(protonated) analyte ion competes for the negative site of the sulfonate ion. This altered equilibrium in effect imparts a change in the solubility of the analyte sample which in turn alters the retention as the analyte is now "attached" to the adsorbent so that it is eluted at a later time.

The pH of the mobile phase is closely associated with ion-pairing, whether the ion-pair reagent is positively charged (tetrabutylammonium, TBA^+) or negatively charged (C_5- or C_6- sulfonate/C_5- or C_6-SO_3^-), and dependent on whether the analyte is an acid or a base. Cationic samples (protonated base) or bases use the pentane, hexane, or a higher hydrocarbon sulfonate ion-pair reagent. Anionic samples (ionized acids) or acids commonly use tetraethylammonium or tetrabutylammonium hydroxide as the ion-pair reagent. Their optimization is pH dependent. For example, Waters® Chromatography ion-pair reagents operate in the low and intermediate pH ranges: PIC™ A (tetrabutylammonium phosphate) for acids operates at pH 7.5 and PIC™ B5 to B8 (pentane to octane sulfonic acid) for bases operate at pH 3.5.

For selection of the proper ion-pair reagent, alkyl chain lengths must be considered. The length of the alkyl chain enables selective separation of the analyte. The longer the chain, the more hydrophobic the counterion, and therefore, the greater the retention due to equilibrium between the counterion and the column adsorbent. Thus by selecting a reagent with a longer chain, selective solubility is obtained, enhancing the resolution.

10.8. Role of the Column

The HPLC column is the heart of the method, critical in performing the separation. The column must possess the selectivity, efficiency, and reproducibility to provide a good separation. All of these characteristics are dependent on the column manufacturer's production of good quality columns and packing materials. Properties of the silica (backbone) such as metal content and silanol activity produced in the manufacturing and bonding processes determine the properties of the finished bonded phase. A good silica and bonding process will provide the reproducible and symmetrical peaks necessary for accurate quantitation.

Commonly used reversed phases are C18 (octadecylsilane, USP L1), C8 (octylsilane, USP L7), phenyl (USP L11), and cyano (USP L18) (24). They are chemically different bonded phases and demonstrate significant changes in selectivity using the same mobile phase. Their properties vary from manufacturer to manufacturer, but given the state-of-the-art character of the vendor's manufacturing process, they show good quality control and provide batch-to-batch reproducibility. For example, no two L1 columns are the same, they vary from manufacturer to manufacturer relative to their pore volumes, pore sizes, surface areas, particle sizes (average range), carbon loads, whether end-capped or not, and the amount of bonded-phase coverage, as well as varying in their basicity and acidity characteristic. With state-of-the-art developments in column technology, most columns on the market exhibit good quality control and provide excellent column-to-column reproducibility and batch-to-batch reproducibility (25), and in some cases they give the chromatographer the option of using column selectivity as an alternative tool (besides mobile phase selectivity) to optimize the HPLC method development (26).