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ordering **note**

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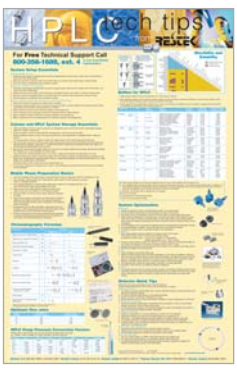

HPLC Tech Tips Wall Chart
Almost everything you need to remember about HPLC, condensed into 3 feet by 2 feet: mobile phase basics, buffers (types, pKa values, pH ranges, formula masses, more), miscibility and solubility chart (invaluable!), system setup and optimization, detector tips, pressure conversion factors, most-used chromatographic equations, and column storage essentials.

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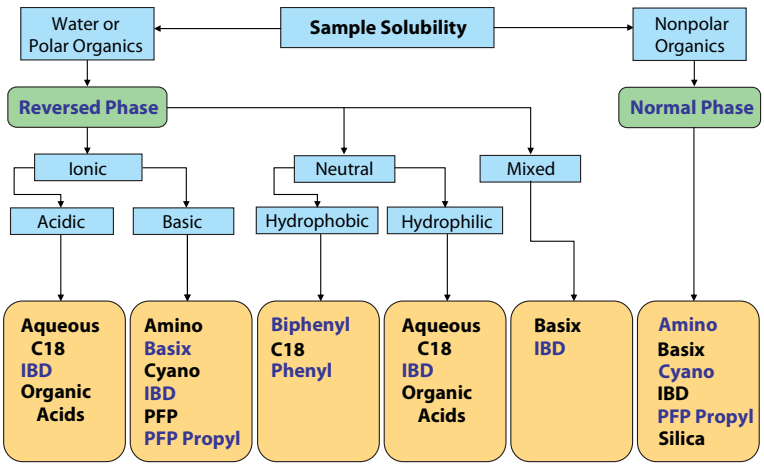
Selecting an HPLC Column

Choosing the best column for your application requires consideration of stationary phase chemistry, retention capacity, particle size, and column dimensions. Identifying the best stationary phase for your separation is the most critical step of column selection, and your decision should be based on sample solubility and the chemical differences among the compounds of interest. Figure 1 is a handy tool for stationary phase selection.

Figure 1 Stationary phase selection.



Reversed phase columns (e.g. alkyl, phenyl, cyano) work well for water-soluble hydrophobic compounds. Some stationary phases incorporate both polar and nonpolar functionality and can be used in either reversed phase or normal phase modes (e.g. Ultra IBD, Allure® Basix, and Allure® PFP Propyl). While straight chain alkyl stationary phases (e.g. C18) are historically the most commonly used, many newer phases provide better separations. Alkyl phases are best suited for analyzing neutral compounds with a high ratio of carbon:heteroatoms where the major distinction among analytes is their hydrophobicity. However, for analyzing compounds that are highly polar, aromatic, or halogenated, nonalkyl stationary phases often provide significantly better selectivity (Figure 2).

Retention capacity is another important consideration and is influenced by surface area and carbon load (% carbon in the packing material). Allure® columns were designed for maximum retention using a high density of ligands bonded to a large surface area silica. Ultra, Kromasil®, Pinnacle™ II and Pinnacle™ DB columns have the same high ligand density, but are more moderately retentive due to their lower surface areas. Surface area is inversely proportional to pore size; thus, larger pore sizes result in less retention. However, wide pore (e.g. 300Å) packings, such as Viva, are ideal when analyzing larger molecules, as a larger pore size is necessary to allow the analytes to 'fit' into the pores.

Particle size and column dimensions also influence column choice. In selecting a particle size, the primary consideration is efficiency (plates/meter) versus column pressure. A 3µm column will have approximately 50% more efficiency than a 5µm column, if all other conditions are constant for both columns. As particle size is further decreased (e.g. <2µm), theoretically, efficiencies will increase proportionally, based on the Van Deemter equation (and the usable flow rate range is much wider). Please note that column backpressure also increases as particle size decreases. Column dimensions include internal diameter and length, where the most commonly used internal diameter (ID) for HPLC columns is 4.6mm. In theory, resolution and pressure should be independent of column ID as long as flow rate is adjusted to maintain the same mobile phase linear velocity (flow rate is proportional to column cross-sectional area). Table I shows the approximate optimum flow rates for four column IDs.

Table I Approximate optimum flow rates for various analytical column IDs.

	5µm Particles	3µm Particles
ID (mm)	Optimum Flow Rate (mL/min.)	Optimum Flow Rate (mL/min.)
4.6	1.00	1.5
3.2	0.50	0.73
2.1	0.20	0.31
1.0	0.05	0.07