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Study of retention in reversed-phase liquid chromatography using linear solvation energy relationships I. The stationary phase

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Abstract

In contrast to almost all previous work in which data sets of retention for reversed-phase liquid chromatography were limited to aromatic solutes, in this work the retention of 87 highly variegated, aliphatic and aromatic solutes have been studied using linear solvation energy relationships. Results show excellent statistical fits for these retention data obtained in 50:50 (v/v) acetonitrile–water, on five bonded phase columns differing in silanol group acidity. The fits are equally good when aliphatic and aromatic solute subsets were examined separately. The most important retention-governing solute parameters are the solute volume and hydrogen bond acceptor basicity. The solute dipolarity/polarizability and hydrogen bond donor acidity are statistically significant but chemically minor factors. The relative importance of the solute parameters in explaining the data does not differ for the various bonded phases studied, but there are some subtle differences between the aliphatic and aromatic solute subsets.

Keywords: Retention behavior; Linear solvation energy relationships; Stationary phases, LC; Retention mechanisms; Aliphatic solutes; Aromatic solutes

1. Introduction

Despite the widespread application of reversed-phase liquid chromatography (RPLC) in analytical and preparative HPLC, the underlying principles and retention mechanism of RPLC are still the subject of long-standing study and current debate [1]. The present work examines the molecular interactions of solutes with the mobile and stationary phases in RPLC based on linear solvation energy relationships (LSERs). The following questions will be addressed: (1) Which solute properties govern the retention in RPLC? (2) Does the retention mechanism for non-silanophilic solutes in RPLC vary upon changes in the residual silanol group acidity of the stationary phase? (3) Do the retention mechanisms for octyl and octadecyl bonded phase differ? (4) Do the retention mechanisms for aliphatic and aromatic compounds differ?

1.1. Reversed-phase liquid chromatography

In RPLC, the commonly used mobile phases are binary solvent systems comprised of water and an

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organic modifier. The bulk properties of these aqueous organic mobile phases have been extensively studied [2–11]. Many early studies of RPLC suggested that the mobile phase plays the dominant role in establishing retention and selectivity [12–16]. However, more recent studies have recognized the active role of the stationary phase [17–19]. Some studies indicate that the net interaction (the sum of disfavorable cavity formation and attractive intermolecular forces) in the stationary phase outweighs the net interaction in the mobile phase [1,20].

The bonded phase is a complicated, heterogeneous media, whose chemical composition and configuration vary with the mobile phase composition, the nature of the silica support, the bonding density, and the alkyl chain length [15,18,21–23]. Despite the fact that the bonded phase is comprised of alkyl chains, the chemical properties of bonded phases differ greatly from those of bulk alkane phases equilibrated with common mobile phase mixtures, as suggested by several chromatographic and spectroscopic studies [15,21,22,24–29]. Mobile phase molecules (water and organic) are intercalated among the bonded chains. These chains are configurationally strained due to the bonding density and chain length. In addition, the residual silanol groups on the silica support play a significant role in solvent enrichment and retention of basic compounds [21,22,30]. There are many different kinds of chemically bonded RPLC materials, including monomeric [31], and both vertically [31] and horizontally [32] polymerized materials. Because monomeric phases are simpler we focused our attention on them in this work.

1.2. Linear solvation energy relationships

In this study, we used linear solvation energy relationships (LSERs) to rationalize and deconvolute the chemical factors that contribute to the retention mechanism in RPLC. LSERs were developed by Kamlet, Taft and their coworkers [33,34] and have been successfully used to correlate, rationalize and explain more than 600 different chemical systems [35]. The LSER approach has been applied extensively to the study of gas chromatography (GC) [36–41], RPLC [42–52] and to some extent to normal-phase liquid chromatography [53]. Based on this model, a free energy related term in a phase transfer process can be correlated with various fundamental molecular solute descriptor properties. When the LSER method is applied to RPLC, the logarithmic capacity factors, log k', are separated into several molecular interaction terms as shown in Eq. (1):

$$\log k' = \log k'_0 + M(\psi_s - \psi_m)V_2 + S(\pi^*_s - \pi^*_m)\pi^*_2 + A(\beta_s - \beta_m)\alpha_2 + B(\alpha_s - \alpha_m)\beta_2 \quad (1)$$

The subscripts s and m denote bulk stationary and mobile phase properties, respectively; the subscript 2 denotes a solute property such as molecular volume (V_2), dipolarity/polarizability (π^*_2), hydrogen-bond acidity (α_2) or hydrogen-bond basicity (β_2). In the above formalism, each solute property is multiplied by a term that represents the difference in the complementary property between the mobile phase and the stationary phase. In other words, the model recognizes that retention results from the differential interactions of a solute in both chromatographic phases. The coefficients M, S, A and B as well as log k'_0 are fitting parameters which ought to be independent of the solute and nature of the chromatographic phases if the formalism is rigorously correct [42]. However, we must recognize that the phase ratio is part of log k'_0.

The dipolarity/polarizability term, $S(\pi^*_s - \pi^*_m)\pi^*_2$, measures the exoergic (favorable) effects of the dipole–dipole and dipole-induced dipole interactions between solutes and the bulk phases. Solute dipolarity/polarizability, π^*_2, measures the ability of a compound to be stabilized by or to stabilize a neighboring charge or dipole by virtue of non-specific dielectric interactions. The bulk property of a chromatographic phase complimentary to π^*_2 is its dipolarity/polarizability, π^*. The exoergic hydrogen-bonding term, $A(\beta_s - \beta_m)\alpha_2$, measures the effect of complexation between hydrogen-bond donor (HBD) solutes and hydrogen-bond acceptor (HBA) bulk phases. The solute hydrogen bonding acidity, α_2, measures the ability of a solute to share a proton in a solute-bulk phase hydrogen bond interaction. The complimentary property of the bulk phase is its HBA basicity (β). Another hydrogen-bonding term, $B(\alpha_s - \alpha_m)\beta_2$, measures the exoergic effect of complexation between hydrogen-bond acceptor (HBA)

solutes and hydrogen-bond donor (HBD) bulk phases. The β_2 term measures the ability of a solute to share a pair of electrons with a proton donor. The complimentary bulk phase property is the HBD acidity (α).

The $M(\psi_s - \psi_m)V_2$ term is much more complicated than the other terms. Originally, it was called the cavity term, and was viewed solely as a measure of the endoergic (unfavorable) process of separating solvent or stationary phase molecules to provide a suitably sized enclosure for the solute [42,43]. Thus, it should reflect the cohesiveness between the phases. More recently it has been suggested that this term arises from a combination of cavity effects and dispersive interactions between the solute and the solvent [54]. Recent work from Carr et al. [1] has shown that the overall free energy of transfer of a methylene group from a bulk mobile phase mixture, such as methanol–water, to bulk hexadecane is controlled more by the net interactions in the nonpolar phase, and not the net interactions in the mobile phase. Furthermore, the work determined that the free energy of transfer of a methylene group from common mobile phases to a bonded phase is nearly equal to the free energy of transfer to bulk hexadecane. Thus we infer that dispersive interactions between the solute and the bonded phase are important to both the retention and selectivity of nonpolar moieties. Therefore, the $M(\psi_s - \psi_m)V_2$ term must be comprised of at least two terms: a cavity term, $M_1(\delta^2_{H,s} - \delta^2_{H,m})V_2$, where δ^2_H is the Hildebrand solubility parameter which measures the cohesiveness of the chromatographic phases (both the mobile and the stationary phases) including a dispersive contribution to the cohesivity; and a separate dispersive term, $M_2(D_2 - D_m)V_2$, where D is the ability of the chromatographic phases to interact with a solute via dispersive forces. Unfortunately, solute size descriptors (volume, area) and solute descriptors of dispersion interaction (molar refraction, polarizability) are strongly covariant and thus cannot be separated using regression methods. This leads to the need to lump both processes together in a single descriptor taken here as V_2 [55,56]. The solute size, V_2, has proven to be a very useful predictor in previous LSER studies of RPLC [43–46,48–50]. However, V_2 is not a good solute descriptor in gas liquid chromatography. Instead Abraham et. al. [57,58] used the gas to hexadecane partition coefficient, L_{16}, as the solute descriptor of the lumped cavity-dispersion process.

In the context of RPLC, direct spectroscopic measurement of bulk alkanes lead us to believe that the bonded alkyl chains per se have zero or rather small values for π^*, α and β [2]. However, upon sorption of water and organic modifier, the stationary phase is known to act as if it were quite polar. Both the chromatographic and spectroscopic measurements show that the π^*, α and β values of the stationary phase can be significantly greater than zero [2,15,24–27,59–61]. Typical values for π^*_s range from 0.7 to 1.1 depending on the mobile phase composition [24]. However, the role of accessible silanol groups in establishing the π^*, α and β of the stationary phase in RPLC has not been well established.

When applied to a fixed pair of mobile and stationary phases, Eq. (1) reduces to Eq. (2) where m, s, a and b are fitting coefficients characteristic of the pair of chromatographic phases. The coefficients are obtained from multifactor simultaneous least square regressions [43]. Comparison of Eq. (2) to Eq. (1) shows that each coefficient reflects the difference in a specific bulk property between the mobile phase and the stationary phase.

$$\log k' = \log k'_0 + mV_2 + s\pi^*_2 + a\alpha_2 + b\beta_2 \qquad (2)$$

Many approaches have been put forward to either measure, calculate, or estimate the solute parameters V_2, π^*_2, α_2, and β_2. In this study, we use V_x, π^{*H}_2, $\Sigma\alpha^H_2$ and $\Sigma\beta^H_2$ for V_2, π^*_2, α_2, and β_2, respectively. V_x is a calculated solute hard core molar volume using McGowan's method [62,63], while π^{*H}_2, $\Sigma\alpha^H_2$ and $\Sigma\beta^H_2$ are corresponding solute parameters obtained from GC measurements by Abraham et al. [64]. Superscript H simply denotes the origins of the parameters. The Σ symbol in $\Sigma\alpha^H_2$ (or $\Sigma\beta^H_2$) signifies that the parameter represents the summation of hydrogen bond acidity (or hydrogen bond basicity) contributed by each donor site (or acceptor site) on a solute. The $\Sigma\alpha^H_2$ and $\Sigma\beta^H_2$ scales pertain to systems in which the solute molecule is surrounded by an excess of solvent, in our case, bulk mobile or stationary phase which act as hydrogen bond donors or acceptors.

Table 1
Test solutes and solute parameters

	Solute	$V_x/100$[a]	π_2^{*H}	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$		Solute	$V_x/100$[a]	π_2^{*H}	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$
1	1-Butanol	0.7309	0.42	0.37	0.48	45	3-Phenyl propanol	1.1978	0.90	0.30	0.67
2	1-Hexanol	1.0127	0.38	0.37	0.48	46	Benzaldehyde	0.8730	1.00	0	0.39
3	1-Octanol	1.2945	0.34	0.37	0.48	47	N-Benzyl formamide	1.1137	1.80	0.40	0.63
4	2-Propanol	0.5900	0.36	0.33	0.56	48	Methyl benzoate	1.0726	0.85	0	0.46
5	Cyclohexanol	0.9041	0.54	0.32	0.57	49	Ethyl benzoate	1.2135	0.85	0	0.46
6	1-Butanal	0.6879	0.65	0	0.45	50	Anisole	0.9160	0.75	0	0.29
7	1-Hexanal	0.9697	0.63	0	0.45	51	Acetophenone	1.0139	1.01	0	0.48
8	1-Heptanal	1.1106	0.61	0	0.45	52	Propiophenone	1.1548	0.95	0	0.51
9	1-Octanal	1.2515	0.59	0	0.45	53	Benzophenone	1.4808	1.50	0	0.50
10	N,N-Dimethyl formamide	0.6468	1.31	0	0.74	54	Benzonitrile	0.8711	1.11	0	0.33
11	N,N-Diethyl formamide	0.9286	1.25	0	0.76	55	*m*-Toluenitrile	1.0120	1.10	0	0.34
12	N,N-Dibutyl formamide	1.4922	1.19	0	0.80	56	Benzyl cyanide	1.0120	1.15	0	0.45
13	N,N-Dimethyl acetamide	0.7877	1.33	0	0.78	57	Nitrobenzene	0.8906	1.11	0	0.28
14	N,N-Diethyl acetamide	1.0695	1.30	0	0.78	58	*m*-Nitrotoluene	1.0315	1.10	0	0.25
15	*n*-Propyl formate	0.7466	0.63	0	0.38	59	*o*-Nitrotoluene	1.0315	1.11	0	0.27
16	*n*-Butyl acetate	1.0284	0.60	0	0.45	60	*p*-Nitrotoluene	1.0315	1.11	0	0.28
17	*n*-Amyl acetate	1.1693	0.58	0	0.45	61	*p*-Nitrobenzyl bromide	1.2065	1.50	0	0.40
18	*n*-Hexyl acetate	1.3102	0.56	0	0.45	62	*p*-Nitrobenzyl chloride	1.1539	1.34	0	0.40
19	Ethyl propionate	0.8875	0.58	0	0.45	63	Fluorobenzene	0.7341	0.57	0	0.10
20	Ethyl butyrate	1.0284	0.58	0	0.45	64	Chlorobenzene	0.8388	0.65	0	0.07
21	Ethyl ether	0.7309	0.25	0	0.45	65	Bromobenzene	0.8914	0.73	0	0.09
22	*n*-Propyl ether	1.0127	0.23	0	0.45	66	Iodobenzene	0.9746	0.82	0	0.12
23	*n*-Butyl ether	1.2945	0.21	0	0.45	67	Benzyl bromide	1.0323	0.98	0	0.20
24	Dioxane	0.6810	0.75	0	0.64	68	*p*-Chlorotoluene	0.9797	0.67	0	0.07
25	Acetone	0.5470	0.70	0.04	0.49	69	*p*-Bromotoluene	1.0323	0.74	0	0.09
26	2-Butanone	0.6879	0.70	0	0.51	70	*p*-Dichlorobenzene	0.9612	0.75	0	0.02
27	2-Hexanone	0.9697	0.68	0	0.51	71	Benzene	0.7164	0.52	0	0.14
28	2-Heptanone	1.1106	0.66	0	0.51	72	Toluene	0.8573	0.52	0	0.14
29	2-Nonanone	1.3924	0.62	0	0.51	73	Ethylbenzene	0.9982	0.51	0	0.15
30	Cyclopentanone	0.7202	0.86	0	0.52	74	*n*-Propylbenzene	1.1391	0.50	0	0.15
31	*n*-Propionitrile	0.5451	0.90	0.02	0.36	75	*n*-Butylbenzene	1.2800	0.51	0	0.15
32	*n*-Valeronitrile	0.8269	0.90	0	0.36	76	*tert.*-Butylbenzene	1.2800	0.49	0	0.16
33	*n*-Hexanitrile	0.9678	0.88	0	0.36	77	*p*-Xylene	0.9982	0.52	0	0.16
34	*n*-Hexyl cyanide	1.1087	0.86	0	0.36	78	Mesitylene	1.1391	0.52	0	0.19
35	*n*-Heptyl cyanide	1.2496	0.84	0	0.36	79	Biphenyl	1.3242	0.99	0	0.22
36	*n*-Octyl cyanide	1.3905	0.82	0	0.36	80	Naphthalene	1.0854	0.92	0	0.20
37	*n*-Nitropropane	0.7055	0.95	0	0.31	81	Anthracene	1.4544	1.34	0	0.26
38	*n*-Nitrobutane	0.8464	0.93	0	0.31	82	Phenol	0.7751	0.89	0.60	0.30
39	*n*-Nitropentane	0.9873	0.91	0	0.31	83	*m*-Cresol	0.9160	0.88	0.57	0.34
40	Methylene chloride	0.4943	0.57	0.10	0.05	84	*p*-Cresol	0.9160	0.87	0.57	0.31
41	Chloroform	0.6167	0.49	0.15	0.02	85	*o*-Cresol	0.9160	0.86	0.52	0.30
42	Dibromomethane	0.5995	0.67	0.10	0.10	86	*p*-Ethylphenol	1.0569	0.90	0.55	0.36
43	Benzylalcohol	0.9160	0.87	0.33	0.56	87	*p*-Chlorophenol	0.8975	1.08	0.67	0.20
44	2-Phenyl ethanol	1.0569	0.91	0.30	0.64						

[a] Values of V_x were obtained from ref. [62,63], while values of π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ were obtained from ref. [64].

1.3. Focus of the present work

In contrast to the previous application of the LSER method to RPLC data [42–52], the present work was designed to really probe the LSER model in three ways. First, a large number of aliphatic and aromatic compounds were chosen as test solutes to see if LSERs could simultaneously encompass a wide range of both aliphatic and aromatic compounds. With only two exceptions [44,45], all prior studies of

LSER in RPLC centered on easily detected aromatic compounds [42–52]. There are some significant questions in this regard since previous efforts to model aqueous solubilities required the use of separate regressions for aliphatic and aromatic subsets [65]. On the other hand, the octanol–water partition coefficient of both aliphatic and aromatic solutes can be accommodated to the same LSER regression [65] despite the fact that aqueous solubilities and octanol–water partition coefficients are closely related [66]. It should be noted that LSER studies of GC quite successfully simultaneously handle both aliphatic and aromatic compounds [36,37,64].

Second, in contrast to previous RPLC–LSER studies, a large number of solutes were examined. These solutes were judiciously chosen to span a wide range in solute properties in terms of size, dipolarity/polarizability and hydrogen bond donor/acceptor characteristics. Table 1 lists the 87 test solutes studied here with their corresponding V_x, π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ values. The test solutes include both aliphatic and aromatic alcohols, aldehydes, amides, esters, ethers, ketones, nitriles, nitro and halogenated compounds, alkylbenzenes, phenols and polyaromatic hydrocarbons. Notice that the solute set was not unduly loaded with a relatively high percentage of low polarity solutes whose retentions are easily correlated with solute size. Nor is the data set loaded with congeners which differ only slightly in their physico-chemical properties. We feel that the use of a large number of very different compounds will allow a much more meaningful test of the application of LSER concepts to RPLC. However, we have excluded both amine and pyridine solutes to avoid confounding the data set with strongly silanophilic solutes.

Third, five monomeric alkyl bonded phases which differ in their acidity (silanol accessibility) were used in this study. The bonded phases used are Zorbax SB-C_{18}, Zorbax Rx-C_{18}, Hypersil C_{18}, Hypersil C_8 and Zorbax C_8. According to the relative silanol acidity ranking proposed by Snyder et al. [30], Zorbax Rx is the least acidic bonded phase among those classified, while the Hypersil columns have intermediate acidity, and the Zorbax phases are among the most acidic phases. These four stationary phases were bonded with dimethyl-alkyl silane. Bonded phase Zorbax SB-C_{18} which was bonded with diisobutyl-alkyl silane was not included in Snyder's list. However, based on the fact that the underlying silica support used for both the Zorbax SB-C_{18} and Rx-C_{18} were specially treated to minimize the number of reactive acidic silanol groups [67–69], and the isobutyl groups on Zorbax SB should impede some access to SiOH groups [70,71], we believe that Zorbax SB-C_{18} is slightly less acidic than Zorbax Rx-C_{18}. Nevertheless, the bulky side chains do alter the bonded phase density and this may have an affect on the phase properties. Notice the Zorbax and Hypersil phases have been ranked as being highly similar in terms of shape selectivity and hydrophobicity [70,72].

To avoid statistical problems involved in variance inflation in multiple regression analysis, and to make chemical sense of the fitting coefficients, the solute parameters must not covary. The degree of orthogonality among the solute variables used in the linear regression is demonstrated by the variance–covariance matrix shown in Table 2, for all solutes, the aliphatic subset and the aromatic subset. Notice that the covariances are extremely weak except between π_2^{*H} and $\Sigma\beta_2^H$. Solute parameters π_2^{*H} and $\Sigma\beta_2^H$ are somewhat correlated, especially when the data sets are subdivided into the aliphatic and aromatic subsets. Given the weak covariances, we believe that the data sets used here are generally free of significant

Table 2
Correlation coefficient matrix of solute variables used in the derivation of LSER equations

	V_x	π_2^{*H}	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$
All Compounds				
V_x	1.000	0.179	0.128	0.124
π_2^{*H}		1.000	0.001	0.283
$\Sigma\alpha_2^H$			1.000	0.081
$\Sigma\beta_2^H$				1.000
Aliphatic only				
V_x	1.000	0.041	0.142	0.246
π_2^{*H}		1.000	0.430	0.414
$\Sigma\alpha_2^H$			1.000	0.056
$\Sigma\beta_2^H$				1.000
Aromatic only				
V_x	1.000	0.344	0.237	0.286
π_2^{*H}		1.000	0.142	0.588
$\Sigma\alpha_2^H$			1.000	0.305
$\Sigma\beta_2^H$				1.000

statistical artifacts, but still some care must be taken when interpreting the s and b coefficients.

2. Experimental

All liquid chromatographic measurements were made at 25.0 (±0.05) °C in 50/50 (v/v) acetonitrile–water. The reported capacity factors were averages of at least triplicate determinations. The peak produced by D_2O was taken as the void-volume of the system. All measurements were made with a Hewlett-Packard 1090 liquid chromatograph. Two detectors were used. A UV detector, built into the HP 1090, was used at a wavelength of 254 nm to detect aromatic compounds; an external refractive index detector (HP 1047A) was used to detect aliphatic compounds. Retention times were taken at the peak maximum reported by a Hewlett-Packard 9153 data system. HPLC-grade solvents were used for the preparation of the mobile phase. Water and acetonitrile were obtained from Baker (Phillipsburg, NJ, USA) and EM Science (Cherry Hill, NJ, USA), respectively. All test solutes were obtained commercially. Most samples were prepared in the mobile phase under study. For a few which were not sufficiently soluble, acetonitrile was added in order to dissolve the solutes to a concentration that could be detected.

Five commercial bonded phases were used in this study: Zorbax SB-C_{18} and Zorbax Rx-C_{18} (Rockland Technologies, pore size = 80Å), Hypersil C_{18} and C_8 (Phenomenex, pore size = 120Å), and Zorbax C_8 (Du Pont, pore size = 100Å). The particle size for all phases was 5 μm. Columns were packed by a pressurized upward-slurry technique with 2-propanol as the packing solvent at 4500 psi packing pressure. Columns were flushed with pure acetonitrile and then brought to the analytical mobile phase composition via a gradient. A very shallow gradient was used to achieve the final mobile phase composition so as to ensure complete equilibration of the mobile and stationary phase. Typically a column was flushed with 50 column volumes of mobile phase per each percentage change in composition from pure modifier to the analytical composition. Columns of different dimensions were packed from the same lot of packing material in order to accommodate the very wide range in k' values encountered with this highly variegated set of solutes.

3. Results and discussions

The log k' values obtained on the various reversed-phase columns in acetonitrile–water (50:50, v/v) are given in Table 3. As a preliminary study, we have applied principal component-factor analysis methods to our data base of 87 solutes on the five phases studied here. We find that 99.79% of all the variance can be explained by a single solute dependent factor (variable), 99.96% of the variance is fit by using two factors to represent each solute, and three solute factors are needed to correlate 99.99% of the variability in the data set. Given that there are both statistical and random errors in the determination of k' we believe that we really only need one or at most two factors to explain the retention behavior of all of the solute on these five phases. Despite the fact that these phases span a considerable range in silanophilicity they are very similar as far as these 87 test solutes are concerned. We presume that if silanophilic probes were used we would see considerable difference between the five phases of different silanol acidity.

The LSER-based regression equations of log k' against the solute parameters V_x, π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are assembled in Table 4. Overall, the statistical measures of the goodness-of-fit for all the equations are equally excellent. The average residuals are about 0.06 and the correlation coefficients are better than 0.99. The fits are as good as those obtained in LSER studies of aqueous solubility [65,73], octanol–water partition coefficients [74–76] and other bulk phase partition coefficients [57,58,77,78]. We must, however, point out that these fits are not as good as those obtained in GC [64]. The comparison to GC is not completely fair since several of the parameters (π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$) were obtained by fitting gas chromatographic data or by back-calculation from gas chromatographic data. Nonetheless, the fits shown in Table 4 are good enough to indicate that LSER method is a very useful approach to identifying the chemical interactions in RPLC, even when applied to a very wide variety of solutes. It must be

Table 3
Log k' values in acetonitrile–water (50:50, v/v) at 25.0 °C

	Solute	log k'				
		Zorbax SB-C_{18}	Zorbax Rx-C_{18}	Hypersil C_{18}	Hypersil C_8	Zorbax C_8
1	1-Butanol	−0.300	−0.360	−0.383	−0.415	−0.351
2	1-Hexanol	0.189	0.136	0.081	0.026	0.085
3	1-Octanol	0.669	0.618	0.547	0.441	0.507
4	2-Propanol	−0.658	−0.710	−0.717	−0.746	−0.675
5	Cyclohexanol	−0.127	−0.192	−0.233	−0.279	−0.212
6	1-Butanal	0.031	−0.029	−0.091	−0.141	−0.077
7	1-Hexanal	0.514	0.459	0.379	0.269	0.382
8	1-Heptanal	0.749	0.702	0.599	0.480	0.583
9	1-Octanal	0.987	0.949	0.831	0.678	0.756
10	N,N-Dimethyl formamide	−0.781	−0.890	−1.017	−0.974	−0.951
11	N,N-Diethyl formamide	−0.388	−0.502	−0.560	−0.557	−0.488
12	N,N-Dibutyl formamide	0.485	0.365	0.327	0.260	0.331
13	N,N-Dimethyl acetamide	−0.705	−0.821	−0.986	−0.925	−0.896
14	N,N-Diethyl acetamide	−0.341	−0.452	−0.537	−0.502	−0.463
15	*n*-Propyl formate	0.105	0.045	0.023	−0.036	0.039
16	*n*-Butyl acetate	0.432	0.377	0.331	0.238	0.314
17	*n*-Amyl acetate	0.661	0.609	0.554	0.436	0.524
18	*n*-Hexyl acetate	0.899	0.855	0.690	0.639	0.731
19	Ethyl propionate	0.195	0.139	0.109	0.034	0.113
20	Ethyl butyrate	0.431	0.382	0.342	0.242	0.323
21	Ethyl ether	0.044	0.018	−0.056	−0.143	−0.066
22	*n*-Propyl ether	0.644	0.637	0.548	0.383	0.483
23	*n*-Butyl ether	1.164	1.163	1.057	0.824	0.935
24	Dioxane	−0.517	−0.587	−0.658	−0.689	−0.625
25	Acetone	−0.479	−0.543	−0.554	−0.582	−0.514
26	2-Butanone	−0.215	−0.277	−0.290	−0.338	−0.262
27	2-Hexanone	0.268	0.205	0.175	0.091	0.172
28	2-Heptanone	0.501	0.444	0.400	0.296	0.385
29	2-Nonanone	0.980	0.936	0.860	0.700	0.798
30	Cyclopentanone	−0.178	−0.257	−0.281	−0.334	−0.251
31	*n*-Propionitrile	−0.259	−0.335	−0.318	−0.348	−0.282
32	*n*-Valeronitrile	0.193	0.119	0.114	0.054	0.133
33	*n*-Hexanitrile	0.422	0.353	0.338	0.255	0.333
34	*n*-Hexyl cyanide	0.643	0.578	0.555	0.453	0.536
35	*n*-Heptyl cyanide	0.872	0.813	0.780	0.651	0.736
36	*n*-Octyl cyanide	1.106	1.055	1.010	0.848	0.937
37	*n*-Nitropropane	0.123	0.053	0.056	0.001	0.082
38	*n*-Nitrobutane	0.350	0.283	0.275	0.201	0.281
39	*n*-Nitropentane	0.576	0.513	0.494	0.399	0.481
40	Methylene chloride	0.202	0.160	0.134	0.061	0.149
41	Chloroform	0.448	0.414	0.370	0.277	0.367
42	Dibromomethane	0.333	0.292	0.255	0.163	0.251
43	Benzylalcohol	−0.208	−0.284	−0.261	−0.308	−0.239
44	2-Phenyl ethanol	−0.091	−0.166	−0.154	−0.206	−0.139
45	3-Phenyl propanol	0.071	−0.004	−0.003	−0.064	−0.001
46	Benzaldehyde	0.193	0.121	0.104	0.026	0.108
47	N-Benzyl formamide	−0.352	−0.460	−0.414	−0.446	−0.380
48	Methyl benzoate	0.435	0.380	0.344	0.233	0.319
49	Ethyl benzoate	0.646	0.592	0.560	0.418	0.520

(*Continued on p. 8*)

Table 3 (*continued*)

	Solute	log k'				
		Zorbax SB-C_{18}	Zorbax Rx-C_{18}	Hypersil C_{18}	Hypersil C_8	Zorbax C_8
50	Anisole	0.490	0.451	0.402	0.288	0.371
51	Acetophenone	0.217	0.143	0.126	0.039	0.122
52	Propiophenone	0.479	0.415	0.387	0.264	0.350
53	Benzophenone	0.783	0.708	0.690	0.531	0.649
54	Benzonitrile	0.256	0.175	0.174	0.100	0.184
55	*m*-Toluenitrile	0.463	0.386	0.372	0.270	0.358
56	Benzyl cyanide	0.252	0.168	0.178	0.108	0.187
57	Nitrobenzene	0.389	0.317	0.306	0.212	0.302
58	*m*-Nitrotoluene	0.608	0.540	0.526	0.394	0.499
59	*o*-Nitrotoluene	0.555	0.485	0.475	0.356	0.459
60	*p*-Nitrotoluene	0.586	0.519	0.506	0.375	0.477
61	*p*-Nitrobenzyl bromide	0.648	0.564	0.561	0.436	0.537
62	*p*-Nitrobenzyl chloride	0.592	0.509	0.505	0.384	0.489
63	Fluorobenzene	0.528	0.498	0.455	0.336	0.432
64	Chlorobenzene	0.739	0.728	0.657	0.503	0.605
65	Bromobenzene	0.802	0.793	0.718	0.550	0.654
66	Iodobenzene	0.917	0.911	0.826	0.639	0.747
67	Benzyl bromide	0.740	0.689	0.653	0.518	0.615
68	*p*-Chlorotoluene	0.968	0.972	0.880	0.689	0.800
69	*p*-Bromotoluene	1.034	1.040	0.942	0.738	0.848
70	*p*-Dichlorobenzene	0.978	0.983	0.888	0.691	0.804
71	Benzene	0.527	0.504	0.453	0.318	0.412
72	Toluene	0.746	0.736	0.664	0.500	0.604
73	Ethylbenzene	0.957	0.948	0.867	0.681	0.786
74	*n*-Propylbenzene	1.196	1.195	1.097	0.880	0.993
75	*n*-Butylbenzene	1.433	1.436	1.326	1.077	1.203
76	*tert.*-Butylbenzene	1.287	1.274	1.182	0.965	1.083
77	*p*-Xylene	0.971	0.979	0.882	0.682	0.788
78	Mesitylene	1.197	1.206	1.101	0.866	0.982
79	Biphenyl	1.133	1.121	1.036	0.825	0.937
80	Naphthalene	0.911	0.904	0.819	0.637	0.740
81	Anthracene	1.331	1.379	1.232	0.965	1.078
82	Phenol	−0.086	−0.157	−0.131	−0.177	−0.083
83	*m*-Cresol	0.085	0.015	0.029	−0.029	0.049
84	*p*-Cresol	0.083	0.014	0.027	−0.031	0.051
85	*o*-Cresol	0.139	0.073	0.081	0.017	0.107
86	*p*-Ethylphenol	0.283	0.218	0.220	0.147	0.213
87	*p*-Chlorophenol	0.181	0.118	0.121	0.063	0.154

Table 4
Coefficients of LSER equations of entire data set

Column	log k'_0	m	s	a	b	n[a]	$\overline{sd}$[a]	r[a]
Zorbax SB-C_{18}	−0.26±0.03	1.63±0.03	−0.31±0.02	−0.62±0.04	−1.68±0.04	87	0.058	0.9935
Zorbax Rx-C_{18}	−0.28±0.03	1.68±0.03	−0.36±0.02	−0.64±0.04	−1.79±0.04	87	0.063	0.9931
Hypersil C_{18}	−0.32±0.03	1.62±0.03	−0.31±0.02	−0.53±0.04	−1.79±0.04	87	0.061	0.9930
Hypersil C_8	−0.38±0.03	1.44±0.03	−0.26±0.02	−0.46±0.03	−1.56±0.03	87	0.055	0.9927
Zorbax C_8	−0.30±0.03	1.48±0.03	−0.27±0.02	−0.47±0.04	−1.63±0.04	87	0.058	0.9925

[a] n = number of test solutes; $\overline{sd}$ and r indicate the average residual and correlation coefficient of the fit, respectively.

noted that in previous studies of RPLC by LSER [66,74,76], the solute parameters (π_2^*, α_2 and β_2) were chosen so as to optimize the goodness of fit to the RPLC and other liquid–liquid data. That was not done here. All solute parameters were defined by a priori computation (V_x), or are gas chromatographic in origin (π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$). This greatly improves our confidence in the overall validity of the LSER concept and approach. That is, the solute descriptor are very general; they can be applied to both GC and RPLC despite the fact that different descriptors (L_{16}, V_2) are needed to model the cavity-dispersion interaction in GC and RPLC.

In Fig. 1a-c, the experimental log k' obtained on the Zorbax Rx-C_{18} phase are plotted against the calculated log k' based on the LSER equation presented in Table 4. Fig. 1a contrasts the aliphatic compounds with the aromatic compounds; Fig. 1b, the HB donors (with $\Sigma\alpha_2^H > 0$) with non-HB donors; and Fig. 1c, the strong HB acceptors (with $\Sigma\beta_2^H \geq 0.5$) with the remaining solutes. The Zorbax Rx-C_{18} phase gave the poorest fit among all the columns

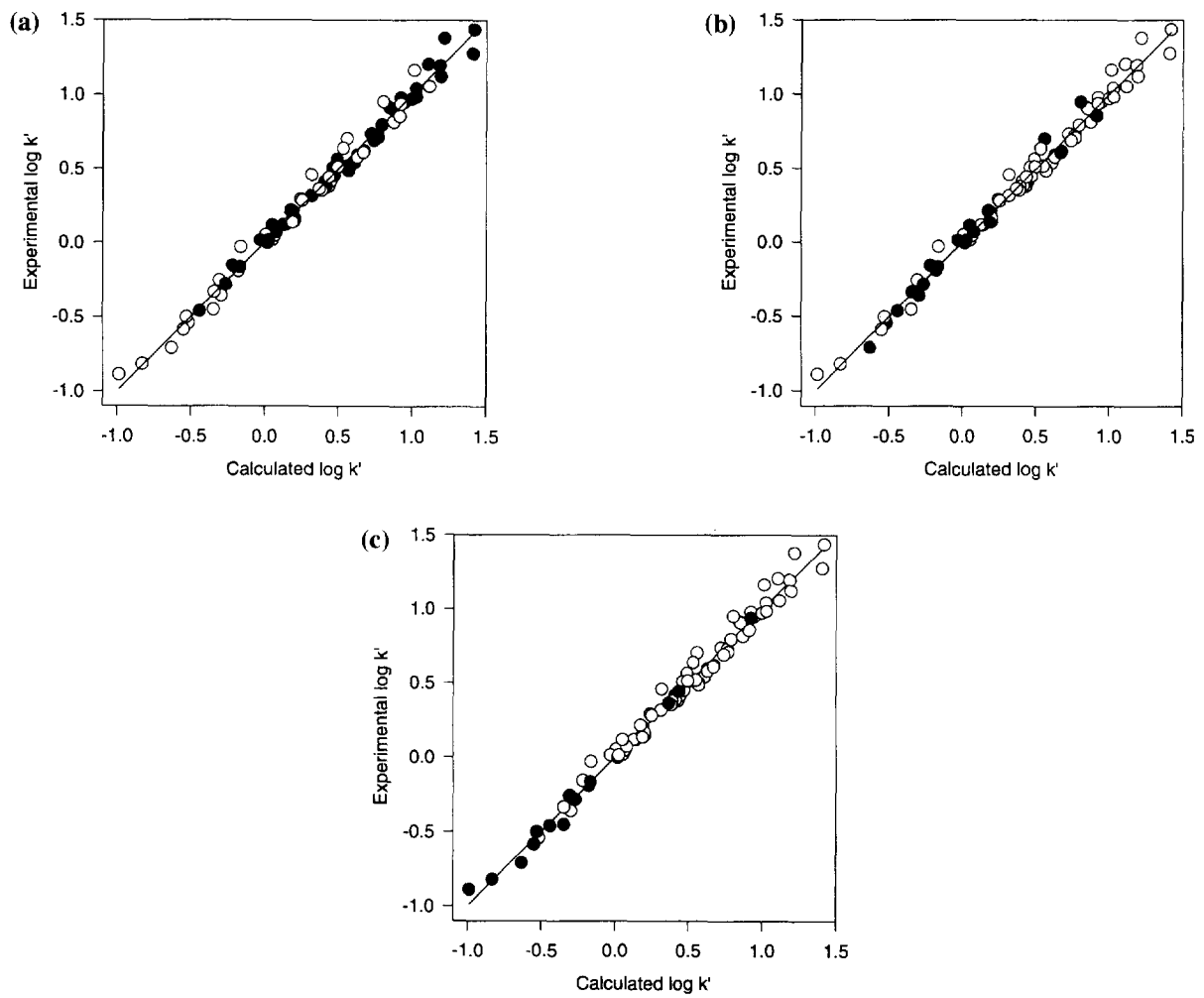

Fig. 1. (a) Plots of experimental vs. calculated log k' for aromatic (●) and aliphatic (○) compounds, obtained on Zorbax Rx-C_{18}; (b) plots of experimental vs. calculated log k' for HB donors (●) and non-HB donors (○), obtained on Zorbax Rx-C_{18}; (c) plots of experimental vs. calculated log k' for strong HB acceptors (●) and the remainders (○), obtained on Zorbax Rx-C_{18}.

studied. A few important points can be drawn from these figures. First, the excellent fits shown by the low average residual and high correlation coefficient are graphically confirmed (see Table 4). Second, compounds from both the aliphatic and aromatic subsets are well dispersed over the entire log k' range. Neither subset fits distinctly better than does the other. Third, the HB donors and the strong HB acceptors are also well dispersed over the entire log k' range, and both subsets fit equally well. However, notice that most compounds having small log k' values are HB donors and strong HB acceptors.

Table 5 shows the percentage variance in log k' contributed by each solute term in Eq. (2). For the data set as a whole, the solute size (V_x) and hydrogen bond basicity ($\Sigma\beta_2^H$) are clearly the most important retention-governing solute parameters in RPLC, in accord with the results of previous studies [42–49].

Table 5
% Variance of log k' accounted for by each solute term

Column	% variance[a] of log k' accounted for by			
	mV_x	$s\pi_2^{*H}$	$a\Sigma\alpha_2^H$	$b\Sigma\beta_2^H$
All Solutes				
Zorbax SB-C_{18}	54.5	3.6	4.6	39.4
Zorbax Rx-C_{18}	52.3	4.4	4.5	40.5
Hypersil-C_{18}	52.5	3.4	3.4	43.6
Hypersil-C_8	53.7	3.2	3.2	43.0
Zorbax C_8	52.8	3.1	3.2	44.0
Average	53.2	3.5	3.8	42.1
Aliphatic solutes				
Zorbax SB-C_{18}	72.5	3.7	3.7	31.8
Zorbax Rx-C_{18}	69.2	5.2	4.0	31.5
Hypersil-C_{18}	67.3	4.5	3.4	36.1
Hypersil-C_8	70.4	2.8	2.9	38.2
Zorbax C_8	68.6	3.3	3.2	38.1
Average	69.6	3.9	3.4	35.1
Aromatic solutes				
Zorbax SB-C_{18}	40.8	4.0	7.1	44.1
Zorbax Rx-C_{18}	39.8	4.8	6.4	44.8
Hypersil-C_{18}	41.0	4.0	6.3	45.7
Hypersil-C_8	40.7	3.8	6.2	46.8
Zorbax C_8	40.4	3.5	6.1	48.0
Average	40.5	4.0	6.4	45.9

[a] % variance due to x is computed through $\frac{100\,C_x^2 \sum (x-\bar{x})^2}{\sum (\log k' - \overline{\log k'})^2}$

where x denotes the solute parameter (V_x, π_2^{*H}, $\Sigma\alpha_2^H$ or $\Sigma\beta_2^H$), C_x, the corresponding fitting coefficient (m, s, a and b), $\bar{x}$ and $\overline{\log k'}$, the average value for x and log k', respectively.

mV_x accounts for about 50%; and $\Sigma\beta_2^H$ accounts for about 40% of the variance in log k' values. Solute dipolarity/polarizability (π_2^{*H}) and hydrogen bond acidity ($\Sigma\alpha_2^H$) both have minor influences, each accounting for less than 5% of the variance in log k' and thus are chromatographically unimportant. The minor contribution of the $s\pi_2^{*H}$ and $a\Sigma\alpha_2^H$ terms means that we do not require accurate π_2^* and α_2 to obtain good fits for LSER regression of RPLC data. In predicting retention in RPLC, one only needs the solute size and hydrogen bond basicity. The % variances in Table 5 do not add up to exactly 100 because of the inefficacy of LSER to explain 100% of the retention values (correlation coefficients less than 1.00).

Notice the % variance contributed by each individual solute term is similar for all the five phases. This result supports the principal component analysis findings that the retention processes on all five columns are very similar. Although LSER analysis shows that two explanatory parameters ($\Sigma\beta_2^H$ and V_x) are needed to adequately define log k', the ratio of their coefficients (b/m) for the five phases are very similar: −1.03 for Zorbax SB-C_{18}, −1.07 for Zorbax Rx-C_{18}, −1.10 for Hypersil C_{18}, −1.08 for Hypersil C_8 and −1.10 for Zorbax C_8. Thus, mathematically only one abstract explanatory parameter is needed to explain the column-to-column variations with a fixed mobile phase.

3.1. Underlying meaning of the LSER coefficients

Fig. 2 shows the fitting coefficients (m, s, a and b) displayed versus the phases arranged in the order of increasing silanol group acidity. The error bars indicate the 95% confidence intervals. Given that the random experimental errors in log k' are less than 3%, the deviations from the fit are mainly due to the LSER model. We will investigate the underlying meaning of the coefficients against the various bulk phase properties (solubility parameter, refractive index, dipolarity/polarizability, HB acidity and basicity) shown in Table 6.

3.2. m coefficient

We mentioned earlier that the mV_x term arises from a combination of cavity formation and disper-

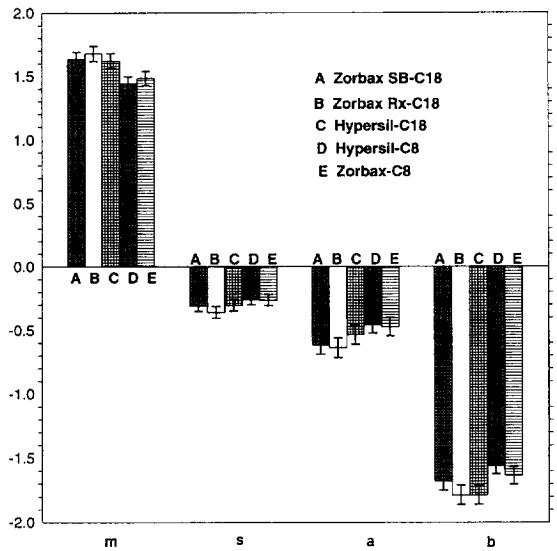

Fig. 2. Plots of the *m*, *s*, *a* and *b* coefficients vs. reversed-phase packing.

sion interactions. We will discuss these two effects separately and show that a large positive m coefficient can be rationalized in either case. The bulk phase property complimentary to solute cavity size is the solvent cohesiveness. A mobile phase of acetonitrile–water (50:50, v/v) is highly cohesive, mainly due to the presence of water molecules ($\delta_H^2 = 554$ cal/ml). Acetonitrile ($\delta_H^2 = 147$ cal/ml) is far less cohesive. According to the solvophobic theory [13,81], water molecules in the mobile phase tend to form molecular networks, which are clusters of water molecules bound tightly to one another via hydrogen bonding. Therefore, to create a cavity in this molecular network so as to accommodate a solute molecule requires an enormous amount of free energy. This rationale is in accord with studies which have shown that solute molecular volume[2] plays an important role in defining solubility in aqueous solution [86,87]. In contrast, the stationary phase, which is composed of bonded alkyl chains and sorbed mobile phase solvents, is much less cohesive. The bonded chains which are alkane-like have very low cohesivity; δ_H^2 of octane is 55 cal/ml and δ_H^2 of octadecane is 66 cal/ml. Moreover, the average density of the bonded alkyl chains is much less, nearly a factor of two lower, than the bulk density of liquid alkanes due to the conformational and steric effects imposed during the bonding process. Consequently, the effective cohesiveness contributed by the alkyl chains is in fact very small. Nevertheless, the sorbed organic modifier and water contribute to the cohesiveness of the stationary phase. Yonker et al. [22] reported that at a mobile phase of acetonitrile–water (50:50, v/v), about 0.29 ml of acetonitrile and 0.09 ml of water are sorbed per g of bonded phase. Considering the 0.29:0.09 (v/v) ratio of acetonitrile to water sorbed into the bonded phase, we infer that the stationary phase is far less cohesive than is the acetonitrile–water (50:50, v/v) mobile phase based on two factors. First, the volume fraction of water (the major contributor to cohesivity) in the bonded phase is less than that in the mobile phase. Second, and more importantly, so few water molecules sorb to the bonded phase that they lose their network structure upon sorbing into the stationary phase. As a result, the free energy required to create a cavity in the mobile phase is much greater than that in the

Table 6
Various properties of bulk phase solvents

Complimentary solvent property	δ_H^2 [a] (cal/ml)	n_D [b]	π^* [c]	β [d]	α [e]
Octane	57	1.395	~0[g]	0[g]	0[g]
Octadecane	66[f]	1.44[f]	~0[g]	0[g]	0[g]
Water	554	1.333	1.09[h]	0.47[i]	1.17[h]
Acetonitrile	147	1.342	0.75[h]	0.40[i]	0.19[h]

[a] Square of Hildebrand solubility parameter, obtained from [79].
[b] Refractive index, obtained from [79].
[c] Bulk phase dipolarity/polarizability.
[d] Bulk phase hydrogen bond basicity.
[e] Bulk phase hydrogen bond acidity.
[f] Extrapolated value from values of lower alkanes.
[g] Estimated values.
[h] Obtained from Ref. [66].
[i] Obtained from Ref. [80].

[2] A great deal of modern computational work [82,83] uses the solvent accessible surface area as the best measure of solute size. The approach is based almost entirely on two experimental studies, i.e., Hermann's work on the solubility of hydrocarbons in water [84], and Higuchi et al.'s work on the solubility of ion pair distribution coefficients between water and organic solvents [85]. Due to the high covariance between V_x and solvent accessible surface area we consider this to be of minor importance at this juncture.

stationary phase; thus, the coefficient for V_x ought to be and is observed to be large and positive.

From the perspective of dispersive interactions, the aqueous mobile phase is less dispersive than is the alkyl bonded phase. Both water and acetonitrile have lower refractive indices than do octane and octadecane (see Table 6). This leads to stronger dispersion interactions between the alkyl bonded phase and the solute than between the aqueous mobile phase and the solute. Previous studies [1,88] strongly suggest dispersion as the major interaction involved in retaining a methylene group, and the net interactions of a solute with the stationary phase are stronger than those with the mobile phase. Such differences in these exoergic dispersive effects lead to a positive m coefficient.

3.3. s coefficient

We turn now to the dipolar interactions. The solvent property complimentary to the solute's π_2^{*H} is the solvent's dipolarity/polarizability (π^*). The mobile phase is a highly dipolar medium as both of its components, water ($\pi^*=1.17$) and acetonitrile ($\pi^*=0.75$), are strongly dipolar substances. As for the stationary phase, although the bonded alkyl chains are almost incapable of dipole/induced-dipole interactions ($\pi^* \cong 0$), the sorbed modifier and water molecules substantially increase its π^* value. Various spectroscopic and thermodynamic studies [2,15,24–27,59–61] have shown that the bonded phase is a moderately polar medium; it is much more polar than pure bulk alkane, or bulk alkane equilibrated with the common mobile phase mixture. Jones and Rutan [24] reported π^* to be 1.23 for a acetonitrile–water (50:50, v/v) mixture, and 0.955 for a solvated octadecyl bonded. π^* values for bonded phases are difficult to measure unambiguously. Even though one can compare results for one bonded phase to another, comparison of bonded phase π^* values to bulk phase values must be done with great caution. Our point is that the difference in dipolarity between the bonded phase and the mobile phase is very small. Based solely on its dipolarity/polarizability, a solute would have only a slight preference for the mobile phase to the stationary phase; thus, a small and negative coefficient for π_2^{*H} is predicted and observed (see Table 4).

3.4. a coefficient

The solvent HBA basicity (β) is the complimentary property to the solute hydrogen acidity ($\Sigma\alpha_2^H$). The acetonitrile–water (50:50, v/v) mobile phase is only moderately basic; pure water ($\beta=0.47$) and pure acetonitrile ($\beta=0.40$) are both only modestly basic. The bonded alkyl chains per se have no HBA basicity ($\beta=0$). The HBA basicity of the stationary phase is established by the sorbed mobile phase components and residual silanol groups. Based on a solvatochromic study of the HBA basicity of mixtures using an -OH hydrogen bond donor, Dallas [2] determined the β value for an acetonitrile–water (50:50, v/v) mixture to be 0.68, and that for bulk hexadecane saturated with the same mixture to be 0.66. Due to the larger amount of organic modifier and water present in a bonded phase relative to bulk hexadecane, the β value for a bonded phase is expected to be even closer to that of an organic aqueous mixture. Although these β values are on a different scale from those reported in Table 6 due to the use of a different HB donor, they are still valuable in indicating the relative HB acceptor strength. Dallas's studies clearly indicate that the mobile phase is only slightly more basic than is the bonded phase. Based solely on its HB donor acidity, a solute would have only a slight preference for the mobile phase to the stationary phase, and thus a small and negative coefficient for $\Sigma\alpha_2^H$ is predicted and observed.

3.5. b coefficient

The solvent HBD acidity (α) is complimentary to the solute hydrogen basicity ($\Sigma\beta_2^H$). The mobile phase is a highly acidic medium as bulk water ($\alpha=1.17$) is an extremely strong HBD acid. Acetonitrile ($\alpha=0.19$) is a relatively weak HBD acid. The bonded alkyl chains per se have no HBD acidity ($\alpha=0$). The large negative b coefficient obtained on all five columns (see Table 4) suggests that the bonded phase is a much weaker HBD acid compared to the mobile phase. This is consistent with Yonker et al.'s report that the ratio of acetonitrile–water sorbed into the bonded phase at a mobile phase of acetonitrile–water (50:50, v/v) is 29:9 [22]. Only a small amount of water sorbs into the bonded phase

relative to the amount of acetonitrile, and thus the HBD acidity of the bonded phase is rather weak. Moreover, the sorbed water molecules can hydrogen bond to the residual silanol groups on the silica surface and to the sorbed acetonitrile molecules, and thus their effective HB acidity is greatly reduced. The sorbed acetonitrile molecules are weak HB donors and do not contribute greatly to the acidity of the bonded phase.

3.6. Use of other solute parameters

In preliminary studies, we attempted to correlate our RPLC data with a variety of different solute parameters. We tried various combinations of volume parameters such as the molar volume, the Bondi volume [89] and the Leahy volume [45,90], with the π_2^*, α_2 and β_2 scales of Kamlet and Taft [33,34,54,66,74,76,91–94], Li and Carr [95,96], and the monomer solute HB acidity and basicity scales of Abraham [97–100]. In general, the LSER regressions using these alternative parameters yield results similar to those reported above. However, the regression fits were poorer than those shown in Table 4. We finally chose V_x, π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ for analysis of our data based on three reasons. First, this parameter combination gave the best regression fits. The regression fits are almost as good as those obtained in the GC experiments from which π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are derived [64]. Second, these parameters have been successfully applied to the correlation of many other physical properties such as retention in various gas–liquid chromatographic stationary phases [36–41,64], toxicity of gases and vapors, water solubility of gaseous solutes, gas–liquid partition coefficients and octanol–water partition coefficients [101]. Third, unlike many of the Kamlet–Taft parameters which were back-calculated from RPLC data or obtained from parameter estimation rules [66,74,76], π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ were derived from gas chromatographic measurements, independent of the reversed-phase system that is being examined.

3.7. General comparison of the five columns

Fig. 2 shows the fitting coefficients m, s, a and b plotted against the columns arranged in the order of increasing silanol group acidity. No significant trend is observed for any of the coefficients in terms of column acidity. Even the b coefficients which represent the differential bulk phase HBD acidity do not show any trend with stationary phase silanol acidity. This result suggests that, with our choice of test solutes, that the stationary phase silanol group acidity does not have a significant effect on the retention process with these phases. The peak shape for strong HB acceptors compounds such as amides are good, i.e., symmetric. Again note that we deliberately avoided including amines, pyridines, carboxylic acids, and other species which strongly ionize, in this study.

3.8. Comparison of C_8 and C_{18} phases

Next, we studied the LSER fitting coefficients vs. the alkyl chain length of the bonded phase. Three C_{18} and two C_8 packings were studied. As a whole, the fitting coefficients for C_{18} and C_8 media are similar at the 95% confidence intervals, except for the m and b coefficients. Because the performance of a bonded phase depends on both the bonded phase chain length and the base silica, we compare bonded phases made on the same silica: the Hypersil C_{18} and C_8. The Hypersil C_{18} phase gave slightly larger m and b coefficients than did the C_8 phase (see Fig. 2). Park et al. also observed larger m coefficients for bonded phases of longer chain lengths [49].

We actually expect the m coefficient to be slightly but significantly larger for a C_{18} vs. a C_8 phase based on prior work of others on the effect of bonded phase chain length on the slopes of plots of log k' vs. solute homolog number [102–106]. The m coefficient must increase with the bonded chain length because only the solute size parameter (V_x) changes substantially upon addition of a methylene group to a solute, while π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ remain virtually constant. Thus, there is an increase in the coefficient of V_x, i.e., the m coefficient, as a function of bonded phase chain length. This begs the issue as to why this is so. Unpublished work from this laboratory shows that slopes of log $K_{gas\rightarrow solvent}$ for a series of n-alkane solutes are virtually independent of the size of alkane solvents [107]. This lead us to postulate that the smaller value of m and b on C_8 vs C_{18} in the same mobile phase must result from differences in the amounts of sorbed mobile phase modifiers with more

Table 7
Coefficients of LSER equations of modified data sets for Zorbax SB-C_{18}

	SP_0	m	s	a	b	n^a	$\overline{sd}^a$	r^a
Exclude HB donor ($\Sigma\alpha_2^H > 0$)								
All	−0.25±0.04	1.63±0.03	−0.34±0.03		−1.62±0.04	67	0.060	0.9925
Aliphatic only	−0.20±0.07	1.62±0.05	−0.36±0.05		−1.67±0.10	32	0.071	0.9914
Aromatic only	−0.29±0.05	1.63±0.05	−0.29±0.04		−1.69±0.08	35	0.049	0.9900
Exclude strong HB acceptor ($\Sigma\beta_2^H > 0.5$) *which are non-HB donor* ($\Sigma\alpha_2^H = 0$)								
All	−0.27±0.03	1.64±0.03	−0.31±0.02	0.61±0.04	−1.68±0.04	76	0.059	0.9917
Aliphatic only	−0.23±0.06	1.65±0.05	−0.35±0.06	−0.86±0.11	−1.69±0.10	32	0.069	0.9893
Aromatic only	−0.28±0.05	1.63±0.05	−0.29±0.03	−0.54±0.04	−1.75±0.06	44	0.046	0.9949

[a] n = number of test solutes; $\overline{sd}$ and r indicate the average residual and correlation coefficient of the fit, respectively.

modifier being sorbed in the C_8 phase. This postulation is in accord with sorption studies which show that sorption of organic modifier and water (mol/% carbon) into a C_8 phase is more extensive than into a C_{18} phase [21]. In other words, in acetonitrile–water (50:50, v/v), a C_8 phase is chemically more similar to the mobile phase than is a C_{18} phase.

3.9. Robustness of the LSER coefficients

The efficacy of the LSER model and the explanatory parameters can be tested through the robustness of the fitting coefficients. Here, we test the robustness of the coefficients by modifying the data set in two ways. First, we exclude the HB donor solutes ($\Sigma\alpha_2^H > 0$); and second, we exclude strong HB acceptor solutes that are non-HB donors ($\Sigma\beta_2^H > 0.5$ and $\Sigma\alpha_2^H = 0$). Table 7 shows the LSER equations for the Zorbax SB-C_{18} phase upon modifications 1 and 2, respectively, for the whole data set, and for the aliphatic and aromatic subsets examined separately. Notice, $\Sigma\alpha_2^H$ was not used as an explanatory parameter in the first three equations because solutes with $\Sigma\alpha_2^H > 0$ were excluded. Compared to the original data sets with all 87 solutes included (Table 4), the LSER coefficients of the modified data sets remain constant within the standard deviation, even with aliphatic and aromatic compounds examined separately. In addition, the regression fits remain excellent. The robustness test was also carried with the other columns with the same conclusion.

3.10. Comparison of aliphatic and aromatic subsets

As stated above, one of our principal goals was to investigate the need to separate aliphatic and aromatic compounds into two classes in RPLC–LSER studies. The LSER regression results for aliphatic and aromatic subsets on all phases are summarized in Tables 8 and 9, respectively. Overall, the statistical goodness of fit for the aliphatic and aromatic subsets are as good as those obtained prior to separating the data. In some cases the fits improved but only slightly. Good regression fits are required to allow valid comparisons between subsets. Notice the $\overline{sd}$ values for the aromatic subsets are consistently smaller than those for the aliphatic subsets. Bear in

Table 8
Coefficients of LSER equations of aliphatic compounds

Column	SP_0	m	s	a	b	n^a	$\overline{sd}^a$	r^a
Zorbax SB-C_{18}	−0.22±0.05	1.64±0.04	−0.34±0.04	−0.85±0.10	−1.70±0.07	42	0.064	0.9928
Zorbax Rx-C_{18}	−0.21±0.05	1.65±0.04	−0.42±0.05	−0.90±0.10	−1.74±0.07	42	0.066	0.9928
Hypersil C_{18}	−0.21±0.05	1.62±0.04	−0.39±0.05	−0.83±0.10	−1.85±0.07	42	0.065	0.9930
Hypersil C_8	−0.32±0.04	1.49±0.03	−0.28±0.04	−0.68±0.08	−1.71±0.06	42	0.052	0.9946
Zorbax C_8	−0.21±0.05	1.51±0.04	−0.31±0.04	−0.75±0.09	−1.76±0.06	42	0.057	0.9938

[a] n = number of test solutes; $\overline{sd}$ and r indicate the average residual and correlation coefficient of the fit, respectively.

Table 9
Coefficients of LSER equations of aromatic compounds

Column	SP_0	m	s	a	b	n^a	$\overline{sd}^a$	r^a
Zorbax SB-C_{18}	−0.28±0.05	1.63±0.05	−0.29±0.03	−0.55±0.04	−1.73±0.06	45	0.046	0.9949
Zorbax Rx-C_{18}	−0.34±0.05	1.73±0.06	−0.34±0.03	−0.56±0.04	−1.87±0.06	45	0.054	0.9938
Hypersil C_{18}	−0.34±0.05	1.59±0.05	−0.29±0.03	−0.50±0.04	−1.72±0.05	45	0.045	0.9947
Hypersil C_8	−0.34±0.04	1.36±0.04	−0.24±0.03	−0.42±0.03	−1.49±0.05	45	0.039	0.9946
Zorbax C_8	−0.28±0.04	1.41±0.04	−0.24±0.03	−0.44±0.03	−1.56±0.05	45	0.040	0.9947

[a] n = number of test solutes; $\overline{sd}$ and r indicate the average residual and correlation coefficient of the fit, respectively.

mind that most of the previous studies which reported excellent regression fits were restricted to aromatic compounds [42,43,46–49].

The LSER fitting coefficients m, s, a and b are plotted in pairs (aliphatic and aromatic subsets) for each phase in Figs. 3–6, respectively. Again, the error bars in the figures indicate the value of the 95% confidence interval of the corresponding coefficients. Given that the random experimental errors in log k' are less than 3%, the deviation from the fits are mainly due to the LSER model. The m, s and b coefficients for the aliphatic and the aromatic subsets are the same within the standard deviation (see Figs. 3 and 4,6). However, the a coefficients for the aliphatic subsets are consistently higher than those for the aromatic subsets for all five stationary phases (see Fig. 5). We do not attach much practical significance to this finding because the $a\Sigma\alpha_2^H$ terms are only of minor importance (see Table 5).

On the other hand, variance studies show that the relative fractional variance accounted by mV_x and $b\Sigma\beta_2^H$ in both subsets are rather different, and in opposite directions. The mV_x term accounts for more variance in the aliphatic subset than in the aromatic subset (see Table 5). The reason for the deviation is unclear to us. However, we suspect that it is due to the nature of the calculation of the solute volume term (V_x). The calculation of V_x considers the number and type of atoms in a molecule but neglects the effects of molecular conformation. Thus, the more compact and smaller aromatic compound is assigned the same value of V_x as its aliphatic counterpart (which has the same number and types of atoms). The size parameter V_x was not able to discriminate

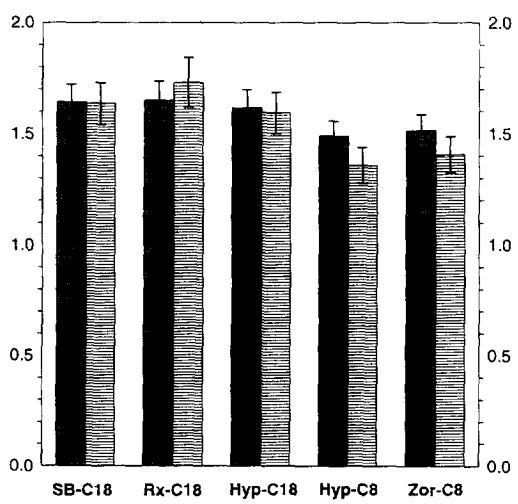

Fig. 3. Plots of the m coefficients for aliphatic subset (darker shade) and aromatic subsets (lighter shade) for the reversed-phase packings.

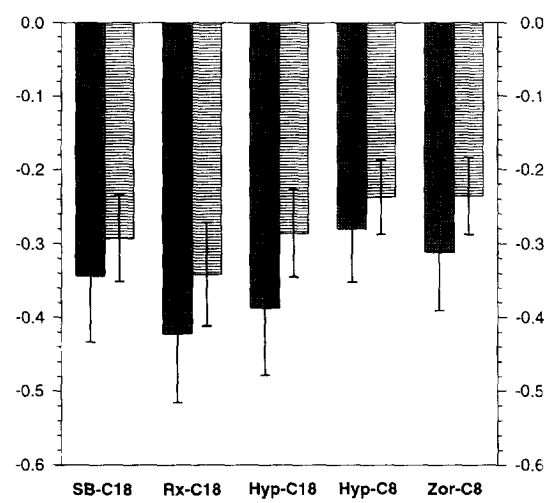

Fig. 4. Plots of the s coefficients for aliphatic subset (darker shade) and aromatic subsets (lighter shade) for the reversed-phase packings.

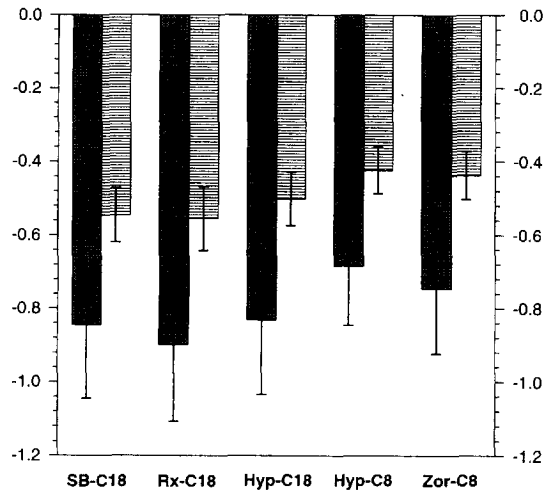

Fig. 5. Plots of the a coefficients for aliphatic subset (darker shade) and aromatic subsets (lighter shade) for the reversed-phase packings.

between isomer pairs, nor to account for shape selectivity. Different volume parameters have been explored but none can effectively account for the observed deviation. We suspect that the difference in the $b\Sigma\beta_2^H$ term for the aliphatic and aromatic subsets arises from compensation for the differences in the mV_x terms.

In conclusion, mV_x and $b\Sigma\beta_2^H$ are the two vital terms needed to explain the retention mechanism in RPLC, for the data set as a whole, or as aliphatic and aromatic subsets. Although the relative contribution of mV_x and $b\Sigma\beta_2^H$ terms in aliphatic and aromatic subsets are different, a single LSER equation is adequate for explaining the retention behavior of both the aliphatic and aromatic subsets.

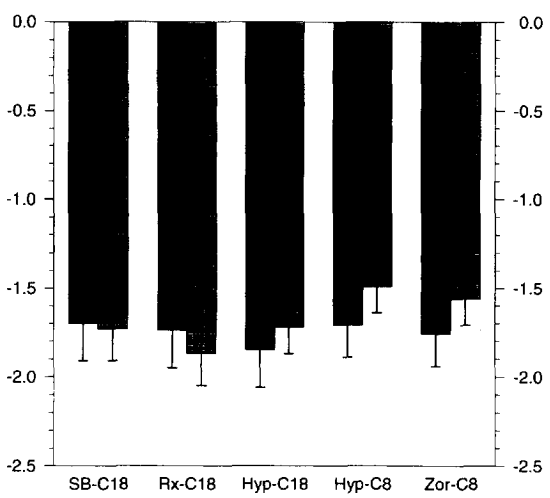

Fig. 6. Plots of the b coefficients for aliphatic subset (darker shade) and aromatic subsets (lighter shade) for the reversed-phase columns.

4. Conclusions

LSERs are shown to be a powerful and robust approach to analyzing solute interactions in the mobile and stationary phases in RPLC, even when applied to a chemically highly diverse data set. The statistical goodness of fit is excellent in correlating retention data on five reversed-phase materials differ in silanol acidity, with either the entire data set or when aliphatic and aromatic subsets are examined separately. The most important retention governing factors are the solute size and hydrogen bond acceptor basicity, while solute dipolarity/polarizability and hydrogen bond donor acidity are of secondary importance. The results confirm the findings of many previous studies that the bonded phase acts as a rather dipolar environment [2,15,24–27,60,61]. The solute parameters used here for RPLC have also been successfully applied to the LSER studies of GC, octanol–water partition coefficients, alkane–water partition coefficients and supercritical fluid chromatography. The chemical interactions of five bonded phases having very different silanol acidity are quite similar for nonsilanophilic solutes. This conclusion has considerable practical impact: we infer that nonsilanophilic solutes will have similar selectivities even on bonded phases of different silanol acidity. The interactions of aliphatic and aromatic compounds with the chromatographic phases are very similar. Separate equations are not required to model the retention behavior of the aliphatic and aromatic subsets.

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