Hydrophilic Interaction
Chromatography
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Hydrophilic Interaction Chromatography
A Guide for Practitioners

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The popularity of hydrophilic interaction chromatography (HILIC) has grown rapidly in recent years. The HILIC mode can provide retention and separation of polar compounds that are difficult to analyze by reversed-phase high-performance liquid chromatography (RP-HPLC) or other means. HILIC has been utilized for a wide variety of applications including drugs and metabolites in biological fluids, biochemicals, pharmaceuticals (from drug discovery to quality control), foods, and environmental. Multiple HILIC stationary phases have been developed, and methods employing mass spectrometric detection or HILIC as part of two-dimensional separation systems are becoming more common.

Many researchers do not have an extensive background or experience with HILIC, particularly as compared with RP-HPLC. Several established references are available for information on RP-HPLC theory, mechanisms, and method development. Despite the recent growth in the use of HILIC, less information is available to guide potential practitioners in the understanding and development of robust HILIC separations. The lack of familiarity with HILIC can lead to trial-and-error method development and perhaps less-than-optimal results for a given application.

We sought to compile a book that would be a good reference for HILIC fundamentals as well as to provide a broad overview of popular areas of application. Our goal was to provide a resource for several important topics to those who want to explore HILIC as a separation mode. We believe that a basic understanding of retention mechanisms and the impact of stationary phase and mobile phase properties on separations can lead to more efficient and effective development of robust separation methods.

The first three chapters of the book are devoted to HILIC retention mechanisms, stationary phases, and general aspects of method development. These chapters provide a foundation for subsequent chapters dealing with different areas of application. The application chapters focus on specific areas of interest to workers in the respective fields being addressed. Unique separation challenges are presented for bioanalytical, environmental, pharmaceutical, and biochemical applications, as well as a thorough discussion of HILIC in two-dimensional chromatography. Illustrative examples of several HILIC methods and development approaches are highlighted, and references for further details are provided.
We are indebted to all the authors who contributed to the book. We believe they provided discussions of their subject area in a concise fashion with minimal redundancy with the other chapters. Research to understand the fundamentals of HILIC separations and further application of HILIC to analytical problems will certainly continue. Our goal is that this book will be a useful reference for current and future HILIC practitioners.

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1.1 Introduction

Hydrophilic interaction chromatography (HILIC) is a technique that has become increasingly popular for the separation of polar, hydrophilic, and ionizable compounds, which are difficult to separate by reversed-phase chromatography (RP) due to their poor retention when RP is used. HILIC typically uses a polar stationary phase such as bare silica or a polar bonded phase, together with an eluent that contains at least 2.5% water and >60% of an organic solvent such as acetonitrile (ACN). However, these values should not be regarded as definitive of the rather nebulous group of mobile and stationary phase conditions that are considered to constitute HILIC. Figure 1.1 shows the number of publications on HILIC between the years 1990 (when the term was first employed) and 2010 according to the Web of Knowledge [1] using the search terms “HILIC” or “hydrophilic interaction (liquid) chromatography.” For the first 12 years or so, the number of publications remained between 1 and 15, but after this period, interest increased rapidly from 19 publications...
in 2003 to 267 in 2010. While HILIC has unique retention characteristics for hydrophilic compounds, this increase in interest also reflects the advantages of HILIC over RP methods in situations where either technique is applicable. These advantages result mostly from the high organic content of typical mobile phases and their resultant high volatility and low viscosity. A particular advantage is in coupling HILIC to mass spectrometry (MS) as mobile phases are more efficiently desolvated in interfaces such as electrospray, giving rise to better sensitivity than with RP methods. Thus, Grumbach and coworkers demonstrated sensitivity increases of 3–4 orders of magnitude when comparing the analysis of the drugs salbutamol and bamethan by HILIC on a bare silica column using a gradient analysis starting at 90% ACN with that on a C18 RP column using a gradient starting at 0% ACN [2]. Columns can be used at considerably lower pressures than in RP; the viscosity of 80–90% ACN mixtures with water as typically used in HILIC is only about half that of 20–30% ACN mixtures that might be used in RP separations [3]. Alternatively, longer columns can be used at pressures typically found in RP analysis, allowing high efficiencies to be obtained [4]. For example, when combining the low viscosity of HILIC with the efficiency gains shown by superficially porous (shell) particle columns, it is possible to generate column efficiencies in excess of 100,000 plates with reasonable analysis times, and using pressures that are well within the capabilities of conventional HPLC systems (pressure $< 400$}

![Figure 1.1. Yearly publications containing the terms “hydrophilic interaction chromatography” or “hydrophilic interaction liquid chromatography” or HILIC according to Thomson Web of Knowledge [1].](image-url)

...
bar). Low viscosity also results in increased solute diffusion in the mobile phase, giving rise to smaller van Deemter C terms and improved mass transfer, and the possibility of operating columns at high flow rates with reduced losses in efficiency for fast analysis [5]. Surprisingly good peak shapes can be obtained for some basic compounds. For example, efficiencies of around 100,000 plates/m with asymmetry factors ($A_s$) close to 1.0 were reported for basic drugs such as nortriptyline ($pK_a \sim 10$) using a 5-µm particle size bare silica HILIC phase. In comparison, such solutes often give rise to peak asymmetry in RP separations.

A separate advantage of HILIC is its compatibility with sample preparation methods using solid-phase extraction (SPE). Some such methods incorporate an elution step that uses a high concentration of an organic solvent, which gives rise to a potential injection solvent of the eluate that is stronger than typical RP mobile phases [2]. This mismatch in solvent strengths can give rise to peak broadening or splitting, necessitating evaporation of the SPE eluate and reconstitution in the mobile phase. SPE eluates with high organic solvent concentrations can be injected directly in HILIC, as they are weak solvents in this technique. The combination of different retention mechanisms in sample purification and analysis steps (HILIC/RP) can be advantageous in giving extra selectivity compared with an RP/RP procedure, where in some cases the SPE column may act merely as a sort of filter for the analytical column [6].

While HILIC is simple to implement in practice, some recent papers have concluded that the separation mechanism is a complex multiparametric process that may involve partition of solutes between a water layer held on the surface and the bulk mobile phase, adsorption via interactions such as hydrogen bonding and dipole–dipole forces, ionic interactions, and even nonpolar retention mechanisms (similar to RP interactions), depending on the stationary and mobile phases [7–9]. In this chapter, we will consider in some detail the various mechanisms that contribute to HILIC separations.

1.2 HISTORICAL BACKGROUND: RECOGNITION OF THE CONTRIBUTION OF PARTITION, ION EXCHANGE, AND RP INTERACTIONS TO THE RETENTION PROCESS

The term “hydrophilic interaction chromatography” was coined in 1990 by Alpert [10]. He carefully avoided the acronym HIC to avoid confusion with the technique of hydrophobic interaction chromatography, the latter being an adaptation of the RP technique where decreasing salt concentrations are used to progressively elute large biomolecules from the stationary phase. However, it is possible that HILIC dates back to the earliest days of liquid chromatography, when Martin and Synge separated amino acids on a silica column using water-saturated chloroform as the mobile phase. These authors explained the separation mechanism as being the partitioning of the solutes between a water layer held on the column surface and the chloroform [11].
The silica was considered to act merely as a mechanical support. It later became clear that use of a solvent that is immiscible with water, such as chloroform, is not an essential requirement. Lindon and Lawhead [12] discussed the separation of sugars such as fructose, glucose, sucrose, melibiose, and raffinose on a micro-Bondapak carbohydrate column (an aminosilica column, 10 µm particle size). The mobile phase was ACN–water (75:25, v/v); the authors showed that increasing the concentration of water reduced the retention times of the sugars. The authors noted that while the α- and β-anomers of sugars are readily separated by gas-liquid chromatography, they were not separated by this LC method, removing an unnecessary complication. However, no explanation for this lack of separation, or for the retention mechanism, was presented. It was shown later that aminopropyl silica in the presence of ACN–water greatly increased the mutarotation rate of the sugars compared with the effect of bare silica [13]. This effect is due to the basic environment generated in the column pores by the presence of the amino groups [14]. With a refractive index detector, it was shown that water was retained on the aminopropyl silica when pumping mobile phases of ACN–water and that the volume fraction of water in the liquid associated with the stationary phase was much higher than that in the corresponding eluent. The extent of water enrichment in the stationary liquid was found to be relatively high when the eluent contained a low water concentration. The separation of the sugars was explained as being due to their partition between the water-rich liquid in the stationary phase and the bulk mobile phase. Using a similar experimental procedure, other workers showed a reduced uptake of water on an aminosilica column when methanol–water was used as the mobile phase compared with ACN–water, as the competition between water and methanol for polar sites on the column was increased [15].

While the reports on sugar analysis were clearly classical HILIC separations in their use of a polar phase together with an ACN–water mobile phase containing a high concentration of organic solvent, a number of other early papers using bare silica columns demonstrated separations that contain at least some of the mechanisms that are now considered contributory to HILIC. Bidlingmeyer and coworkers [16] separated organic amines on a silica column using “reversed-phase eluents” consisting typically of ACN–water (60:40, v/v) containing ammonium phosphate buffer, pH 7.8. They showed that increasing the salt concentration decreased the retention of ionized basic compounds, indicating the contribution of ionic retention to the overall mechanism. It was demonstrated that over the range 70–30% ACN, if buffer strength and pH were held constant, retention decreased with increasing proportion of ACN as would be expected in a reversed-phase separation. Good peak symmetry was obtained for basic compounds on these bare silica columns. The authors concluded from a comparison with RP that the key to good peak shapes with these solutes was not the presence or absence of silanols but more probably the accessibility of these surface groups. Nevertheless, the concentrations of
ACN employed in this work were at the lowest end of the range generally used for HILIC separations, and it is questionable whether the important partition element of the HILIC mechanism was involved to any extent in such separations, as the mobile phase becomes more hydrophilic and thus competitive with the stationary phase. Other early work by Flanagan and Jane also showed the separation of basic drugs on bare silica columns, but this time using nonaqueous ionic eluents [17,18]. The nonaqueous, primarily methanolic eluents, contained additives such as perchloric acid or ammonium perchlorate of appropriate pH and ionic strength. The authors demonstrated that the retention of quaternary compounds increased with eluent pH, particularly in the pH range of 7–9, whereas the retention of bases decreased steadily at a high pH, where they were unprotonated. The observations were consistent with an ionic retention mechanism on ionized silanols. However, in the absence of water in the mobile phase, the conditions are clearly not consistent with those of HILIC. Euerby and coworkers [19] separated a variety of basic analytes on bare silica columns of varying metal content using buffered methanol and ACN mobile phases of again rather low organic concentration (typically 20–40%). Their experimental conditions were somewhat similar to those of Bidlingmeyer (organic solvent concentrations were lower than classic HILIC conditions), and their conclusions were also that ionic and hydrophobic mechanisms were the main contributors to retention.

Cox and Stout [20] studied the retention of a set of nitrogenous bases such as thiamine and morphine on some bare silica columns. Their work was inspired by the difficulties that were encountered in the separation of basic compounds using typical RP columns available at that time (mid-1980s), which often gave long and variable retention, poor separation efficiency, and excessive peak tailing. While their studies indicated that ion exchange was a major contributor to retention in these systems, they reported that the mechanism appeared to be more complex, incorporating more than a single retention process. Linear plots of retention factor versus the reciprocal of buffer cation concentration (see Section 1.3.3.4) were obtained with retention decreasing as the concentration of the buffer increased, indicative of an ion-exchange mechanism. These experiments were performed at low concentrations of methanol (15% or 30%). These are not typical HILIC conditions, and little, if any, contribution of a classical HILIC partitioning mechanism seems likely. However, all of the plots showed a positive intercept on the y-axis. For a pure ion-exchange mechanism, straight lines passing through the origin should be generated. The positive intercept of the plot was cited as evidence for a competing mechanism, which existed at an infinite buffer concentration (i.e., when the reciprocal of the buffer concentration is zero). The authors first considered changes in the ionization of the solutes with addition of the organic solvent that could have influenced the results, but discounted this hypothesis on the basis that morphine was a strong base and should be completely ionized. The authors therefore concluded that some nonionic interaction of the solute with
silanol groups might occur. It seems that this contributory mechanism might in fact be of the same nature as that suggested in Bidlingmeyer’s work; that is, it is hydrophobic in origin [16]. Most of this work was carried out with low concentrations of methanol, that is, remote from classical HILIC conditions. However, a plot of $k$ derived from retention at an infinite buffer concentration for thiamine and morphine against methanol concentration from 15–75% v/v showed a U-shaped plot with retention maxima shown at 15% and 75% methanol, the maximum at 75% methanol in hindsight perhaps being indicative of the onset of a HILIC retention process.

While the paper of Alpert [10] was clearly not the first to demonstrate analysis using HILIC conditions, it was certainly a landmark publication because of the quality of the separations demonstrated for peptides, nucleic acids, and other polar compounds, and its careful discussion of the separation mechanism. Alpert showed that retention of peptides on hydrophilic columns, including a strong cation exchange material, PolySulfoethyl A, and a (largely) uncharged material, PolyHydroxyethyl A, increased dramatically when concentrations of ACN greater than 70% were used and that the order of their elution was from the least to the most hydrophilic, that is, the opposite from the order in RP separations. For the cation exchange material, electrostatic effects were superimposed on the HILIC mechanism. In agreement with the conclusions of previous workers [13,15], Alpert interpreted the earlier retention of sugars on amino columns as being not due to any electrostatic effects but caused by the hydrophilic nature of the basic column groups, demonstrating that the separation of carbohydrates could also be performed on the neutral PolyHydroxyl A phase, albeit giving elution in doublets corresponding to the $\alpha$- and $\beta$-anomers. Clearly, this neutral phase could not generate the alkaline mobile phase environment required to speed up the mutarotation of sugars. However, the problem was overcome by addition of a small amount of amine to the mobile phase to speed up the mutarotation process. In analogy with the partition mechanism that had previously been suggested for the separation of sugars, Alpert proposed that the same mechanism could also explain the separation of other classes of polar solutes, such as peptides and amino acids. He also cited the relatively small differences obtained in the separations of peptides between uncharged and charged stationary phases as the organic content of the mobile phase was increased as further evidence that partition was the dominant mechanism. As the partition contribution to retention is increased, the proportional contribution of ion exchange to the total retention is reduced. It was, nevertheless, clearly shown on the cation exchange phase that ionic retention effects could be superimposed on HILIC retention and could give useful selectivity effects. Alpert noted distinct similarities in the separation of thymidilic acid oligomers between HILIC and classical partitioning systems, citing this result as being further indicative of a partition mechanism in the chromatographic technique. He speculated that some form of dipole–dipole interactions might be involved, although retention of sugars had been shown to correlate better with their hydration number than with their potential to form hydrogen bonds [15].
1.3 RECENT STUDIES ON THE CONTRIBUTORY MECHANISMS TO HILIC RETENTION

1.3.1 Overview

As HILIC retention depends on the hydrophilicity of the solutes, attempts have been made to correlate this retention with physical descriptors of this property. Log P values represent the log of the partition coefficient when a solute is distributed between an aqueous phase and \( n \)-octanol, which in simple terms (using concentrations as an approximation for activities) can be written as

\[
\log P = \log([C_o]/[C_w]),
\]

where \( C_o \) is the concentration of the compound in octanol, and \( C_w \) is the concentration of the compound in water. Strictly, \( \log P \) refers to the distribution of the nonionized form of ionogenic compounds. Alternatively, the distribution coefficient \( D \) is defined as the equilibrium concentration ratio of a given compound in both its ionized and nonionized forms between octanol and water. The use of \( \log D \) instead of \( \log P \) requires knowledge or estimation of the \( pK_a \) of the compound to calculate its ionization at a particular pH. Kadar and coworkers [21] studied the application of \( \log D \) values produced by the ACD (Advanced Chemistry Development Inc.) calculation program to the prediction of a compound’s suitability for HILIC analysis. The ACD program generates estimations of \( \log D \) for mono- or polyprotic acids and bases over the pH range of 0–14 with increments of 0.1 pH units [22]. The authors tested the hypothesis that a relationship exists between the analyte’s retention factor, \( k \), and its \( \log D \) at pH 3.0. The value of pH 3.0 was chosen due to the consideration that the majority of active pharmaceutical ingredients are basic amines that will be protonated under acidic conditions, and that these conditions are frequently used for their analysis. In this work the authors assumed that a partially immobilized layer of water existed on the phase and that the pH of this immobilized water layer was 3.0. The authors debated at some length whether aqueous pH data (\( \bar{w}\text{pH} \)) should be used rather than \( \bar{\omega}\text{pH} \) values (where the pH is measured in the aqueous–organic solution). There is a considerable difference in these quantities when large concentrations of ACN are utilized, as in typical HILIC separations. However, due to the paucity of data concerning \( pK_a \) values in aqueous–organic mixtures, the authors decided to use \( \bar{w}\text{pH} \) and \( \bar{\omega}\text{pK} \) data. A further consideration not mentioned by the authors is that if a water layer exists on the column surface in HILIC, it is possible that data measured in water are more appropriate. For this work, the authors selected 30 probe compounds representative of pharmaceutical compounds used in the therapeutic areas of anti-infectives, cancer, cardiovascular and metabolic disease, and the central nervous system. They determined the retention factor of each compound experimentally using a bare silica HILIC column and a mobile phase consisting of 85%, 90%, or 95%...
ACN containing a total ammonium formate buffer concentration of 10 mM at pH 3.0. Linear regression analysis of log $k$ versus log D produced correlation coefficients of 0.751, 0.696, and 0.689 for 85%, 90%, and 95% ACN concentrations, respectively, giving relationships (for example with 85% ACN) of the form

$$\log k = -0.132 \cdot \log D - 0.234.$$ 

These equations could then potentially be used to predict the value of $k$ for a given calculated log D value. The authors interpreted the deviations of the correlation coefficients from unity as being due to secondary interactions in addition to the partitioning that was initially assumed in the hypothesis to be the only retention mechanism on the bare silica column. In particular, they considered that electrostatic interactions would occur with negatively charged silanols, giving increasing retention for charged basic compounds relative to that expected for a pure partition mechanism. Conversely, charged acidic compounds should experience repulsion and therefore give retention less than expected from a pure partitioning mechanism. Indeed, they showed that the predicted $k$ from log D values of several compounds that contained at least one basic functional group that was fully protonated at the experimental pH was significantly underestimated compared with the experimentally measured retention. The authors concluded that there is a direct correlation between a compound’s HILIC retention and its distribution ratio, although an accurate prediction of $k$ could not be made due to these secondary interactions. They concluded that the work also supports Alpert’s theory of a partition mechanism to describe HILIC separations. Bicker and coworkers [23], who studied the retention of nucleosides and nucleobases on a series of silica packings bonded with neutral trimethoxysilylpropylurea ligands, obtained variable results with prediction of retention based on log D values. They cautioned that these predictions should only be regarded as a simplistic concept for estimating the relative strength of HILIC-type interactions because the underlying molecular processes of retention and their correlations with solute polarity are not sufficiently understood as yet. They reported severe limitations of the predictions in the case of charged solutes, where other types of interaction than a partition mechanism come into effect. West and coworkers [24] acquired retention data for 76 model compounds using two zwitterionic phases and pH 4.4 ammonium acetate buffer in 80% ACN, with an overall salt concentration of 20 mM. The coefficients of determination ($r^2$) of 0.70 and 0.87 for ZIC-HILIC and a Nucleodor phase, respectively, gave evidence according to the authors that hydrophilic partitioning was only one of the mechanisms involved in HILIC separations, and thus log D values could only give a rough estimate of retention. They argued that the relatively high salt concentrations used should have suppressed some ionic interactions of ionized stationary phase groups, possibly improving the correlation with log D. No particular groups of solutes (neutral, anionic, cationic, or zwitterionic) appeared to be responsible for the poor
correlation, as all were scattered more or less uniformly about the regression line. However, it seemed that the fit was poorest for solutes with low retention, where the accuracy of the measurement could be a factor.

Some reports have shown the separation of the same mixture of solutes on a number of different stationary phases, in studies designed to contribute to elucidation of the separation mechanism. Guo and Gaiki examined the retention characteristics of four polar silica-based stationary phases (amide, amino, silica, and sulfobetaine—a zwitterionic phase containing quaternary amine and sulfonic acid groups) using small polar compounds as solutes. The solutes studied included salicylic acid and derivatives, some nucleosides and nucleic acid bases, selected because they are usually difficult to retain on RP columns [25]. Figure 1.2 shows the separation of the salicylic acid derivatives on the four columns using ACN–water (85:15 v/v) containing 20 mM ammonium acetate as the mobile phase. The retention and elution order clearly varied

![Figure 1.2](image-url)

**Figure 1.2.** Separation of acidic compounds on four different columns. Mobile phase ACN–water (85:15, v/v) containing 20 mM ammonium acetate. Column temperature 30°C. Flow rate 1.5 mL/min. Ultraviolet (UV) detection. Compound identities: 1 = salicylamide, 2 = salicylic acid, 3 = 4-amino salicylic acid, 4 = acetylsalicylic acid, 5 = 3,4-dihydroxyphenylacetic acid. All columns 25 × 0.46 cm containing 5-µm particle size packing. Reprinted from Reference 25 with permission from Elsevier.
from column to column. The acids were most retained on the amino column. As this column contained positively charged groups with the mobile phase conditions used, the negatively charged acids could undergo ionic interactions, increasing their retention. The acids had weaker retention on the amide column, and aspirin and 4-aminosalicylic acid (peaks 4 and 3) were only partially resolved. In contrast, the resolution of these two solutes was greatly improved on the zwitterionic column, however with a reversed order of elution compared with the amino column. On the bare silica column, the peaks were also well resolved, but their elution order was more similar to that on the amino column. The authors considered that the different elution patterns of the acids on the four columns indicated that the polar stationary phases had significant differences in retention and selectivity. Similar differences in selectivity were noted for a mixture of nucleic acid bases and nucleosides on the four columns. Specific interactions between the solutes and surface functional groups were thought to be most likely to be responsible for these selectivity differences. Such interactions could not be considered under a pure partition model, nor would they be accounted for in predictions using log D values. The authors also investigated the contribution of ionic processes to the overall retention, examining the effect of different ammonium salts (ammonium acetate, formate, and bicarbonate) on the retention of the acid compounds. They showed some differences in retention of the solutes, which they attributed in part to different eluting strengths of the competing anions in ionic interactions with the positively charged column groups. They also investigated the effect of salt concentration by varying the concentration of ammonium acetate from 5 to 20 mM in a mobile phase of ACN–water (85:15, v/v). For salicylic acid and aspirin, they showed increases in retention on the amide, bare silica, and zwitterionic column of 20–40% as the buffer strength increased. The authors considered the possibility that an increase in the buffer strength could be reducing repulsive effects of the acids from negatively charged silanol groups on the silica-based phases. However, they observed smaller but significant (8–20%) increases in the retention time of cytosine on all four columns. As cytosine was not charged under the mobile phase conditions used, electrostatic effects could not contribute to retention increases for this solute. The authors concluded that in this case, the retention increase might be related to increased hydrophilic partitioning, instead of any specific interactions with the functional groups on the stationary phases. Higher salt concentrations should drive more solvated salt ions into the water-rich liquid layer on the column surface, resulting in an increase in volume or hydrophilicity of the liquid layer, leading to stronger retention of the solutes. The authors suggested that this experiment provided indirect evidence to support the retention mechanism of HILIC that had been proposed by Alpert [10]. Nevertheless, increases in the salt concentration produced considerable decreases in the retention of salicylic acid and aspirin on the amino column. The ion exchange interactions of the acids on this phase were reduced by increasing competition of the buffer ions. It was interesting to note that no such decreases in retention were observed
on the zwitterionic phase. The authors speculated that electrostatic repulsion from the negatively charged sulfonic groups was balanced by the influence of the quaternary amine groups on this phase.

A comparison of the retention properties of HILIC phases using a rather different set of solutes was performed recently by McCalley [26]. Figure 1.3 shows the separation of a mixture of two neutral compounds (phenol and caffeine) two strong acids (\(p\)-xylene-2-sulfonic acid and naphthalene-2-sulfonic acid) and four basic compounds (nortriptyline, diphenhydramine, benzylamine, and procainamide) on five different silica-based HILIC phases of the same dimensions and particle size (5 \(\mu\)m). The mobile phase was 5 mM ammonium formate, pH 3.0, in either 85\% ACN (Fig. 1.3a) or 95\% ACN (Fig. 1.3b). The structure of the bonded groups and the physical characteristics of

![Figure 1.3.](image)

Figure 1.3. (a) Chromatograms of eight solutes on five different HILIC columns (all 25 \(\times\) 0.46 cm, 5 \(\mu\)m particle size). Mobile phase ACN–water (95:5, v/v) containing 5 mM ammonium formate, pH 3.0, 1 mL/min. Peak identities: (1) phenol, (2) naphthalene-2-sulfonic acid, (3) \(p\)-xylene-sulfonic acid, (4) caffeine, (5) nortriptyline, (6) diphenhydramine, (7) benzylamine, (8) procainamide.
these phases are given in Table 1.1. These were zwitterionic sulfobetaine, bare silica, diol, amide, and a mixed mode phase. The mixed mode phase was developed to exhibit both hydrophilic and reversed-phase characteristics [27], consisting of a long carbon chain with a diol grouping on the outlying carbon atoms. It was suggested that this phase has a dual operation mode. For example, the separation of cytosine and naphthalene could be achieved in the RP mode using ACN–ammonium acetate buffer pH 5 (52:48 v/v) with naphthalene eluting last, and in the HILIC mode using ACN–buffer (92:8, v/v) with naphthalene eluting first. It is immediately clear from this comparison that considerable differences exist in the selectivity of the various columns toward this group of solutes. For the basic solutes (peaks 5–8), the silica column is much more retentive than the other phases (note that the time axis is about double that for the other phases). This high retention is likely to result from ionic

Figure 1.3. (b) Mobile phase ACN–water (85:15, v/v) containing 5 mM ammonium formate, pH 3. Reprinted from Reference 26 with permission from Elsevier.