HPLC Separation Modes

Liquid chromatography (LC) uses liquid as mobile phase (eluent). It is an analytical method that separates a mixture of compounds based on their physical and chemical differences. High performance liquid chromatography (HPLC) is a method that introduces the mobile phase under high-pressure conditions resulting in rapid and high-performance separations. The various interactions between the analyte, stationary phase (packing material), and mobile phase are the key factors for the separation. A wide variety of separation modes can be achieved by using particular combinations of stationary and mobile phases.

Separation mode	Characteristics
Reversed Phase Chromatography (RPC)	• Separation is based on the partition equilibrium between stationary phase and mobile phase. • The polarity of the stationary phase is lower than that of the mobile phase. • Typically the mobile phase contains a mixture of organic solvents (methanol, acetonitrile, or THF) and aqueous solvents (water or buffer). • Using the lower polarity mobile phase causes a faster elution.
Hydrophilic Interaction Chromatography (HILIC)	• Separation is based on hydrophilic interaction. • A high polarity stationary phase is used. • Typically the mobile phase contains a mixture of organic solvents such as acetonitrile and aqueous solvents (water or buffer). • Using the higher polarity mobile phase causes a faster elution. • Applicable for the analysis of high polar substances.
Normal Phase Chromatography (NPC)	• Separation is based on the partition equilibrium between the stationary phase and the mobile phase. • The polarity of the stationary phase is higher than that of the mobile phase. • Typically the mobile phase contains a mixture of organic solvents with different polarities such as hexane and isopropanol. • Using the higher polarity mobile phase causes a faster elution.
Ligand Exchange Chromatography (LEX)	• Separation is based on differences in analytes' coordination complex. • Stationary phase modified with metal sulfonate complex ion. • Works in combination with size exclusion or HILIC modes.
Ion Exclusion Chromatography (IEX)	• Separation is based on electrostatic interaction (repulsion) between the ion exchanger and ionic solutes. • Dissociated ionic molecules elute faster than non-dissociated forms. • Used mainly for the analysis of organic acids.
Ion Chromatography (IC)	• Separation is based on electrostatic interaction (bonding) between the ion exchanger and ionic solutes. • Has a relatively small ion exchange capacity. • Electrical conductivity detector can be used with low-salt concentration mobile phase. • Used mainly for the analysis of inorganic compounds.
Size Exclusion Chromatography (SEC)	• Network or pores on the surface of the packing material works as molecular sieve to separate molecules based on their sizes. • To separate molecules solely based on their sizes, it requires an analytical condition without any analyte and packing gel interaction. • The bigger the molecule size, the faster the elution sequence. • Used for molecular weight or molecular distribution determination of macromolecules and qualification of oligomers.
Ion Exchange Chromatography (IEC)	• Separation is based on electrostatic interactions between the ion exchanger and ionic solutes. • The mobile phase of choice should have a sufficient buffering capacity at the pH that produces the largest charge differences between the analyte of interest. • The elution position is optimized by varying the pH, salt concentration, and/or ionic strength of the mobile phase.
Hydrophobic Interaction Chromatography (HIC)	• Separation is based on hydrophobic interaction. • Hydrophobic functional group is modified on the stationary phase. • Adsorption of analytes generally occurs at a high salt concentration and they are released by lowering the salt concentration. • Used mainly for the analysis of proteins.
Affinity Chromatography (AFC)	• Separation is based on adsorption of the analyte to the specific biologically derived ligand pair. • Highly selective. • A buffer solution with the appropriate pH and ionic strength is selected based on the type of ligand, analytes, and their interaction. • Used mainly for the purification and concentration of biological active substances.
Chiral Separation Chromatography (CS)	• Separation of optical isomers using chiral selectors. • Highly selective.
Multimode Chromatography	• Separation is based on the combination of different modes.

Column Selection by Sample Character and Separation Mode

Sample Solubility	Sample MW	Separation Mode
Aqueous soluble	≥ 2,000	RPC
		LEX
		IEX
		SEC
		IEC
		HIC
		AFC
	≤ 2,000	RPC
		HILIC
		LEX
		IEX
		IC
		SEC
		IEC
		AFC
		CS

Sample Solubility	Sample MW	Separation Mode
Organic soluble	≥ 2,000	SEC
	≤ 2,000	RPC
		NPC
		SEC

RPC : Reversed Phase Chromatography
HILIC : Hydrophilic Interaction Chromatography
NPC : Normal Phase Chromatography
LEX : Ligand Exchange Chromatography
IEX : Ion Exclusion Chromatography
IC : Ion Chromatography
SEC : Size Exclusion Chromatography
IEC : Ion Exchange Chromatography
HIC : Hydrophobic Interaction Chromatography
AFC : Affinity Chromatography
CS : Chiral Separation Chromatography

Column Selection (Application)

Pharmaceuticals, Cosmetics

		Separation Mode	Page
Pharmaceuticals Metabolites Additives	Hydrophobic substances	RPC	8, 10, 12, 16, 17
	Hydrophilic substances	HILIC	20, 22
		IEC+RPC	12
		LEX+SEC	24, 25
	Substances in bio-fluid (serum·plasma·urine)	RPC	8
		SEC+RPC	42, 44, 72
	Polymer	SEC	38, 44, 50, 56
Moisturizers	Polyalcohols	RPC	12
		LEX+SEC	24
		LEX+HILIC	24
		SEC	38, 44
	Protein hydrolysates	RPC	10, 12
		SEC	36
	Mucopolysaccharides	SEC	38
Emulsifiers	Surfactants	SEC+RPC	44
		SEC	46, 52
Preservatives	Paraben Dehydroacetic acid	RPC	10, 12, 16, 17
Optical active materials		CS	68

Foods

		Separation Mode	Page
Nutritional ingredients	Monosaccharides Disaccharides Sugar alcohols	HILIC	20, 22
		LEX+SEC	24
		LEX+HILIC	24
	Oligosaccharides	HILIC	20, 22
		LEX+HILIC	24
		SEC	24, 38, 42
	Low molecular water-soluble dietary fiber	SEC	42
	Polysaccharides	SEC	24, 38
	Organic acids	RPC	8, 12
		IEX+RPC	28
		IC	30
	Water-soluble vitamins	RPC	8, 10, 12
		IEC+RPC	12
		HILIC	20, 22
	Fat-soluble vitamins	RPC	10
		NPC	17
		SEC	46, 52
	Fatty acids	RPC	12, 16, 17
		SEC	44, 46, 48, 52
	Nucleic acids (umami)	IEC+SEC	42
	Amino acids	IEC+IEX+RPC	12
		IC	32
		IEC	66
Food safety	Food additives	RPC	10, 12, 68
		HILIC	20, 22
	Pesticides	RPC	12
		IEC+RPC	12
		HILIC	20
		IC	30
	Mycotoxin	RPC	16
	Pretreatment of residual pesticides	SEC GPC (Clean-up)	70

New Materials

		Separation Mode	Page
Synthetic polymers	Organic solvent soluble	SEC	44, 46, 48, 52, 56
	Polar organic solvent soluble		38, 44, 50, 52, 56
	High temperature/ Ultra high temperature		58
	Water-soluble		36, 38, 42, 44
Additives Oligomers		RPC	10, 12, 16, 17
	Organic solvent soluble	SEC	44, 46, 48, 52
	Polar organic solvent soluble		38, 44, 50, 52
	Water-soluble		36, 38, 42, 44

Separation Mode (Page 4 and Page 5)

RPC : Reversed Phase Chromatography
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Biotechnology

		Separation Mode	Page
Genomics	Nucleobases Nucleotides Nucleosides	RPC	12
		IEC+SEC	12, 42
		IEC	64
	Oligo nucleic acids	RPC	12
		IEC+SEC	42
		IEC	64
	DNA/RNA	SEC	38, 44
Proteomics	Amino acids	RPC	10
		IEC+IEX+RPC	12
		IEC	66
		IEC+SEC	42
	Peptides Proteins	RPC	10, 12
		SEC	36, 38, 42, 44
		IEC	64, 66
		HIC	68
Glycomics	Glycoproteins	RPC	10, 12
		SEC	36, 38, 42, 44
		IEC	64, 66
		HIC	68
		AFC	68
	Sugar chains	HILIC	20, 22
		AFC	68
	Monosaccharides	HILIC	20, 22
		LEX+SEC	24
		LEX+HILIC	24
	Sialic acids Uronic acids Aldonic acids	IEX+RPC	28
Hormones	Amines	RPC	8, 10, 12
		IEC	66
	Steroids	RPC	10
		HILIC	20, 22
		SEC	38, 44
Lipids	Phospholipids	NPC	17
		SEC	44, 46, 52
	Lipoproteins	SEC	38
		AFC	68

Environment

		Separation Mode	Page
Water quality	Anions	IC	30
	Oxyhalides	IC	30
		IEC+HILIC	20
	Cyanide Cyanogen chloride	IEX	28
	Cations	IC	32
	Surfactants	RPC	10, 16
		SEC+RPC	44
	Perchloric acids	IC	30
		IEC+HILIC	20
	Pesticides	RPC	12, 16, 17
		IEC+RPC	12
		HILIC	20
		IC	30
Soil	Anions	IC	30
	Heavy metals	IC	32
	Humic substances	SEC	38
	Organic arsenic	IEX+RPC	12
	Pesticides	RPC	12, 16, 17
		IEC+RPC	12
		HILIC	20
		IC	30
Environmental hormones	Pretreatment of Phthalates PCBs Benzo [a] pyrene	SEC GPC (Clean-up)	70
Bioethanols	Monosaccharides Oligosaccharides	HILIC	20, 22
		LEX+SEC	24
	Oligosaccharides Alcohols Furfural	LEX+SEC	24
	Saccharides Organic acids Alcohols Furfural	IEX+RPC+SEC	28
	Hemicelluloses Celluloses	SEC	50, 56
Biodiesels	Cations	IC	32
	Fatty acid glycerides	SEC	44
	Fatty acid methyl esters	RPC	12
	Organic acids	IC	30