Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1026 (2004) 77–89
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Column selectivity in reversed-phase liquid chromatography V. Higher metal content (type-A) alkyl-silica columns

Jonathan J. Gilroy [b], John W. Dolan [b], P.W. Carr [c], Lloyd R. Snyder [a,*]

[a] *Rheodyne LLC/LC Resources Group, 26 Silverwood Court, Orinda, CA 94563, USA*
[b] *BASi Northwest Laboratory, 3138 NE Rivergate, Bldg. 301C, McMinnville, OR 97128, USA*
[c] *Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA*

Received 4 June 2003; received in revised form 10 November 2003; accepted 11 November 2003

Abstract

Retention measurements involving 16 test solutes have been carried out for 38 type-A alkyl-silica columns and three bonded-zirconia columns. These measurements have been analyzed in terms of a model previously developed for type-B columns, so as to yield values of five column selectivity parameters (**H**, **S***, **A**, **B**, **C**) for each type-A column. Overall differences in selectivity between type-A and -B columns can be related to the average values of **H**, **S***, etc. for each column type. Compared to type-B columns, type-A columns provide generally stronger retention for carboxylic acids, while solutes that are more hydrophobic or less bulky are more retained on type-B columns. Hydrogen-bond acceptors (e.g. aliphatic amides) and cations (e.g. protonated bases) are strongly retained on type-A versus type-B columns. Compared to type-B columns, bonded-zirconia columns show much increased retention of cations and reduced retention of hydrogen-bond acceptors. Because of relatively large differences in the selectivity of bonded-zirconia, type-A, and type-B columns, it will prove difficult to find columns of different type (e.g. a type-A and a type-B column) which have equivalent selectivity. Type-A columns also tend to be more different from each other (in terms of selectivity) than is the case for type-B columns. As a result, the replacement of a given type-A column by an "equivalent" type-A column also appears unlikely, except for samples that do not contain ionized compounds.

Keywords: Column seletivity; Stationary phases, LC; Selectivity; Alkyl-silica column; Bonded-zirconia column

1. Introduction

Previous work [1–4] has shown that the selectivity of type-B (higher purity, low metal content [5]) alkyl-silica columns for reversed-phase liquid chromatography (RP-LC) can be characterized quantitatively by means of Eq. (1):

$$\log(k/k_{\text{ref}}) = \log\alpha = \underset{(i)}{\eta'\mathbf{H}} + \underset{(ii)}{\sigma'\mathbf{S}} + \underset{(iii)}{\beta'\mathbf{A}} + \underset{(iv)}{\alpha'\mathbf{B}} + \underset{(v)}{\kappa'\mathbf{C}} \quad (1)$$

Here, k is the retention factor of any solute, k_{ref} the value of k for a nonpolar reference solute (ethylbenzene), and the remaining selectivity-related symbols represent empirical, eluent-dependent properties of the solute (η', σ', β', α', κ') or eluent-independent properties of the column (**H**, **S**, **A**, **B**, **C**). Terms i–v of Eq. (1) represent contributions to solute retention and column selectivity from various solute-column interactions. Thus, the various column parameters measure the following column properties: **H**, *H*ydrophobicity; -**S**, *S*teric resistance to insertion of bulky solute molecules into the stationary phase (similar to, but not the same as "shape selectivity" [6]); **A**, column hydrogen-bond *A*cidity, mainly attributable to non-ionized silanols; **B**, column hydrogen-bond *B*asicity, believed to be the result of sorbed water in the stationary phase; and **C**, column *C*ation-exchange activity, due to ionized silanols. The parameters η', σ', etc. denote complementary properties of the solute (see Section 6. Nomenclature). If values of the column parameters **H**, **S**, etc. quantitatively describe column selectivity and are available for a large number of different columns, it becomes easy and convenient to select columns of either similar or very different selectivity [4]. Similar columns are required when a column already in use requires replacement. Very different columns are useful in method development work to create deliberate changes in selectivity.

* Corresponding author. Tel.: +1-925-254-6334; fax: +1-925-254-2386.
E-mail address: synder0036@attbi.com (L.R. Snyder).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.11.043

In the present investigation, we have extended our previous studies [1–4] of 92 type-B columns to two new column types: 38 type-A alkyl-silica and three bonded-zirconia columns. Type-A columns are typically of older design and are more acidic than are type-B columns, due to the presence of contaminating metals (e.g. Fe, Al) and/or isolated silanols in the silica matrix [5]. Bonded-zirconia columns are advantageous for operating at extreme conditions of temperature or pH. It is of interest to (a) determine if Eq. (1) is applicable to the latter columns and (b) compare the selectivities of these columns with that of type-B columns. Values of **H**, **S**, etc. for the 41 columns of the present study can be combined with similar data for the 92 type-B columns of [4] to yield a preliminary column-selectivity database for immediate practical application. Ongoing work in our laboratory is intended to extend this database to several hundred RP-LC columns of different kinds. The following paper (Part VI [7]) adds 21 columns which contain polar-groups that are either embedded or used to end-cap the column.

2. Background and theory

2.1. Revised equation 1

While Eq. (1) has so far proved useful for characterizing column selectivity [1–4], in one respect it is conceptually confusing. Whereas terms i and iii–v represent attractive interactions between the solute and column, steric interaction (term ii, $\sigma'\mathbf{S}$) is repulsive in nature. As a consequence, the solute parameter σ' increases for increasing steric interaction (more "bulky" solutes), while the column parameter **S** decreases (less penetrable stationary phases). A more consistent formulation of Eq. (1) is to replace term ii with $-\sigma'\mathbf{S}^*$:

$$\log\alpha = \underset{(i)}{\eta'\mathbf{H}} - \underset{(ii)}{\sigma'\mathbf{S}^*} + \underset{(iii)}{\beta'\mathbf{A}} + \underset{(iv)}{\alpha'\mathbf{B}} + \underset{(v)}{\kappa'\mathbf{C}} \quad (2)$$

where, $\mathbf{S}^* \equiv -\mathbf{S}$. The value of term ii is the same in both Eqs. (1) and (2), which are in turn equivalent in all respects. Values of $\mathbf{S}^*$ now measure steric resistance to insertion of bulky solute molecules into a given stationary phase, and steric interaction now increases with increasing values of both $\mathbf{S}^*$ and σ'.

2.2. Type-A versus type-B alkyl-silica columns

Since 1990, most newly-introduced, analytical-scale, RP-LC columns have been made from highly-purified, "type-B" silica. A recent study [8] has illustrated the change in column ionization (-SiOH → $-SiO^-$) as a function of mobile phase pH for a type-A and a type-B column (Resolve C_{18} and Symmetry C_{18}, respectively). Fig. 1 illustrates the change in negative charge (cation-exchange capacity) for each column type as a function of mobile phase pH. The negative charge on the type-A column of Fig. 1 is always greater (increased silanol ionization) than for the type-B column. The behavior of individual type-A or -B columns as in Fig. 1 is likely to vary over wide limits, as suggested by data reported in Fig. 6 of [2], as well as by very different values of **C** in Table 1 and in [4] for different type-A or -B columns.

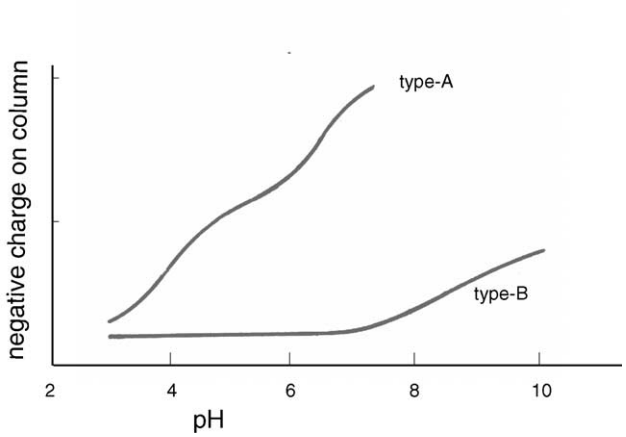

Fig. 1. Cation-exchange capacity of type-A and -B columns as a function of mobile phase pH. Adapted from Fig. 4 of [8] for Resolve C_{18} (type-A) and Symmetry C_{18} (type-B) columns. See text for details.

Improved column manufacturing processes over the last 15 years have also allowed an increase in the concentration of alkyl ligands, so that the surface coverage of the newer type-B columns tends to be greater versus that of the older type-A columns. Thus, the 38 type-A columns of Table 1 have an average surface coverage $C_L = 2.9 \pm 0.3$ (1 S.D.) μmol/m^2 for C_8 columns, and 2.5 ± 0.7 for C_{18} columns. Corresponding values of $\mathbf{C}_L$ for the type-B columns reported previously [4] are on average 28% higher: 3.7 ± 0.7 for C_8 and 3.2 ± 0.7 for C_{18} (the latter average values exclude some intentionally underbonded phases, as well as atypical phases such as StableBond and XTerra).

As a result of the lower ligand surface density of type-A columns, column hydrophobicity **H** should decrease, and bulky solute molecules should have generally easier access to the stationary phase (illustrated in Fig. 2b versus a), resulting in lower values of **H** and $\mathbf{S}^*$ for type-A versus type-B columns. Steric hindrance can also interfere with other solute-column interactions ([3], and see Fig. 2c and d), with possible consequences for the application of Eq. (2) to type-A versus type-B columns. In addition, values of the column parameter **C** of Eq. (2) increase with the amount of negative charge on the column [3]; thus, we anticipate larger values of **C** for type-A versus type-B columns. Finally, more acidic (type-A) silanols should also be stronger hydrogen-bond donors when not ionized, and this suggests that values of the column parameter **A** will also be larger for type-A versus type-B columns. These major differences in the properties of type-A versus type-B columns will have important consequences for the accuracy of Eq. (2) when applied to these two column types (Section 4.1).

Table 1
Properties and selectivity of columns used in the present study. Column parameter values calculated from Eq. (2) with solute parameters of Table 2 (Section 3.4)

Column	Properties		Selectivity parameters							S.D.
	d_{pore}[a]	C_L[b]	**H**	**S***	**A**	**B**	**C** (2.8)	**C** (7.0)	log k_{ref}	
Agilent										
1a. Zorbax (C_8)	7	3.1	0.974	0.041	0.216	0.176	0.974	1.051	0.902	0.061
2a. Zorbax (C_{18})	7	2.4	1.089	−0.055	0.474	0.060	1.489	1.566	0.996	0.114
Alltech										
3a. Adsorbosphere (C_{18})	8	3.4	0.989	0.073	0.070	−0.044	1.496	1.683	0.885	0.023
4a. Allsphere ODS-1	8	1.6	0.733	0.160	0.387	0.002	0.846	1.142	0.67	0.039
5a. Allsphere ODS-2	8	3.1	1.004	0.040	0.243	−0.028	0.960	1.281	0.901	0.018
6a. Econosil (C_{18})	6	1.9	0.966	0.066	0.376	−0.032	1.026	1.339	0.912	0.025
7a. AlphaBond (C_{18})	12.5	1.8	0.845	0.094	0.061	0.001	0.579	1.760	0.722	0.014
8a. Econosphere (C_{18})	8	3.4	0.818	0.128	0.036	−0.017	1.046	1.522	0.708	0.018
Eprogen										
9a. SynChropak RP8	30	3.5	0.639	0.099	0.109	0.029	0.223	0.639	0.065	0.012
10a. SynChropak RP (C_{18})	30	3.5	0.746	0.115	0.230	0.033	0.259	0.746	0.248	0.026
11a. SynChropak RP100 (C_{18})	10	3.5	0.918	0.059	−0.072	0.123	0.225	0.918	0.728	0.035
Jones										
12a. Apex (C_8)	10	2.9	0.869	0.069	0.235	0.168	1.368	1.376	0.643	0.064
13a. Apex (C_{18})[d]	10	2.8	0.985	0.035	0.013	0.042	1.246	2.311	0.783	0.025
14a. Apex II (C_{18})[c]	10	3.2	1.008	0.074	0.235	0.123	2.039	2.690	0.828	0.054
Phenomenex										
15a. Nucleosil (C_8)[d]	10	2.49	0.575	0.134	0.038	0.017	0.282	1.122	0.429	0.014
16a. Nucleosil (C_{18})	10	2.06	0.906	0.052	0.012	−0.030	0.321	0.730	0.862	0.016
17a. Partisil (C_8)	8.5	2.33	0.749	0.071	−0.099	0.074	0.035	0.546	0.649	0.023
18a. Partisil ODS-3	8.5	1.45	0.810	0.079	−0.007	0.002	0.317	0.902	0.732	0.016
19a. Bondaclone (C_{18})	14.8	1.61	0.824	0.056	−0.125	0.044	0.078	0.347	0.652	0.016
20a. Sphericlone ODS-2	8	2.9	0.975	0.045	0.278	−0.051	0.866	1.326	0.882	0.019
Supelco										
21a. Supelcosil LC-8-DB	12	3.2	0.819	0.036	−0.072	0.143	0.446	0.554	0.536	0.039
22a. Supelcosil LC-18-DB	12	3.1	0.979	0.026	0.047	0.114	0.481	0.531	0.755	0.037
23a. Supelcosil LC-8	12	3.2	0.834	0.048	−0.027	0.086	1.117	1.094	0.561	0.030
24a. Supelcosil LC-18	12	3.1	1.018	0.047	0.181	0.162	1.595	1.752	0.768	0.065
Thermo										
25a. Hypersil ODS	12	2.83	0.974	0.026	−0.122	0.020	0.913	0.974	0.744	0.017
26a. Hypersil ODS-2	8	2.45	0.985	−0.016	0.139	−0.011	0.254	0.370	0.749	0.007
27a. Hypersil BDS (C_{18})	13	3.17	0.993	−0.016	−0.095	−0.009	0.337	0.281	0.745	0.006
28a. Hypersil 100 (C_{18})	10	2.84	1.033	−0.013	−0.005	−0.022	0.339	0.639	0.941	0.007
29a. Hypersil PAH[d] (C_{18})	12	4.23	0.949	0.057	0.234	−0.017	1.439	1.724	0.721	0.038
Grace/Vydac										
30a. Vydac 201TP[d] (C_{18})			0.901	0.022	0.409	−0.004	0.394	1.026	0.331	0.022
Waters										
31a. MicroBondapak (C_{18})	12.5	1.4	0.798	0.077	−0.030	0.016	0.285	0.854	0.662	0.007
32a. Nova-Pak (C_8)	6	3.0	0.899	0.028	−0.094	0.006	0.611	0.621	0.595	0.015
33a. Nova-Pak (C_{18})	6	2.8	1.049	−0.004	0.098	−0.027	0.546	0.563	0.811	0.009
34a. Spherisorb (C_8)	8	3.0	0.763	0.091	−0.032	0.053	0.737	1.142	0.691	0.021
35a. Spherisorb ODS-1	8	1.4	0.682	0.186	0.323	0.018	0.843	1.297	0.659	0.036
36a. Spherisorb ODS-2	8	2.9	0.962	0.076	0.070	0.034	0.908	1.263	0.919	0.024
37a. Spherisorb ODS-B	8	2.9	0.975	−0.027	0.240	0.384	−0.642	1.680	0.836	0.139
38a. Resolve (C_{18})	8	2.5	0.968	0.127	0.335	−0.046	1.921	2.144	0.897	0.045
Zirchrom										
39a. ZirChrom-PBD	24	[g]	1.34	0.14	−0.21	−0.02	2.26	2.31	−0.01	0.16
40a. ZirChrom-EZ	24	[g]	1.11	0.10	−0.77	0.07	2.17	2.10	0.06	0.22
41a. ZirChrom-PS	24	[g]	0.64	−0.28	−0.30	0.09	1.82	1.52	−0.54	0.11
Results for addition of 10 mM TEA to mobile phase (Alltech columns)										
3a. Adsorbosphere (C_{18})	8	3.4	0.995	0.051	0.077	−0.038	0.974	[f]	0.884	0.017
4a. Allsphere ODS-1	8	1.6	0.735	0.145	0.408	0.000	0.729	1.073	0.640	0.040
5a. Allsphere ODS-2	8	3.1	1.004	0.029	0.224	−0.034	0.755	1.076	0.897	0.016

Table 1 (*Continued*)

Column	Properties		Selectivity parameters							S.D.
	d_{pore} [a]	C_L [b]	**H**	**S***	**A**	**B**	**C** (2.8)	**C** (7.0)	log k_{ref}	
6a. Econosil (C_{18})	6	1.9	0.967	0.060	0.392	−0.032	0.837	1.150	0.910	0.026
8a. Econosphere (C_{18})	8	3.4	0.816	0.117	0.051	−0.013	0.803	[f]	0.704	0.012
"Special" columns (no TEA addition)										
Alltech										
83. Platinum EPS (C_8)	10	2.9	0.420	0.152	0.151	0.026	0.509	1.369	0.022	0.018
84. Platinum EPS (C_{18})	10	2.5	0.616	0.168	0.335	0.026	0.718	1.728	0.417	0.039
85. Prevail (C_8)	10	1.2	0.618	0.089	0.040	0.041	0.081	1.072	0.530	0.015
86. Prevail (C_{18})	10	1.4	0.889	0.070	0.316	0.022	0.107	1.205	0.975	0.037
GL sciences										
87. Inertsil ODS-P[d]	10	≈2.7	0.978	0.028	0.612	−0.038	0.234	[f]	0.825	0.033

[a] Pore diameter (nm).
[b] Ligand concentration (μmol/m^2) .
[c] Not end-capped.
[d] Polymeric phase.
[f] Berberine not eluted from column at pH 7.0.
[g] Thin layer of cross-linked polymer coating; PBD refers to polybutadiene; PS refers to polystyrene, and EZ is a Lewis-acid deactivated polybutadiene phase.

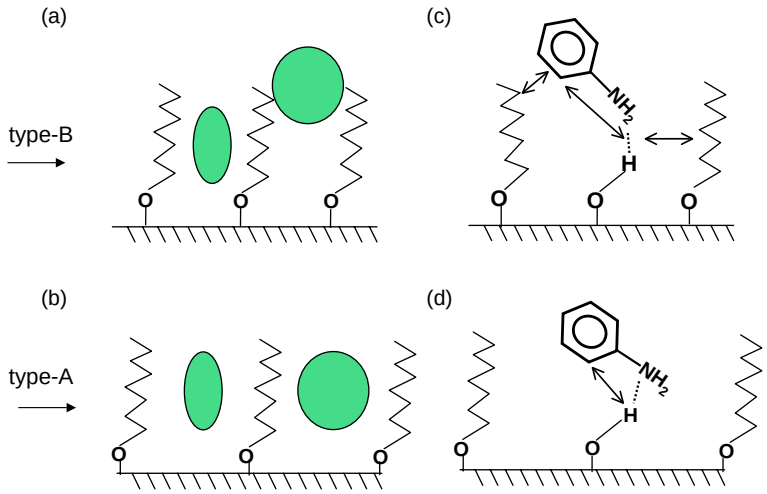

Fig. 2. Illustration of different steric hindrance effects in interactions of solute and column. (a, c) type-B columns with more closely-spaced ligand groups (larger **H** and **S***); (b, d) type-A columns with less closely-spaced ligand groups (smaller **H** and **S***). Arrows indicate steric hindrance between solute and column.

A reviewer has raised an important question with regard to the above discussion. If silanols are increasingly ionized at higher pH (as in Fig. 1), then the relative concentration of non-ionized silanols must decrease at higher pH—in which case **A** should be a function of pH (which we have so far failed to recognize in the development of Eq. (2).). For the case of type-B columns, the extent of silanol ionization for pH ≤ 7 is usually small—as seen in Fig. 1 and confirmed in Fig. 6 of [2] for other type-B columns. This is less likely to be true for type-A columns, so that the use of values of **A** measured at pH 2.8 (as in the present procedure) for separations at higher-pH could result in a reduction in the reliability of values of **H**, **S***, etc. at higher pH. However, it should be noted that the type-A column of Fig. 1 (Resolve C_{18}, #38a) is quite acidic, as measured by very large values of **C** at both pH 2.8 and 7.0 (see Table 1 and Table 4 of [4]). That is, most other alkyl-silica column will be considerably less ionized over the pH range 2.8–7.0 (and values of **A** will therefore be less dependent on pH).

3. Experimental

3.1. Equipment, materials and procedures

These were as described previously [1,4]. The mobile phase was 50% (v/v) acetonitrile/buffer, and the buffer was 60 mM potassium phosphate at either pH 2.80 or (for berberine as solute only) pH 7.0. Other conditions were 35 °C, flowrate equal 2.00 ml/min, 500 ng injection of each solute,

and UV detection at 205 nm. In some additional experiments (Section 4.4), 20 mM triethyamine was added to the buffer (while maintaining the pH constant). In every experiment, columns were equilibrated prior to sample injection as described in [4].

3.2. Columns

The type-A alkyl-silica and bonded-zirconia columns used in the present study are described in Table 1. These 41 columns were each the generous gift of the manufacturer and include two type-A columns (#29a, 30a) that were identified as polymeric rather than monomeric. The properties of the columns in Table 1 were provided by the manufacturer; in the case of type-A columns, it is not known if there are other columns in Table 1 which are also prepared from polyfunctional silanes, i.e. might be considered as "polymeric" columns. The bonded-zirconia columns were in each case prepared by coating zirconia with a polymeric stationary phase.

3.3. Samples

The same 16 solutes used in a preceding study [4] to characterize the selectivity of 92 type-B columns were used in the present study to characterize the columns of Table 1. These solutes are listed in Table 2 with values of η' σ', etc. from [4]. Because amitriptyline and nortriptyline are more strongly retained on some of these columns, additional injections of the individual solutes were made for purposes of peak identification.

3.4. Calculations

Values of the retention factor k were determined as $k = (t_R - t_0)/t_0$, where t_0 equals the retention time for thiourea. Resulting values of k for different solutes and columns are not reported here but are available from the authors. Given values of k for each of the 16 test solutes of Table 2 and 41 columns of Table 1, corresponding values of α were calculated, equal to the ratio of k-values for the compound in question and ethylbenzene, respectively. Resulting values of α were then fit (multiple linear regression) to Eq. (2) using values of the solute parameters (η', σ', etc.) that were reported previously for type-B columns [4] and are shown in Table 2. This application of Eq. (2) leads to the values of **H**, **S***, etc. shown in Table 1 for columns #1a–41a.Values of **C** at pH 7.0 were determined [2] from:

$$\mathbf{C}(7.0) = \mathbf{C}(2.8) + \log(k_{7.0}/k_{2.8}) \quad (3)$$

where $k_{7.0}$ and $k_{2.8}$ refer to values of k for berberine (a quaternary ammonium salt) at pH 7.00 and 2.80, respectively.

4. Results and discussion

4.1. Fit of retention data for type-A columns to Eq. (2)

The previous application [1,3,4] of Eq. (1) to 90 test solutes and 87 type-B C_{18} columns yielded a correlation with an average standard deviation (S.D.) of 0.005 in log α (±1% in α). Values of the solute parameters η', σ', etc. of Eq. (1) were also derived in [4] for the 16 test solutes of Table 2 (listed in Table 2). With values of k (or α) for these test solutes and a given column, plus corresponding values of the solute parameters from Table 2, a multiple linear regression of values of log α in terms of Eq. (2) provides corresponding values of the column parameters **H**, **S***, etc. for a given column. When this approach was applied to retention data for the 38 type-A columns of Table 1, the average S.D. of the fit to Eq. (2) was 0.032 log units, or ±8% in α, i.e. a poorer fit for type-A columns than for type-B columns. The relative failure of Eq. (2) for type-A columns could arise from either of two possibilities: (a) type-A columns might involve some

Table 2
Solute parameter values for the test compounds of the present study (best-fit values for type-B columns [4])

Solute	η'	σ'	β'	α'	κ'
1. Acetophenone	−0.744	0.133	0.059	−0.152	−0.009
2. Benzonitrile	−0.703	0.317	0.003	0.080	−0.030
3. Anisole	−0.467	0.062	0.006	−0.156	−0.009
4. Toluene	−0.205	−0.095	0.011	−0.214	0.005
5. Ethylbenzene	0	0	0	0	0
6. 4-Nitrophenol	−0.968	0.040	0.009	0.098	−0.021
7. 5-Phenylpentanol	−0.495	0.136	0.030	0.610	0.013
8. 5,5-Diphenylhydantoin	−0.940	0.026	0.003	0.568	0.007
9. *cis*-chalcone	−0.048	0.821	−0.030	0.466	−0.045
10. *trans*-chalcone	0.029	0.918	−0.021	−0.292	−0.017
11. *N*,*N*-Dimethylacetamide	−1.903	0.001	0.994	−0.012	0.001
12. *N*,*N*-Diethylacetamide	−1.390	0.214	0.369	−0.215	0.047
13. 4-*n*-Butylbenzoic acid	−0.266	−0.223	0.013	0.838	0.045
14. Mefenamic acid	0.049	0.333	−0.049	1.123	−0.008
15. Nortriptyline	−1.163	−0.018	−0.024	0.289	0.845
16. Amitriptyline	−1.094	0.163	−0.041	0.300	0.817

new solute-column interaction not included in terms i–v of Eq. (2), or (b) the approximate nature of Eq. (2) might lead to decreased accuracy when solute parameters (η', σ', etc.) derived from data for type-B columns are applied to a very different family of columns (i.e. type-A phases).

The possibility of solute-column interactions not included in Eq. (1) for type-A columns can be tested, using a similar approach as in the derivation of the solute parameters of Table 2 for type-B columns. Thus, the fit of retention data for type-B columns by Eq. (1) [1,4] was the result of repetitive multiple regressions, so as to derive best-fit values of both the solute and column parameters. The close agreement of experimental and calculated value of α ($\pm$1%) was in turn argued as evidence against the presence of any additional solute-column interactions other than those represented in Eq. (1). When the same procedure (repetitive multiple regression versus Eq. (1) was applied to values of α for type-A columns, somewhat changed values of the solute parameters η', σ', etc. resulted, and the final fit to Eq. (1) was improved ($\pm$3% in α). This result was checked by dividing the columns of Table 1 into two groups (#1a–19a and 20a–38a), with repetitive multiple regression applied to columns #1a–19a (the training set) in order to calculate new values of η', σ', etc. The latter values of η', σ', etc. were then applied (multiple regression) to each group of columns. Similar values of average S.D. were found for each column set: training set (#1a–19a), $\pm$3% in α; test set (#20a–38a), $\pm$4% in α.

As for the case of type-B columns [1,4], the latter result for type-A columns suggests that no significant solute-column interactions are present other than those represented in Eq. (1). A possible exception to the latter conclusion might be provided by chelating solutes, which have been reported to bind to RP-LC columns that are metal-contaminated [9,10]. However, sample-column chelation usually results in badly tailed peaks which tend to preclude useful separations of chelating compounds. In the remainder of this paper we will ignore chelating interactions.

The reduced accuracy of Eq. (2) for type-A columns (using best-fit solute parameters for type-B columns) appears due to the approximate nature of this equation, rather than a failure to recognize all significant solute-column interactions for the solutes of Table 2. See the further discussion of Appendix A, where it is suggested that the poorer agreement of Eq. (2) for type-A columns (using the solute parameters of Table 2) may arise from changed steric hindrance in the RP-LC interactions of different solutes with type-A versus type-B columns (as in Fig. 2c and d).

4.1.1. Practical consequences of error in the application of Eq. (2) to different column types

Although the iterative regression procedure for the type-A columns of Table 1 (with final S.D. = 0.013) resulted in significant changes in the final values of the solute parameters (η', σ', etc.), the changes in values of **H**, **S***, etc. from initial (Table 1) to best-fit values were quite minor. Average differences in values of **H**, **S***, etc. from the first to the last regression are: $\mathbf{H} = 0.001 \pm 0.005$; $\mathbf{S}^* = 0.001 \pm 0.006$; $\mathbf{A} = 0.003 \pm 0.014$; $\mathbf{B} = 0.001 \pm 0.007$; $\mathbf{C} = 0.001 \pm 0.005$. The resulting effect of these varying differences in **H**, **S***, etc. (e.g. $\pm$0.005 for **H**) on predicted values of α via Eq. (2) is 1% or less for each parameter (see discussion of Table 5 of [4]). The ("non-best-fit") values of **H**, **S***, etc. in Table 1 for type-A columns can therefore be considered as chromatographically equivalent to best-fit values of these parameters.

4.2. Comparison of type-A and -B columns in terms of selectivity

The selectivity of both type-A and -B columns is determined by the properties of the packing [3,4]: (a) the silica used to make the column—especially its acidity as in Fig. 1; (b) the pore diameter of the particle; (c) the bonding process (ligand length and concentration), and (d) whether the column has been end-capped or not. Section 2.2 has shown that the average ligand concentration for the present type-A columns is 28% less for type-A versus type-B columns, while the acidity of type-A columns tends to be greater than that of type-B columns (Fig. 1). If we ignore the small average difference in pore diameter and ligand length between the type-A columns of the present study and the type-B columns of [4], the resulting average values of **H**, **S***, etc. for C_8 and C_{18} columns of each type are summarized in Table 3. Also shown in Table 3 are difference in values of each column parameter for type-A versus type-B columns. Values of **H** and **S*** tend to be slightly lower for type-A columns, as expected from their lower average ligand coverage (Section 2.2.). Values of **B** are somewhat larger for type-A columns; see the discussion of the following paper [7]. Values of **A** and especially **C** for type-A columns are considerably higher, as expected from the greater acidity of type-A columns. See the further discussion of following Section 4.2.1. for the dependence of column selectivity on packing properties such as ligand length and concentration, pore diameter and end-capping.

We also see in Table 3 that differences among type-A columns are generally greater than are those for type-B columns, as indicated by larger values of S.D. for most type-A column parameters. As a consequence of this greater variability, it is more difficult to replace a type-A column with a second type-A column from a different source (see Section 4.5)—compared to the similar replacement of a type-B column by a type-B column [4]. The greater variation among type-A versus type-B columns is likely a consequence of (a) the use of less optimized (and more variable) processes for column manufacture prior to 1990 [11] and (b) the greater variability of older silicas from manufacturer to manufacturer (i.e. varying silica purity).

4.2.1. Selectivity comparisons for related columns introduced by various manufacturers at different times

Previous papers [3,4] dealing with type-B columns have correlated values of **H**, **S***, etc. with such column proper-

Table 3
Comparison of selectivity for end-capped type-A vs. -B columns; average values and standard deviation of each column parameter from Table 1 (type-A) or [4] (type-B)

	H[b]	**S***	**A**	**B**	**C**(2.8)	**C**(7.0)
C_8 columns						
Type-A	0.84 ± 0.16	−0.05 ± 0.07	0.06 ± 0.20	0.07 ± 0.06	0.75 ± 0.49	1.01 ± 0.32
Type-B	0.83 ± 0.06	−0.01 ± 0.04	−0.16 ± 0.13	0.02 ± 0.02	0.02 ± 0.20	0.31 ± 0.42
Difference[a]	0.01	−0.04	0.23	0.05	0.73	0.70
C_{18} columns						
Type-A	0.84 ± 0.28	−0.06 ± 0.07	0.12 ± 0.16	0.05 ± 0.11	0.78 ± 0.55	1.13 ± 0.55
Type-B	1.00 ± 0.07	0.01 ± 0.03	−0.07 ± 0.11	−0.01 ± 0.02	0.05 ± 0.18	0.17 ± 0.23
Difference[a]	−0.15	−0.07	0.19	0.06	0.72	0.96

[a] Type-A vs. type-B.
[b] Uncertainty figures are ±1S.D., e.g. for the type-A C_8 columns, the variation in **H** is 0.16 (1 S.D.).

Table 4
Summary of effect of column properties on selectivity

Column property	Effect of property on column selectivity[a]					
	H	**S***	**A**	**B**	**C**(2.8)	**C**(7.0)
Ligand length	++	+	++	o	++	o
Ligand concentration (mol/m^2)	++	+	++	−	++	−
Pore diameter (nm)	−	o	−−	o	++	o
End-capping	o	o	−−	o	−−	−−

Approximate trends from [4].
[a] ++: large relative increase with increase in property value; +: small increase; o: little effect; −: small decrease; −−: large decrease.

ties as ligand length and concentration, pore diameter and end-capping. Table 4 provides a summary of the relative effects of each column property on each column parameter, assuming a similar starting silica acidity. It is interesting to compare values of **H**, **S***, etc. for "related" columns developed by a given manufacturer at different times. Several examples are summarized in Table 5 and interpreted below in light of Table 4.

In a first example (Table 5), the Allsphere ODS-1 and ODS-2 columns from Alltech are compared. For the ODS-2 column, there is a large increase in **H**, a moderate increase in **S***, **C**(2.8) and **C**(7.0), a moderate decrease in **A** and little change in **B**. From Table 1 it is known that the ODS-2 column has about twice the ligand concentration as ODS-1, which is consistent (Table 4) with most of the changes in values of **H**, **S***, etc. for these two columns.

In a second example, consider the C_8 and C_{18} Supelcosil and Supelcosil-DB ("base deactivated") columns, whose ligand concentrations are about the same for all four columns (Table 1). The average change in **H**, **S***, etc. for the two DB columns is shown in Table 5, and it is seen that there

Table 5
Changes in column selectivity over time for different column manufacturers

Company	Column	**H**	**S***	**A**	**B**	**C**(2.8)	**C**(7.0)
Alltech	4a. Allsphere ODS-1	0.74	−0.16	0.40	0.00	0.85	1.15
	5a. Allsphere ODS-2	1.00	−0.04	0.24	−0.03	0.96	1.28
	Change	+0.26	+0.12	−0.16	−0.03	+0.11	+0.13
Supelco	23a. Supelcosil LC-8	0.84	−0.05	−0.02	0.08	1.12	1.10
	21a. Supelcosil LC-8-DB	0.82	−0.04	−0.07	0.14	0.45	0.56
	Change	−0.02	0.01	−0.04	0.06	−0.67	−0.54
	24a. Supelcosil LC-18	1.02	−0.05	0.20	0.17	1.60	1.76
	22a. Supelcosil LC-18-DB	0.98	−0.03	0.05	0.12	0.48	0.53
	Change	−0.04	0.02	−0.15	−0.06	−1.12	−1.22
	Average change	−0.03	0.02	−0.10	0.00	−0.90	−0.88
Thermo	25a. Hypersil ODS	0.98	−0.03	−0.11	0.02	0.92	0.98
	26a. Hypersil ODS-2	0.99	0.01	0.14	−0.02	0.26	0.37
	Change	0.01	0.05	0.25	−0.03	−0.66	−0.61
Waters	35a. Spherisorb ODS-1	0.68	−0.19	0.33	0.02	0.84	1.30
	36a. Spherisorb ODS-2	0.96	−0.07	0.07	0.04	0.91	1.26
	Change	0.28	0.12	−0.26	0.02	0.06	−0.04
	31a. MicroBondapak (C_{18})	0.80	−0.08	−0.03	0.02	0.28	0.85
	33a. Nova-Pak (C_{18})	1.05	0.00	0.11	−0.03	0.55	0.57
	74. Symmetry (C_{18})	1.05	0.06	0.02	−0.02	−0.30	0.16
	77. Xterra MS (C_{18})	0.99	0.01	−0.14	−0.01	0.13	0.05

is a moderate decrease in **A** (−0.10) and a large decrease in **C**(−0.90, −0.88), while other column parameters are essentially unchanged. This implies that silica acidity has been decreased for the two DB columns, which is consistent with the "base deactivation" of the silica used for the DB columns.

A comparison of the Thermo Hypersil ODS and ODS-2 columns in Table 5 shows mainly a large increase in **A** (+0.25) and very large decrease in **C**(−0.66, −0.61), i.e. H-bond acidity (**A**) increases, while Bronsted acidity (**C**) decreases. Referring to Table 1, the ODS-2 column has about the same ligand concentration (2.45 versus 2.83), but a smaller pore diameter (8 nm versus 12 nm). These changes in **A** and **C** are in line with the predictions of Table 4 for a reduction in pore diameter.

A comparison of Spherisorb ODS-1 and ODS-2 in Table 5 shows a similar pattern of change in **H**, **S***, etc. as noted above for the two Allsphere columns. In both cases (Spherisorb and Allsphere), the column that was introduced later (ODS-2) has about twice the ligand concentration, which accounts for the differences between the ODS-1 and ODS-2 versions.

Finally, four C_{18} columns from Waters which were introduced 5–10 years apart are listed in chronological order in Table 5. There is no regular progression of values of **H**, **S***, etc. with time, but the latest column (XTerra MS C_{18}) compared with the earliest column (MicroBondapak C_{18}) shows an increase in **H** and **S***, combined with a general decrease in column acidity (values of **A** and **C**), i.e. typical of type-B versus type-A columns (Section 4.2.).

4.3. Other reversed-phase columns

A variety of other reversed-phase columns are commercially available, as illustrated by the following examples.

4.3.1. "Special" type-B columns

Five, so-called "special" columns (#83–87 of Table 1) were evaluated in our previous study of type-B columns [4]. These columns differed from other type-B columns in their manufacture and also exhibited much poorer agreement (±7% in k) with Eq. (1) than did the remaining 87 ("non-special") type-B columns of [4]. We have previously noted that the poorer fit of these "special" columns with Eq. (1) might be due to their greater acidity versus the average type-B column, which suggests that these "special" columns might correlate better with Eq. (1) or (2) when best-fit (from repeated regression) solute parameters for type-A columns are used, instead of values of η', σ', etc. derived from type-B columns. This is in fact the case; the use of best-fit ("type-A") solute parameters gives S.D. = 0.008 (±2% error in α) for columns #83–87, i.e. a similar S.D. as for the 38 type-A columns of Table 1 (0.013) when best-fit solute parameters are used.

4.3.2. Polymeric alkyl-silica columns

One of the "special" type-B columns of Section 4.3.1 (Inertsil ODS-P) is classified by the manufacturer as polymeric, while two other columns in Table 1 (#29a, 30a) are also reported as polymeric phases. When best-fit solute parameters (for type-A columns) are used with Eq. (2) for these columns, an average S.D. = 0.012 log units results. Thus, these columns can be regarded as type-A columns, and their selectivity can be described (at least approximately) by their values of **H**, **S***, etc.

4.3.3. Bonded-zirconia columns

Bonded-zirconia columns are commercially available as alternatives to the more common alkyl-silica columns. The zirconia surface differs in important ways from silica, which results in strong interactions with oxyanion solutes [12,13], analogous to the strong interaction of cations with the type-A silica surface. We therefore examined three columns of this type (#39a, 40a, 41a), as summarized in Table 1. It should be noted that these columns also differ from those discussed here and previously [4] in that the stationary phase is a polymer that is coated onto zirconia.

Correlations of retention data with Eq. (2) for bonded-zirconia columns #39a, 40a, 41a gave generally poor agreement (average S.D. = 0.16 or ±41% in α). The selectivity parameters for these columns (Table 1) showed some striking differences when compared with alkyl-silica columns. Thus, values of **A** are relatively low (average **A** = −0.43), while values of **C** are quite high (average **C** = 2.0). Bonded-zirconia column #40a has the lowest value of **A** (−0.77), presumably as a result of the intentional deactivation of the zirconia so as to reduce the activity of Lewis-acid (hydrogen-bond donor) sites. The relative values of **A** and **C** for bonded-zirconia columns are consistent with what we know about this stationary phase [12], i.e. an absence of proton-donor silanols and the ability of phosphate (in the buffer) to strongly adsorb to the zirconia surface with creation of a large negative charge on the column. As also seen in Table 1 (next to last column), values of log k for ethylbenzene and the bonded-zirconia columns are generally low (a consequence of the much lower surface area of wide-pore columns #39a–41).

4.4. Effect of triethylamine on column selectivity

In order to counteract the frequent tailing of basic solutes when separated on type-A columns, silanol suppressors such as triethylamine (TEA) have been added to the mobile phase [14]. TEA has a pK_a of 11 in water, and this value will be lower by about two units in a mobile phase that contains 50% organic [15]. The effect of added TEA on the retention of various nonionizable solutes in unbuffered 50% methanol/water mobile phase has been reported for two polymeric columns [16]. It was found that TEA addition leads to decreased column hydrophobicity and hydrogen-bond acidity, i.e. a decrease in values of **H** and

A. For these conditions, the TEA in the mobile phase is expected to be a mixture of protonated and unprotonated species. Consequently, unprotonated TEA should be sorbed by the polymeric stationary phase (resulting in an increase in column polarity, or decrease in **H**), as well as interact by hydrogen bonding with non-ionized silanols (with a decrease in **A**). Because no acidic or basic solutes were included in the study of [15], the effect of TEA on values of **B** or **C** cannot be inferred.

For five type-A columns from Table 1 (#3a–6a,8a), the separation of the test solutes of Table 2 was repeated with mobile phase that also contained 10 mM of TEA (final pH adjusted to 2.8). Resulting values of **H**, **S***, etc. due to added TEA were calculated (Table 1) by regression of values of α versus Eq. (2), using the solute parameters of Table 2. The average change in values of **H**, **S***, etc. as a result of added TEA was: **H**, 0.00; **S***, −0.01; **A**, 0.01; **B**, 0.00; **C**(2.8), −0.26. Thus, **C** is the only column selectivity parameter that is significantly affected by TEA addition at low pH. A logical interpretation is that a buffered, pH 2.8 mobile phase results in complete ionization of the TEA. If that is so, TEA can interact with ionized silanols, thereby suppressing the negative charge on the column – with a decrease in **C**. Protonated TEA should not be sorbed appreciably by the nonpolar ligands, nor hydrogen-bond to non-ionized silanols, i.e. values of **H**, **S***, **A** and **B** should remain unchanged (unlike the case above [16] where an unbuffered mobile phase was used). At pH 7, the average change in **C** is somewhat smaller (−0.17), which may reflect the greater ionization of silanol groups at higher pH (with a proportionately smaller effect on **C** from added TEA), as well as possibly reduced ionization of the TEA.

Because the average increase in **C** for type-A versus type-B columns is 0.7–1.0 (Table 3), and the decrease in **C** for type-A columns after addition of 10 mM TEA is only about 0.3 units, it appears unlikely that TEA addition to type-A columns can lower the value of **C** enough to eliminate the difference in **C**-values for type-A versus type-B columns (see the further discussion of Section 4.5.1).

4.5. Comparing columns in terms of selectivity

The major goal of the present series of studies is to facilitate the convenient selection of a replacement column, i.e. a column that can provide chromatographically equivalent sample resolution and run time as the column which it replaces—with no change in separation conditions. While column efficiency (plate number N) should be comparable for two "equivalent" columns, similar values of k and α are usually a more critical requirement. Average values of k for different columns will be approximately proportional to values of k_{ref} (values of k for ethylbenzene) and retention time can be further adjusted by a change in flowrate. This suggests that the value of k_{ref} for the replacement column should not differ by more than about ±25%.

The most critical property in determining column equivalency is selectivity. A replacement column with identical values of **H**, **S***, etc. as the original column will be equivalent in terms of selectivity. However, identical values of **H**, **S***, etc. for two different columns are both unlikely and unnecessary for "equivalent" separations of a given sample. We have previously developed a quantitative means of comparing the selectivity of two columns 1 and 2 in terms of the function F_s [4], where:

$$F_s = \{[f_{ch}(\mathbf{H}_2 - \mathbf{H}_1)]^2 + [f_{cs}(\mathbf{S}^*_2 - \mathbf{S}^*_1) + [f_{ca}(\mathbf{A}_2 - \mathbf{A}_1)]^2 + [f_{cb}(\mathbf{B}_2 - \mathbf{B}_1)]^2 + [f_{cc}(\mathbf{C}_2 - \mathbf{C}_1)]^2\}^{1/2} \quad (4)$$

Here, $\mathbf{H}_1$ and $\mathbf{H}_2$ refer to values of **H** for columns 1 and 2, respectively (and similarly for values of **S***, **A**, etc.), and the weighting factors f_{ch}, f_{cs}, etc. have the following approximate values: $f_{ch} = 12.5$, $f_{cs} = 100$, $f_{ca} = 30$, $f_{cb} = 143$ and $f_{cc} = 83$. It was found [4] that if $F_s \leq 3$ for two columns, they are likely to provide equivalent selectivity and separation for different samples and conditions. The application of Eq. (4) to the 87 ("non-special") type-B columns described in [4] suggests for a mobile phase pH = 2.8 that on average there will be two other type-B columns with $F_s \leq 3$ which could serve as equivalent replacements in terms of selectivity. However, for 36% of these type-B columns there are no replacement columns with $F_s \leq 3$ (which means that for many type-B columns, there are >2 other columns with $F_s \leq 3$). Strikingly, a similar comparison among the 43 type-A columns of Table 1 (including "special" columns #83–87) yields no two columns with $F_s \leq 3$, suggesting that type-A columns differ much more from each other than do type-B columns (as suggested in Section 4.2). Consequently, the probability of finding two equivalent type-A columns for a given sample appears low.

4.5.1. Replacing a type-A column with an "equivalent" type-B column

Aside from lot-to-lot changes in column selectivity, or the non-availability of a previously used column, there are several possible reasons one might want to change from an older, type-A column to a newer type-B column: to achieve higher values of N or improved peak shape, better batch-to-batch reproducibility [12], improved column stability, better resolution or a shorter run time. For the same reasons, a change from a type-B to a type-A column would normally not be considered. When the 43 type-A columns of Table 1 are compared with the 87 type-B columns of [4] by means of Eq. (4), it is found that $F_s \leq 3$ for only one type-A column (Hypersil BDS C_{18}, which is equivalent to type-B column #27 of [4]). For every other type-A column of Table 1, it appears that no equivalent type-B column exists with $F_s \leq 3$.

However, values of F_s calculated from Eq. (4) are "worst case" values that assume that the sample contains every possible kind of compound. As discussed in [4], if acids, bases and other ionic solutes are absent from the sample, values of

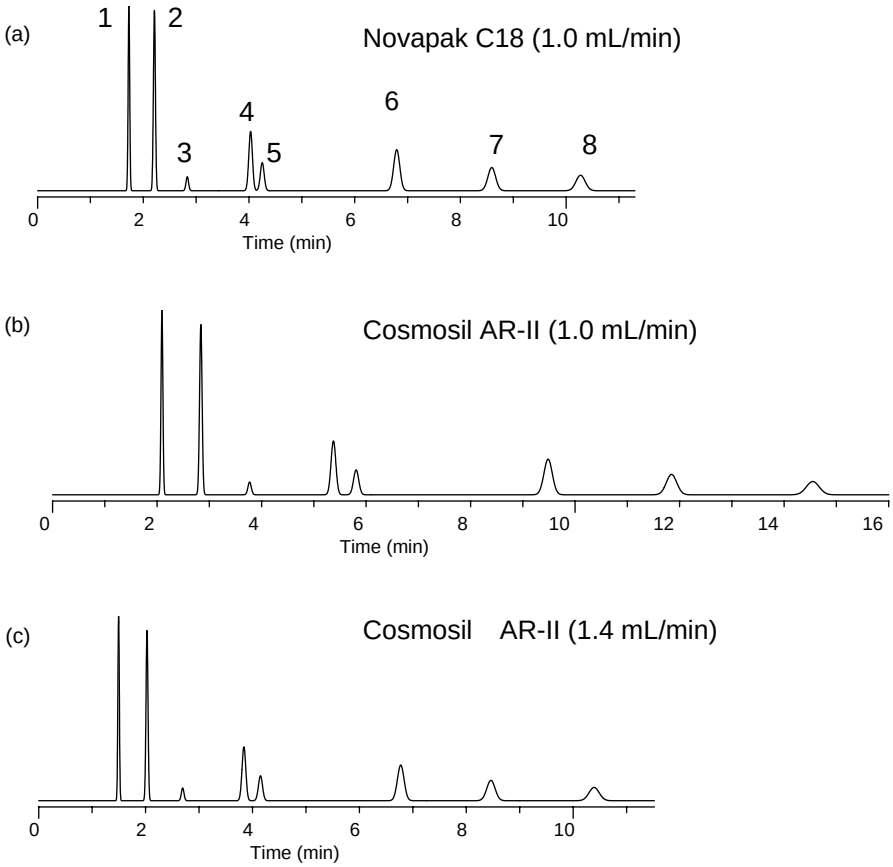

Fig. 3. An "equivalent" column for Nova-Pak C_{18}, assuming a sample without acidic or basic solutes. Sample: (1) *N*,*N*-diethylacetamide; (2) 5,5-diphenylhydantoin; (3) acetophenone; (4) 5-phenylpentanol; (5) anisole; (6) toluene; (7) *cis*-4-nitrochalcone; (8) *trans*-chalcone. Experimental conditions for each separation are the same (except for flowrate) and are given in Section 3; columns and flowrate identified in the figure. See text for details.

B and **C** no longer affect column selectivity, and the function F_s then simplifies to:

$$F_s(-\mathbf{B}, -\mathbf{C}) = \{[f_{ch}(\mathbf{H}_2 - \mathbf{H}_1)]^2[f_{cs}(\mathbf{S}^*_2 - \mathbf{S}^*_1)]^2 + [f_{ca}(\mathbf{A}_2 - \mathbf{A}_1)]^2\}^{1/2} \quad (5)$$

Values of $F_s(-\mathbf{B}, -\mathbf{C})$ are generally much smaller than values of F_s, which means that the possibility of finding an equivalent replacement column (i.e. with $F_s \leq 3$) for a sample that is free of acids and bases becomes much greater. For samples that contain neither acids nor bases, the percentage of equivalent type-B columns (with $F_s \leq 3$) increases from 70 to 94%, and from 0 to 90% for type-A columns.

As an example, consider the separation of a mixture of neutral solutes by a Nova-Pak C_{18} column (Fig. 3a). The best match of the latter type-A column with a type-B column via Eq. (4) gives $F_s = 20.3$, i.e. there is no equivalent type-B column for samples that contain acids, bases and neutrals. However, Eq. (5) applies for the example of Fig. 3 (no acids or bases in this sample), and a comparison of $F_s(-\mathbf{B}, -\mathbf{C})$ values for the latter type-A column with various type-B columns yields a good potential match: Cosmosil AR-II with $F_s(-\mathbf{B},-\mathbf{C}) = 1.2$ and similar k_{ref} values (6.5 and 8.1, respectively). As seen in 3b, the resulting separation on the Cosmosil column provides a reasonable match in terms of selectivity, but the run time for the Cosmosil column is somewhat longer. An increase in flowrate for the Cosmosil column from 1.0 to 1.4 ml/min (Fig. 3c) eliminates the run time difference, giving a final separation that is quite close to that of Fig. 3a for the Nova-Pak column. A caveat to keep in mind when comparing type-A and -B columns for any sample is the greater error in such comparisons (±8% in α), versus comparing two type-A or two type-B columns (±1–3% in α) (Section 4.1.2).

It should be noted in passing that if two columns (A and B) have equal values of F_s relative to a third column (C), it cannot be concluded that columns A and B must be similar to each other. Thus, column A might differ from C in terms of the column parameter **H**, while column B might differ from C in terms of $\mathbf{S}^*$, in which case columns A and B would differ from each other in terms of both **H** and $\mathbf{S}^*$.

5. Conclusions

Column selectivity for type-B RP-LC columns can be described (±1% in α, 1 S.D.) by five column parameters

[4], i.e. values of **H**, **S***, **A**, **B** and **C** in Eq. (2):

$$\log(k/k_{ref}) \equiv \log\alpha = \eta'\mathbf{H} - \sigma'\mathbf{S^*} + \beta'\mathbf{A} + \alpha'\mathbf{B} + \kappa'\mathbf{C} \quad (2)$$

The application of Eq. (2) to retention data for 38 type-A alkyl-silica columns resulted in a poorer fit with Eq. (2) (±8% in α). Resulting values of **H**, **S***, etc. were obtained for each type-A column, allowing comparisons of column selectivity for these columns with each other and with the 92 type-B columns reported previously [4]. The greater error in Eq. (2) when applied to type-A columns appears due to the approximate nature of Eq. (2), combined with substantial differences in the properties of type-A versus type-B columns. That is, the five terms of Eq. (2) appear to account for all solute-column interactions that contribute significantly to retention and column selectivity for type-A columns, and values of **H**, **S***, etc. quantitatively define column selectivity for both type-A and -B columns.

Column selectivity depends on various properties of the stationary phase. On average, type-A columns differ from type-B columns in two respects: greater silica acidity and lower ligand concentrations for type-A columns. As a result, values of **H** (hydrophobicity) and **S*** (steric hindrance) tend to be smaller for type-A columns, while **A** (hydrogen-bond acidity) and **C** (cation-exchange activity) are usually much larger. Values of **B** (hydrogen-bond basicity) are somewhat greater, for reasons discussed in the following paper [7]. The addition of triethylamine to low-pH buffered mobile phases results in a decrease in values of the column parameter **C**, while values of the other column parameters are essentially unchanged. Apparently, ionized TEA interacts with ionized silanols so as to reduce the negative charge on the column.

The selectivity of bonded-zirconia columns is quite different from that of other RP-LC column types reported by us [1–4,7]. Bonded-zirconia columns are characterized by very low hydrogen-bond acidity (low values of **A**) and very high cation-exchange activity (large values of **C**). These properties can be reconciled with the stationary-phase composition of these columns, i.e. an absence of acidic silanols and the presence of cation-exchange sites due to phosphate adsorption. The agreement of retention data for bonded-zirconia columns with Eq. (2) was found to be poor (±41% in α), so resulting values of **H**, **S***, etc. for these columns must be considered as approximate.

A previously derived function (F_s [4]) can quantitatively compare any two columns in terms of selectivity, based on their respective values of **H**, **S***, etc. Two columns with $F_s \leq 3$ should give "equivalent" separations for any sample or experimental conditions. For samples that contain acids and bases, as well as neutral solutes, it is possible to find "equivalent" columns for about 70% of the type-B columns described in [4]. Because type-A columns as a group are much more diverse in terms of values of **H**, **S***, etc. there is a much smaller likelihood of matching two columns in terms of selectivity, when one or both columns are type-A. However, for samples of restricted composition (e.g. containing no acidic or basic solutes), the possibility of finding "equivalent" type-A and -B columns becomes much greater (90 and 94% probability for a type-A or -B column, respectively).

6. Nomenclature

The following list contains all symbols defined in the present and following papers (Parts V and VI). Reference to a defining equation (e.g. Eq. V-4) indicates both the paper (e.g. Part V) and equation number (e.g. 4).

a, *b*, ...	fitting constants
A	column hydrogen-bond acidity, related to number and accessibility of silanol groups in the stationary phase; also "type-A" column based on metal-containing silica; Eq. V-2
$\mathbf{A}_b$	average value of **A** for 87 type-B columns in [4] (columns #1–82c); Eq. V-A.1
B	column hydrogen-bond basicity; also "type-B" column based on pure silica; Eq. V-2
$\mathbf{B}_b$	average value of **B** for 87 type-B columns in [4] (columns #1–82c); Eq. V-A.1
C	column cation-exchange activity, related to number and accessibility of ionized silanols in stationary phase; Eq. V-2
$\mathbf{C}_b$	average value of **H** for 87 type-B columns in [4] (columns #1–82c): Eq. V-A.1
C(2.8)	value of **C** for pH = 2.8 (Eq. V-3)
C(7.0)	value of **C** for pH = 7.0 (Eq. V-3)
C_L	ligand concentration (μmol/m^2)
d_{pore}	particle pore diameter (nm)
EPG	embedded or end-capping polar group
f_{ch}, f_{cs}, etc.	weighting factors in Eq. V-4
F_s	column selectivity comparison function; a function of differences in **H**, **S***, **A**, **B** and **C** for two columns (Eq. V-4); assumes a sample that contains acidic and basic solutes
$F_s(-\mathbf{B},-\mathbf{C})$	value of F_s for a sample with no acids or bases present (Eq. V-5)
H	column hydrophobicity; Eq. V-2
$\mathbf{H}_b$	average value of **H** for 87 type-B columns in [4] (columns #1–82c); Eq. V-A.1
$\mathbf{H}_1$, $\mathbf{H}_2$	value of **H** for columns 1 and 2
k	retention factor, equal to $(t_R\text{-}t_0)/t_0$
k_{ref}	value of *k* for ethylbenzene
$k_{2.8}$, $k_{7.0}$	values of *k* for berberine at pH 2.8 and 7.0 respectively (Eq. V-3)
r	correlation coefficient
RP-LC	reversed-phase liquid chromatography
S*	steric resistance to insertion of bulky solute molecules into the stationary phase (Eq. V-2); as **S*** increases, bulky solute

	molecules experience greater difficulty in penetrating the stationary phase and being retained previously; **S** is defined in Eq. V-1 and previously to be equal to $-\mathbf{S}^*$; see Section 2.1
S	equal to $-\mathbf{S}^*$; see Section 2.1
$\mathbf{S}^*_b$	average value of $\mathbf{S}^*$ for 87 type-B columns in [4] (columns #1–82c); Eq. V-A.1
S.D.	standard deviation
TEA	triethylamine
t_0	column dead time (min)
t_R	retention time (min)
α	separation factor for two solutes
α'	solute relative hydrogen-bond acidity, measured for a given mobile phase and temperature
β'	solute relative hydrogen-bond basicity, measured for a given mobile phase and temperature
η'	solute relative hydrophobicity, measured for a given mobile phase and temperature
κ'	relative charge on solute molecule (positive for cations, negative for anions), measured for a given mobile phase and temperature
σ'	relative steric resistance of solute molecule to penetration into stationary phase (σ' is larger for more bulky molecules), measured for a given mobile phase and temperature

Acknowledgements

The present study (including following paper [7]) was supported in part by a Small Business Innovation Research (SBIR) grant from the National Institutes of Health (US Department of Health and Human Services). We are also much indebted for the advice, and critical comments of Dr. Uwe Neue (Waters Corp.) and Dr. Colin Poole (Wayne State University), as well as the support of the various manufacturers who donated the columns of Table 1.

Appendix A. The origin of errors in Eq. (1) for type-A columns when using solute parameters derived for type-B columns

Although terms i–v of Eq. (2) have a physico-chemical basis, similar to that of the well known solvation equation [17], Eq. (2) is completely empirical and therefore approximate. Approximate relationships of this kind are most accurate for systems (e.g. columns) which do not vary much from each other (and especially for those systems used to "calibrate" the solute parameters); however, as noted in Sections 2.2 and 4.2 there are marked differences between type-A and -B columns. The results of Section 4.1.1. suggest that the reduced accuracy of Eq. (2) for type-A columns (using best-fit solute parameters for type-B columns) is due to the approximate nature of this equation, rather than a failure to include significant, additional solute-column interactions. If this is so, we should expect to see a correlation between values of S.D. for each column and differences in values of **H**, $\mathbf{S}^*$, etc. for that column versus average values of **H**, $\mathbf{S}^*$, etc. for type-B columns (since the solute parameters of Table 2 were derived from retention data for type-B columns). In fact, such a relationship is found for several different column types:

$$\begin{aligned}\text{S.D.} = &-0.006 - 0.001|\mathbf{H} - \mathbf{H}_b| + 0.030|\mathbf{S}^* - \mathbf{S}^*_b| \\ &+ 0.041|\mathbf{A} - \mathbf{A}_b| + 0.311|\mathbf{B} - \mathbf{B}_b| \\ &+ 0.010|\mathbf{C} - \mathbf{C}_b| \\ &(r^2 = 0.923;\ \text{S.D.} = 0.008) \qquad \text{(A.1)}\end{aligned}$$

Here, $\mathbf{H}_b$, $\mathbf{S}^*_b$, etc. refer to average values of **H**, $\mathbf{S}^*$, etc. for type-B columns [4], and values of **H**, $\mathbf{S}^*$, etc. are values for (a) 87 type-B columns from [4]; (b) 38 type-A columns from the present study, (c) 21 columns with embedded or end-capping polar groups reported in the following paper [7]; (d) five "special" type-B columns from [4] (see Section 4.3.1.), and (e) three bonded-zirconia columns from the present study. Values of S.D. in Eq. i-1 are in each case from regressions versus Eq. (2) which use the type-B solute parameters of Table 2. A plot of the data described by Eq. (A.1) is shown in Fig. 4 (calculated versus experimental values of S.D.; the correlation of

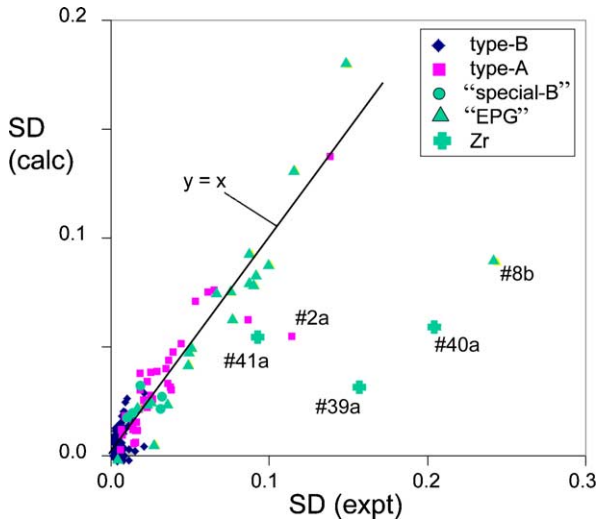

Fig. 4. Correlation of standard deviation (S.D.) values for various columns with Eq. (A.1) (using solute parameter values for type-B columns [4]). Calculated S.D.-values are from Eq. (4); experimental S.D. values are the fit to Eq. (1) as in Table 1. "EPG" refers to columns with an embedded or end-capping polar group [7]; "Zr" refers to bonded-zirconia columns. See Appendix A for details.

Eq. (A.1) omits the five outlier solutes (out of 154) identified in Fig. 4 [#2a,39a–41a,5b]), three of which are for the bonded-zirconia columns.

We believe that steric hindrance plays a major role in limiting the general accuracy of Eq. (2), as well as related relationships for RP-LC retention such as the solvation equation (see discussion of [3]). The importance of steric hindrance in solute-column interactions has also been demonstrated recently for the retention of protonated bases by bonded-zirconia columns [18]. Fig. 2c and d illustrate solute-column interactions that might be affected by steric hindrance (arrows). Changes in ligand density $\mathbf{C}_L$ and silanol acidity for type-A versus type-B columns might each affect solute-column steric hindrance, thereby leading to changes in the configuration of the retained solute molecule and variable solute-column interactions as described by terms i and iii–v of Eq. (2). Eq. (2) and other attempts to describe RP-LC column selectivity generally ignore the effects of variable steric hindrance on these solute-column interactions. As example, consider the hydrogen-bond interaction of a strong acceptor solute (e.g. an aliphatic amide) with a silanol group, as described by term iii ($\beta'\mathbf{A}$) of Eq. (2). In solution (e.g. the mobile phase), there is little difference in the acceptor-strength (β_2^H) of an *N*,*N*-dialkylamide which is substituted by methyl, ethyl or *n*-butyl groups [19,20], whereas acceptor-strength in the stationary phase (β') decreases by a factor of more than four in going from the dimethyl to the dibutyl derivative [3]. That is, with increasing steric hindrance around the amide group, the interaction of this group with a stationary phase silanol becomes weaker. It should also be noted that steric effects similar to these have been observed in solution [21], although to a much smaller degree than in reversed-phase stationary phases.

References

[1] N.S. Wilson, M.D. Nelson, J.W. Dolan, L.R. Snyder, R.G. Wolcott, P.W. Carr, J. Chromatogr. A 961 (2002) 171.
[2] N.S. Wilson, M.D. Nelson, J.W. Dolan, L.R. Snyder, P.W. Carr, J. Chromatogr. A 961 (2002) 195.
[3] N.S. Wilson, M.D. Nelson, J.W. Dolan, L.R. Snyder, P.W. Carr, L.C. Sander, J. Chromatogr. A 961 (2002) 217.
[4] J.J. Gilroy, J.W. Dolan, L.R. Snyder, J. Chromatogr. A 1000 (2003) 757.
[5] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, second ed., Wiley/Interscience, New York, 1997, p. 178–182.
[6] L.C. Sander, S.A. Wise, J. Chromatogr. A 656 (1993) 335.
[7] N.S. Wilson, J.J. Gilroy, J.W. Dolan, L.R. Snyder, J. Chromatogr. A 1026 (2004) 91.
[8] A. Mendez, E. Bosch, M. Roses, U.D. Neue, J. Chromatog. A 986 (2003) 33.
[9] H. Engelhardt, T. Lobert, Anal. Chem. 71 (1999) 1885.
[10] M.R. Euerby, C.M. Johnson, I.D. Rushin, D.A.S.S. Tennekoon, J. Chromatogr. A 705 (1995) 229.
[11] U.D. Neue, E. Serowik, P. Iraneta, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 87.
[12] J. Nawrocki, M.P. Rigney, A. McCormick, P.W. Carr, J. Chromatrogr. A 657 (1993) 229.
[13] M.R. Buchmeiser, J. Chromatrogr. A 918 (2001) 233.
[14] M.A. Stadalius, J.S. Berus, L.R. Snyder, LCGC North Am. 6 (1988) 494.
[15] D. Sykora, E. Tesarova, D.W. Armstrong, LCGC North Am. 20 (2002) 974.
[16] J.H. Park, Y.K. Ryu, J.H. Lim, H.S. Lee, J.K. Park, Y.K. Lee, M.D.D. Jang, J.K. Suh, P.W. Carr, Chromatographia 49 (1999) 635.
[17] C.F. Poole, S.K. Poole, J. Chromatogr. A 965 (2002) 26.
[18] X. Yang, J. Dai, P.W. Carr, Anal. Chem. 75 (2003) 3153.
[19] M.A. Abraham, J.A. Platts, J. Org. Chem. 66 (2001) 3484.
[20] L.C. Tan, P.W. Carr, M.H. Abraham, J. Chromatogr. A 752 (1996) 1.
[21] M.H. Abraham, P.L. Grellier, D.V. Prior, P.P. Duce, J.J. Morris, P.J. Taylor, J. Chem. Soc. Perkin Trans. II (1989) 699.