationary phase and an aqueous, polar mobile phase. Separations based on an analytes relative affinity for the mobile phase versus the stationary phase. In RP-HPLC, hydrophobic molecules tend to interact with the stationary phase, whereas hydrophilic molecules elute first.

Creative Proteomics' analytical scientists are very experienced in HPLC analysis applied to food and beverages to pharmaceuticals and many other industries. We provide fast-turnover customized services and concise and concise written reports to help customers solve your analysis and technical problems.

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