system. It is important to note that while altering the temperature of a separation can lower back pressure, it also lowers retention and can change selectivity. pH can also be used to control the selectivity and retention of ionizable compounds. Acid-base equilibrium can be employed to directly affect the retention characteristics of acidic and basic compounds, mainly in reversed phase chromatography (RPC). The pH limit of most silica columns is between 2 and 8.

Stationary Phases

Stationary phase, or the specific chemical ligand bonded to the silica support, plays a primary role in resolving compounds. Through selectivity, the major contributor to resolution, a stationary phase can control the retention characteristics of the solutes. Identifying the appropriate stationary phase can greatly ease method development and create less need for mobile phase additives. The decision tree in Figure 2 (next page) can help analysts select appropriate stationary phases, based upon analyte solubility and polarity. Liquid chromatography employs specific modes of separation which are denoted by the polarity distinction between the stationary and mobile phases; the most common are reversed phase, normal phase and HILIC.

Reversed phase chromatography (RPC) consists of a nonpolar stationary phase and a polar mobile phase. RPC is the most commonly used mode and works well for the analysis of water-soluble hydrophobic compounds. The most common types of columns used in RPC are alkyls (most often a C18, also known as octadecyl or ODS). End-capping is often employed in reversed phase columns. End-capping refers to the dense bonding or modification of the silica surface to further limit silanol activity. This acts to provide better peak symmetry, especially for basic compounds. Alternate ligands and bonding chemistries can be applied to RPC columns to incorporate phenyl, cyano, amino, and other polar groups into the stationary phase, providing alternate selectivity to a C18.

Normal phase chromatography (NPC), named because it was the first type of liquid chromatography, not for being more common, employs a polar stationary phase and a nonpolar mobile phase. NPC is suited for the analysis of fat soluble compounds and can also provide more selectivity for positional isomers than is commonly observed in RPC. Bare silica columns are most commonly used for NPC. Other phases for NPC include cyano and amino.

Hydrophilic Interaction Chromatography (HILIC) employs a polar stationary phase and a less polar mobile phase. HILIC differentiates itself from RPC and NPC as it uses traditional NPC stationary phases and RPC mobile phases. HILIC is recommended for the analysis of very polar compounds, often having negative log P values, and for analysis by LC/MS. Bare silica, cyano and amino columns are also commonly used in HILIC mode. Some stationary phases, like IBD, PFP propyl and cyano, incorporate both nonpolar and polar functionality and can be used in multiple or mixed-mode separation mechanisms.

Restek stationary phases and recommended uses are presented in Figure 3 (page 149). For additional help selecting a column, contact Restek at **support@restek.com** or call your local Restek representative.

Figure 1 Relative retention capabilities of commercially available columns for hydrophobic compounds.