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Characterization of some silica-based reversed-phase liquid chromatographic columns based on linear solvation energy relationships

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Abstract

The characterization of six silica-based reversed-phase liquid chromatographic columns was attempted by calculating characteristic interaction constants for the stationary phases based on linear solvation energy relationships. Four interaction properties of the stationary phase, m (the cavity formation/dispersive interaction strength), s (dipolarity/polarizability), b (hydrogen bond donating acidity) and a (hydrogen bond accepting basicity), are readily determined by multiple regression analyses of logarithmic capacity factors (k') for a set of test solutes measured on it in an aqueous mobile phase of a given organic content *versus* the solute properties represented by the Van der Waals molar volume, Kamlet–Taft dipolarity/polarizability, π^*, hydrogen bond accepting basicity, β, and hydrogen bond donating acidity, α. The magnitudes of the four constants vary with the type of bonded ligand and with brand in the case of stationary phases having the same ligand, while they generally decrease in the order $m > b > a > s$, regardless of the type of the organic modifier in the mobile phase for all six columns. Although the four interaction strength constants are not as general as the widely used Rohrschneider and McReynolds constants for GLC stationary phases, it is believed that they will be useful in choosing the best column for a given separation among a number of nominally equivalent columns and columns with different bonded functionality.

1. Introduction

Practising chromatographers have noted the significant differences in retention characteristics between nominally equivalent reversed-phase liquid chromatographic (RPLC) columns such as C_{18} [1] and cyano-bonded materials [2]. This variability is to be expected, as retention in RPLC is determined by both solvophobic and chemical interactions between solute molecules and reactive sites of the stationary phase, which involve not only interactions between solute molecules and the organic bonded phase (solvophobic) [3] but also hydrogen bonding interactions with unreacted silanol groups and complexation with trace metals on the silica surface [4]. The relative contribution of these two types of interactions depends on the characteristics of the stationary phase, which include the nature of the base silica particles such as the specific surface area, pore size and volume, the nature of the bonded organic ligand and the bonding process. Chemical interactions are often re-

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garded as undesirable as these types of interactions, in particular silanophilic interactions, are responsible for the excessive peak tailing and long retention times observed for basic solutes. The presence of these types of interactions also leads to poorer control of the column packing, resulting in column-to-column irreproducibility [3,4]. As the diversity in retention properties of various stationary phases due to this variability has caused many difficulties for chromatographers in selecting the best column for a given separation, a knowledge of the nature and relative contributions of possible interactions between the solute and stationary phase to RPLC retention is of great value in that it can be utilized to help chromatographers in the task of selecting the best column and to optimize the selectivity for a given separation.

As direct measurement of interaction properties of bulk material is difficult and often less sensitive than chromatographic measurements, classification of columns has most often been attempted by measuring their retentivity and selectivity for a particular class of compounds. A good summary of chromatographic methods can be found in a book by Unger [5]. Snyder and co-workers characterized, on the basis of gradient elution theory, a number of columns having different bonded functional groups such as C_{18}, C_8, phenyl, C_1 and cyano groups according to their solvophobic retention [3,6]. They combined the phase-volume ratio and the polarity of the stationary phase into a column-strength parameter and determined the retention strength of the columns to be in the order $C_{18} > C_8 >$ phenyl $> C_1 >$ cyano. They also concluded that the contribution of the polarity of the column to the retention strength is small compared with the contribution of the phase-volume ratio. Differences in the solvophobic strength of these columns have been successfully utilized in RPLC method development [7] and in optimizing separations of complex mixtures such as phenylthiohydantoin-amino acid derivatives [8].

Recently, Ying and Dorsey [9] reported a method for characterizing the retentivities of a number of commercial ODS, a phenylpropyl and a cyanopropyl columns for RPLC which utilizes a value of ln k'_w, the retention of a compound with water as eluent 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. Their study was, however, centred only on the determination 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 these solvophobic strength or retentivity data, if information on interaction characteristics of the column, analogous to the Rohrschneider and McReynolds constants for GLC stationary phases [10], is available, the task of choosing the best column and optimizing for a given separation will be much easier.

In this paper, we describe a simple method for characterizing the stationary phase in RPLC in terms of the type and relative strength of the solute–stationary phase interactions by multiple regression analysis of retention data for a set of test compounds based on linear solvation energy relationships (LSERs) [11,12] using the Van der Waals molar volumes and the Kamlet–Taft solvatochromic parameters for the test solutes, π^* (dipolarity/polarizability), β (hydrogen bonding acceptor basicity) and α (hydrogen bonding donor acidity). As will be shown, this approach provides characteristic interaction constants for RPLC stationary phases, which are similar to Rohrschneider and McReynolds constants for GLC stationary phases. The use of column selectivity based on these interaction constants might be useful in method development in RPLC.

2. Theory

Kamlet, Taft and co-workers applied the LSER approach to about 600 processes [12], including a large number of systems of immediate relevance to chromatography, such as Rohrschneider's gas–liquid partition coefficients [13], retention of McReynolds solutes on polymeric silicone oil gas chromatographic phases [14], retention in NPLC [15] and RPLC [16–19], log k'_w in RPLC [20] and surface polarity of

carbon fibres for use in gas–solid chromatography [21]. 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 [11,12]:

$$SP = SP_0 + \text{cavity term} + \text{dipolar term} + \text{hydrogen bonding term(s)} \quad (1)$$

where 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_I) 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 chromatographic retention, SP in Eq. 1 denotes a logarithmic capacity factor and the relevant LSER is given by

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

where the subscript 2 designates a solute property, the subscripts s and m denote the stationary and mobile phases, respectively, and 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

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

The $\log k'_0$ term includes the volume phase ratio and dipolar interactions between the solute and the chromatographic phases when π_2^* is zero. 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 conditions. When capacity factors for a set of solutes measured on a number of different stationary phase columns using a 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. Then any variations in the coefficients m, s, b and a with different columns are due to variations in the properties (δ_s^2, π_2^*, α_s and β_s) of the stationary phases. Modification of the stationary phase by the mobile phase components varies with the type of bonded functional group and bonding density in a given mobile phase. However, when the mobile phase is constant for all the columns studied, what we measure is the actual bonded phase environment which really controls retention. Different magnitudes of these coefficients for different columns are indicative of 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, b and a can therefore be regarded as measures of the relative strength of corresponding interaction properties of the column in a mobile phase of given composition. Eq. 2 does not contain an explicit term for dispersive interactions between the solute and the chromatographic phases. It has been shown that the $M(\delta_s^2 - \delta_m^2)V_{I,2}/100$ term is in fact a combination of cavity formation and dispersive interaction [15]. Different values of the coefficient m for different columns should indicate the relative magnitude of the Hildebrand solubility parameter and dispersive interaction strength of various stationary phases. Similarly, the coefficient s is indicative of the relative magnitude of dipolarity, and the coefficients b and a are indicative of the relative strength of HB donating and HB accepting capability of various stationary phases, respectively.

This approach has been found useful for the characterization of the chromatographic prop-

Table 1
Properties of the selected compounds (from ref. 23)

No.	Compound	$V_I/100$	π^*	β	α
1	Acetophenone	0.690	0.90	0.49	0.03
2	Propiophenone	0.788	0.88	0.49	0
3	Butyrophenone	0.886	0.86	0.49	0
4	Naphthalene	0.753	0.70	0.15	0
5	2-Phenylethanol	0.732	0.97	0.55	0.33
6	Benzyl alcohol	0.634	0.99	0.52	0.35
7	Phenol	0.536	0.72	0.33	0.61
8	1-Naphthol	0.798	0.82	0.33	0.61
9	*p*-Chlorophenol	0.626	0.72	0.23	0.69
10	*p*-Cresol	0.634	0.68	0.34	0.58
11	Chlorobenzene	0.581	0.71	0.07	0
12	Bromobenzene	0.624	0.79	0.06	0
13	Nitrobenzene	0.631	1.01	0.30	0
14	Benzene	0.491	0.59	0.10	0
15	Toluene	0.592	0.55	0.11	0
16	Ethylbenzene	0.687	0.53	0.12	0
17	Pyridine	0.470	0.87	0.44	0
18	Aniline	0.562	0.73	0.50	0.16
19	4-Methylpyridine	0.570	0.84	0.47	0
20	*p*-Bromoaniline	0.695	0.79	0.40	0.20
21	*p*-Toluidine	0.660	0.69	0.52	0.13
22	Phenetole	0.727	0.69	0.30	0
23	Benzaldehyde	0.606	0.92	0.44	0
24	Ethyl benzoate	0.736	0.76	0.39	0

erties of some stationary phases for use in NPLC (silica and alumina) [15] and RPLC (octadecyl-bonded silica and octadecyl-bonded alumina) [22]. Once these interaction constants, which are analogous to Rohrschneider and McReynolds constants for GLC stationary phases, have been determined for a wide variety of columns, the task of choosing the best column and optimizing for a given separation will be much easier. We were careful to choose 24 compounds of widely varying chemical properties to ensure that the results will be broadly applicable to everyday separation problems. Values of the Van der Waals molar volume and Kamlet–Taft solvatochromic parameters for the solutes are given in Table 1.

3. Experimental

Retention measurements were made with an HPLC system composed of a Shimadzu LC-9A pump, a Rheodyne Model 7125 injector equipped with 20-μl sample loop, a Hitachi L-4200 UV–Vis detector set at 254 nm and a Hewlett-Packard 3396 Series II integrator. Columns investigated were Nucleosil C_8 (250 × 4.6 mm I.D., 10 μm) (Alltech, Deerfield, IL, USA), Hypersil CPS-1 (150 × 4.6 mm I.D., 5 μm) (Alltech), Ultrasphere CN (150 × 4.6 mm I.D., 5 μm) (Beckman Instruments, Fullerton, CA, USA), Supelcosil LC-DP (phenyl) (250 × 4.6 mm I.D.) (Supelco, Bellefonte, PA, USA) and μBondapak C_{18} and μBondapak CN (300 × 3.9 × 300 I.D., 10 μm) (Waters–Milipore, Milford, MA, USA). Some of the properties of these columns, as supplied by the manufacturers, are given in Table 2. The column was placed in a water-jacket and the temperature was controlled at 30 ± 0.1°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^{-1}. An aliquot of 10% aqueous sodium

Table 2
Properties of the columns as supplied by the manufacturers

Column No.	Stationary phase	Ligand	Surface area (m^2 g^{-1})	Carbon loading (%)[a]	Bonding density[b] (μmol m^{-2})	End-capped
I	μBondapak C_{18}	C_{18}	330	10 (0.030)	1.43	Yes
II	Nucleosil C_8	C_8	350	8 (0.023)	2.15	–
III	Supelcosil LC-DP	Diphenylmethyl	170	–	–	–
IV	Ultrasphere CN	Cyanopropyl	200	4.4 (0.022)	3.31	Yes
V	Hypersil CPS-1	Cyanopropyl	170	3.5 (0.020)	3.06	No
VI	μBondapak CN	Cyanopropyl	330	6.0 (0.018)	2.82	Yes

[a] Values in parentheses are %C m^{-2}.
[b] Bonding densities in μmol m^{-2} were calculated using an equation by Berendsen and De Galan [24].

nitrate was injected to determine the column void volume. The capacity factors were calculated from the mean retention times of triplicate injections. The relative standard deviation for three replicate retention time measurements was usually less than 1.5% for all solutes. In order to check the stability of the column, we injected toluene before and after a day's measurements and found that the retention times of toluene were reproducible to within 1% for the day. This check was done every day and we observed that the retention times of toluene agreed to within 2% before and after the whole series of experiments.

All the solutes were of analytical-reagent grade from Aldrich (Milwaukee, WI, USA) and used as received. Methanol and acetonitrile were of HPLC grade and from Ajax (Auburn, Australia).

4. Results and discussion

In order to see how to arrive at the best LSER describing retention on various stationary phases, let us examine the capacity factors for the 24 test solutes on a μBondapak C_{18} column in methanol–water (20:80) as an example.

$$\log k' = -0.57(\pm 0.24) + 3.35(\pm 0.27)V_I/100 - 0.003(\pm 0.270)\pi^* - 1.70(\pm 0.23)\beta - 0.47(\pm 0.12)\alpha \quad n = 24,\ r = 0.961,\ \text{S.D.} = 0.131 \quad (4)$$

The coefficient s for the π^* parameter is statistically zero, indicating that dipolar interactions do not affect the retention. The π^* parameter was therefore excluded in the following regression. The resulting triple regression equation is given by

$$\log k' = -0.57(\pm 0.17) + 3.35(\pm 0.26)V_I/100 - 1.70(\pm 0.23)\beta - 0.47(\pm 0.12)\alpha \quad n = 24,\ r = 0.961,\ \text{S.D.} = 0.128 \quad (5)$$

In further regressions for log k' on various columns, other statistically insignificant parameter(s), if there are any, were excluded in a similar manner. Results of multiple linear regressions of log k' on the six columns in aqueous methanol and acetonitrile mixtures *vs.* the solute properties are given in Tables 3 and 4. The correla-

Table 3
Calculated coefficients in regressions of log k' on the six columns in aqueous methanol *vs.* solute parameters

Column[a]	Organic modifier (%, v/v)	Log k'_0	m	s	b	a	r
I	20	−0.57	3.35	NS[b]	−1.70	−0.47	0.961
	40	−0.47	2.51	NS	−1.68	−0.37	0.980
II	20	−0.33	3.16	NS	−1.38	−0.63	0.934
	40	−0.25	2.19	NS	−1.32	−0.48	0.956
III	20	−0.58	3.10	NS	−1.17	−0.47	0.927
	40	−0.53	2.28	NS	−1.18	−0.33	0.978
IV	20	−0.56	2.50	NS	−1.33	NS	0.940
	40	−0.44	1.70	NS	−1.15	NS	0.965
V	20	−0.55	2.15	NS	−1.18	NS	0.930
	40	−0.57	1.53	NS	−1.15	NS	0.971
VI	20	−0.61	2.09	NS	−1.11	−0.12	0.926
	40	−0.45	1.31	NS	−0.90	−0.08	0.927

[a] Column designations as in Table 2.
[b] NS = not significant.

Table 4
Calculated coefficients in regressions of log k' on the six columns in aqueous acetonitrile *vs.* solute parameters

Column[a]	Organic modifier (%, v/v)	Log k'_0	m	s	b	a	r
I	20	−0.34	3.03	−0.22	−1.93	−0.41	0.994
	40	−0.06	1.65	−0.35	−1.14	−0.36	0.992
II	20	−0.11	2.78	−0.10	−1.80	−0.47	0.981
	40	−0.13	1.41	−0.17	−1.07	−0.43	0.984
III	20	−0.32	2.98	−0.14	−1.81	−0.29	0.992
	40	−0.17	1.74	−0.18	−1.26	−0.30	0.990
IV	20	−0.32	2.32	NS[b]	−1.64	−0.10	0.980
	40	−0.18	1.39	NS	−1.19	−0.15	0.977
V	20	−0.40	2.22	NS	−1.60	NS	0.979
	40	−0.24	1.27	NS	−1.09	−0.13	0.979
VI	20	−0.45	1.95	NS	−1.28	−0.11	0.978
	40	−0.20	1.11	NS	−0.92	−0.13	0.983

[a] Column designations as in Table 2.
[b] NS = Not significant.

tion coefficients are mostly close to unity, indicating that the retention behaviour of the solutes on the RPLC columns is well represented by the LSER model.

In order to gain an understanding of the factors responsible for the differences in retention properties of various RPLC columns, let us examine the sign and magnitude of the coefficients in Tables 3 and 4. It is seen that m and b are much larger than a and s. The coefficients s for all six columns with aqueous methanol and for three cyano-bonded columns with aqueous acetonitrile are statistically zero, and are very small for non-polar alkyl- and phenyl-bonded phases with aqueous acetonitrile, indicating that the solute dipolarity plays only a very minor role in determining retention and selectivity and in turn the column dipolarity is not a significant factor in characterizing the column. Other workers have made similar observations on the relative unimportance of the column polarity in RPLC [16,17]. In further discussions we therefore consider only the coefficients m, b and a in detail.

It is seen from the signs of the coefficients that increasing solute size (V_I) causes an increase in retention, *i.e.*, free energy concepts favour solute transfer from the more cohesive mobile phase to the less cohesive stationary phase. The magnitude of the coefficient m decreases with increasing content of organic modifier on a given column. If δ^2 of the stationary phase does not change with changes in the mobile phase composition, m should be proportional to δ^2 of the mobile phase. As water is more cohesive (δ = 23.4 $cal^{1/2}$ $cm^{-3/2}$) than methanol (14.3 $cal^{1/2}$ $cm^{-3/2}$), acetonitrile (11.75 $cal^{1/2}$ $cm^{-3/2}$) and free-form analogues of the stationary phases (δ = 8.02 $cal^{1/2}$ $cm^{-3/2}$ for *n*-hexadecane, 7.54 $cal^{1/2}$ $cm^{-3/2}$ for *n*-octane, 8.64 $cal^{1/2}$ $cm^{-3/2}$ for *n*-propylbenzene and 10.17 $cal^{1/2}$ $cm^{-3/2}$ for butyronitrile), the cavity-forming process in the solvent becomes decreasingly endoergic with decreasing water content.

As the coefficient m indicates a combination of cohesiveness and dispersive interaction strength of the bonded moiety, it is expected to increase with increasing solubility parameter and the amount of the ligand per unit area of the stationary phase surface in a mobile phase of a

given composition. The magnitude of m for an ODS column is greater [$\delta = 8.02$ cal$^{1/2}$ cm$^{-3/2}$, %C m$^{-2} = 0.03$; *e.g.*, $m = 2.51$ in methanol–water (40:60)] than that for an octyl column [$\delta = 7.54$ cal$^{1/2}$ cm$^{-3/2}$, %C m$^{-2} = 0.023$; *e.g.*, $m = 2.19$ in methanol–water (40:60)].

It is interesting to compare the coefficient m with the solvophobic column strength J of Snyder and co-workers [3,6]. They used a set of 22 solutes of widely differing chemical properties but compounds such as acids and bases that were likely to exhibit chemical selectivity were generally excluded. They combined the phase-volume ratio and the polarity of the stationary phase into J. As they excluded the solutes that can undergo HB interactions with the stationary phase, the polarity contribution to J is from solute–stationary phase dipole–dipole and dipole–induced dipole interactions. It has been shown above and from observations by other workers [16,17] that these types of interactions are unimportant in RPLC retention processes. In view of the negligible contribution from the dipolar interaction strength of the stationary phase, the column strength measured by J is mainly solvophobic in nature and is expected to vary in a parallel direction with the coefficient m, which represents, in essence, the non-polar solvophobic interaction strength of the given column. The coefficient m for an ODS column is greater than that for an octyl column and this is in agreement with observations made on Zorbax columns by Snyder and co-workers [3,6] and Ying and Dorsey [9]. Snyder and co-workers [3,6] also noted that retention in RPLC increases with increase in the amount and surface area of bonded phases. The stationary phase volumes decrease in the order $C_{18} > C_8$, but the bonded-phase surface area (and bonding density) tends to increase from C_{18} to C_8. Their J value is a combination of stationary phase volume and stationary phase surface area when the polarity of the stationary phase is unimportant. In view of this, it seems likely that the %C m^{-2} of the stationary phase, rather than bonding density in μmol m^2, better represents the combination of volume and surface area of the bonded ligand. The carbon percentage per unit surface area of our ODS column, which has a greater m value, is greater than that of the octyl column. They also observed that a more polar, cyanopropyl-bonded phase has a smaller J value than C_{18} and C_8 columns. The coefficients m for all cyano columns studied are smaller than those of ODS and octyl columns. For different brands of the cyano-bonded phase columns, the value of m increases with increasing %C m^{-2} (Table 2) in both methanol- and acetonitrile-modified mobile phases.

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 modifiers [25–28]. Different values of the coefficients m for the same column are thus observed in methanol- and acetonitrile-modified eluents.

Opposing this effect, increases in HB donor acidity (α) and HB acceptor basicity (β) lead 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 (10–90 vol.-% methanol, $\alpha = 1.17$–1.02 [29], $\beta = 0.25$–0.60 [30]) than free-form analogues of the stationary phases (alkanes, $\alpha = 0$, $\beta = 0$; butyronitrile, $\alpha = 0$, $\beta = 0.31$ [31]). The magnitude 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 magnitude of each coefficient indicates that the most important factor influencing RPLC retention for the solutes studied is the endoergic cavity formation term. The HB terms are less important and contributions to retention from the type A and type B HB vary with the type of bonded functional group and with brand for the cyano-bonded phases. 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 bonded moiety of the stationary phases were greater than those with the mobile phase, the retention must have been

increased with increasing solute α and β values.

The magnitudes of the coefficients b and a vary with the type of bonding moiety of the column and with brand for stationary phases with the same ligand. If interactions between the solute and the mobile phase dominate retention, what is causing the magnitude of those coefficients (*i.e.*, retention properties) for different brands of cyano-bonded column to vary? As discussed above, the coefficient b, for example, is a cross-product of a constant (B) and the difference in properties of the stationary phase and the mobile phase. It is well known that end-crapping cannot block the surface silanol groups completely. If there is any variability 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, hence yielding different values of coefficients b and a for different brands.

Comparison of the magnitude of the coefficients b (the HB donating acidity) for non-polar alkyl- and phenyl-bonded phases with those for polar cyano-bonded phases indicates that in general b is smaller for polar cyano-bonded phases than for non-polar phases. It seems that polar cyano-bonded phases are better solvated owing to stronger dipolar and HB interactions of cyano groups with the mobile phase components than non-polar alkyl- and phenyl-bonded phases, so residual silanol groups on alkyl- and phenyl-bonded phases are more exposed to solutes and the HB interactions between the solutes and the stationary phase become more significant. This is in agreement with the observation that retention and excessive peak tailing for basic solutes due to strong HB interactions are less prominent on cyano-bonded phase columns [2]. In both methanol- and acetonitrile-modified mixtures the magnitude of b for the stationary phases becomes smaller with increasing amount of organic modifier. This is because the stationary phase is more solvated at higher organic content mobile phases, hence residual silanol groups on the stationary phase might be less exposed to solutes and the HB interaction between the solutes and the stationary phase becomes less significant.

Similar trends in variation of the coefficient a (HB accepting basicity) for columns with the type of bonded ligand and brand in the case of stationary phases having the same ligand are also observed. However, as can be seen from the much smaller magnitudes of a relative to those of b, the HB basicity of the stationary phase is of minor importance in characterizing the column. Similar observations of unimportance of the $a\alpha$ terms have also been made by other workers [16,17].

It might be better to present the coefficients for every column as the characteristic column constants after multiplying by 100 for the sake of convenience of presentation. This will also make the values of the column constants have an order similar to widely used Rohrschneider and McReynolds constants for GLC phases. As we already know well in which direction a given type of interaction between the solute and the stationary phase affects retention, we may also remove the sign for the coefficients. The characteristic interaction constants for the six stationary phases observed in mobile phases containing 40% of organic modifier are given in Table 5. The log k'_0 values are also included as this term includes the volume phase ratio and dipolar interactions between the solute and the stationary phases when π^* is zero and might be useful in characterizing other characteristics of the stationary phase than characterized by the interaction constants. This term is necessary if one wants to predict log k' for a solute with a given combination of the mobile and stationary phase.

By examining the interaction constants for the six columns in Table 5 we can infer the following practically significant results. The much greater magnitudes of the coefficient m and b in both methanol- and acetonitrile-modified mobile phases indicates that the more important interaction characteristics of the column are cavity/dispersion and type A hydrogen bonding, and solute dipolarity and HB acidity are not effectively differentiated in RPLC. In both mobile phases, the coefficients m for ODS-, octyl- and phenyl-bonded phases columns are greater than those for cyano-bonded phases. This indicates that the first three stationary phases have greater discriminating capabilities in the separation of

Table 5
Characteristic interaction constants for the six columns

Mobile phase	Column[a]	Log k'_0	m	b	a	s
Methanol–water (40:60)	I	47	251	168	37	NS[b]
	II	25	219	132	48	NS
	III	53	228	118	33	NS
	IV	44	170	115	NS	NS
	V	57	153	115	NS	NS
	VI	45	131	90	8	NS
Acetonitrile–water (40:60)	I	6	165	114	36	35
	II	23	141	107	43	17
	III	17	174	126	30	18
	IV	18	139	119	15	NS
	V	24	127	109	13	NS
	VI	20	111	92	13	NS

[a] Column designations as in Table 2.
[b] NS = Not significant.

compounds that differ in their size but have similar HB donor and acceptor strengths. In practice, the retention of a solute in RPLC on a given column is controlled simultaneously by all types of interactions. We believe, however, that it is safe to say that if the two solutes have a significant difference in size then the ODS column will produce the greatest chromatographic selectivity. With methanol–water (40:60) as mobile phase, the solute HB basicity is best discriminated by the ODS column. Although minor, the better discrimination of the HB acidity of solutes can be achieved by the three non-polar columns than cyano-bonded columns. The interaction constants for the six columns are, in general, greater in a methanol- than an acetonitrile-modified mobile phase, indicating that differentiation of more subtle differences in the solute properties may be achieved by using a methanol-modified mobile phase.

In conclusion, for RPLC columns the variabilities in the retention properties of apparently equivalent columns have caused many difficulties for practising chromatographers in that choosing the best column for a given separation is most often a trial-and-error process. However, these variabilities can now be a very useful feature in choosing the best column for a given separation. Further, these interaction constant can be readily calculated by simply regressing retention data for a set of solutes *vs.* the solute parameters based on the LSER. Although the four interaction strength constants (m, s, b and a) are, of course, not as general as Rohrschneider and McReynolds constants, we believe they would give helpful information for choosing the best column for a given separation among a number of nominally equivalent columns and columns with different bonded functionalities. In order to ensure that these measures of column strengths are broadly applicable to everyday separation problems, interaction constants for a greater number of RPLC columns with more diverse bonding ligands from various manufacturers than used in this study need to be determined. Work for utilizing these interaction constants in selecting and optimizing separations of practical samples is in progress.

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6. References

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