**Characterization of Hydrophilic Interaction Liquid Chromatography Retention by a Linear Free Energy Relationship. Comparison to Reversed- and Normal-Phase Retentions**

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**Abstract**

The Abraham solvation parameter model, a linear free energy relationship (LFER) approach, has been used to characterize a polymeric zwitterionic (sulfobetaine) column in HILIC mode. When acetonitrile (MeCN) is used in the preparation of mobile phases the main solute characteristics affecting the chromatographic behavior of analytes are the molecular size and the hydrogen-bonding (both acidity and basicity) interactions. The former property is more favorable in the acetonitrile-rich mobile phase, reducing thus the retention, but the latter reveals a higher affinity for the water layer adsorbed on the stationary phase, enhancing retention. However, if the aprotic acetonitrile is replaced by methanol, a hydrogen-bond acidic solvent, solute hydrogen-bond basicity does not contribute any more to retention, quite the opposite. Thus, a slightly different selectivity is observed in methanol/water than in acetonitrile/water. Normal-phase mode and HILIC-MeCN share the same main factors affecting retention. For reversed-phase and immobilized artificial membrane (IAM) chromatography, the solute molecular size increase retention because of the lower amount of energy required in the formation of a cavity in the solvated stationary phase. On the contrary, the analyte hydrogen-bond basicity favors interactions with the hydroorganic mobile phase and reduces retention. The determined parameters justify the reversed selectivity commonly observed in HILIC in reference to reversed-phase. In most instances, the least retained solutes in reversed-phase are the most retained in HILIC.

**Highlights**

The Abraham model is used for the characterization of a HILIC column.

Selectivity depends on the organic solvent used, acetonitrile or methanol.

HILIC is compared to normal-phase, reversed-phase and IAM retention selectivity.

**Keywords**

LFER, HILIC, reversed-phase, normal-phase, IAM

## 1. Introduction

Although Hydrophilic Interaction Liquid Chromatography (HILIC) was introduced by Alpert [1] almost 30 years ago, HILIC has attracted much more interest in the last few years as complementary to the widely used and studied reversed-phase liquid chromatography (RPLC) [2]. Despite that the retention time is very short, HILIC is able to separate polar or ionized solutes on a polar stationary phase with a much less polar mobile phase containing a small amount of water [3]. Numerous applications of HILIC have been developed in the last years (for instance, refs. [4–7]). Often, the HILIC column is coupled to the end of a RPLC column and thus, it allows separation of the most polar components which are almost not retained in the RPLC column. As in normal-phase liquid chromatography (NPLC) and conversely to RPLC, the HILIC stationary phase is more polar than the mobile phase. Thus in HILIC and NPLC, polar analytes are more retained than nonpolar ones, whereas in RPLC the reversed effect is observed [2]. Historically, HILIC has been considered a variant of NPLC, but the presence of a significant amount of water in the mobile phase is a clear differentiating factor. Polar solutes are more soluble in the hydroorganic mobile phase of HILIC, similar to those of RPLC, than in the organic mobile phase of NPLC [2]. Separation mechanisms are also different from those of NPLC and RPLC. Partitioning of solutes between the bulk mobile phase and a water rich layer partially immobilized on the stationary phase surface is considered the main retention mechanism in HILIC, although some secondary polar and ionic interactions may take place [1–3,8,9].

The stationary phase in HILIC must be necessarily polar enough to interact with the water contained in the mobile phase and immobilize part of it on the surface. Bare silica, the classical stationary phase for NPLC, was one of the first supports used in HILIC, but the growing application fields of this chromatographic mode has propitiated the development of many other polar phases such as amino, amide, diol, cyano and especially zwitterionic bonded phases, among others, bonded to different supports [2,3,8,9]. Although all these phases may appear to be very different, they have in common that they are the support for the adsorbed water layer acting as stationary phase, and thus the analytes show in them similar partition properties. In fact, the main retention mechanism is expected to be the partitioning of the solute between the mobile phase and the adsorbed water layer, contributing to retention the supporting bonded phase only by secondary interactions with the solute.

As in RPLC, acetonitrile/water is the mobile phase most used for HILIC, although with a much lower proportion of water (3-40%). Other organic modifiers of different eluotropic strength commonly used in RPLC, such as methanol, isopropanol or tetrahydrofuran, have also been investigated as HILIC mobile phases [8]. Changing the organic solvent in the eluent may give slightly different HILIC selectivities, but they are expected to be very distinct from those of RPLC and even

different from those of NPLC with the common silica/organic solvent combinations. Therefore, it is convenient to investigate the different solute-solvent interactions contributing to the partitioning process in HILIC, the effect of changing the mobiles phase composition on these interactions, and the comparison of HILIC to RP- and NPLC interactions. This can be achieved by means of linear free energy relationships (LFER), which relates retention in these systems (usually measured by the retention factor) to different kinds of solute-solvent interactions (polarity, polarizability, hydrogen bonding...).

In column chromatography, retention is usually characterized and related to partition and interaction processes by means of the retention factor (*k*). This is a measure of the residence time of an analyte in the stationary phase in relation to the time it resides in the mobile phase, and consequently it depends on the equilibrium constant for the distribution of the analyte between both phases. Thus, the solute transfer between mobile and stationary phases can be interpreted as the difference in the free energies of solvation of the compound in the two condensed phases. Solute transfer involves first the creation of a cavity in the receptor solvent big enough to dissolve the solute and the subsequent reorganization of the solvent molecules, followed by the newly established solute-solvent interactions. The cavity formation is an endoergic process depending on the solvent intermolecular forces and the size of the solute, but the free energy corresponding to the reorganization of the solvent molecules is normally residual due to the compensation of enthalpic and entropic contributions. Solute-solvent interactions for non-ionic compounds comprise orientation (dipole-dipole), induction (dipole-induced dipole), dispersion (instantaneous dipole-induced dipole) and hydrogen bonding (acidity and basicity). Thus, Abraham proposed a LFER relating the logarithm of the retention factor (log *k*) to the different contributions involved in the transfer process between mobile and stationary phases [10]. The model, also known as solvation parameter model, is written according to Eq. (1):

$$\log k = c + e \cdot E + s \cdot S + a \cdot A + b \cdot B + v \cdot V \qquad (1)$$

The $v \cdot V$ term accounts for the cavity formation in the solvent together with residual solute-solvent dispersion interactions (with $V$ being the McGowan volume of the solute in $cm^3\ mol^{-1}/100$), $c$ is a system constant, and the rest of the terms are related to solute-solvent interactions. The $e \cdot E$ term models the polarizability contributions from *n*- and π-electron pairs, $s \cdot S$ the dipole-type interactions (orientation and induction), $a \cdot A$ the hydrogen bond donation from the solute to the solvent, and $b \cdot B$ the hydrogen bond donation from solvent to solute. *E*, *S*, *A*, *B*, and *V* are solute descriptors, either experimentally determined or calculated. *e*, *s*, *a*, *b*, and *v* are the system coefficients, reflecting the difference in solute interaction between the solvated stationary phase and the mobile phase. The sign (positive or negative) and magnitude of these coefficients lead to the characterization of

chromatographic systems, finding the key features responsible for retention and allowing the comparison between different retention modes, columns, and mobile phases. These system coefficients are obtained by multiple linear regression from the retention factors of a set of solutes with well known and variated *E*, *S*, *A*, *B*, and *V* molecular descriptors.

Equation (1) was developed for retention of neutral (non-ionic) solutes. Ionic solutes show additional electrostatic interactions and Abraham [11] proposed additional terms to the general LFER model accounting for these interactions, which need two additional solute descriptors (one for anions and one for cations). Also the main *E*, *S*, *A*, *B* and *V* descriptors are different for the neutral and ionic forms of the same compound. The new model is applicable to both neutral and ionic solutes but it cannot be of general application to liquid chromatography because acid-base compounds might only be partially ionized in the mobile phase, in different degrees depending on the pH of the mobile phase and the p$K_a$ of the acid-base compound. Thus, different variations of Eq (1) and descriptors have been proposed to account for the ionization of solutes and model LC retention [12–14]. The new introduced descriptors were mostly based in the ionization degree of the compound or in the difference between the pH of the mobile phase and the p$K_a$ of the compound (which is directly related to the degree of ionization). One of these models was proposed by West group and tested in two HILIC columns [15]. Later, Schuster and Lindner applied the same model to more HLIC columns [16,17]. Good fits to the model were obtained and the fitting coefficients for neutral and partially ionized solutes were reasonably similar to the ones obtained with neutral solutes only [15].

However, as pointed out by West, the values of the descriptors used were a gross simplification of the true ones. Both the buffer pH and the analyte p$K_a$ should be measured in the mobile phase. In fact, pH was measured in the mobile phase, although with pH calibration in water, but the p$K_a$ values in the mobile phase (acetonitrile/water, 80/20 v/v) were not known and the values determined or estimated in water were used instead. Thus, the degree of ionization in the mobile phase could not be properly estimated. In addition, they pointed that the pH, p$K_a$ and thus degree of ionization would be different in the organic-rich mobile phase from that it would be in the water-rich pseudo-stationary phase, and even different in the transition layers between mobile and pseudo-stationary phase. In this scenario, the variation on the p$K_a$ and the degree of ionization would be different for acids and bases [15,18]. Also ionic species could be surrounded by "shells" of solvent of a different composition from the bulk mobile phase [15,19]. Additionally, we have recently observed that the retention of anions in HILIC is also buffer dependent [20]. For instance, retention of fully ionized acids with trimethylamine buffers was much lower than with ammonium buffers (80% of acetonitrile in the mobile phase), which was indicative of ionic interactions with the buffers. In 90% acetonitrile, the differences were much larger.

Because of all this troubles with ion retention and ionic descriptors, we shall limit our study to neutral solutes for which Eq. (1) should be directly applicable and we will focus on the determination of the main factors governing HILIC retention and selectivity of neutral solutes. Column characterization for ionic compounds may be attempted in a further work.

The retention factors used in Eq. (1) are obtained from the ratio of the measured adjusted retention volume $V_R$-$V_M$ (or time $t_R$-$t_M$) of solutes and the hold-up volume $V_M$ (or time $t_M$) of the chromatographic system:

$$k = \frac{V_R - V_M}{V_M} = \frac{t_R - t_M}{t_M} \quad (2)$$

Retention of a particular analyte can be easily obtained from its chromatographic peak, but the proper measurement of hold-up volumes in HILIC is not straightforward. The main retention mechanism in this chromatographic mode is assumed to be the partition of analytes between the mobile phase and a water layer adsorbed onto the stationary phase. The thickness of this immobilized water layer is strongly related to the water content in the mobile phase, and therefore the eluent volume inside the column (and consequently the hold-up volume) depends on the mobile phase composition. This water uptake is especially relevant for polymeric columns such as the zwitterionic (sulfobetaine) ZIC-pHILIC used in the present work [21,22]. Therefore, an accurate measurement of retention factors requires the determination of hold-up volumes (or times) for each and every mobile phase included in the study. In addition to system characterization, the Abraham LFER approach offers a way to determine hold-up volumes through homologous series [23].

The purpose of this study is to characterize HILIC systems through the Abraham model for neutral solutes and to compare them to other well stablished modes such as normal and reversed phases. A zwitterionic ZIC-pHILIC column and two mobile phase systems with different amounts of water in acetonitrile or methanol have been selected for this purpose. The study should point out the main solute-solvent interactions responsible for HILIC retention of neutral solutes and justify the particular HILIC selectivity complementary of reversed-phase one.

## 2. Materials and methods

### 2.1 Instrumentation and chromatographic conditions

HPLC measurements were performed on a Shimadzu (Kyoto, Japan) HPLC system consisting of two LC-10ADvp pumps, a SIL-10ADvp auto-injector, an SPD-M10AVvp diode array detector and a CTO-10ASvp oven at 25 °C and a SCL-10Avp controller. A 5 μm, 150 x 4.6 mm ZIC-pHiLIC (Merck, Darmstadt, Germany) column was employed. Injection volume and flow rate were 1 μL and 0.50 mL $min^{-1}$, respectively.

Aqueous buffers used in the preparation of mobile phases were directly prepared from ammonium acetate at different concentrations in order to provide a total concentration of 5 mM after mixing with the organic solvent.

### 2.2 Chemicals and solvents

Injected analytes and ammonium acetate were purchased from Acros Organics, Alfa Aesar, Baker, Merck, and Sigma-Aldrich; all of high purity grade (≥ 97%). Water was obtained from a Milli-Q plus system (Millipore, Billerica, USA) with a resistivity of 18.2 MΩ cm. Acetonitrile and methanol were HPLC gradient grade and purchased from Fisher.

### 2.3 Methods

Extracolumn volume was determined injecting 1 μL of 0.4 mg $mL^{-1}$ aqueous solution of potassium bromide (Merck, >99%) in absence of column and using water as eluent at two different flow rates, 0.25 and 0.50 mL $min^{-1}$, each in triplicate. The overall extracolumn volume in the particular chromatograph employed was 0.101(±0.003) mL. This volume was subtracted from the gross retention volumes obtained from chromatograms.

Hold-up volumes were measured from the measured retention volumes of the following homologous series: a) *n*-alkyl benzenes: benzene, toluene, ethyl-, propyl-, butyl-, pentyl-, hexyl-, octyl-, and dodecylbenzene; and b) *n*-alkyl phenones: aceto-, propio-, butyro-, valero-, hexano-, heptano-, octano-, nonano-, and decanophenone.

Stock solutions of injected analytes were prepared in methanol at a concentration of 5 mg $mL^{-1}$, and diluted to 1 mg $mL^{-1}$ before injection. All measurements were taken in triplicate. The column was equilibrated for 30 min at working flow rate (0.5 mL $min^{-1}$) after changing the mobile phase composition.

## 3. Results and discussion

### 3.1 Measurement of hold-up volumes: biserial LFER approach

Since the ratio between mobile and stationary phases strongly depends on the eluent composition, hold-up volumes were determined for each mobile phase according to a variation of the recently proposed LFER approach based on homologous series [23]. Homologous series components show very similar solvation parameters, except volume ($V$). Then, as long as chromatographic conditions remains unchanged (i.e. same column and mobile phase composition), for a particular series of homologous compounds the retention volume of an analyte ($V_R$) can be modelled by Eq. (3) that can be easily derived from Eq. (1) and Eq. (2):

$$V_R = V_M + r10^{vV} \quad (3)$$

where $r$ is a constant determined by the particular homologous series employed ($E$, $S$, $A$, and $B$ are nearly constant for the homologues within a series) and the chromatographic system ($V_M$, $c$, $e$, $s$, $a$, and $b$ are constant values for a particular column and mobile phase composition):

$$r = V_M \cdot 10^{c+e\cdot E+s\cdot S+a\cdot A+b\cdot B} \quad (4)$$

In this work two different homologous series have been injected, *n*-alkyl benzenes and *n*-alkyl phenones, and the obtained retention volumes for both series have been jointly fitted to the following expression:

$$V_R = V_M + (r_{benz} f_{benz} + r_{phen} f_{phen})10^{vV} \quad (5)$$

where $r_{benz}$ and $r_{phen}$ are only referred to the particular homologous series (*n*-alkyl benzenes and *n*-alkyl phenones, respectively), and $f_{benz}$ and $f_{phen}$ are just flag descriptors adopting only the binary values of 1 or 0. For instance, in the case of benzene series $f_{benz}$=1 and $f_{phen}$=0, whereas for phenones $f_{benz}$=0 and $f_{phen}$=1. From a mathematical point of view, the retention volume of a particular homologue ($V_R$) is the dependent variable, the flag descriptors ($f_{benz}$ and $f_{phen}$) and the homologue molecular volume ($V$) are the independent variables, and $V_M$, $r_{benz}$, $r_{phen}$, and $v$ are fitted constant values for a particular chromatographic system. Equation 5 allows a joint evaluation of $V_M$, leading to more robust and accurate hold-up volumes in relation to those obtained from the evaluation of single homologous series.

Table 1 show the fitted parameters in Eq. (5) for both studied homologous series obtained in mobile phases containing acetonitrile or methanol from 95% down to 80%. In all cases good fittings were obtained with determination coefficients ($R^2$) not lower than 0.99. As expected for an HILIC behavior, due to the more cohesive nature of the stationary phase water layer in relation to the hydroorganic mobile phase, the $v$ coefficient is always negative. The plots of retention vs. molecular volumes are shown in Figure S1 of the supplementary material. In nearly all cases, with the exception of 80% methanol, for a particular molecular volume the chromatographic retention of phenones is higher than the one of benzenes, decreasing these differences when molecular volume increase. When methanol is used in the mobile phase as organic modifier, the behavior of both series become more similar with increasing the water content. This could be related with the higher hydrogen bond acceptor basicity and polarity/polarizability of phenones ($B \approx 0.50$, $S \approx 0.95$) with respect to benzenes ($B \approx 0.15$, $S \approx 0.60$), the properties of the water layer adsorbed onto the stationary phase and responsible for chromatographic retention, and differences in hydrogen bond donor acidity of bulk mobile phases depending on the organic modifier employed (acetonitrile or methanol).

Figure 1 shows the adjusted retention volumes ($V_R$-$V_M$) of toluene, sometimes used as hold-up marker in a similar way as inorganic salts are employed in RPLC, and decanophenone and

dodecylbenzene as the homologues included in the study with the highest molecular volumes. Subtracted hold-up volumes are the fitted ones from Eq. (5) (Table 1). The adjusted retention volume for dodecylbenzene is below 0.04 mL, suggesting that this compound could be used as a rough marker of hold-up volume in HILIC. On the contrary, the use of toluene as marker should be avoided, since it would lead to a significant overestimation of hold-up volumes.

### 3.2 Characterization of ZIC-pHILIC column

Eight different mobile phases were used to conduct this study differing in the nature (acetonitrile or methanol) and content of organic modifier (95, 90, 85, and 80% in volume). Acetonitrile is by far the most commonly used organic solvent in the preparation of HILIC mobile phases. Contrary to reversed-phase, water is the strongest solvent in HILIC (competition with the water layer onto the stationary phase for the partition of the analytes) and thus it is convenient to mix the aqueous buffer with high volumes of a completely miscible organic solvent of poor elution strength. Acetonitrile is an excellent candidate, with a hydrogen bond acceptor basicity similar to water, but with a significantly reduced polarity and very poor hydrogen bond donor acidity. The eluotropic strength of methanol is in principle much closer to that of water because of its improved hydrogen bond donor acidity in relation to acetonitrile.

Retention factors ($k$) were measured for the compounds of Table 2 in the eight mobile phases commented above (data provided in the supplementary material, Table SP1). Molecular descriptors ($E$, $S$, $A$, $B$, and $V$) and log $k$ values were considered as independent and dependent variables, respectively, in Eq. (1), and then system constants ($c$) and coefficients ($e$, $s$, $a$, $b$, and $v$) were calculated by multilinear regression (Table 3).

The compounds with residuals higher than 2.5 times the standard deviation of the linear regression in any of the studied chromatographic systems were marked as outliers, and these particular compounds were excluded for the correlation of all systems involving the same organic solvent. As a result, the same set of 56 compounds were used to characterize the ZIC-pHILIC column in the range of 80-95% acetonitrile. The same procedure was followed for methanol, but also excluding the compounds lacking experimental retention data at 80% of organic modifier.

The main solute properties affecting the retention in mobile phases containing acetonitrile are the molecular volume and the hydrogen bond capabilities. Since intermolecular interactions in acetonitrile rich mobile phase are weaker than in the aqueous layer adsorbed on the stationary phase, the formation of a cavity in the mobile phase requires a lower amount of energy and therefore the higher the molecular volume the lower the retention ($v < 0$). Regarding solutes with hydrogen bond acidity or basicity, the stationary phase is prone to provide stronger interactions than the hydroorganic mobile phase, increasing thus their chromatographic retention ($a > 0$ and $b > 0$). However, the solute

dipolarity/polarizability properties or the dispersion interactions through π- and *n*-electron pairs show a little effect on the retention behavior ($e \approx 0$ and $s \approx 0$). There is quite a good agreement of these results with those presented by West [15] for neutral solutes in 80% acetonitrile and a ZIC-HILIC and a Nucleodur HILIC columns. The main driven factors of retention in this study were $b$ (>0) and $v$ (<0). $e$ and $s$ were not significantly different from 0, and only $a$ was smaller than in our case, but still clearly positive.

When the protic methanol is used as mobile phase constituent instead of the aprotic acetonitrile, there is a dramatic change in the retention behavior of solutes with hydrogen bond capacities. Analytes with hydrogen bond basicity will be less retained in methanol ($b < 0$) than in acetonitrile ($b > 0$), and the solute hydrogen bond acidity does not significantly contribute to retention ($a \approx 0$) when the alcohol is used as mobile phase. In methanol/water mobile phases both the molecular volume ($v < 0$) and the hydrogen bond basicity ($b < 0$) of the solute are responsible for a reduction in the retention, whereas the analyte dipolarity/polarizability ($s > 0$) and dispersion interactions ($e > 0$) clearly contribute to increase the chromatographic retention.

A close look at Table 3 shows that the $v$ coefficients for acetonitrile/water and methanol/water are very similar in the range 85-95% of organic cosolvent. This suggests that the energy required to transfer the cavity from the mobile phase to the HILIC aqueous stationary phase is similar for acetonitrile/water and methanol/water, but the hydrogen bonding and polarity interactions are indeed different ($b$, $a$, $s$, and $e$ coefficients). Thus, we can observe the influence of these properties on the selectivity of two mobile phases with similar $v$ coefficient value by simply subtracting the two correlations:

$$\log k_2 - \log k_1 = (c_2 - c_1) + (e_2 - e_1)\cdot E + (s_2 - s_1)\cdot S + (a_2 - a_1)\cdot A + (b_2 - b_1)\cdot B + (v_2 - v_1)\cdot V \qquad (6)$$

For instance, if we want to compare HILIC selectivity in 90% methanol to 90% acetonitrile, provided that $v$ is very similar in both systems ($v_{90\%\mathrm{MeOH}} - v_{90\%\mathrm{MeCN}} \approx 0$), we obtain:

$$\log k_{90\%\mathrm{MeOH}} \approx \log k_{90\%\mathrm{MeCN}} + 0.05 + 0.49E + 0.29S - 0.83A - 1.02B \qquad (7)$$

Figure 2 presents the selectivity plot for several combinations of mobile phases, and the dashed line represents equal retention for the two mobile phases compared. Since the cavity term is cancelled, solutes diverge from the dashed line depending on their polarity and hydrogen bonding capabilities. The positive contribution dipolarity/polarizability $E$ and $S$ terms, as well as the small system constant difference, increases retention when methanol is used in the mobile phase instead of acetonitrile, whereas hydrogen-bonding acidity ($A$) and basicity ($B$) terms favors the retention in acetonitrile. It is noteworthy that the homologous series components lay in most cases in an almost parallel line above the dashed line of equal retention. This is because of the similarity of $E$, $S$, $A$, and $B$ molecular descriptors for all members of the series which produces almost the same shift for all homologues.

For alkyl phenones ($E \approx 0.78$, $S \approx 0.96$, $A = 0$, $B \approx 0.50$) and alkyl benzenes ($E \approx 0.59$, $S \approx 0.50$, $A = 0$, $B \approx 0.15$) the expected shifts would be 0.2 and 0.3 log $k$ units, respectively for 90% MeOH vs 90% MeCN comparison, i.e. they are expected to be more retained in mobile phases containing methanol. In the comparison of selectivity between acetonitrile and methanol at 80% of organic modifier the difference in the volume term can not be cancelled, and in fact it shows a negative value of -0.46. Consequently, differences in retention depend on the molecular volume of the solute as well, and thus homologous series do not show anymore the straight tendency parallel to the dashed line.

### 3.3 Comparison of reversed-phase, normal-phase, IAM and HILIC chromatographic modes

For a particular set of compounds used for the characterization of chromatographic systems the range of retention factors depends on the mobile phase composition. Higher proportions of the stronger eluent reduce the retention of analytes. Consequently, the upper range of the solvation property in eq 1 (log $k$) is reduced with the amount of water in the mobile phase in HILIC mode, or with the content of organic solvent (acetonitrile, methanol…) in reversed-phase. Therefore, in our opinion, it is convenient to normalize the system coefficients to gain a broad perspective on the weight of the main factors affecting retention for a particular column and organic modifier used in the mobile phase. To do so, each characterized chromatographic system has been considered as a five-dimensional vector of system coefficients ($e$, $s$, $a$, $b$, and $v$), with a vector's length ($l$) mathematically defined as:

$$l = \sqrt{e^2 + s^2 + a^2 + b^2 + v^2} \tag{8}$$

and each system coefficient has been divided by $l$ in order to obtain the unitary coefficients ($e_u$, $s_u$, $a_u$, $b_u$, and $v_u$) [24].

Normalization is especially relevant when different chromatographic modes are compared, since retention in reversed-phase for neutral compounds is normally much higher than in normal-phase or HILIC. However, this approach has the limitation of a certain loss of information about differences in chromatographic retention related with the content of organic solvent in a particular system. As an example, Figure 3A shows the variation of system coefficients for a reversed-phase Spherisorb ODS-2 column with the acetonitrile content in the mobile phase. When increasing the acetonitrile composition, $v$ decreases and $b$ becomes less negative, but the rest of coefficients remain approximately constant. Consequently, in reversed-phase mode all compounds, but especially those with a significant contribution of molecular size ($V$ is always $\geq 0$) will greatly reduce their retention in acetonitrile-rich mobile phases. This is the case of butylbenzene or benzene in Figure 3B, where the contributions of the $v \cdot V$ term (i.e. mean value of $|v \cdot V| / (|e \cdot E| + |s \cdot S| + |a \cdot A| + |b \cdot B| + |v \cdot V|)$ ) is 75 and 63%, respectively. The volume term for corticosterone and hydrocortisone is less relevant, about 45%, but

the reduction in retention with acetonitrile content is enhanced by the hydrogen-bond basicity term, $b \cdot B$ about 30%. 4-Aminobenzamide shows a similar contribution of the $b \cdot B$ term, but a smaller molecular volume (29%). After normalization of the system coefficients (Figure 3C), no significant variations with mobile phase composition are observed, and a global picture of the behavior of chromatographic systems involving a Spherisorb ODS-2 column and acetonitrile/water mobile phases is obtained. In summary, the variation in chromatographic retention for a particular column with the mobiles phase features can be explained by the system coefficients (Figure 3A) depending on the solute properties, but normalized coefficients (Figure 3C) provide information about the selectivity of the chromatographic system, which in this case remains almost unchanged throughout the studied range.

A similar treatment for the ZIC-pHILIC column when acetonitrile is used as organic modifier leads to an opposite elution order (Figures 3B and 4B) and selectivity (Figures 3C and 4C). Benzene and butylbenzene show a poor contribution to those terms increasing retention (null hydrogen-bond acidity and 10-15% from hydrogen-bond basicity), but a very significant volume term favoring the solute transfer into the mobile phase (75-80%). At the other side of the scale, 4-aminobenzamide is the most retained compound due to its notable hydrogen-bonding features (60%) and reduced molecular size (30%). Interestingly, a different selectivity is observed when methanol is used in the mobile phase, depending in this case on the content of organic solvent (Figure 4D), which is expected to change the elution order (Figure 4E).

With the aim of comparing the retention features of the HILIC column studied in this work with other chromatographic modes, we have selected from the available literature different systems characterized by means of Abraham's solvation parameter model: a) several C18 and C8 reversed-phase columns in mobiles phases containing acetonitrile, methanol or tetrahydrofuran as organic modifier (Table SP2, supplementary material); b) an immobilized artificial membrane column (IAM.PC.DD2) with acetonitrile or methanol (Table SP3); and c) several columns (bare silica, cyano, diol, amino…) and non-aqueous organic solvents used in normal-phase (Table SP4). Normalized system coefficients have been calculated for each chromatographic system and wherever possible averaged values has been obtained.

For reversed-phase mode (Table 4) mean values have been calculated: a) for a particular column and reported mobile phase compositions of using the same organic modifier (e.g. Spherisorb ODS-2 and mobile phases containing acetonitrile from 30 to 80%); and b) for all columns and compositions involving the same organic solvent. The radar plot (Figure 5) show very similar coefficients for all the columns and organic modifiers, with the solute molecular volume ($v_u >> 0$) and the hydrogen bond basicity ($b_u << 0$) being the main parameters affecting retention. The more negative $b_u$ coefficient for THF is consistent with the lack of hydrogen bond acidity of the solvent. Immobilized

artificial membrane (IAM) stationary phases consist of phosphatidylcholine chains bonded to silica surface. These phospholipids are a major component of biological membranes and thus IAM columns seems to mimic better than C18 the interaction of analytes with biological membranes. Retention in IAM is used to estimate drug lipophilicity and cell permeation (e.g. octanol/water partition coefficient or intestinal drug absorption) [25]. However, the results presented in Table 5 and Figure 5, show thatIAM chromatographic systems behave very similarly to reversed-phase ones.

Normal-phase systems are much more difficult to cluster. Seemingly, the nature of the mobile phase plays a key role on defining the system characteristics, more relevant than that of the stationary phase (Table 6 and Figure 5). Similar unitary coefficients are obtained for different columns (bare silica, alkyl amino, alkyl diol, and alkyl cyano) when the same *n*-hexane/methanol (99/1) mobile phase is used. In these cases, the *e* coefficient was reported to be statistically irrelevant and therefore the authors of the original work omitted this coefficient in the correlations. For these systems, solute acidity ($a_u >> 0$), followed by hydrogen bond basicity ($b_u > 0$) and polarity/polarizability ($s_u > 0$) are responsible for retention due to favorable interactions with stationary phase. In contrast to reversed-phase, the higher the solute molecular volume ($v_u < 0$), the lower retention, due to the lower intermolecular interactions of organic solvent molecules in relation to the stationary phase. For the Nucleosil Silica column a slightly different behavior was reported depending on the amount of alcohol in the *n*-hexane/propan-2-ol mobile phases. Above 10% $e_u$ coefficient is still not relevant, but solute hydrogen bond basicity ($b_u >> 0$) takes over at the expense of hydrogen bond acidity ($a_u > 0$). In the range from 1 to 7% of propan-2-ol a similar trend is followed, but with the addition of the descriptor accounting for interactions with lone pair electrons ($e_u > 0$). However, when pure non-polar aprotic solvents (*n*-hexane, *n*-heptane) or mixed with 10% ethyl acetate or 20% diethyl ether were used, the molecular volume term ($v_u$) becomes negligible, suggesting a similar cohesion of both mobile and stationary phases, in addition to a negative value of $e_u$.

The studied HILIC systems, with the significant differences already mentioned depending on whether acetonitrile or methanol are used in the mobile phase, shares with normal-phase the negative value of the molecular volume term ($v_u$, Figure 6). Both reversed-phase and HILIC-methanol show also a relevant negative coefficient for solute hydrogen bond basicity ($b_u$), exactly the opposite of both normal-phase and HILIC-acetonitrile. Notice that the normal-phase system selected in Figure 6 for qualitative comparison purposes corresponds to a silica stationary phase and *n*-hexane/propan-2-ol (1-7% of alcohol) mobile phases. Hydrogen bond acidity ($a_u$) favors the retention in normal-phase and HILIC, especially in the latter when using acetonitrile as mobile phase constituent.

In summary, reversed-phase and IAM show the opposite behavior of normal-phase and HILIC-acetonitrile. The solute-solvent polar-type interactions ($s_u$, $a_u$, and $b_u$) favor the solubility in the hydroorganic mobile phase in reversed mode (negative coefficients) and in the polar stationary phase

(silica, adsorbed water layer…) in normal mode and HILIC-acetonitrile (positive coefficients). HILIC-methanol is a significant exception ($b_u$ negative and similar to normal-phase) due to the hydrogen bond acidity of the alcohol (α=0.98 [26]), 5 times higher than that of pure acetonitrile (0.19) and slightly lower than that of water (1.17). The contribution of solute-solvent polarizability interactions through π- and *n*-electrons generally result in increased retention, with the exception of some HILIC-acetonitrile and normal-phase systems (*e* is statistically negligible or negative).

The much different solvation properties of HILIC systems with regard to reversed-phase explains the well known different selectivity between these chromatographic modes. We cannot subtract directly HILIC and reversed-phase correlations, like in HILIC-MeCN vs HILIC-MeOH comparison to explain the selectivity plot because the *v* coefficients, and thus the contribution of the volume term, are very different and of opposite sign. Nevertheless, we can combine the two compared correlations to eliminate the $v \cdot V$ term and obtain one correlation in function of the other. If we apply eq 1 to two different HPLC systems (subscripts 1 and 2) and combine the two equations to cancel the volume term, the following expression is obtained:

$$\log k_2 = \frac{v_2}{v_1}\log k_1 + \left(c_2 - \frac{v_2}{v_1}c_1\right) + \left(e_2 - \frac{v_2}{v_1}e_1\right)E + \left(s_2 - \frac{v_2}{v_1}s_1\right)S + \left(a_2 - \frac{v_2}{v_1}a_1\right)A + \left(b_2 - \frac{v_2}{v_1}b_1\right)B \quad (9)$$

Figure 7 shows the selectivity plot for two typical HILIC and reversed-phase systems, 95% of acetonitrile in the studied ZIC p-HILIC column and 40% acetonitrile in Spherisorb ODS-2. The correlation calculated from the LFER coefficients of both systems (Table 3 and Table SP2) is:

$$\log k_{\mathrm{HILIC}} = -0.33\log k_{\mathrm{RPLC}} - 0.58 + 0.05E - 0.12S + 0.64A + 0.21B \quad (10)$$

where the subscripts HILIC and RPLC refer to ZIC-pHILIC – 95% MeCN and Spherisorb ODS-2 – 40% MeCN respectively.

The dashed line shows the line for volume term correction, with a slope of -0.33 and intercept of -0.58. This means that we expect a compound to be about three times less retained in HILIC than in reversed-phase, and because of the negative slope in a reversed order with regard to volume size. In other words, large solutes are more retained in reversed-phase, but less in HILIC. Solutes deviate from the line depending on their polarity and hydrogen bonding capabilities. *E* has practically no effect, *S* slightly disfavors retention in HILIC, but on the contrary *B* and especially *A* favors retention in HILIC, as compared with reversed-phase. Solutes with low polarity and hydrogen bonding abilities are almost in line, such as alkyl benzenes and phenones ($A = 0$ and compensation of $-0.12S$ and $0.20B$ terms because usually $S > B$). Benzonitrile is slightly below the volume term correction line because of the more negative contribution of the *S* term, not enough balanced by *B*. Solutes with large hydrogen bond abilities (in particular hydrogen bond acidity, *A*) are above the line being more retained in HILIC as expected from reversed-phase retention, as it is the case of aniline and especially phenol.

## Conclusions

For unionized analytes, the main contribution to retention in reversed-phase and IAM liquid chromatography is the solute molecular size, due to the reduced intermolecular interactions of the stationary phase (C18, lipids…) in relation to the hydroorganic mobile phase. The contrary takes place in HILIC and normal phases, where a highly cohesive water layer adsorbed on the stationary phase is responsible for retention. Generally, polar interactions (polarity, polarizability, hydrogen-bonding…) favor retention in normal phase and HILIC due to the higher polarity of the stationary phase in relation to the eluent, but with the exception of the solute hydrogen-bond basicity in HILIC when methanol is used as mobile phase constituent. The similarity between hydrogen-bond acidity of methanol and water is most probably responsible for this behavior, especially if compared with the aprotic acetonitrile. Regarding this characteristic, HILIC-MeOH and reversed-phase show an unexpected similar behavior. The solute-solvent interactions of HILIC-MeCN, inverse from those of RPLC-MeCN, explain the complementary retention selectivity of the two stationary phases.

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## Conflict of interest statement

The authors declare no conflict of interest.

## TABLES

**Table 1**
Fitted hold-up volumes ($V_M$) and the rest of parameters in Eq. (5) from chromatographic retention of *n*-alkyl benzene and *n*-alkyl phenone homologues (standard errors in brackets).

| Solvent | % | $V_M$ (mL) | $r_{benz}$ | $r_{phen}$ | $v$ | $N$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| MeCN | 95 | 1.373(0.009) | 0.47(0.02) | 0.77(0.07) | -0.54(0.05) | 18 | 0.991 |
| | 90 | 1.310(0.004) | 0.64(0.03) | 0.91(0.06) | -0.72(0.04) | 18 | 0.996 |
| | 85 | 1.240(0.003) | 0.64(0.02) | 0.89(0.04) | -0.68(0.02) | 18 | 0.998 |
| | 80 | 1.162(0.012) | 0.59(0.03) | 0.79(0.06) | -0.51(0.05) | 18 | 0.992 |
| MeOH | 95 | 1.467(0.013) | 1.04(0.04) | 1.74(0.12) | -0.58(0.04) | 18 | 0.995 |
| | 90 | 1.526(0.017) | 1.09(0.06) | 1.58(0.13) | -0.57(0.05) | 18 | 0.993 |
| | 85 | 1.623(0.014) | 1.11(0.05) | 1.30(0.08) | -0.56(0.04) | 18 | 0.995 |
| | 80 | 1.915(0.007) | 1.19(0.12) | 1.17(0.14) | -0.84(0.06) | 18 | 0.990 |

**Table 2**
Compounds included in the study and their corresponding molecular descriptors [27].

| Compound | *E* | *S* | *A* | *B* | *V* | Compound | *E* | *S* | *A* | *B* | *V* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2,3-Dimethylphenol | 0.85 | 0.84 | 0.51 | 0.37 | 1.06 | Geraniol[a,d] | 0.51 | 0.54 | 0.35 | 0.63 | 1.49 |
| 2,4-Dimethylphenol[a] | 0.84 | 0.79 | 0.52 | 0.40 | 1.06 | Heptanophenone | 0.77 | 0.95 | 0.00 | 0.50 | 1.72 |
| 2-Naphthol | 1.52 | 1.08 | 0.61 | 0.40 | 1.14 | Hexanophenone | 0.78 | 0.95 | 0.00 | 0.51 | 1.58 |
| 2-Nitroaniline[b] | 1.18 | 1.37 | 0.30 | 0.36 | 0.99 | Hexylbenzene | 0.59 | 0.50 | 0.00 | 0.15 | 1.56 |
| 2-Nitroanisole | 0.97 | 1.34 | 0.00 | 0.45 | 1.09 | Hydrocortisone | 2.03 | 3.49 | 0.71 | 1.90 | 2.80 |
| 3-Chloroaniline | 1.05 | 1.10 | 0.30 | 0.30 | 0.94 | Hydroquinone[b] | 1.06 | 1.27 | 1.06 | 0.57 | 0.83 |
| 3-Nitroaniline[c] | 1.20 | 1.71 | 0.40 | 0.35 | 0.99 | Methyl benzoate | 0.73 | 0.85 | 0.00 | 0.46 | 1.07 |
| 4-Aminobenzamide | 1.34 | 1.94 | 0.80 | 0.94 | 1.07 | Monuron[c] | 1.14 | 1.50 | 0.47 | 0.78 | 1.48 |
| 4-Chloroacetanilide[b] | 0.98 | 1.47 | 0.64 | 0.51 | 1.24 | Naphthalene | 1.34 | 0.92 | 0.00 | 0.20 | 1.09 |
| 4-Chloroaniline | 1.06 | 1.13 | 0.30 | 0.31 | 0.94 | Nitrobenzene | 0.87 | 1.11 | 0.00 | 0.28 | 0.89 |
| 4-Chlorophenol | 0.92 | 1.08 | 0.67 | 0.20 | 0.90 | Nonanophenone | 0.76 | 0.95 | 0.00 | 0.50 | 2.00 |
| 4-Nitroaniline | 1.22 | 1.92 | 0.46 | 0.35 | 0.99 | Octanophenone | 0.77 | 0.95 | 0.00 | 0.50 | 1.86 |
| Acetanilide[c] | 0.90 | 1.37 | 0.48 | 0.67 | 1.11 | Octylbenzene | 0.58 | 0.48 | 0.00 | 0.15 | 1.84 |
| Acetophenone | 0.82 | 1.01 | 0.00 | 0.48 | 1.01 | *o*-Toluidine | 0.97 | 0.92 | 0.23 | 0.45 | 0.96 |
| Aniline | 0.96 | 0.96 | 0.26 | 0.41 | 0.82 | Pentan-1,5-diol[a,d] | 0.39 | 0.90 | 0.72 | 0.92 | 0.93 |
| Anisole | 0.71 | 0.75 | 0.00 | 0.29 | 0.92 | Pentan-1-ol[d] | 0.22 | 0.42 | 0.37 | 0.48 | 0.87 |
| Antipyrine[d] | 1.30 | 1.83 | 0.00 | 1.37 | 1.48 | Pentan-3-ol[d] | 0.22 | 0.36 | 0.33 | 0.56 | 0.87 |
| α-Pinene[a,d] | 0.44 | 0.20 | 0.00 | 0.14 | 1.26 | Pentylbenzene | 0.59 | 0.51 | 0.00 | 0.15 | 1.42 |
| Benzaldehyde[d] | 0.82 | 1.00 | 0.00 | 0.39 | 0.87 | Phenol[d] | 0.81 | 0.89 | 0.60 | 0.30 | 0.78 |
| Benzamide[a] | 0.99 | 1.50 | 0.49 | 0.67 | 0.97 | Propan-1-ol[d] | 0.24 | 0.42 | 0.37 | 0.48 | 0.59 |
| Benzene | 0.61 | 0.52 | 0.00 | 0.14 | 0.72 | Propan-2-ol[d] | 0.21 | 0.36 | 0.33 | 0.56 | 0.59 |
| Benzonitrile[c] | 0.74 | 1.11 | 0.00 | 0.33 | 0.87 | Propiophenone | 0.80 | 0.95 | 0.00 | 0.51 | 1.15 |
| Benzophenone | 1.37 | 1.34 | 0.00 | 0.50 | 1.48 | Propylbenzene | 0.60 | 0.50 | 0.00 | 0.15 | 1.14 |
| Benzyl benzoate | 1.26 | 1.35 | 0.00 | 0.53 | 1.68 | P-Xylene | 0.61 | 0.52 | 0.00 | 0.16 | 1.00 |
| Bromobenzene | 0.88 | 0.73 | 0.00 | 0.09 | 0.89 | Pyrrole | 0.61 | 0.91 | 0.22 | 0.25 | 0.58 |
| Butan-1-ol[d] | 0.22 | 0.42 | 0.37 | 0.48 | 0.73 | Quinoline[b] | 1.27 | 0.97 | 0.00 | 0.54 | 1.04 |
| Butylbenzene | 0.60 | 0.51 | 0.00 | 0.15 | 1.28 | Resorcinol[a,d] | 0.98 | 1.11 | 1.09 | 0.52 | 0.83 |
| Butyrophenone | 0.80 | 0.95 | 0.00 | 0.51 | 1.30 | Thiourea[b] | 1.00 | 0.83 | 0.80 | 0.90 | 0.57 |
| Caffeine | 1.50 | 1.82 | 0.08 | 1.25 | 1.36 | Thymol[a] | 0.82 | 0.80 | 0.43 | 0.44 | 1.34 |
| Chlorobenzene | 0.72 | 0.65 | 0.00 | 0.07 | 0.84 | Toluene | 0.60 | 0.52 | 0.00 | 0.14 | 0.86 |
| Corticosterone | 1.86 | 3.43 | 0.40 | 1.63 | 2.74 | Valerophenone | 0.80 | 0.95 | 0.00 | 0.50 | 1.44 |
| Cortisone | 1.96 | 3.50 | 0.36 | 1.87 | 2.75 | *Minimum value* | *0.21* | *0.20* | *0.00* | *0.07* | *0.54* |
| Decanophenone | 0.75 | 0.95 | 0.00 | 0.50 | 2.14 | *Maximum value* | *2.03* | *3.50* | *1.09* | *1.90* | *2.80* |
| Dodecylbenzene | 0.57 | 0.47 | 0.00 | 0.15 | 2.41 | *Median* | *0.82* | *0.95* | *0.04* | *0.46* | *1.06* |
| Estradiol | 1.80 | 1.77 | 0.86 | 1.10 | 2.20 | *Mean* | *0.89* | *1.06* | *0.25* | *0.50* | *1.22* |
| Ethylbenzene | 0.61 | 0.51 | 0.00 | 0.15 | 1.00 | *Std. dev. (mean)* | *0.40* | *0.67* | *0.30* | *0.39* | *0.52* |
| Furan | 0.37 | 0.51 | 0.00 | 0.13 | 0.54 | | | | | | |

[a] Excluded from MeCN correlations.
[b] Excluded from MeCN and MeOH correlations.
[c] Excluded from MeOH correlations.
[d] Retention data not available at 80% MeOH, excluded from MeOH correlations.

**Table 3**

Fitted constants (*c*) and system coefficients (*e*, *s*, *a*, *b*, and *v*) of eq 1 for ZIC-pHILIC and studied mobile phases (standard errors of fitted coefficients in brackets). Number of solutes (*N*), determination coefficients ($R^2$) and standard error of the fittings (*SE*) are also reported.

| Mobile phase | | *c* | *e* | *s* | *a* | *b* | *v* | *N* | $R^2$ | *SE* |
|---|---|---|---|---|---|---|---|---|---|---|
| MeCN | 95% | -0.55(0.04) | -0.05(0.08) | 0.06(0.06) | 0.82(0.07) | 0.75(0.08) | -0.57(0.04) | 56 | 0.944 | 0.10 |
| | 90% | -0.51(0.05) | -0.04(0.09) | 0.09(0.08) | 0.83(0.08) | 0.77(0.09) | -0.71(0.04) | 56 | 0.930 | 0.13 |
| | 85% | -0.50(0.05) | 0.08(0.09) | 0.01(0.07) | 0.74(0.08) | 0.71(0.09) | -0.67(0.04) | 56 | 0.922 | 0.12 |
| | 80% | -0.50(0.03) | 0.07(0.06) | 0.02(0.05) | 0.55(0.05) | 0.44(0.06) | -0.48(0.03) | 56 | 0.928 | 0.08 |
| MeOH | 95% | -0.44(0.04) | 0.39(0.06) | 0.37(0.05) | 0.04(0.05) | -0.10(0.07) | -0.65(0.03) | 47 | 0.967 | 0.07 |
| | 90% | -0.46(0.04) | 0.45(0.06) | 0.38(0.05) | 0.00(0.05) | -0.25(0.07) | -0.65(0.03) | 47 | 0.967 | 0.07 |
| | 85% | -0.55(0.04) | 0.51(0.05) | 0.41(0.04) | 0.00(0.04) | -0.46(0.06) | -0.62(0.02) | 47 | 0.974 | 0.06 |
| | 80% | -0.82(0.08) | 0.95(0.12) | 0.64(0.10) | -0.16(0.10) | -1.09(0.15) | -0.94(0.05) | 47 | 0.944 | 0.14 |

**Table 4**

Mean normalized system coefficients for some reversed-phase columns and mobile phases containing acetonitrile, methanol, or tetrahydrofuran as organic modifier (standard errors in brackets).

| Column | *Mobile phase* | $e_u$ | $s_u$ | $a_u$ | $b_u$ | $v_u$ | *N* | Ref. |
|---|---|---|---|---|---|---|---|---|
| ERC-1000 (ODS) | 50-90% MeCN | 0.00(0.01) | -0.11(0.04) | -0.28(0.02) | -0.65(0.02) | 0.70(0.01) | 5 | [28,29] |
| Spherisorb ODS-2 | 30-80% MeCN | 0.11(0.01) | -0.23(0.03) | -0.26(0.05) | -0.65(0.01) | 0.67(0.02) | 6 | [28][a] |
| Unisil C18 | 30-90% MeCN | 0.20(0.08) | -0.17(0.04) | -0.13(0.01) | -0.54(0.03) | 0.78(0.01) | 7 | [28,30] |
| XTerra MSC18 | 20-60% MeCN | 0.04(0.04) | -0.19(0.01) | -0.16(0.04) | -0.65(0.02) | 0.71(0.03) | 3 | [24] |
| XTerra RP18 | 20-60% MeCN | 0.10(0.01) | -0.20(0.03) | -0.10(0.04) | -0.67(0.01) | 0.70(0.02) | 3 | [24] |
| Zorbax C8 | 20-50% MeCN | 0.00(0.02) | -0.08(0.01) | -0.14(0.02) | -0.72(0.03) | 0.67(0.03) | 4 | [31] |
| | Mean MeCN | 0.09(0.09) | -0.16(0.06) | -0.19(0.07) | -0.63(0.07) | 0.71(0.05) | 28 | |
| Nucleosil 5-C18 | 45-80% MeOH | 0.10(0.02) | -0.22(0.02) | -0.21(0.03) | -0.66(0.01) | 0.68(0.03) | 8 | [28,32] |
| Spherisorb ODS-2 | 40-80% MeOH | 0.13(0.04) | -0.29(0.06) | -0.21(0.05) | -0.57(0.02) | 0.72(0.03) | 5 | [33] |
| Zorbax C8 | 10-50% MeOH | 0.05(0.02) | -0.15(0.04) | -0.09(0.02) | -0.49(0.05) | 0.85(0.03) | 5 | [31] |
| | Mean MeOH | 0.09(0.04) | -0.21(0.06) | -0.17(0.06) | -0.60(0.08) | 0.73(0.08) | 18 | - |
| Spherisorb ODS-2 | 30-60% THF | -0.05(0.02) | -0.13(0.02) | -0.11(0.07) | -0.78(0.01) | 0.59(0.03) | 4 | [28,34] |
| Zorbax C8 | 20-50% THF | 0.06(0.06) | -0.10(0.01) | 0.00(0.02) | -0.78(0.01) | 0.62(0.01) | 4 | [31] |
| | Mean THF | 0.00(0.07) | -0.12(0.02) | -0.06(0.08) | -0.78(0.01) | 0.61(0.03) | 8 | - |

[a]System coefficients calculated from retention factors in refs. [35–40].

**Table 5**

Normalized system coefficients for an immobilized artificial membrane column in mobile phases containing acetonitrile and methanol.

| Column | *Mobile phase* | $e_u$ | $s_u$ | $a_u$ | $b_u$ | $v_u$ | *N* | Ref. |
|---|---|---|---|---|---|---|---|---|
| IAM.PC.DD2 | 10-60% MeCN | 0.21(0.06) | -0.22(0.04) | 0.10(0.07) | -0.64(0.04) | 0.69(0.04) | 4 | [24] |
| | 10-60% MeOH | 0.18(0.01) | -0.09(0.01) | 0.07(0.02) | -0.72(0.05) | 0.65(0.06) | 6 | [41] |

**Table 6**
Normalized system coefficients for some columns and non-aqueous mobile phases used in normal-phase mode (standard errors of mean values in brackets).

| Column | Mobile phase | | $e_u$ | $s_u$ | $a_u$ | $b_u$ | $v_u$ | Ref. |
|---|---|---|---|---|---|---|---|---|
| LiChrospher Diol | *n*-pentane | | -0.11 | 0.31 | - | 0.95 | - | [42,43] |
| | *n*-pentane/diethyl ether (80/20) | | -0.33 | 0.56 | 0.30 | 0.70 | - | [42,43] |
| Spherisorb $NH_2$ | *n*-hexane | | -0.21 | 0.47 | - | 0.86 | - | [43] |
| | *n*-hexane/ethyl acetate (90/10) | | -0.18 | 0.53 | 0.77 | 0.29 | - | [43] |
| Nucleosil Silica | *n*-hexane/propan-2-ol (99/1) | | 0.14 | 0.15 | 0.42 | 0.75 | -0.47 | [43,44] |
| | *n*-hexane/propan-2-ol (98/2) | | 0.18 | 0.21 | 0.31 | 0.73 | -0.55 | [43,44] |
| | *n*-hexane/propan-2-ol (95/5) | | 0.11 | 0.24 | 0.24 | 0.77 | -0.54 | [43,44] |
| | *n*-hexane/propan-2-ol (93/7) | | 0.10 | 0.27 | 0.20 | 0.78 | -0.53 | [43,44] |
| | | Mean | 0.13 (0.04) | 0.22 (0.05) | 0.29 (0.10) | 0.75 (0.02) | -0.52 (0.03) | |
| Nucleosil Silica | *n*-hexane/propan-2-ol (90/10) | | - | 0.30 | 0.20 | 0.83 | -0.42 | [43,44] |
| | *n*-hexane/propan-2-ol (85/15) | | - | 0.33 | 0.14 | 0.81 | -0.47 | [43,44] |
| | *n*-hexane/propan-2-ol (80/20) | | - | 0.33 | 0.11 | 0.82 | -0.45 | [43,44] |
| | | Mean | - | 0.32 (0.02) | 0.15 (0.04) | 0.82 (0.01) | -0.45 (0.02) | |
| Hypersil APS $NH_2$ | *n*-hexane/MeOH (99/1) | | - | 0.28 | 0.87 | 0.35 | -0.21 | [45] |
| Hypersil CN | *n*-hexane/MeOH (99/1) | | - | 0.39 | 0.76 | 0.47 | -0.25 | [45] |
| Hypersil Silica | *n*-hexane/MeOH (99/1) | | - | 0.35 | 0.73 | 0.51 | -0.27 | [45] |
| LiChrospher CN | *n*-hexane/MeOH (99/1) | | - | 0.40 | 0.78 | 0.42 | -0.26 | [45] |
| LiChrospher Diol | *n*-hexane/MeOH (99/1) | | - | 0.34 | 0.76 | 0.47 | -0.27 | [45] |
| Spherisorb CN | *n*-hexane/MeOH (99/1) | | - | 0.33 | 0.71 | 0.56 | -0.28 | [45] |
| Ultrasphere CN | *n*-hexane/MeOH (99/1) | | - | 0.40 | 0.77 | 0.42 | -0.24 | [45] |
| | | Mean | - | 0.36 (0.04) | 0.77 (0.05) | 0.46 (0.07) | -0.25 (0.02) | |

**Table 7**
Normalized system coefficients for HILIC columns and mobile phases containing acetonitrile or methanol as organic modifier (standard errors in brackets).

| Column | Mobile phase | $e_u$ | $s_u$ | $a_u$ | $b_u$ | $v_u$ |
|---|---|---|---|---|---|---|
| ZIC-pHILIC | 95% MeCN | -0.04 | 0.06 | 0.72 | 0.55 | -0.41 |
| | 90% MeCN | -0.03 | 0.05 | 0.72 | 0.51 | -0.46 |
| | 85% MeCN | 0.05 | 0.00 | 0.67 | 0.54 | -0.50 |
| | 80% MeCN | 0.18 | 0.04 | 0.68 | 0.41 | -0.57 |
| | Mean MeCN | 0.04(0.10) | 0.04(0.03) | 0.70(0.03) | 0.51(0.06) | -0.48(0.07) |
| ZIC-HILIC[a] | 80% MeCN | - | - | 0.26 | 0.70 | -0.67 |
| Nucleodur HILIC[a] | 80% MeCN | - | - | 0.35 | 0.67 | -0.65 |
| ZIC-pHILIC | 95% MeOH | 0.30 | 0.55 | 0.19 | -0.51 | -0.56 |
| | 90% MeOH | 0.29 | 0.52 | 0.14 | -0.66 | -0.43 |
| | 85% MeOH | 0.25 | 0.48 | 0.13 | -0.78 | -0.27 |
| | 80% MeOH | 0.21 | 0.46 | 0.09 | -0.82 | -0.26 |
| | Mean MeOH | 0.26(0.05) | 0.50(0.04) | 0.14(0.04) | -0.69(0.14) | -0.38(0.14) |

[a]From ref. [15].

**FIGURES**

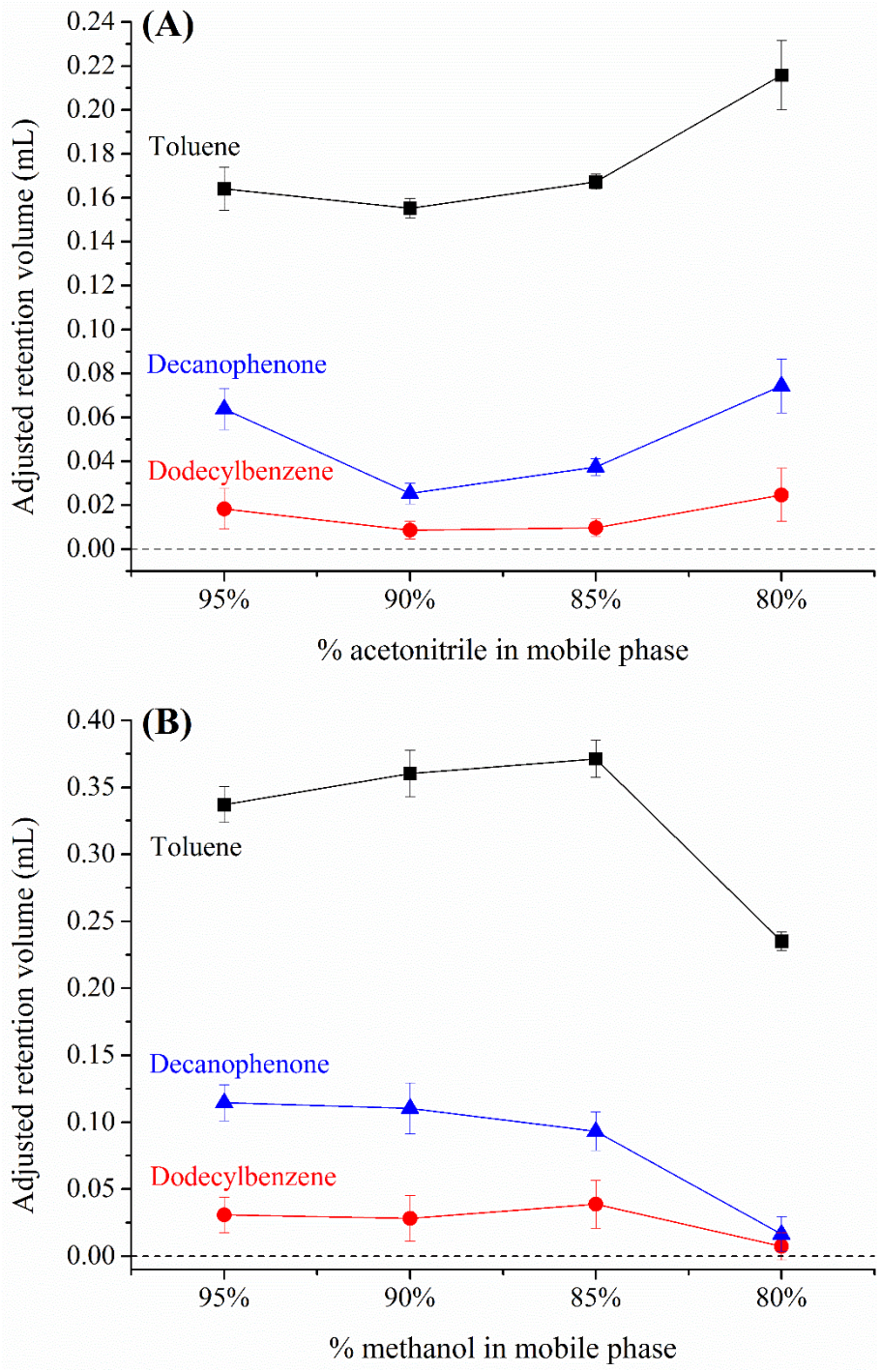


**Figure 1.** Adjusted retention volumes ($V_R$-$V_M$) for toluene, dodecylbenzene, and decanophenone in mobile phases containing (A) acetonitrile or (B) methanol (hold-up volume ($V_M$) determined by the homologous series approach (Table 1)).

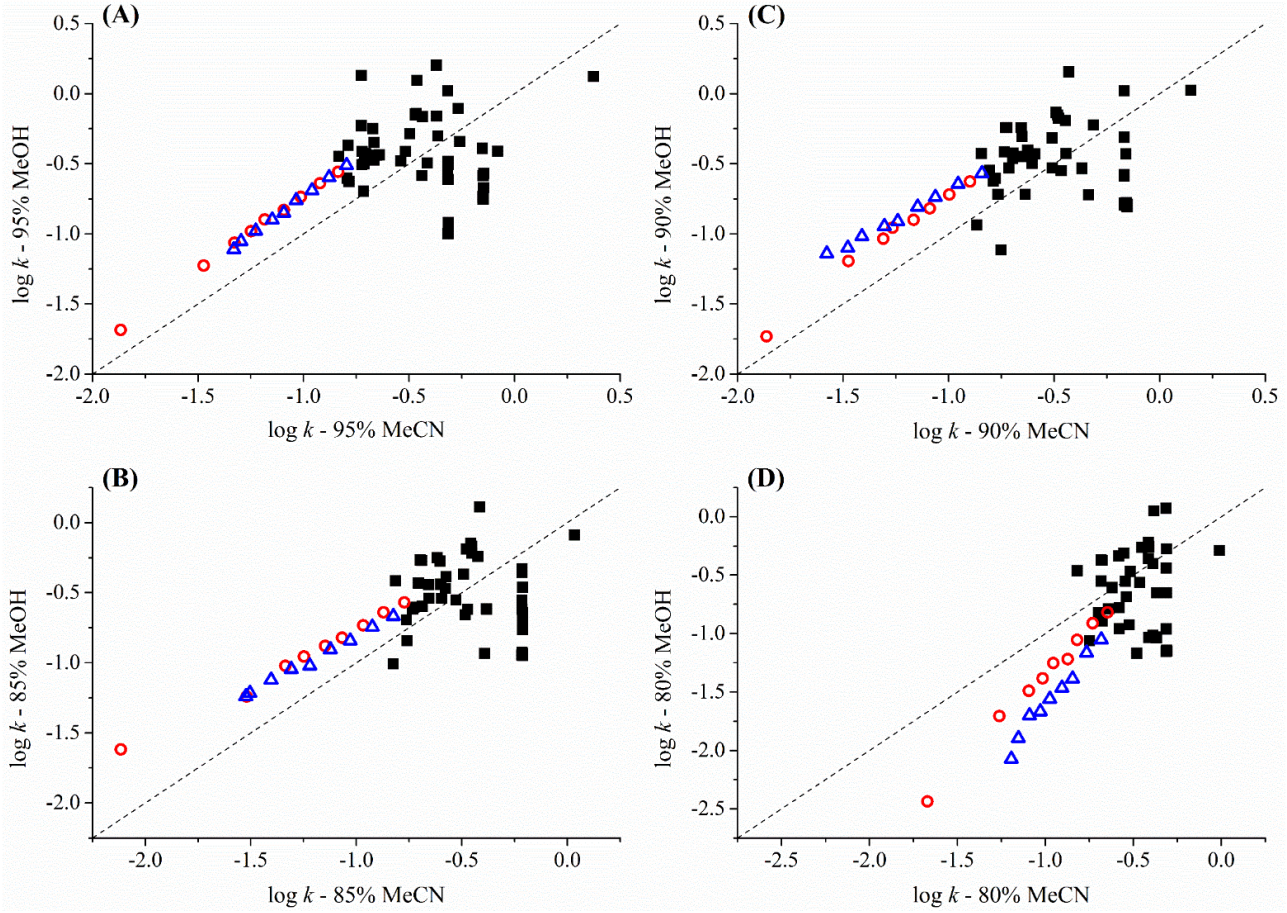


**Figure 2.** Selectivity plot for ZIC-pHILIC depending on the nature of organic modifier (acetonitrile or methanol) in the mobile phase: (A) 95% methanol vs 95% acetonitrile; (B) 85% methanol vs 85% acetonitrile; (C) 90% methanol vs 90% acetonitrile; and (D) 80% methanol vs 80% acetonitrile. Dashed line of zero intercept and unitary slope represents the line of equal retention for both chromatographic systems. Legend: (○) alkyl benzenes; (△) alkyl phenones; and (■) rest of the studied solutes.

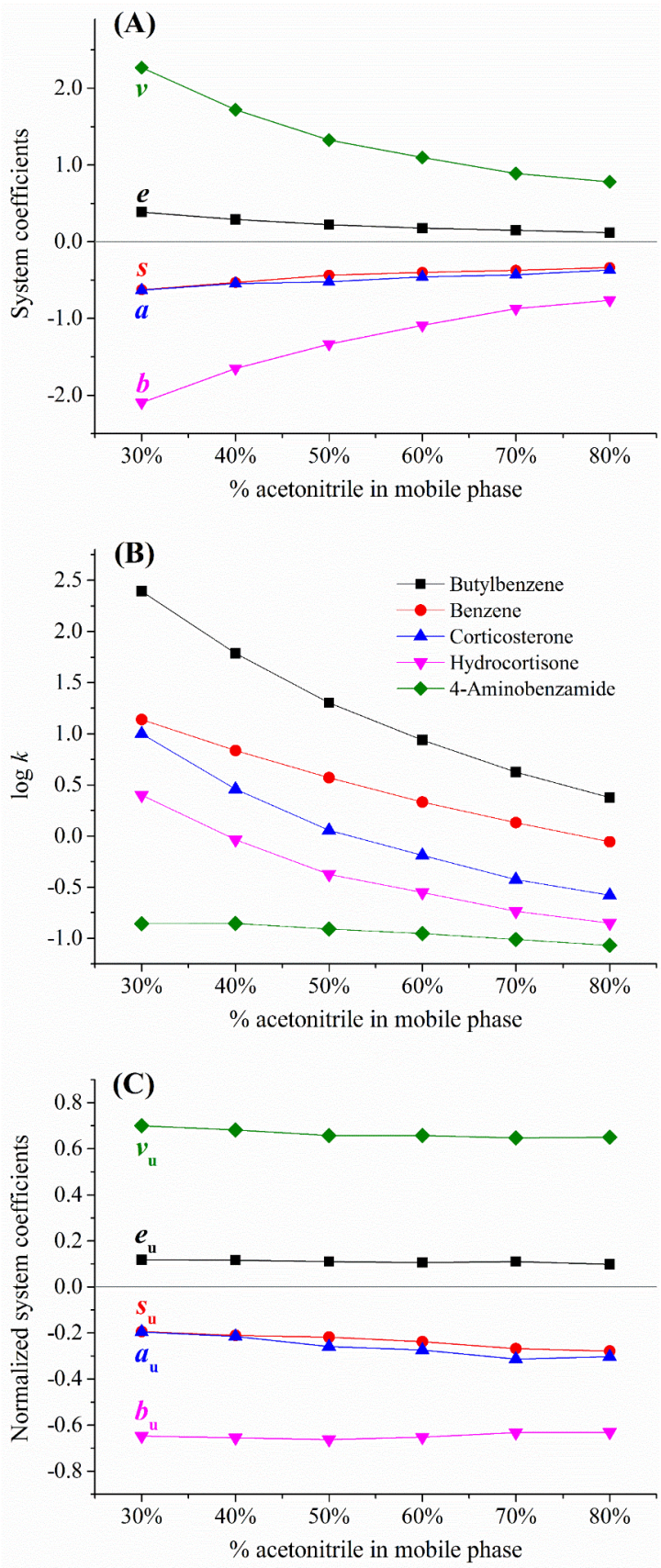


**Figure 3.** Characterization (Eq. (1)) of a Spherisorb ODS2 column with the acetonitrile content of the mobile phase [28]. (A) System coefficients; (B) estimated retention factors for butylbenzene, benzene, corticosterone, hydrocortisone, and 4-aminobenzamide; (C) Normalized system coefficients.

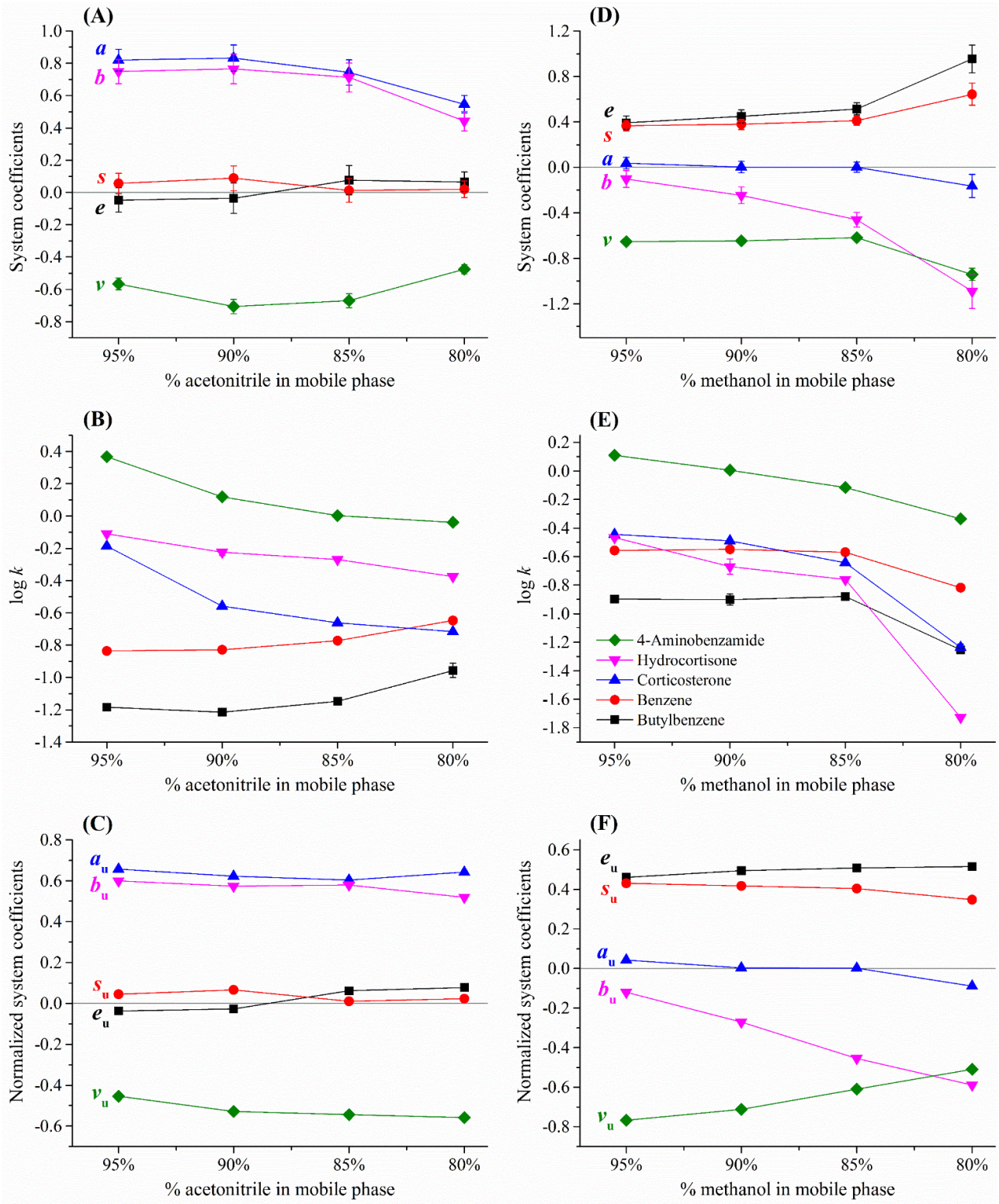


**Figure 4.** Characterization (Eq. (1)) of a ZIC-pHILIC column with mobile phases containing acetonitrile (A, B, C) and methanol (D, E, F). (A, D) System coefficients; (B, E) estimated retention factors for butylbenzene (■), benzene (●), corticosterone (▲), hydrocortisone (▼), and 4-aminobenzamide (◆); (C, F) Normalized system coefficients.

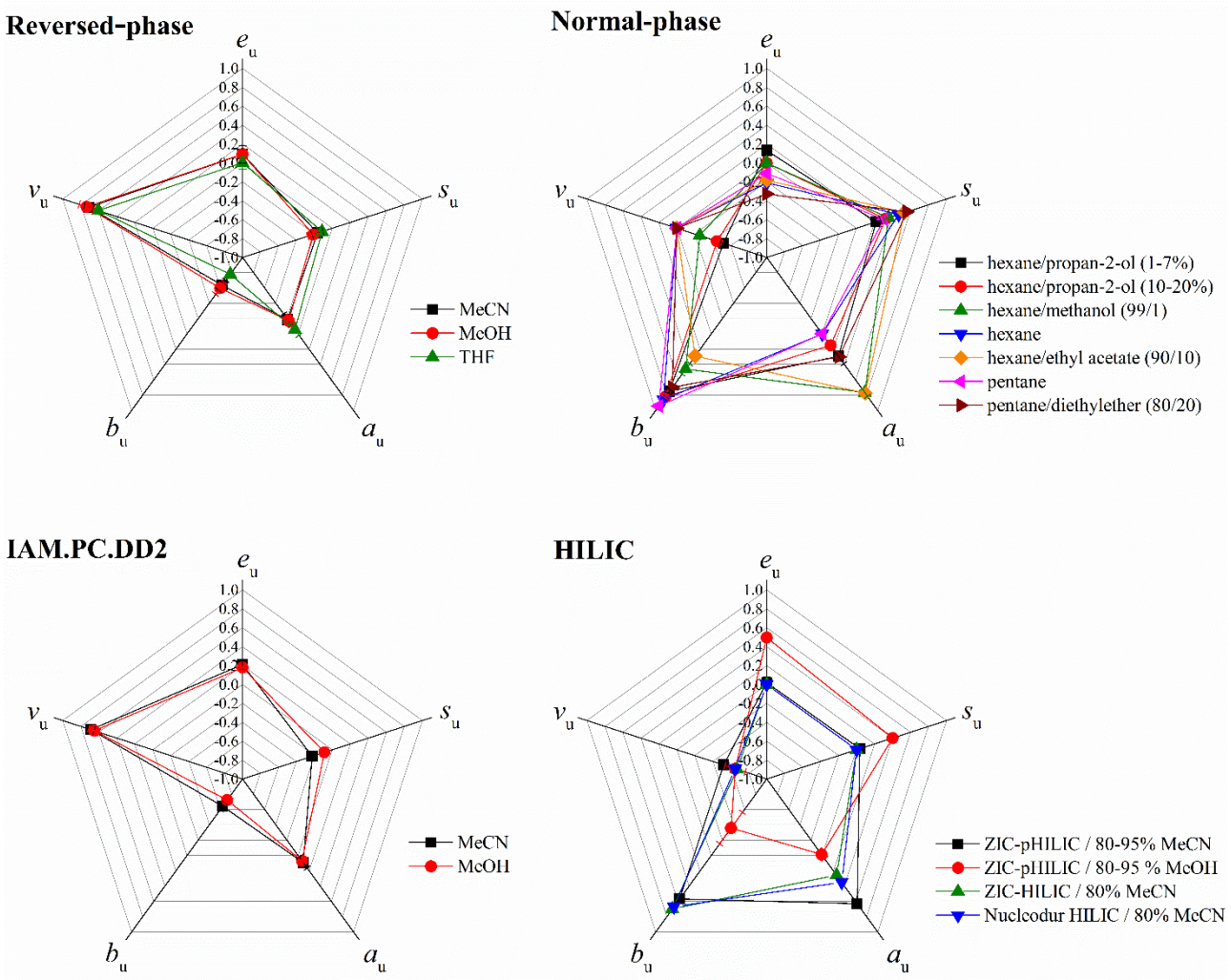


**Figure 5.** Radar plot of normalized system coefficients for some reversed-, normal-phase, IAM and HILIC chromatographic systems.

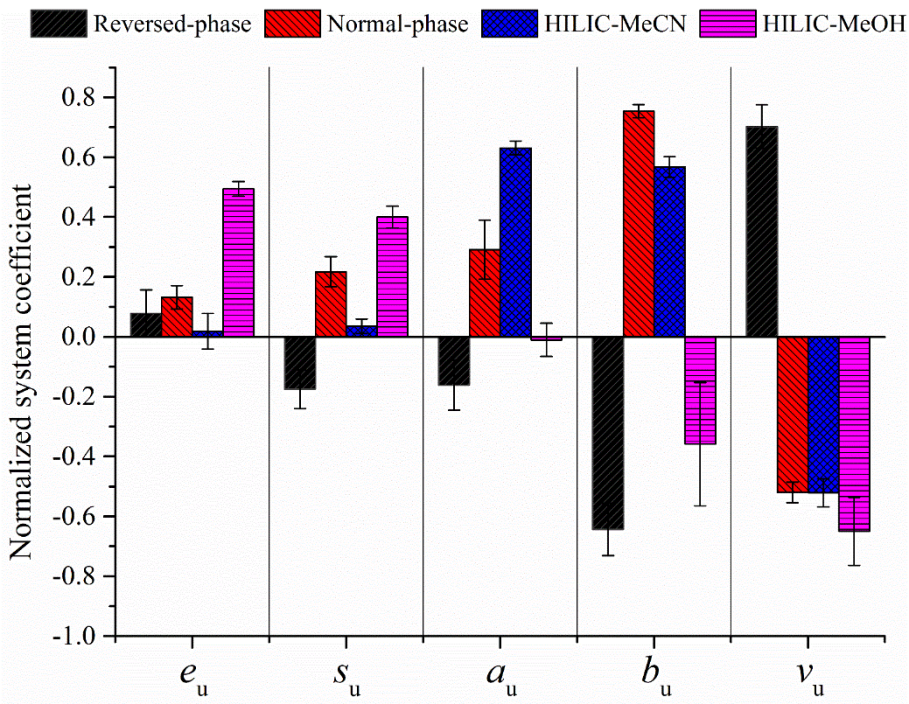


**Figure 6.** Mean values of normalized system coefficients. Reversed-phase: all systems in Table 4 (acetonitrile, methanol, and tetrahydrofuran); Normal phase: Nucleosil Silica with *n*-hexane/propan-2-ol from 1 to 7% of alcohol (Table 6); HILIC-MeCN: ZIC-pHILIC with 80 to 95% acetonitrile (Table 5); and HILIC-MeOH: ZIC-pHILIC with 80 to 95% methanol (Table 5).

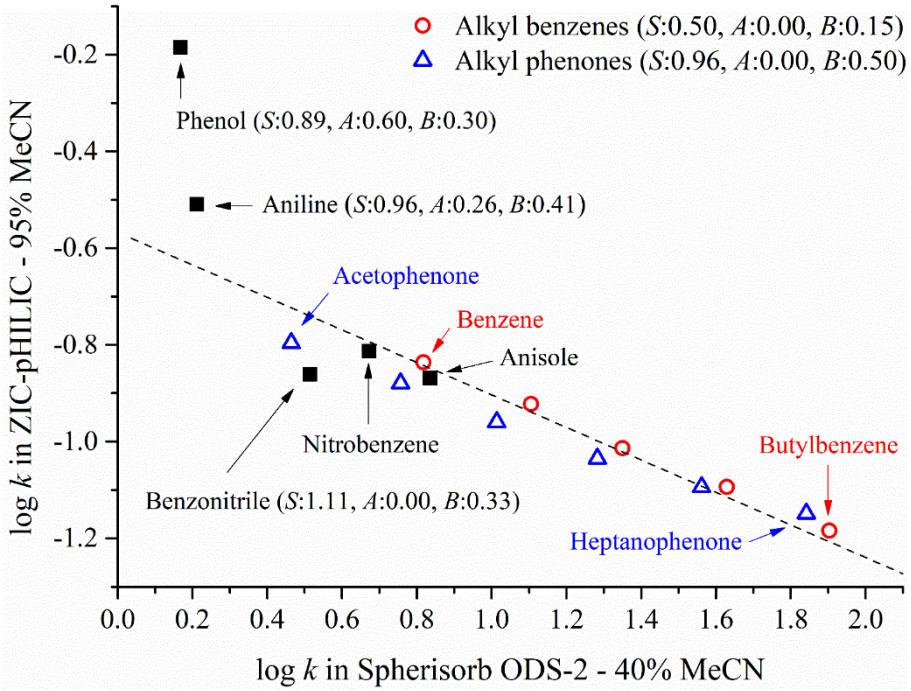


**Figure 7.** Selectivity plot for ZIC-pHILIC column with a mobile phase consisting of 95% acetonitrile and a reversed-phase ODS2 column and 40% acetonitrile. Dashed line accounts for the volume term correction (log $k_{HILIC}$ = -0.33 log $k_{RPLC}$ – 0.58).

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## SUPPLEMENTARY TABLES

**Table SP1**. Retention factors measured in a ZIC-pHILIC (150 x 4.6 mm) using 5 mM ammonium acetate mobile phases (standard errors in brackets).

| | Acetonitrile content in mobile phase (v/v) | | | | Methanol content in mobile phase (v/v) | | | |
|---|---|---|---|---|---|---|---|---|
| Compound | 95% | 90% | 85% | 80% | 95% | 90% | 85% | 80% |
| 2,3-Dimethylphenol | -0.609(0.007) | -0.761(0.015) | -0.272(0.003) | -0.376(0.004) | -0.468(0.002) | -0.492(0.004) | -0.533(0.003) | -0.766(0.002) |
| 2,4-Dimethylphenol | -0.636(0.006) | -0.782(0.017) | -0.757(0.004) | -0.716(0.008) | -0.549(0.002) | -0.574(0.002) | -0.629(0.006) | -0.942(0.009) |
| 2-Naphthol | -0.432(0.004) | -0.604(0.011) | -0.595(0.001) | -0.548(0.006) | -0.190(0.002) | -0.206(0.001) | -0.224(0.005) | -0.304(0.005) |
| 2-Nitroaniline | -0.370(0.001) | -0.225(0.019) | -0.272(0.001) | -0.470(0.082) | 0.006(0.002) | -0.950(0.022) | -1.192(0.016) | -1.354(0.005) |
| 2-Nitroanisole | -0.890(0.011) | -0.985(0.019) | -0.902(0.015) | -0.869(0.014) | -0.263(0.002) | -0.282(0.003) | -0.315(0.003) | -0.427(0.005) |
| 3-Chloroaniline | -0.553(0.007) | -0.620(0.010) | -0.568(0.002) | -0.501(0.004) | -0.170(0.018) | -0.163(0.001) | -0.181(0.000) | -0.260(0.005) |
| 3-Nitroaniline | -0.541(0.001) | -0.463(0.005) | -0.586(0.002) | -0.499(0.042) | 0.083(0.004) | -0.613(0.004) | -0.731(0.004) | -1.395(0.019) |
| 4-Aminobenzamide | 0.367(0.004) | 0.119(0.001) | 0.002(0.001) | -0.039(0.001) | 0.111(0.005) | 0.007(0.002) | -0.117(0.003) | -0.336(0.004) |
| 4-Chloroacetanilide | -0.514(0.004) | -0.645(0.009) | -0.962(0.082) | -0.639(0.014) | -0.674(0.194) | -0.612(0.003) | -0.708(0.002) | -1.177(0.008) |
| 4-Chloroaniline | -0.545(0.007) | -0.609(0.010) | -0.563(0.002) | -0.497(0.004) | -0.169(0.002) | -0.181(0.002) | -0.204(0.005) | -0.301(0.004) |
| 4-Chlorophenol | -0.424(0.003) | -0.225(0.021) | -0.273(0.002) | -0.378(0.001) | -0.344(0.001) | -0.356(0.001) | -0.381(0.005) | -0.508(0.006) |
| 4-Nitroaniline | -0.433(0.003) | -0.542(0.011) | -0.515(0.003) | -0.463(0.001) | 0.196(0.005) | 0.144(0.002) | 0.095(0.001) | 0.030(0.000) |
| Acetanilide | -0.482(0.022) | -0.588(0.005) | -0.602(0.004) | -0.585(0.006) | -0.566(0.003) | -0.638(0.002) | -0.778(0.001) | -1.798(0.063) |
| Acetophenone | -0.796(0.012) | -0.867(0.008) | -0.825(0.002) | -0.682(0.004) | -0.511(0.002) | -0.567(0.001) | -0.667(0.003) | -1.051(0.024) |
| Aniline | -0.509(0.006) | -0.561(0.006) | -0.524(0.004) | -0.471(0.002) | -0.192(0.004) | -0.224(0.002) | -0.282(0.005) | -0.462(0.003) |
| Anisole | -0.868(0.016) | -0.923(0.008) | -0.848(0.003) | -0.775(0.011) | -0.488(0.004) | -0.485(0.002) | -0.512(0.005) | -0.708(0.009) |
| Antipyrine | -0.369(0.001) | -0.424(0.004) | -0.486(0.005) | -0.515(0.004) | -0.711(0.010) | -0.861(0.007) | -1.208(0.019) | - |
| α-Pinene | -0.368(0.001) | -1.273(0.047) | -1.148(0.012) | -1.084(0.003) | -1.166(0.016) | -1.204(0.003) | -1.362(0.019) | - |
| Benzaldehyde | -0.988(0.016) | -0.830(0.013) | -1.020(0.006) | -0.706(0.004) | -0.421(0.003) | -0.855(0.007) | -1.052(0.012) | - |
| Benzamide | -0.892(0.009) | -0.974(0.012) | -0.914(0.001) | -0.859(0.006) | -0.263(0.003) | -0.279(0.002) | -0.311(0.004) | -0.429(0.006) |
| Benzene | -0.836(0.009) | -0.829(0.003) | -0.772(0.004) | -0.647(0.021) | -0.556(0.002) | -0.548(0.002) | -0.569(0.002) | -0.818(0.003) |
| Benzonitrile | -0.861(0.014) | -0.928(0.018) | -0.845(0.008) | -0.804(0.010) | -0.490(0.001) | -0.531(0.002) | -0.629(0.017) | -0.955(0.004) |
| Benzophenone | -0.886(0.018) | -0.996(0.019) | -0.927(0.006) | -0.869(0.012) | -0.469(0.003) | -0.475(0.002) | -0.499(0.007) | -0.639(0.008) |
| Benzyl benzoate | -1.061(0.016) | -1.218(0.042) | -1.127(0.013) | -1.102(0.017) | -0.509(0.003) | -0.490(0.004) | -0.478(0.012) | -0.533(0.008) |
| Bromobenzene | -0.770(0.010) | -0.812(0.010) | -0.725(0.001) | -0.633(0.005) | -0.498(0.001) | -0.460(0.003) | -0.445(0.003) | -0.542(0.005) |
| Butan-1-ol | -0.181(0.012) | -0.210(0.002) | -0.272(0.001) | -0.378(0.002) | -0.901(0.005) | -0.960(0.013) | -1.214(0.023) | - |

**Table SP1**. (cont.)

| | Acetonitrile content in mobile phase (v/v) | | | | Methanol content in mobile phase (v/v) | | | |
|---|---|---|---|---|---|---|---|---|
| Compound | 95% | 90% | 85% | 80% | 95% | 90% | 85% | 80% |
| Butylbenzene | -1.184(0.001) | -1.215(0.006) | -1.147(0.015) | -0.957(0.044) | -0.897(0.004) | -0.901(0.039) | -0.879(0.004) | -1.252(0.010) |
| Butyrophenone | -0.960(0.008) | -1.102(0.033) | -1.028(0.007) | -0.845(0.007) | -0.690(0.002) | -0.737(0.004) | -0.843(0.006) | -1.388(0.061) |
| Caffeine | -0.314(0.002) | -0.397(0.011) | -0.273(0.001) | -0.444(0.009) | -0.128(0.007) | -0.258(0.004) | -0.408(0.008) | -0.769(0.006) |
| Chlorobenzene | -0.806(0.011) | -0.852(0.012) | -0.761(0.003) | -0.671(0.005) | -0.542(0.002) | -0.515(0.004) | -0.509(0.003) | -0.644(0.005) |
| Corticosterone | -0.185(0.009) | -0.558(0.009) | -0.662(0.004) | -0.716(0.002) | -0.444(0.002) | -0.489(0.002) | -0.644(0.008) | -1.237(0.024) |
| Cortisone | -0.306(0.003) | -0.217(0.006) | -0.274(0.002) | -0.377(0.007) | -0.387(0.005) | -0.493(0.005) | -0.643(0.008) | -1.241(0.022) |
| Decanophenone | -1.330(0.011) | -1.712(0.043) | -1.524(0.023) | -1.193(0.013) | -1.110(0.012) | -1.141(0.037) | -1.239(0.021) | -2.161(0.357) |
| Dodecanophenone | -1.181(0.020) | -1.387(0.033) | -1.301(0.023) | -1.248(0.027) | -0.903(0.012) | -0.927(0.012) | -0.989(0.011) | -1.763(0.086) |
| Dodecylbenzene | -1.867(0.018) | -2.173(0.050) | -2.124(0.108) | -1.670(0.010) | -1.685(0.015) | -1.734(0.062) | -1.632(0.142) | -2.258(0.172) |
| Estradiol | -0.368(0.002) | -0.463(0.005) | -0.473(0.004) | -0.445(0.003) | -0.581(0.016) | -0.618(0.001) | -0.723(0.006) | -1.387(0.016) |
| Ethylbenzene | -1.014(0.004) | -1.030(0.001) | -0.967(0.003) | -0.820(0.026) | -0.736(0.002) | -0.719(0.004) | -0.732(0.003) | -1.053(0.004) |
| Furan | -0.787(0.013) | -0.798(0.002) | -0.729(0.002) | -0.663(0.003) | -0.502(0.000) | -0.507(0.002) | -0.544(0.001) | -0.809(0.001) |
| Geraniol | -0.369(0.001) | -1.025(0.021) | -1.014(0.012) | -1.015(0.001) | -1.319(0.002) | -1.638(0.013) | - | - |
| Heptanophenone | -1.149(0.006) | -1.375(0.046) | -1.308(0.006) | -1.029(0.006) | -0.898(0.004) | -0.944(0.007) | -1.045(0.001) | -1.672(0.094) |
| Hexanophenone | -1.094(0.017) | -1.300(0.023) | -1.220(0.004) | -0.974(0.005) | -0.852(0.022) | -0.909(0.010) | -1.021(0.006) | -1.561(0.032) |
| Hexylbenzene | -1.328(0.012) | -1.378(0.008) | -1.336(0.012) | -1.096(0.042) | -1.062(0.003) | -1.035(0.017) | -1.022(0.007) | -1.489(0.011) |
| Hydrocortisone | -0.109(0.006) | -0.224(0.020) | -0.270(0.001) | -0.374(0.003) | -0.467(0.004) | -0.670(0.053) | -0.760(0.013) | -1.728(0.009) |
| Hydroquinone | -0.891(0.011) | -0.224(0.020) | -0.275(0.002) | -0.375(0.003) | 0.118(0.005) | 0.004(0.001) | -1.229(0.034) | -0.319(0.002) |
| Methanol | -0.184(0.010) | -0.212(0.002) | -0.273(0.001) | -0.490(0.194) | -0.668(0.200) | -0.980(0.006) | -1.312(0.034) | - |
| Methyl benzoate | -0.889(0.011) | -0.958(0.017) | -0.896(0.005) | -0.859(0.009) | -0.581(0.003) | -0.614(0.004) | -0.703(0.010) | -1.121(0.011) |
| Monuron | -0.369(0.002) | -0.225(0.020) | -0.271(0.002) | -0.379(0.005) | -0.646(0.003) | -0.683(0.047) | -0.826(0.008) | -1.742(0.050) |
| Naphthalene | -0.806(0.011) | -0.854(0.016) | -0.767(0.002) | -0.681(0.003) | -0.396(0.004) | -0.350(0.002) | -0.319(0.005) | -0.361(0.006) |
| Nitrobenzene | -0.813(0.007) | -0.860(0.014) | -0.786(0.004) | -0.720(0.015) | -0.286(0.004) | -0.281(0.003) | -0.292(0.004) | -0.385(0.006) |
| Nonanophenone | -1.297(0.047) | -1.582(0.029) | -1.504(0.012) | -1.153(0.004) | -1.053(0.010) | -1.099(0.008) | -1.218(0.048) | -1.967(0.312) |
| Octanophenone | -1.227(0.007) | -1.500(0.040) | -1.404(0.027) | -1.090(0.008) | -0.978(0.007) | -1.017(0.006) | -1.122(0.008) | -1.726(0.185) |
| Octylbenzene | -1.474(0.023) | -1.578(0.035) | -1.521(0.041) | -1.262(0.028) | -1.226(0.004) | -1.194(0.015) | -1.245(0.073) | -1.708(0.042) |
| *o*-Toluidine | -0.584(0.005) | -0.647(0.010) | -0.613(0.002) | -0.563(0.005) | -0.327(0.003) | -0.363(0.003) | -0.425(0.004) | -0.654(0.003) |
| Pentan-1,5-diol | -0.874(0.008) | -0.213(0.001) | -0.271(0.001) | -0.378(0.003) | -0.569(0.319) | -0.976(0.015) | -1.215(0.014) | - |
| Pentan-1-ol | -0.179(0.009) | -0.214(0.002) | -0.271(0.002) | -0.377(0.002) | -0.788(0.201) | -0.941(0.057) | -1.211(0.005) | - |

**Table SP1**. (cont.)

| | Acetonitrile content in mobile phase (v/v) | | | | Methanol content in mobile phase (v/v) | | | |
|---|---|---|---|---|---|---|---|---|
| Compound | 95% | 90% | 85% | 80% | 95% | 90% | 85% | 80% |
| Pentan-3-ol | -0.180(0.009) | -0.210(0.001) | -0.272(0.002) | -0.380(0.002) | -0.785(0.205) | -0.977(0.010) | -1.215(0.036) | - |
| Pentylbenzene | -1.249(0.013) | -1.329(0.045) | -1.248(0.014) | -1.017(0.045) | -0.981(0.008) | -0.955(0.010) | -0.955(0.004) | -1.383(0.018) |
| Phenol | -0.185(0.008) | -0.225(0.021) | -0.273(0.001) | -0.378(0.007) | -0.878(0.026) | -0.969(0.018) | -0.730(0.005) | - |
| Propan-1-ol | -0.182(0.013) | -0.210(0.002) | -0.271(0.001) | -0.379(0.001) | -0.676(0.208) | -0.985(0.049) | -1.236(0.002) | - |
| Propan-2-ol | -0.182(0.013) | -0.212(0.001) | -0.272(0.002) | -0.384(0.003) | -0.903(0.005) | -0.977(0.006) | -1.212(0.010) | - |
| Propiophenone | -0.880(0.003) | -0.985(0.000) | -0.925(0.001) | -0.766(0.006) | -0.597(0.001) | -0.645(0.003) | -0.743(0.003) | -1.165(0.028) |
| Propylbenzene | -1.094(0.007) | -1.131(0.017) | -1.067(0.004) | -0.872(0.022) | -0.830(0.003) | -0.818(0.006) | -0.821(0.005) | -1.218(0.002) |
| *p*-Xylene | -0.981(0.015) | -1.074(0.018) | -0.972(0.003) | -0.893(0.008) | -0.730(0.004) | -0.705(0.004) | -0.725(0.007) | -1.007(0.014) |
| Pyrrole | -0.551(0.005) | -0.606(0.003) | -0.560(0.004) | -0.499(0.003) | -0.180(0.004) | -0.207(0.001) | -0.256(0.006) | -0.413(0.002) |
| Quinoline | -0.369(0.002) | -0.464(0.001) | -0.473(0.003) | -0.446(0.002) | -0.554(0.006) | -0.617(0.001) | -0.731(0.008) | -1.401(0.005) |
| Resorcinol | -0.891(0.008) | -0.223(0.019) | -0.273(0.002) | -0.376(0.005) | -0.261(0.004) | -0.968(0.011) | -1.203(0.019) | - |
| Thiourea | -0.179(0.009) | -0.212(0.001) | -0.274(0.001) | -0.381(0.003) | -0.652(0.222) | -0.961(0.029) | -1.209(0.008) | 0.053(0.003) |
| Thymol | -0.876(0.014) | -1.051(0.021) | -0.272(0.002) | -0.375(0.004) | -0.821(0.009) | -0.856(0.011) | -0.926(0.009) | -1.693(0.024) |
| Toluene | -0.922(0.008) | -0.926(0.006) | -0.871(0.003) | -0.731(0.020) | -0.639(0.003) | -0.627(0.007) | -0.640(0.003) | -0.911(0.002) |
| Valerophenone | -1.035(0.008) | -1.194(0.016) | -1.123(0.008) | -0.906(0.004) | -0.761(0.002) | -0.807(0.001) | -0.904(0.003) | -1.467(0.073) |

**Table SP2**. System coefficients for some reversed-phase columns and mobile phases containing acetonitrile, methanol, or tetrahydrofuran as organic modifier (standard errors in brackets).

| Column | Mobile phase | *c* | *e* | *s* | *a* | *b* | *v* | *n* | *R* | *SD* | *F* | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spherisorb ODS-2 | 30% MeCN | -0.11(0.06) | 0.38(0.06) | -0.63(0.04) | -0.63(0.04) | -2.10(0.05) | 2.27(0.05) | 103 | 0.993 | 0.083 | 1320 | [1][a] |
| | 40% MeCN | -0.08(0.05) | 0.29(0.05) | -0.53(0.04) | -0.54(0.02) | -1.65(0.05) | 1.72(0.05) | 112 | 0.991 | 0.079 | 1155 | [1][a] |
| | 50% MeCN | -0.11(0.04) | 0.22(0.04) | -0.44(0.03) | -0.52(0.03) | -1.34(0.04) | 1.33(0.04) | 127 | 0.990 | 0.065 | 1222 | [1][a] |
| | 60% MeCN | -0.21(0.04) | 0.18(0.03) | -0.40(0.02) | -0.46(0.02) | -1.09(0.03) | 1.10(0.03) | 127 | 0.990 | 0.055 | 1259 | [1][a] |
| | 70% MeCN | -0.29(0.04) | 0.15(0.03) | -0.37(0.02) | -0.43(0.02) | -0.87(0.03) | 0.89(0.03) | 127 | 0.998 | 0.053 | 933 | [1][a] |
| | 80% MeCN | -0.41(0.04) | 0.12(0.03) | -0.34(0.02) | -0.37(0.02) | -0.76(0.03) | 0.78(0.03) | 127 | 0.985 | 0.053 | 771 | [1][a] |
| ERC-1000 (ODS) | 50% MeCN | -0.20(0.04) | 0.02(0.04) | -0.18(0.04) | -0.58(0.03) | -1.50(0.05) | 1.60(0.04) | 44 | 0.995 | 0.035 | 766 | [1,2] |
| | 60% MeCN | -0.26(0.04) | -0.02(0.03) | -0.17(0.04) | -0.52(0.03) | -1.34(0.04) | 1.37(0.03) | 51 | 0.996 | 0.034 | 1278 | [1,2] |
| | 70% MeCN | -0.39(0.03) | -0.01(0.03) | -0.18(0.04) | -0.50(0.03) | -1.19(0.04) | 1.24(0.02) | 57 | 0.997 | 0.035 | 1623 | [1,2] |
| | 80% MeCN | -0.52(0.03) | -0.01(0.03) | -0.19(0.05) | -0.47(0.04) | -1.02(0.04) | 1.10(0.02) | 60 | 0.996 | 0.041 | 1294 | [1,2] |
| | 90% MeCN | -0.62(0.04) | 0.01(0.04) | -0.22(0.06) | -0.39(0.05) | -0.80(0.05) | 0.92(0.02) | 62 | 0.992 | 0.053 | 717 | [1,2] |
| Unisil C18 | 30% MeCN | -0.24(0.13) | 0.21(0.12) | -0.30(0.06) | -0.31(0.04) | -1.53(0.06) | 2.15(0.08) | 34 | 0.994 | 0.041 | 475 | [1,3] |
| | 40% MeCN | -0.32(0.12) | 0.28(0.12) | -0.26(0.06) | -0.26(0.04) | -1.18(0.06) | 1.66(0.05) | 37 | 0.992 | 0.041 | 362 | [1,3] |
| | 50% MeCN | -0.33(0.10) | 0.28(0.10) | -0.24(0.05) | -0.22(0.04) | -0.90(0.05) | 1.27(0.05) | 37 | 0.989 | 0.036 | 279 | [1,3] |
| | 60% MeCN | -0.36(0.09) | 0.28(0.09) | -0.21(0.04) | -0.18(0.03) | -0.69(0.04) | 1.00(0.05) | 37 | 0.987 | 0.031 | 228 | [1,3] |
| | 70% MeCN | -0.34(0.08) | 0.24(0.08) | -0.19(0.04) | -0.13(0.03) | -0.53(0.04) | 0.78(0.04) | 37 | 0.982 | 0.028 | 167 | [1,3] |
| | 80% MeCN | -0.35(0.07) | 0.21(0.07) | -0.15(0.03) | -0.10(0.02) | -0.38(0.03) | 0.60(0.03) | 37 | 0.978 | 0.024 | 138 | [1,3] |
| | 90% MeCN | -0.30(0.05) | 0.17(0.05) | -0.13(0.02) | -0.06(0.02) | -0.28(0.02) | 0.41(0.02) | 37 | 0.976 | 0.017 | 126 | [1,3] |
| Zorbax C8 | 20% MeCN | -0.25(0.05) | 0.11(0.06) | -0.29(0.05) | -0.47(0.05) | -2.59(0.07) | 2.68(0.07) | 57 | 0.996 | 0.07 | - | [4] |
| | 30% MeCN | -0.27(0.05) | -0.02(0.05) | -0.23(0.05) | -0.46(0.05) | -2.54(0.07) | 2.36(0.07) | 57 | 0.995 | 0.07 | - | [4] |
| | 40% MeCN | -0.25(0.04) | -0.02(0.05) | -0.26(0.04) | -0.43(0.04) | -2.16(0.06) | 1.90(0.06) | 57 | 0.994 | 0.06 | - | [4] |
| | 50% MeCN | -0.30(0.04) | -0.04(0.04) | -0.24(0.04) | -0.41(0.04) | -1.80(0.05) | 1.56(0.05) | 57 | 0.993 | 0.05 | - | [4] |

**Table SP2**. (cont.)

| Column | Mobile phase | *c* | *e* | *s* | *a* | *b* | *v* | *n* | *R* | *SD* | *F* | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XTerra MSC18 | 20% MeCN | -0.15(0.05) | 0.27(0.08) | -0.67(0.05) | -0.43(0.04) | -2.35(0.06) | 2.76(0.06) | 49 | 0.993 | 0.09 | 606 | [5] |
| | 40% MeCN | -0.15(0.04) | 0.15(0.06) | -0.46(0.04) | -0.42(0.03) | -1.72(0.04) | 1.76(0.04) | 55 | 0.994 | 0.07 | 764 | [5] |
| | 60% MeCN | -0.30(0.03) | 0.00(0.05) | -0.33(0.04) | -0.32(0.03) | -1.11(0.04) | 1.19(0.03) | 58 | 0.991 | 0.07 | 551 | [5] |
| XTerra RP18 | 20% MeCN | -0.28(0.03) | 0.38(0.05) | -0.58(0.04) | -0.21(0.03) | -2.35(0.05) | 2.56(0.04) | 48 | 0.996 | 0.06 | 1189 | [5] |
| | 40% MeCN | -0.17(0.03) | 0.23(0.05) | -0.46(0.03) | -0.26(0.03) | -1.57(0.04) | 1.58(0.03) | 55 | 0.996 | 0.06 | 1121 | [5] |
| | 60% MeCN | -0.33(0.02) | 0.12(0.03) | -0.34(0.02) | -0.20(0.02) | -1.00(0.03) | 1.03(0.02) | 55 | 0.996 | 0.04 | 1229 | [5] |
| Spherisorb ODS-2 | 40% MeOH | -0.36(0.05) | 0.37(0.05) | -0.83(0.04) | -0.49(0.03) | -2.07(0.04) | 2.70(0.04) | 112 | 0.995 | 0.069 | 2069 | [1][a] |
| | 50% MeOH | -0.24(0.05) | 0.25(0.05) | -0.69(0.04) | -0.46(0.03) | -1.84(0.05) | 2.14(0.04) | 114 | 0.993 | 0.077 | 1551 | [1][a] |
| | 60% MeOH | -0.32(0.05) | 0.25(0.04) | -0.65(0.03) | -0.43(0.03) | -1.53(0.04) | 1.77(0.04) | 126 | 0.992 | 0.072 | 1408 | [1][a] |
| | 70% MeOH | -0.36(0.04) | 0.28(0.04) | -0.58(0.03) | -0.44(0.02) | -1.23(0.04) | 1.35(0.03) | 126 | 0.991 | 0.062 | 1337 | [1][a] |
| | 80% MeOH | -0.45(0.04) | 0.28(0.04) | -0.55(0.03) | -0.40(0.02) | -0.90(0.04) | 1.03(0.03) | 126 | 0.987 | 0.061 | 919 | [1][a] |
| Zorbax C8 | 10% MeOH | -0.94(0.08) | 0.10(0.08) | -0.34(0.07) | -0.25(0.09) | -1.77(0.11) | 3.80(0.10) | 39 | 0.992 | 0.10 | - | [4] |
| | 20% MeOH | -0.67(0.07) | 0.23(0.07) | -0.55(0.07) | -0.42(0.08) | -1.72(0.10) | 3.26(0.10) | 39 | 0.992 | 0.09 | - | [4] |
| | 30% MeOH | -0.64(0.06) | 0.21(0.06) | -0.56(0.06) | -0.34(0.08) | -1.78(0.09) | 2.99(0.09) | 39 | 0.993 | 0.08 | - | [4] |
| | 40% MeOH | -0.66(0.06) | 0.17(0.06) | -0.55(0.05) | -0.27(0.07) | -1.65(0.08) | 2.63(0.08) | 39 | 0.992 | 0.07 | - | [4] |
| | 50% MeOH | -0.72(0.05) | 0.16(0.06) | -0.53(0.05) | -0.27(0.07) | -1.51(0.08) | 2.27(0.07) | 39 | 0.992 | 0.07 | - | [4] |
| Nucleosil 5-C18 | 45% MeOH | 0.10(0.06) | 0.20(0.05) | -0.56(0.05) | -0.44(0.03) | -1.76(0.07) | 2.02(0.09) | 31 | 0.992 | 0.046 | 304 | [1,6] |
| | 50% MeOH | 0.12(0.05) | 0.19(0.04) | -0.51(0.04) | -0.44(0.03) | -1.62(0.06) | 1.78(0.07) | 34 | 0.994 | 0.042 | 502 | [1,6] |
| | 55% MeOH | 0.11(0.05) | 0.22(0.04) | -0.48(0.04) | -0.43(0.03) | -1.48(0.06) | 1.55(0.07) | 35 | 0.994 | 0.043 | 523 | [1,6] |
| | 60% MeOH | 0.10(0.05) | 0.20(0.04) | -0.42(0.04) | -0.40(0.03) | -1.31(0.06) | 1.34(0.07) | 35 | 0.994 | 0.041 | 455 | [1,6] |
| | 65% MeOH | 0.13(0.05) | 0.20(0.04) | -0.34(0.04) | -0.37(0.03) | -1.13(0.06) | 1.10(0.07) | 35 | 0.992 | 0.041 | 351 | [1,6] |
| | 70% MeOH | 0.09(0.04) | 0.16(0.03) | -0.32(0.03) | -0.33(0.02) | -0.96(0.05) | 0.94(0.06) | 35 | 0.992 | 0.035 | 343 | [1,6] |
| | 75% MeOH | 0.09(0.04) | 0.15(0.03) | -0.28(0.03) | -0.29(0.02) | -0.77(0.05) | 0.76(0.06) | 32 | 0.991 | 0.031 | 299 | [1,6] |
| | 80% MeOH | 0.08(0.04) | 0.12(0.03) | -0.23(0.03) | -0.25(0.02) | -0.65(0.04) | 0.62(0.05) | 33 | 0.989 | 0.029 | 235 | [1,6] |

**Table SP2**. (cont.)

| Column | Mobile phase | *c* | *e* | *s* | *a* | *b* | *v* | *n* | *R* | *SD* | *F* | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spherisorb ODS-2 | 30% THF | 0.19(0.07) | -0.07(0.07) | -0.33(0.08) | -0.12(0.08) | -2.38(0.10) | 1.95(0.06) | 30 | 0.996 | 0.06 | 0.19 | [1,7] |
| | 40% THF | 0.14(0.06) | -0.10(0.06) | -0.26(0.07) | -0.18(0.06) | -1.75(0.08) | 1.37(0.05) | 30 | 0.995 | 0.05 | 0.14 | [1,7] |
| | 50% THF | 0.03(0.04) | -0.10(0.05) | -0.20(0.05) | -0.20(0.05) | -1.30(0.07) | 0.96(0.04) | 30 | 0.994 | 0.04 | 0.03 | [1,7] |
| | 60% THF | -0.09(0.04) | -0.09(0.04) | -0.20(0.05) | -0.26(0.05) | -0.98(0.06) | 0.69(0.03) | 30 | 0.993 | 0.04 | -0.09 | [1,7] |
| Zorbax C8 | 20% THF | -0.18(0.09) | 0.49(0.10) | -0.41(0.10) | 0.01(0.10) | -2.88(0.13) | 2.35(0.13) | 57 | 0.984 | 0.14 | - | [4] |
| | 30% THF | -0.20(0.07) | 0.23(0.08) | -0.30(0.07) | 0.08(0.07) | -2.52(0.09) | 1.97(0.09) | 57 | 0.987 | 0.10 | - | [4] |
| | 40% THF | -0.28(0.06) | 0.07(0.07) | -0.27(0.07) | 0.00(0.07) | -2.12(0.09) | 1.66(0.09) | 57 | 0.981 | 0.10 | - | [4] |
| | 50% THF | -0.37(0.05) | 0.00(0.06) | -0.25(0.05) | -0.06(0.05) | -1.70(0.07) | 1.35(0.07) | 57 | 0.983 | 0.07 | - | [4] |

[a]Calculated from retention factors in refs. [8–13].

**Table SP3**. System coefficients for an immobilized artificial membrane column in mobile phases containing acetonitrile and methanol.

| Column | Mobile phase | *c* | *e* | *s* | *a* | *b* | *v* | *n* | *R* | *SD* | *F* | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IAM.PC.DD2 | 10% MeCN | -0.73(0.04) | 0.76(0.06) | -0.77(0.04) | 0.13(0.04) | -1.96(0.05) | 2.40(0.04) | 49 | 0.994 | 0.07 | 708 | [5] |
| | 20% MeCN | -0.70(0.04) | 0.50(0.06) | -0.58(0.04) | 0.19(0.03) | -2.00(0.06) | 2.13(0.04) | 52 | 0.994 | 0.07 | 742 | [5] |
| | 40% MeCN | -0.71(0.03) | 0.29(0.04) | -0.34(0.03) | 0.14(0.02) | -1.19(0.04) | 1.16(0.03) | 51 | 0.991 | 0.05 | 480 | [5] |
| | 60% MeCN | -0.98(0.03) | 0.27(0.04) | -0.26(0.03) | 0.19(0.02) | -0.59(0.04) | 0.62(0.03) | 55 | 0.963 | 0.05 | 125 | [5] |
| IAM.PC.DD2 | 10% MeOH | -1.24(0.07) | 0.66(0.06) | -0.28(0.05) | 0.23(0.06) | -2.52(0.09) | 2.73(0.10) | 34 | 0.995 | 0.07 | 592 | [14] |
| | 20% MeOH | -1.20(0.06) | 0.65(0.05) | -0.31(0.04) | 0.22(0.04) | -2.50(0.07) | 2.57(0.08) | 34 | 0.997 | 0.06 | 965 | [14] |
| | 30% MeOH | -1.14(0.07) | 0.60(0.05) | -0.29(0.05) | 0.23(0.05) | -2.38(0.08) | 2.25(0.09) | 34 | 0.995 | 0.07 | 615 | [14] |
| | 40% MeOH | -1.08(0.05) | 0.53(0.05) | -0.31(0.04) | 0.22(0.04) | -2.28(0.07) | 2.00(0.07) | 34 | 0.996 | 0.05 | 765 | [14] |
| | 50% MeOH | -1.12(0.07) | 0.49(0.06) | -0.28(0.05) | 0.20(0.05) | -2.07(0.08) | 1.66(0.09) | 34 | 0.993 | 0.07 | 372 | [14] |
| | 60% MeOH | -1.22(0.08) | 0.45(0.07) | -0.21(0.06) | 0.24(0.07) | -1.86(0.11) | 1.32(0.11) | 31 | 0.984 | 0.08 | 148 | [14] |

**Table SP4**. System coefficients for some columns and non-aqueous mobile phases used in normal-phase mode (standard errors in brackets).

| Column | Mobile phase | *c* | *e* | *s* | *a* | *b* | *v* | *n* | *R* | *SD* | *F* | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spherisorb $NH_2$ | *n*-hexane | -1.31(0.13) | -0.55(0.14) | 1.23(0.26) | - | 2.25(0.33) | - | 36 | 0.909 | 0.30 | 51 | [15] |
| | *n*-hexane/ethyl acetate (90/10) | -1.35(0.16) | -0.38(0.14) | 1.10(0.22) | 1.60(0.20) | 0.60(0.30) | - | 42 | 0.871 | 0.30 | 29 | [15] |
| LiChrospher Diol | *n*-pentane | -0.83(0.22) | -0.23(0.14) | 0.63(0.30) | - | 1.94(0.42) | - | 35 | 0.727 | 0.15 | 12 | [15][a] |
| | *n*-pentane/diethylether (80/20) | -0.23(0.10) | -0.34(0.11) | 0.58(0.12) | 0.31(0.10) | 0.72(0.13) | - | 44 | 0.751 | 0.11 | 13 | [15][a] |
| Nucleosil Silica | *n*-hexane/propan-2-ol (99/1) | -0.63(0.25) | 0.51(0.17) | 0.54(0.22) | 1.52(0.21) | 2.69(0.35) | -1.69(0.31) | 57 | 0.869 | 0.36 | 31 | [15][b] |
| | *n*-hexane/propan-2-ol (98/2) | -0.80(0.22) | 0.64(0.16) | 0.74(0.22) | 1.08(0.17) | 2.54(0.30) | -1.91(0.27) | 51 | 0.898 | 0.29 | 37 | [15][b] |
| | *n*-hexane/propan-2-ol (95/5) | -0.92(0.26) | 0.36(0.18) | 0.83(0.23) | 0.81(0.21) | 2.62(0.36) | -1.83(0.32) | 56 | 0.839 | 0.37 | 24 | [15][b] |
| | *n*-hexane/propan-2-ol (93/7) | -1.08(0.24) | 0.33(0.17) | 0.90(0.22) | 0.67(0.20) | 2.62(0.34) | -1.78(0.30) | 57 | 0.847 | 0.35 | 26 | [15][b] |
| | *n*-hexane/propan-2-ol (90/10) | -1.41(0.26) | - | 0.99(0.24) | 0.64(0.24) | 2.71(0.40) | -1.37(0.25) | 54 | 0.799 | 0.41 | 22 | [15][b] |
| | *n*-hexane/propan-2-ol (85/15) | -1.34(0.23) | - | 1.00(0.23) | 0.42(0.23) | 2.46(0.38) | -1.42(0.24) | 55 | 0.787 | 0.40 | 20 | [15][b] |
| | *n*-hexane/propan-2-ol (80/20) | -1.46(0.22) | - | 0.99(0.22) | 0.33(0.22) | 2.44(0.37) | -1.35(0.22) | 56 | 0.794 | 0.38 | 22 | [15][b] |
| Hypersil Silica | *n*-hexane/MeOH (99/1) | -1.21(0.10) | - | 1.06(0.10) | 2.23(0.10) | 1.56(0.13) | -0.83(0.09) | 34 | 0.990 | 0.11 | 356 | [16] |
| Hypersil APS $NH_2$ | *n*-hexane/MeOH (99/1) | -1.14(0.09) | - | 0.94(0.08) | 2.94(0.09) | 1.20(0.11) | -0.72(0.08) | 36 | 0.993 | 0.10 | 217 | [16] |
| LiChrospher Diol | *n*-hexane/MeOH (99/1) | -0.94(0.09) | - | 1.07(0.07) | 2.37(0.09) | 1.47(0.11) | -0.85(0.07) | 35 | 0.993 | 0.09 | 535 | [16] |
| LiChrospher CN | *n*-hexane/MeOH (99/1) | -0.89(0.10) | - | 0.99(0.12) | 1.94(0.08) | 1.04(0.13) | -0.64(0.10) | 35 | 0.991 | 0.09 | 418 | [16] |
| Ultrasphere CN | *n*-hexane/MeOH (99/1) | -1.10(0.09) | - | 1.03(0.11) | 1.97(0.08) | 1.08(0.12) | -0.60(0.09) | 35 | 0.992 | 0.09 | 446 | [16] |
| Hypersil CN | *n*-hexane/MeOH (99/1) | -1.16(0.08) | - | 0.95(0.10) | 1.86(0.07) | 1.15(0.11) | -0.61(0.08) | 35 | 0.993 | 0.08 | 503 | [16] |
| Spherisorb CN | *n*-hexane/MeOH (99/1) | -0.99(0.10) | - | 1.00(0.08) | 2.13(0.09) | 1.69(0.12) | -0.84(0.08) | 35 | 0.991 | 0.10 | 396 | [16] |

[a] Calculated from retention factors in ref. [17].

[b] Calculated form data provided by Prof. Cheong [18].

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