

Chromatographic Selectivity of Cyano-Bonded Silica Columns in RPLC Based on the Linear Solvation Energy Relationships

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Differences in chromatographic properties in RPLC of four brands of cyano bonded silica stationary phases are rationalized in terms of the type and relative strength of the solute-stationary phase interactions, which can be readily inferred from multiple linear regression analyses of retention data for a set of standard compounds on the stationary phases under study based on the linear solvation energy relationships (LSERs). Although four brands of cyano bonded columns studied (CPS-Hypersil, Ultrasphere cyano, Spherisorb-CN and μ -Bondapak-CN) have similar bonding density and have been prepared from monofunctional cyanopropylsilane reagents, they possess quite different, relative hydrogen bonding (HB) donor and acceptor strengths. Comparison of the retention behavior on a cyano-bonded silica column with that on an ODS column shows that there are significant differences in the strength of HB interactions between the solute and the stationary phase on the two columns with different functionalities. Information on the differences in the interaction characteristics among brands of the cyano-bonded silica columns and between the ODS and cyano-bonded columns can be utilized to optimize the selectivity for a given separation on these columns.

Introduction

Recently Smith and Miller¹ compared the retention properties in RPLC of three brands of cyano-bonded silica stationary phases by measuring retention of a series of test compounds. They found considerable differences in elution behavior of the test solutes on different brands of cyano-bonded phases although the cyano-bonded columns (CPS-Hypersil, Ultrasphere cyano, and Spherisorb-CN) used have similar carbon loadings and have been prepared from monofunctional cyanopropylsilane reagents. They ascribed these different chromatographic properties of the three cyano-bonded phases to so-called 'specific cyano (interaction) effects' without providing a detailed explanation for the observed differences, which are believed to occur due to differences in the types and strengths of interactions between the solute and cyano-bonded stationary phase.

In this paper, we rationalize the 'specific cyano effect' in terms of differences in the types and relative strengths of the solute-stationary phase interactions, which can be readily inferred from multiple linear regression analyses of retention data based on the linear solvation energy relationships (LSERs)^{2,3} using the van der Waals molar volumes and the solvatochromic parameters for the test solutes π^* (dipolarity/polarizability), β (hydrogen bonding acceptor basicity) and α (hydrogen bonding donor acidity). Kamlet, Taft and their coworkers have applied the LSER approach to some 600 processes³ including a large number of systems of immediate relevance to chromatography, such as Rohrschneider's gas-liquid partition coefficients⁴, retention of McReynold's solutes on polymeric silicone oil gas chromatographic phases⁵, and retention in normal⁶ and reversed phase liquid chromatography⁷⁻¹⁰. According to the LSER formalism, when applied to phase transfer processes, a general solute or solvent property (SP) can be correlated *via* the use of three types of terms as follows^{2,3}:

$$SP = SP_0 + \text{cavity term} + \text{dipolar term} +$$

$$\text{hydrogen bonding term(s)} \quad (1)$$

SP_0 denotes the value of SP when all the three terms in the equation are zero. The cavity term is usually taken as the product of the solute van der Waals molar volume (V_l) and the square of the Hildebrand solubility parameter (δ) of the solvent. The dipolar term is the product of the solute π^* and the solvent π^* . The π^* parameter measures a combination of dipolarity/polarizability of a compound. The hydrogen bonding (HB) terms are written as a cross product of the solute α and the solvent β (type B HB) and the product of the solute β and the solvent α (type A HB). The parameters α and β measure HB donor acidity and HB acceptor basicity of the compound, respectively. In the case of the chromatographic retention, SP in the equation below denotes a logarithmic capacity factor and the subscript 2 designates a solute property. The subscripts s and m denote the stationary and mobile phases, respectively.

$$\log k' = \log k'_0 + M(\delta_s^2 - \delta_m^2)V_{l,2}/100 + S(\pi_s^* - \pi_m^*)\pi_2^* + B(\alpha_s - \alpha_m)\beta_2 + A(\beta_s - \beta_m)\alpha_2 \quad (2)$$

The coefficients M , S , B , and A are the fitting parameters.

When a system with a fixed pair of mobile and stationary phases is considered, Eq. (2) is reduced to

$$\log k' = \log k'_0 + mV_{l,2}/100 + s\pi_2^* + b\beta_2 + a\alpha_2 \quad (3)$$

The coefficients m , s , b , and a are obtained by multiple linear regression of $\log k'$ vs. the solute parameters. The sign and magnitude of the coefficients measure the direction and relative strength of different types of solute-stationary (and mobile) phase interactions affecting retention for a given pair of mobile-stationary phase condition. When capacity factors for a given series of solutes measured on different brands of cyano-bonded stationary phase columns using the mobile phase of the same composition are examined, the mobile phase parameters in Eq. (2). (δ_m^2 , π_m^* , α_m and β_m) are fixed. Any variations in the coefficients m , s , b , and a with brands

Table 1. Properties and Capacity Factors of the Compounds on μ -Bondapak CN Column

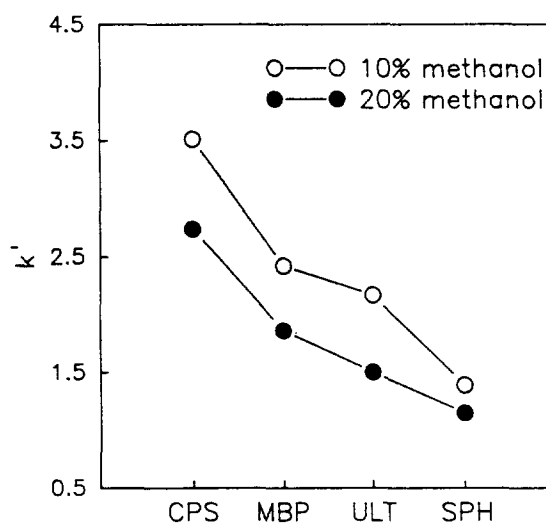
| Compound | $V_i/100^a$ | π^{*a} | β^a | α^a | % Methanol | | | | % Acetonitrile | | |
|------------------|-------------|------------|-----------|------------|------------|------|------|------|----------------|------|------|
| | | | | | 10 | 20 | 30 | 40 | 10 | 20 | 30 |
| Acetophenone | 0.690 | 0.90 | 0.49 | 0.03 | 2.27 | 1.36 | 0.93 | 0.79 | 1.82 | 1.43 | 1.13 |
| Propiophenone | 0.788 | 0.88 | 0.49 | 0 | 3.57 | 2.04 | 1.33 | 1.03 | 2.90 | 2.22 | 1.60 |
| Butyrophenone | 0.886 | 0.86 | 0.49 | 0 | 5.63 | 3.03 | 1.88 | 1.38 | 4.64 | 3.41 | 2.20 |
| Valerophenone | 0.984 | 0.84 | 0.49 | 0 | 9.51 | 4.96 | 2.82 | 1.99 | 7.96 | 5.44 | 3.09 |
| 2-Phenylethanol | 0.732 | 0.97 | 0.55 | 0.33 | 1.12 | 0.88 | 0.67 | 0.50 | 1.21 | 1.05 | 0.81 |
| N-Methylaniline | 0.660 | 0.73 | 0.47 | 0.12 | 1.44 | 1.07 | 0.83 | 0.60 | 1.58 | 1.50 | 1.30 |
| <i>p</i> -Cresol | 0.634 | 0.68 | 0.34 | 0.58 | 1.63 | 1.19 | 0.86 | 0.74 | 1.63 | 1.39 | 1.18 |
| Nitrobenzene | 0.631 | 1.01 | 0.30 | 0 | 2.02 | 1.51 | 1.16 | 1.08 | 2.21 | 2.07 | 1.81 |
| Toluene | 0.592 | 0.55 | 0.11 | 0 | 2.42 | 1.86 | 1.40 | 1.08 | 2.73 | 2.87 | 2.52 |
| Methyl benzoate | 0.736 | 0.76 | 0.39 | 0 | 3.07 | 1.88 | 1.33 | 0.87 | 2.77 | 2.20 | 1.62 |

^aData from ref. 21.

of the columns are determined only by the variations in the stationary phase properties (δ_s^2 , π^* , α , and β). The differences in these coefficients then indicate the differences in the extent of contributions to retention from various types of interactions of the stationary phase with the solute. The values of the coefficients m , s , h , and a , thus, can be regarded as measures of relative strength of corresponding interaction properties of the column. This approach has been found useful for characterization of chromatographic properties of some stationary phases for use in normal⁶ or reversed phase LC¹¹. Examination of retention data on the cyano-bonded phases using the above approach has shown that although the four brands of cyano-bonded columns studied (CPS-Hypersil, Ultrasphere cyano, Spherisorb-CN and μ -Bondapak-CN) are of not very different bonding density and have been prepared from monofunctional cyanopropylsilane reagents, they possess quite different, relative HB donor and acceptor strengths.

Experimental

Values of capacity factors for a set of standard compounds (acetophenone, propiophenone, butyrophenone, valerophenone, toluene, nitrobenzene, N-methylaniline, methyl benzoate, 2-phenylethanol, and *p*-cresol) on CPS-Hypersil (abbreviated CPS), Spherisorb-CN (abbreviated SPH) and Ultrasphere cyano (abbreviated ULT) columns were taken from Smith and Miller¹. Retention measurements on a μ -Bondapak-CN column (3.0 \times 300 mm, 10 μ m, Waters, Milford, U.S.A.) (abbreviated MBP) were obtained for the same solutes as used by Smith and Miller¹, with Waters HPLC system (Milford, U.S.A.) composed of a Model 510 pump, a Model U6K injector equipped with 10- μ l sample loop, a Model 441 UV-detector set to a wavelength of 254 nm and a Model 730 Data module. The column was placed in a water-jacket, and the temperature was controlled at 30 \pm 0.1 $^\circ$ C. The eluents used were methanol-pH 7 buffer or acetonitrile-pH 7 buffer in different proportions. The eluent flow rate was 1 ml/min. An aliquot of 10% aqueous sodium nitrate was injected to determine the column void volume. The capacity factors were calculated from the mean retention times of triplicate injections and are given in Table 1. All the solutes were reagent grade from Aldrich (Milwaukee, U.S.A.) and used

**Figure 1.** Variation of retention of toluene with brands of cyano-bonded phase columns.

without further purification. Methanol and acetonitrile were HPLC grade and from Ajax (Auburn, Australia).

Results and Discussion

Figure 1 shows variation of retention of toluene with brands of cyanobonded phase columns. At a given methanol composition (e.g., 10%) retention on the CPS column is much longer than those on the remaining three columns even though bonding densities of the columns are quite the same (see Table 2). This seemingly anomalous retention behavior of toluene on the cyano-bonded phase columns can not be ascribed solely to differences in bonding density of the columns. This indicates that there are considerable differences in retention effects due to the differences in solute-stationary phase interactions even though all four columns have the same bonded functionality. In order to gain understanding of factors causing the differences in retention properties of the cyano columns, multiple linear regression of $\log k'$ on the CPS column in 10% methanol-water vs. the solute properties was performed at first based on Eq. (3). The resu-

Table 2. Properties of the Cyano-Bonded Columns as Supplied by the Manufacturer

| Column | Surface area (m ² /g) | Carbon loading (%) | Bonding density (% C/m ²) | End-capped |
|--------|----------------------------------|--------------------|---------------------------------------|------------|
| CPS | 170 | 3.5 | 0.021 | yes |
| SPH | 220 | 3.9 | 0.018 | yes |
| ULT | 200 | 4.4 | 0.022 | yes |
| MBP | 330 | 6.0 | 0.018 | yes |

ling equation is given by Eq. (4).

$$\log k' = -0.99(\pm 0.10) + 2.82(\pm 0.13)V_f/100 - 0.07(\pm 0.10)\pi^* - 1.05(\pm 0.15)\beta - 0.73(\pm 0.13)\alpha$$

$$n = 10, r = 0.998, \text{ S.D.} = 0.004 \quad (4)$$

The coefficient s for the π^* parameter is statistically zero, indicating that dipolar interactions do not affect retention. For retention data on the remaining columns in both methanol- and acetonitrile-modified eluents, we observed the same results. Thus the π^* parameter was excluded in subsequent regressions. Data point for *p*-cresol turned out to be an outlier based upon Cook's distance and Student's *t*-test¹² in regressions of k' data on all four columns in both methanol- and acetonitrile-modified eluents, and thus were not included in regressions. The results of triple regressions for retention data on the four columns in methanol-water eluents are listed in Table 3. Correlation coefficients are mostly very close to unity, indicating that retention behavior of the solutes on the cyano-bonded columns is well represented by the LSER model. It is seen from the signs of the coefficients, as might be expected from *a priori* considerations, that increasing solute size (V_f) causes an increase in retention, *i.e.*, free energy concepts favor solute transfer from the more cohesive mobile phase to the less cohesive stationary phase.

The size of the coefficient m is decreasing with increasing content of organic modifier, which is less cohesive than water. Opposing this effect, increase in HB donor acidity (α) and HB acceptor basicity (β) leads to lower $\log k'$ values because the solutes have greater affinities for the more strongly hydrogen bonding aqueous mobile phase. Values of HB donor acidity and HB acceptor basicity for aqueous organic mobile phases are generally greater than those for butyronitrile, a free-form analog of the cyanopropyl group bonded to silica (10-90 vol % methanol, $\alpha = 1.17 - 1.02^{13}$, $\beta = 0.26 - 0.60^{14}$; butyronitrile, $\alpha = 0$, $\beta = 0.31^{15}$). The size of the coefficient b is greater than that of the coefficient a . This indicates that type A HB interactions between the solute and the mobile phase predominate over type B HB interactions. Comparison of the size of each coefficient indicates that the most important factor influencing RPLC retention for the solutes studied is endoergic cavity formation term. HB terms are less important and contributions to retention from type A and type B HB are varied with brands. The negative sign of both the coefficients b and a also indicates that both type A and type B HB interactions occur mainly between the solute and the mobile phase. If HB interactions of the solute with the cyanopropyl groups of the stationary phase were ever greater than those with the mobile phase, retention must have increased with increasing the solute's α and β .

Table 4 lists values of the three coefficients on the four cyano-bonded columns in 20% methanol- and acetonitrile-pH 7 buffer. The size of the coefficients are quite different for different brands. If interactions between the solute and the mobile phase are dominating retention, what are then causing the size of those coefficients (*i.e.*, retention properties) for different brands of cyano-bonded column to vary? Eq. (2) indicates that the coefficient (*e.g.*, the coefficient b) is cross product of a constant (B) and the differences in properties of the stationary phase and the mobile phase (*i.e.*, $b = B(\alpha_s - \alpha_m)$). It is well known that end-capping can not block the surface silanol groups completely. If there is any variabi-

Table 3. Coefficient Estimates in Regressions of $\log k'$ on the CPS Column vs. Solute Parameters^a

| Column | % Methanol | Intercept | m | b | a | r | S.D. |
|--------|------------|-------------|------------|-------------|-------------|-------|-------|
| CPS | 10 | -1.03(0.07) | 2.84(0.12) | -1.09(0.12) | -0.72(0.12) | 0.998 | 0.030 |
| | 20 | -0.87(0.05) | 2.40(0.10) | -1.10(0.10) | -0.61(0.10) | 0.998 | 0.024 |
| | 30 | -0.72(0.06) | 1.97(0.10) | -1.13(0.10) | -0.34(0.10) | 0.996 | 0.025 |
| | 40 | -0.66(0.07) | 1.62(0.13) | -1.06(0.13) | -0.33(0.13) | 0.991 | 0.032 |
| MBP | 10 | -0.82(0.06) | 2.11(0.11) | -0.59(0.11) | -1.07(0.11) | 0.997 | 0.004 |
| | 20 | -0.74(0.04) | 1.85(0.08) | -0.81(0.08) | -0.65(0.08) | 0.998 | 0.002 |
| | 30 | -0.66(0.04) | 1.52(0.07) | -0.82(0.07) | -0.51(0.07) | 0.997 | 0.002 |
| | 40 | -0.63(0.13) | 1.28(0.24) | -0.73(0.23) | -0.64(0.23) | 0.971 | 0.015 |
| ULT | 10 | -1.02(0.14) | 2.31(0.26) | -0.20(0.19) | -1.45(0.18) | 0.993 | 0.042 |
| | 20 | -0.88(0.06) | 1.84(0.11) | -0.36(0.11) | -1.08(0.11) | 0.997 | 0.026 |
| | 30 | -0.73(0.04) | 1.30(0.07) | -0.32(0.07) | -0.77(0.07) | 0.998 | 0.016 |
| | 40 | -0.62(0.04) | 0.97(0.07) | -0.32(0.07) | -0.61(0.07) | 0.995 | 0.017 |
| SPH | 10 | -0.92(0.14) | 1.86(0.26) | -0.03(0.25) | -1.46(0.26) | 0.987 | 0.062 |
| | 20 | -0.77(0.11) | 1.42(0.19) | -0.11(0.19) | -1.68(0.19) | 0.991 | 0.047 |
| | 30 | -0.73(0.07) | 1.14(0.13) | -0.22(0.13) | -0.99(0.13) | 0.992 | 0.031 |
| | 40 | -0.48(0.07) | 0.59(0.13) | -0.16(0.13) | -0.58(0.13) | 0.972 | 0.032 |

^aStandard deviations in the coefficient estimates are given in parentheses.

Table 4. Comparison of Coefficient Estimates on Different Cyano-Bonded Columns at 20% Organic Modifier

| Modifier | Column | Intercept | m | b | a | b/a |
|--------------|--------|-----------|------|-------|-------|-------|
| Methanol | CPS | -0.87 | 2.40 | -1.10 | -0.61 | 1.80 |
| | MBP | -0.74 | 1.85 | -0.81 | -0.65 | 1.25 |
| | ULT | -0.88 | 1.84 | -0.36 | -1.08 | 0.25 |
| | SPH | -0.77 | 1.42 | -0.11 | -1.68 | 0.07 |
| Acetonitrile | CPS | -0.59 | 2.20 | -1.36 | -0.43 | 3.16 |
| | MBP | -0.46 | 1.77 | -1.17 | -0.45 | 2.60 |
| | ULT | -0.63 | 1.47 | -0.70 | -0.51 | 1.37 |
| | SPH | -0.57 | 1.13 | -0.46 | -0.62 | 0.74 |

Table 5. Ratio of the Coefficient Estimates on Different Cyano-Bonded Columns Relative to the Coefficient Estimates on the CPS Column

| Modifier | Column | m/m_{CPS} | b/b_{CPS} | a/a_{CPS} |
|--------------|--------|-------------|-------------|-------------|
| Methanol | CPS | 1 | 1 | 1 |
| | MBP | 0.77 | 0.74 | 1.09 |
| | ULT | 0.77 | 0.33 | 1.77 |
| | SPH | 0.59 | 0.10 | 2.75 |
| Acetonitrile | CPS | 1 | 1 | 1 |
| | MBP | 0.80 | 0.86 | 1.05 |
| | ULT | 0.67 | 0.52 | 1.19 |
| | SPH | 0.51 | 0.34 | 1.44 |

lity in the concentration of surface silanol groups on the initial silica, which might affect the HB properties of the stationary phase (α_s and β_s), this will cause these ostensibly equivalent columns to show different HB interaction strengths toward the solute and hence yielding different values of b and a coefficients for different brands. As described above the value of each coefficient can thus be regarded as measures of strengths of different types of interactions for a given brand of stationary phase column. In eluents containing a fixed amount of organic modifier, the coefficient m is the greatest on the CPS column and is decreasing in the order, MBP>ULT>SPH column. In view of the fact that the $mV_i/100$ term also approximates an increase in dispersive interactions between the stationary phase and the solute as the solute increase in size and hence the polarizability⁶, it is somewhat unexpected that the ULT column, which has about the same bonding density as the CPS column, show much smaller m coefficient than the CPS column. The absolute size of the b and a coefficient decrease in the order, CPS>MBP>ULT>SPH. It seems that even though the CPS column has the lowest surface area it possesses a greater number of unreacted surface silanol groups exposed. Examination of relative size of the coefficient b to a (b/a) reveals that on the four brands of cyano-bonded columns contributions of two types of HB interactions to retention are quite different. On the CPS and MBP column type A HB interactions affect retention to a greater extent than type B HB while on the ULT and SPH column the reverse is true.

It is well known that the stationary phase is preferentially solvated by the organic component in the mobile phase and the extent of this solvation is different for different modifier¹⁶⁻¹⁹. Different values of the coefficients, m , b , and a for the same column are thus observed in methanol and acetonitrile-modified eluents (see Table 4).

For ODS columns variabilities in retention properties of apparently equivalent columns have caused many difficulties for the practicing chromatographers in that choosing the best column for a given separation is most often a trial and error process. However, these variabilities could be a very useful feature in choosing the best column for a given separation in the case of cyano-bonded phases columns studied here because variations in interaction strengths of cyano-bonded phase columns among brands are much greater than are in ODS columns. Further, quantitative measures of these interaction strengths can be readily estimated by simply re-

gressing retention data for a set of solutes *vs.* the solute parameters based on the LSER. For example, on the CPS column, the coefficient m is greater than those on the remaining columns. This indicates that the CPS column may possess a greater discriminating capability in the separation of compounds which differ in their size but have similar HB donor and acceptor strength. On the CPS and MBP columns the ratio b/a is greater than unity, but the reverse is true on the ULT and SPH columns. This tells us that the CPS and MBP columns may be more effective in separating compounds which differ in their HB acceptor basicity than the ULT and SPH columns while the ULT and SPH columns better discriminate compounds of different HB donor acidity than do the CPS and MBP columns if the other properties of the compounds are similar. In the acetonitrile-modified eluents the b/a ratios become greater than in methanol-modified eluents, indicating that differences in the HB acceptor basicity of compounds may be better discriminated in the aqueous acetonitrile than aqueous methanol. Retention of a compound on the cyano-bonded column is, in practice, controlled by all three types of interactions and thus the above statement can be viewed only in a limited sense. Despite this limitation, information obtained by the LSER approach on the differences in interaction properties is still useful in characterizing the columns and thus in choosing the best column for a given separation. Table 5 lists the ratio of each of the three coefficients on the MBP, ULT and SPH columns, relative to those on the CPS column as a reference. We may use the values of this ratio as McReynold-type constants for strength of each type of interactions for a given column. Alternatively, it would be simpler and better to use the values of the three coefficients *per se* in Table 4 as the interaction strength constants since in this way no reference or standard column is necessary.

In view of the fact that chromatographic selectivities of different brands of the cyano-bonded phase columns are more widely varying than ODS columns, changing brands of the cyano-bonded phase columns could offer greater optimization potential than changing brands of ODS columns commonly used in RPLC. Further, selectivity characteristics of the cyano bonded phase and ODS columns are also quite different. Table 6 shows comparison of selectivities of a cyano-bonded phase column (MBP column) with an ODS column (Hypersil ODS). We can see that on the cyano-bonded phase column dipolarity of a compound do not affect reten-

Table 6. Comparison of Selectivity of the μ -Bondapak CN Column and an Hypersil ODS Column^a

| Column | % Modifier | <i>m</i> | <i>s</i> | <i>b</i> | <i>a</i> |
|--------|------------|----------|-------------------|----------|----------|
| MBP | 40% MeOH | 1.28 | N.E. ^b | -0.73 | -0.64 |
| | 30% MeCN | 1.21 | N.E. | -1.12 | -0.45 |
| ODS | 40% MeOH | 3.71 | -0.67 | -2.29 | N.E. |
| | 30% MeCN | 2.64 | -0.49 | -1.77 | N.E. |

^aThe *k'* data on the ODS column used in regressions are from refs. 22 and 23. ^bNo effect on retention.

tion of the compound while on the ODS column it plays a minor but appreciable role in the determination of retention. On the ODS column one may differentiate only differences in the HB acceptor basicity of the compounds whereas on the cyano-bonded phase column both the HB acceptor basicity and HB donor acidity of the compounds may be discriminated. This better discriminating power of the cyano-bonded phase column will certainly provide increased optimization potential.

In conclusion, the so-called "specific cyano effects" of cyano-bonded phase columns reported by Smith and Miller¹ are found to be due to the differences in the interaction strengths of the stationary phases. Recently Dorsey and Ying²⁰ reported a method of characterizing retentivities of a number of commercial ODS, a phenylpropyl and a cyanopropyl columns for RPLC which utilizes a value of $\ln k'_w$, retention of a compound in an eluent of 100% water and the slope of the plot of $\ln k'$ vs. E_T (30), a measure of solvent polarity. They used 26 solutes of widely varying size and chemical properties in their study. Their study was, however, centered only on the estimation of the retentivity of the column. Information on the retentivity of a column will be useful in the characterization of a column. In addition to this retentivity data, information on interaction characteristics of the column, analogous to McReynold's constants for GC stationary phases, is given in hand, the task of choosing the best column and optimizing for a given separation will be much easier. Although interaction strength constants approximated by the four coefficients (*m*, *s*, *b*, and *a*), are, of course, not as extensive as McReynold's constants, we believe they would be quite helpful information for choosing the best brand for a given separation among a number of nominally equivalent columns. In order to ensure these measures of column strengths to be broadly applicable to everyday separation problems, a greater number of solutes of widely varying chemical properties than used in this study must be employed in the study. Work is in progress in our laboratory on establishing the interaction strength constants for various polar RPLC columns with different bonded functionalities.

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