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Column selectivity in reversed-phase liquid chromatography IV. Type-B alkyl-silica columns

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Abstract

Columns for reversed-phase HPLC (RP-LC) can be characterized by five, retention-related parameters: **H** (hydrophobicity), **S** (steric selectivity), **A** (hydrogen-bond acidity), **B** (hydrogen-bond basicity), and **C** (cation-exchange behavior). In the present study, values of the latter parameters have been measured for 92 type-B (low metals content) alkyl-silica columns and compared to column properties such as ligand length, ligand concentration, pore diameter, and the presence or absence of end-capping. With the exception of five columns of unusual design, retention factors, *k*, for 16 representative test compounds were correlated with values of **H**, **S**, etc., within an average ±1.2% (1 standard deviation, SD), suggesting that all significant solute–column interactions are recognized by these five column parameters. A single-valued function F_s is proposed to measure differences in selectivity for any two RP-LC columns whose values of **H**, **S**, etc., are known. This allows the easy selection of columns whose selectivity is desired to be either similar to or different from a starting column, for application in either routine analysis or method development.

Keywords: Column selectivity; Selectivity; Stationary phases, LC; Alkyl-silica columns, type B; Hydrophobicity; Steric selectivity; Hydrogen bond acidity; Hydrogen bond basicity; Cation-exchange behavior

1. Introduction

Previous work [1–3] suggests that the selectivity of alkyl-silica columns for reversed-phase liquid chromatography (RP-LC) can be characterized quantitatively by means of Eq. (1):

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

Here, *k* is the retention factor of any solute, k_{ref} is the value of *k* for a reference solute (ethylbenzene), and the remaining symbols represent selectivity-related properties of the solute (η', σ', β', α', κ') or 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 (**H**, **S**, etc.) measure the following column properties: **H**, Hydrophobicity; −**S**, Steric resistance to insertion of bulky solute molecules into the stationary phase (similar to, but not the same as ''shape selectivity'' [4]); **A**, column hydrogen-bond Acidity, mainly attributable to non-ionized silanols; **B**, column hydrogen-bond Basicity; and **C**, column Cation-exchange activity due to ionized silanols. Values of **H**, **S**, etc., are relative rather than absolute measures of

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column selectivity, which still allows comparisons of columns selectivity in terms of these parameters (Section 4.3). The parameters η', σ', etc., denote complementary properties of the solute (see Section 6. Nomenclature).

The previous application [1] of Eq. (1) to 90 test solutes and 10 monomeric type-B C_{18} columns yielded a correlational accuracy of ± 0.004 units in $\log \alpha$ ($\pm 1\%$ in α, 1 standard deviation, SD). Terms (i)–(v) of Eq. (1) have been further related to solute structure and properties of the column [3], suggesting that each term of Eq. (1) predominantly represents a single solute–column interaction. Together these findings indicate that (a) all important contributions to column selectivity are represented in Eq. (1) (for the 10 columns originally studied), and (b) five column parameters (**H**, **S**, etc.) completely characterize column selectivity for these 10 columns.

In the present study, we have carried out similar measurements as in Ref. [1] for a more diverse group of alkyl-silica RP-LC columns; i.e., one polymeric and 91 monomeric columns made from type-B (low metal content [5]) silica, columns differing in alkyl chain length (C_3–C_{30}), ligand concentration, particle pore diameter, and the presence or absence of end-capping. Our aim was to further test the applicability of Eq. (1) for the latter columns, and in the process to identify any exceptions to Eq. (1). A further goal was the measurement of values of **H**, **S**, etc., for a large number of different columns, hence providing chromatographers with a practical basis for the selection of columns of either similar or different selectivity.

2. Background and theory

2.1. Derivation of Eq. (1)

The original development of Eq. (1) can be summarized as follows. Initially, it was recognized that the main contribution to RP-LC retention is due to hydrophobic interaction between solute and column (term (i) of Eq. (1)). For many solutes, especially non-ionized, less polar molecules of similar ''shape'', hydrophobic interactions account almost completely for solute retention and values of *k*. For these so-called ''ideal'' solutes [1], values of α can be approximated within ± 2–3% by:

$$\log \alpha \approx \eta' \mathbf{H} \quad (2)$$

Eq. (2) allowed a determination of *relative* values of **H** for each of the 10 columns in the original study. Eq. (2) can also be applied to retention data for other ''non-ideal'' solutes in order to isolate contributions Δ to retention and selectivity which arise from terms (ii)–(v) of Eq. (1); i.e., for non-ideal solutes,

$$\Delta = \log \alpha - \eta' \mathbf{H} \quad (3)$$

The application of Eq. (3) to values of *k* for solutes of diverse molecular structure allowed the identification of four solute groups, for each of which values of Δ depend mainly on just one of terms (ii)–(v) of Eq. (1). Average values of Δ for each of the latter solute groups were then determined for each column and equated to relative values of the column parameters **S**, **A**, **B,** and **C** of Eq. (1). Once values of **H**, **S**, etc., were determined in this way for each of the 10 columns of the original study, the solute parameters η', σ', etc., could be determined by multiple regression of values of $\log \alpha$ versus **H**, **S**, etc.

Solute retention (as described by Eq. (1)) depends on the solute, column and separation conditions (mobile phase composition and temperature). Eq. (1), which is based on properties of the solute and column, could assume that *either* the solute parameters (η', σ', etc.) *or* column parameters (**H**, **S**, etc.) change with conditions. Because the primary goal of the present study is the classification of columns according to selectivity, it is logical to allow values of η', σ', etc., to vary with conditions—rather than values of **H**, **S**, etc. Such a convention is compatible with both theory and experiment [2], leads to no decrease in the reliability of Eq. (1), and is much more practical for purposes of selecting columns of either similar or different selectivity, *regardless* of separation conditions. Because a change in mobile phase pH changes silanol ionization and the negative charge on the column, the column parameter **C** varies with mobile phase pH (see Section 3.6). Values of **H**, **S**, etc., are otherwise constant for any sample or separation conditions.

2.2. Practical application of Eq. (1)

For a reversed-phase liquid chromatography (RP-

LC) assay that is to be used over several months or years, replacement columns with equivalent selectivity must be available during that time. ''Equivalent'' selectivity implies differences in individual separation factors α of $\leq$3% [6]. The need for equivalent column selectivity means that the supplier should be able to guarantee batch-to-batch column uniformity, or the user must be able to locate a column with equivalent selectivity from another source. In either case, column selectivity must be measurable in such a way that changes $>$3% in α for two columns (and any sample or separation conditions) can be easily and reliably anticipated. Thus, if a *reliable* column characterization based on appropriate test solutes (*not* the sample of interest) and a standard set of separation conditions shows two columns to be equivalent, those two columns should give similar separations for other samples and separation conditions.

For method development, as opposed to the routine use of an RP-LC assay, columns of very *different* selectivity may be needed in order to achieve acceptable sample resolution [7]. The availability of a quantitative description of column selectivity for different commercial columns would allow the user to select one or more columns for a maximum change in selectivity. Columns of very different selectivity are also required for the development of *orthogonal* separations, which serve to minimize the possibility of some unexpected sample component overlapping a peak of interest—and hence being overlooked. In either of these two method development situations, there is less need for a *precise* measurement of differences in column selectivity, as compared to a requirement for equivalent columns in routine analysis. That is, the use of Eq. (1) to identify equivalent columns requires deviations in Eq. (1) of $\leq$3% in α, whereas the identification of columns of very *different* selectivity (for method development) can be achieved despite greater errors in Eq. (1) (or uncertainty in values of **H**, **S**, etc.) for a given column.

Until recently, it appears that no general column test or tests have been described which can guarantee that two columns will give equivalent separations for any sample or experimental conditions [8]. On the basis of recent work for 10 different C_{18} columns [1–3], we believe that the column parameters of Eq. (1) *are* able to define column selectivity with the accuracy and precision required for the recognition of equivalent column selectivity. What remains to be shown is the applicability of Eq. (1) for a wider range of RP-LC columns, e.g., the 92 type-B alkyl-silica columns of the present study. At a later time, results will be reported for other column types; e.g., columns made from type-A silica, columns with an embedded or end-capped polar group, phenyl and cyano columns, etc.

2.3. Some potential complications in the use of Eq. (1) for characterizing column selectivity

If Eq. (1) accurately describes retention for any RP-LC column, values of η', σ', etc., reported in Ref. [1] can be used (multiple regression via Eq. (1)) to measure values of **H**, **S**, etc., for other columns. However, certain issues must first be addressed. Thus, values of k obtained in Ref. [1] and used there to derive values of η', σ', etc., employed acetonitrile–water (50%, v/v) as mobile phase for non-ionizable solutes, and acetonitrile–buffer (50%, v/v) for acidic or basic solutes. For reasons of convenience, it is preferable to carry out measurements (as in the present study) with a single organic/buffer mobile phase. Therefore, it is necessary to correct values of η', σ', etc., reported in [1–3] for the use of buffered mobile phases in the present study (Appendix A). We also assumed [1–3] that virgin columns stored as received from the manufacturer would maintain their original retention properties over the 2 years during which the data of [1–3] were collected. We have since found that small changes in k and derived values of **H**, **S**, etc., can occur during long-term storage of the column as in the study of [1–3]. Similar changes in stored columns with time have been reported by others [9] and also confirmed to us by one column manufacturer.

Finally, as discussed below (Section 3.5), column equilibration in RP-LC separation can be slower than previously appreciated, which also contributed to some uncertainty in values of k and related solute parameters reported previously [1,3]. The collective impact of the above three considerations is that values of η', σ', etc., reported in Refs. [1–3] require minor revision for the accurate measurement of values of **H**, **S**, etc., via Eq. (1). In the present study,

we have redetermined values of η', σ', etc., for a select group of test solutes, based on data for a large number of RP-LC columns. In this way it was possible to (a) measure values of **H**, **S**, etc., for these 92 columns and (b) assess the general accuracy of Eq. (1) for a wider range of RP-LC column properties.

3. Experimental

3.1. Equipment and materials

These were essentially as described in Ref. [1]. Detection at 205 nm was employed.

3.2. Columns

The columns used in the present study are described in Table 1. Columns were 15×0.46 cm with 5-μm particles, if available. One to three columns of each type were the generous gift of the manufacturer.

3.3. Samples

Eighteen test solutes were distributed among seven sample mixtures, as summarized in Table 2. The very different retentions of solutes within a given mixture minimized the possibility of band overlap or reversal when these mixtures were separated on different columns. The sample mixtures of Table 2 contain 50 μg/ml of each solute, and 10 μl volumes were injected (500 ng). Values of the separation factor α were determined for 16 solutes (exclusive of thiourea) and interpreted in terms of Eq. (1).

3.4. Procedure

Separations were carried out with two different mobile phases, having pH values of 2.80 and 7.00, respectively. Other conditions were a temperature of 35 °C, and a flow-rate of 2.0 ml/min for 15×0.46-cm columns. Flow rates were changed if necessary for columns of other dimensions to maintain acceptable pressure. The mobile phase consisted of acetonitrile–buffer (50%, v/v) (equal volumes of acetonitrile and buffer were combined). The buffer was 60 m*M* potassium phosphate, and its pH (either 2.80 or 7.00) was adjusted prior to addition of acetonitrile by combining 60 m*M* mixtures of phosphoric acid with monobasic potassium phosphate (for pH 2.8) or dibasic potassium phosphate (for pH 7.0). The resulting phosphate concentration in the final mobile phase was 30 m*M*.

Measurements were initially carried out with the pH 2.8 mobile phase. Prior to sample injections, each column was filled with pH 2.8 mobile phase and stored for 16–24 h just prior to use. After connection of the column to the HPLC system, the column was further flow-equilibrated for 20 min, followed by injection of the seven samples of Table 2 at intervals of 10 min (in a few cases, longer run times were required). Injection of mixture #1 was then repeated. Following retention measurements for the pH 2.8 mobile phase, the column was equilibrated with the pH 7.0 mobile phase for 20 min and sample #4 (berberine) was injected in triplicate.

3.5. Column equilibration

When carrying out isocratic measurements of retention time in RP-LC systems, the retention time of each sample component usually becomes constant (±0.002 min) after 10–20 min of column equilibration (flow of mobile phase through the column). However, column equilibration can require a much longer time for the combination of ionized solutes, low-pH mobile phase, and certain commercial alkyl-silica columns. An example is shown in Fig. 1, for values of k as a function of injection time in the case of the ionized strong base, amitriptyline (a), and the neutral solute ethylbenzene (b). We have observed a similar slow equilibration (same sample and conditions) for eight of 19 commercial alkyl-silica columns. When another Symmetry C_{18} column was first flushed with pH 2.8 mobile phase and stored for 16 h (‘‘static’’ equilibration), reattachment of the column to the system followed by repeated injections of amitriptyline over a 9-h interval gave constant values of $k = 0.363 \pm 0.003$ (1 SD; 14 injections). In the present study, all columns were subjected to ‘‘static’’ equilibration for 16–24 h prior to the collection of data at pH 2.8. As a check on complete equilibration for each of the columns of Table 1, mixture #1 of Table 2 (containing amitriptyline) was injected at intervals of 20 and 90 min after prior,

Table 1
Properties and selectivity of columns used in the present study

Column	Properties		Selectivity parameters							SD
	d_{pore} [a]	C_L [b]	**H**	**S**	**A**	**B**	**C**(2.8)	**C**(7.0)	log k_{ref}	
Agilent										
1. Zorbax RX-C_8 [c]	8	2.0	0.792	0.076	0.117	0.018	0.012	0.948	0.703	0.006
2. Zorbax Rx-18[c]	8	3.5	1.077	−0.040	0.310	−0.037	0.096	0.415	0.886	0.010
3. Zorbax StableBond 80A C3[c]	8	2.0	0.601	0.124	−0.080	0.038	−0.084	0.810	0.450	0.011
3a. Zorbax StableBond 80A C_8[c,d]	8	2.0	0.795	0.079	0.138	0.018	0.014	1.020	0.710	0.006
4. Zorbax StableBond 80A C_{18}[c]	8	2.0	1.008	0.021	0.215	−0.002	0.077	0.822	0.884	0.003
5. Zorbax StableBond 300A C3[c]	30	2.0	0.526	0.122	−0.194	0.047	0.057	0.711	−0.151	0.012
6. Zorbax StableBond 300A C_8[c]	30	2.0	0.701	0.085	0.002	0.047	0.146	0.820	0.106	0.008
7. Zorbax StableBond 300A C_{18}[c]	30	2.0	0.906	0.050	0.045	0.043	0.253	0.700	0.344	0.009
8. Zorbax Eclipse XDB-C_8	8	3.8	0.919	−0.025	−0.219	−0.008	0.003	0.012	0.823	0.008
9. Zorbax Eclipse XDB-C_{18}	8	4.0	1.077	−0.024	−0.062	−0.033	0.055	0.089	0.958	0.005
Akzo-Nobel										
10. Kromasil 100-5C4	11	3.8	0.734	−0.002	−0.334	0.015	0.009	−0.003	0.700	0.005
11. Kromasil 100-5C_8	11	3.7	0.864	−0.013	−0.212	0.019	0.054	−0.001	0.881	0.003
12. Kromasil 100-5C_{18}	11	3.5	1.051	−0.035	−0.070	−0.022	0.039	−0.057	1.098	0.003
Alltech										
13. Alltima C_{18}	10	2.8	0.993	0.014	0.036	−0.013	0.092	0.390	1.062	0.005
Bischoff Chromatography										
14. ProntoSIL 60-5 C_8 SH	6	3.2	0.929	0.015	0.162	−0.017	−0.313	1.005	0.922	0.014
15. ProntoSIL 120-5 C_8 SH	12	3.2	0.739	0.062	−0.081	0.013	0.076	0.526	0.687	0.003
16. ProntoSIL 200-5 C_8 SH	20	3.2	0.761	0.026	−0.194	0.024	0.125	1.443	0.439	0.004
17. ProntoSIL 300-5 C_8 SH	30	3.2	0.739	0.041	−0.130	0.027	0.156	0.405	0.26	0.007
18. ProntoSIL 120-5 C_{18} SH	12	3.0	1.032	−0.018	−0.108	−0.024	0.114	0.403	0.938	0.021
19. ProntoSIL 120-5-C_{18}-AQ	12	2.1	0.974	0.007	−0.083	0.003	0.137	0.224	0.910	0.003
20. ProntoSIL 60-5-C_{18} H	6	2.9	1.158	−0.041	0.067	−0.078	0.102	0.262	1.087	0.021
21. ProntoSIL 120-5-C_{18} H	12	2.9	1.005	−0.008	−0.105	−0.004	0.125	0.987	0.873	0.003
22. ProntoSIL 200-5-C_{18} H	20	2.9	0.956	0.002	−0.121	0.016	0.163	0.218	0.679	0.006
23. ProntoSIL 300-5-C_{18} H	30	2.9	0.956	0.012	−0.089	0.015	0.238	0.249	0.511	0.005
Dionex										
24. Acclaim C_8	12	3.7	0.857	−0.004	−0.274	0.012	0.086	0.016	0.780	0.005
25. Acclaim C_{18}	12	3.2	1.032	−0.018	−0.142	−0.027	0.086	−0.002	1.002	0.003
25a. Acclaim300 C_{18}	30		0.957	0.018	−0.170	0.019	0.261	0.222	0.462	0.006
ES Industries										
26. Chromegabond WR C_8	12	3.5	0.855	−0.025	−0.279	0.024	0.200	0.144	0.554	0.003
27. Chromegabond WR C_{18}	12	3.4	0.979	−0.026	−0.159	−0.003	0.320	0.282	0.732	0.003
GL Sciences										
28. Inertsil C_8-3	10	1.6	0.830	0.004	−0.267	−0.017	−0.334	−0.362	0.849	0.003
29. Inertsil ODS-3	10	1.3	0.990	−0.022	−0.145	−0.023	−0.474	−0.334	1.037	0.004
Hichrom/ACT										
30. Ace5 C_8	10	3.2	0.834	−0.007	−0.218	0.025	0.109	0.145	0.693	0.002
31. Ace5 C_{18}	10	2.6	1.000	−0.026	−0.096	−0.006	0.143	0.096	0.895	0.001
										0.000
Argonaut/Jones Chromatography										0.000
32. Genesis C_8 120A[c]	12	3.68	0.829	0.017	−0.081	0.018	0.055	0.300	0.795	0.006
33. Genesis C_{18} 120A	12	3.87	1.005	−0.004	−0.068	−0.007	0.139	0.125	0.993	0.005
34. Genesis C4 EC 120A	12	3.48	0.646	0.058	−0.330	0.027	0.063	0.400	0.526	0.009

Table 1. Continued

Column	Properties		Selectivity parameters							SD
	d_{pore} [a]	C_L [b]	**H**	**S**	**A**	**B**	**C**(2.8)	**C**(7.0)	log k_{ref}	
35. Genesis EC C_8 120A	12	3.85	0.864	−0.005	−0.173	0.023	0.064	0.142	0.837	0.005
36. Genesis C4 300A	30	4.8	0.615	0.057	−0.397	0.036	0.143	0.249	0.059	0.007
37. Genesis C_{18} 300A	30	3.85	0.975	−0.005	−0.086	0.013	0.266	0.270	0.543	0.005
38. Genesis AQ 120A (C_{18})	12	4.03	0.960	0.036	−0.157	0.007	0.060	0.233	0.981	0.007
MAC-MOD/Higgins Analytical										
39. PRECISION C_8	12	3.1	0.821	0.014	−0.179	0.022	0.095	0.241	0.692	0.002
40. PRECISION C_{18}	12	2.8	1.003	−0.003	−0.041	−0.009	0.079	0.340	0.976	0.002
Merck										
41. Purospher STAR RP18e	12	3.0	1.003	−0.012	−0.070	−0.036	0.018	0.045	1.023	0.003
42. Chromolith RP18e	13	3.6	1.003	−0.029	0.009	−0.014	0.103	0.187	0.493	0.002
Nacalai Tesque										
43. COSMOSIL AR-II (C_{18})	12	3.4	1.017	−0.010	0.127	−0.028	0.116	0.494	0.907	0.006
44. COSMOSIL MS-II (C_{18})	12	2.8	1.031	−0.040	−0.131	−0.014	−0.118	−0.027	0.908	0.003
Nomura										
45. Develosil ODS-UG-5 (C_{18})	14	3.2	0.997	−0.025	−0.145	−0.004	0.150	0.154	0.926	0.004
46. Develosil ODS-HG-5 (C_{18})	14	3.4	0.980	−0.015	−0.171	−0.008	0.187	0.221	0.911	0.002
47. Develosil ODS-MG-5 (C_{18})	10	1.6	0.963	0.036	−0.164	−0.003	−0.012	0.051	1.051	0.011
48. Develosil C30-UG-5 (C_{30})	14	1.8	0.976	0.036	−0.195	0.011	0.158	0.177	0.892	0.015
Phenomenex										
49. Luna C_8(2)	10	5.5	0.889	−0.041	−0.221	−0.001	−0.299	−0.169	0.859	0.003
50. Luna C_{18}(2)	10	3.00	1.002	−0.024	−0.123	−0.007	−0.269	−0.174	0.983	0.003
51. Prodigy ODS (3)	10	3.30	1.023	−0.025	−0.130	−0.012	−0.195	−0.134	1.003	0.002
52. Synergi Max-RP	8	3.21	0.989	−0.028	−0.008	−0.013	−0.133	−0.034	0.976	0.005
53. Luna C5	10	7.85	0.800	−0.030	−0.251	0.003	−0.277	0.115	0.770	0.008
54. Jupiter300 C_{18}	30	5.50	0.945	−0.031	−0.224	0.008	0.234	0.218	0.467	0.005
55. Jupiter300 C5	30	5.30	0.729	−0.021	−0.382	0.016	0.129	0.331	0.183	0.007
56. Jupiter300 C4	30	6.30	0.698	−0.008	−0.426	0.019	0.153	0.142	0.126	0.008
Restek										
57. Allure C_{18}	6	3.6	1.116	−0.04	0.114	−0.044	−0.047	0.066	1.195	0.008
58. Restek Ultra C_8	10	3.6	0.876	−0.030	−0.229	0.018	0.043	0.011	0.883	0.008
59. Restek Ultra C_{18}	10	3.6	1.055	−0.030	−0.068	−0.021	0.009	−0.066	1.101	0.003
Supelco										
60. Discovery C_8	18	3.4	0.832	−0.011	−0.237	0.029	0.119	0.143	0.522	0.002
61. Discovery C_{18}	18	3.0	0.984	−0.027	−0.128	0.004	0.176	0.153	0.683	0.003
62. Discovery BIO Wide pore C5	30	4.1–5.0	0.655	0.019	−0.305	0.029	0.091	0.220	0.059	0.005
63. Discovery BIO Wide pore C_8	30	3.8–4.3	0.840	−0.018	−0.224	0.034	0.206	0.195	0.345	0.003
64. Discovery BIO Wide pore C_{18}	30	3.3–4.0	0.836	−0.014	−0.253	0.028	0.121	0.119	0.528	0.002
ThermoHypersil										
65. Hypersil Beta Basic-8	15	3.9	0.834	−0.016	−0.248	0.029	0.110	0.114	0.619	0.003
66. Hypersil Beta Basic-18	15	3.6	0.993	−0.032	−0.099	0.002	0.163	0.126	0.808	0.003
67. Hypersil Bio Basic-8	30	5.5	0.821	−0.011	−0.232	0.029	0.231	0.211	0.253	0.003
68. Hypersil Bio Basic-18	30	4.9	0.975	−0.025	−0.099	0.007	0.253	0.217	0.512	0.002
69. Hypersil BetamaxNeutral (C_{18})	6	3.0	1.099	−0.035	0.068	−0.031	−0.038	0.012	1.231	0.005
70. Hypurity C_8			0.833	−0.010	−0.200	0.034	0.157	0.161	0.546	0.003
71. Hypurity C_{18}			0.981	−0.020	−0.090	0.002	0.192	0.168	0.744	0.003
Varian										
72. Varian OmniSpher 5 C_{18}^c	11	3.5	1.055	−0.051	−0.033	−0.029	0.122	0.058	1.035	0.008

Table 1. Continued

Column	Properties		Selectivity parameters							SD
	d_{pore} [a]	C_L [b]	**H**	**S**	**A**	**B**	**C**(2.8)	**C**(7.0)	log k_{ref}	
Waters										
73. Symmetry C_8	9	3.55	0.893	−0.05	−0.205	0.021	−0.508	0.283	0.843	0.006
74. Symmetry C_{18}	9	3.17	1.052	−0.063	0.019	−0.021	−0.302	0.162	0.993	0.003
75. DeltaPak C_{18} 100A	10	3.03	1.028	−0.019	−0.017	−0.011	−0.051	0.024	0.956	0.004
76. Xterra MS C_8	12.4	2.75	0.803	−0.004	−0.292	−0.005	0.058	−0.009	0.571	0.006
77. Xterra MS C_{18}	12.5	2.25	0.985	−0.012	−0.142	−0.015	0.134	0.051	0.803	0.003
77a. Symmetry300 C4	25	3.19	0.659	0.017	−0.428	0.014	0.102	0.185	0.157	0.007
78. Symmetry 300 C_{18}	25	3.5	0.984	−0.031	−0.051	0.003	0.228	0.202	0.549	0.002
79. DeltaPak C_{18} 300A	30	3.21	0.955	0.013	−0.104	0.016	0.235	0.286	0.481	0.006
80. Atlantis dC_{18} [e]	9.6	1.52	0.917	0.031	−0.192	0.001	0.036	0.087	0.908	0.008
81. YMC Pro C_8	12.5	3.19	0.890	−0.014	−0.214	0.007	−0.322	0.020	0.814	0.005
82. YMC Pro C_{18}	12.5	2.54	1.015	−0.013	−0.117	−0.006	−0.154	−0.005	0.939	0.008
82a. J'Sphere L80	8	0.9	0.762	0.036	−0.216	−0.001	−0.400	0.345	0.764	0.011
82b. J'Sphere M80	8	1.6	0.926	0.026	−0.123	−0.004	−0.294	0.139	0.957	0.007
82c. J'Sphere H80	8	2.9	1.132	−0.059	−0.023	−0.068	−0.242	−0.161	1.124	0.009
Column (''Special'' type-B columns (see text for details)										
Alltech										
83. Platinum EPS C_8 [c]	10	2.9	0.420	0.152	0.151	0.026	0.509	1.369	0.022	0.018
84. Platinum EPS C_{18} [c]	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	10	≈2.7	0.978	0.028	0.612	−0.038	0.234	– [f]	1.048	0.033

[a] Pore diameter (μm).
[b] Ligand concentration (μmol/m^2).
[c] Not end-capped.
[d] Also labeled ''Zorbax Rx-C_8''.
[e] Formerly called ''Polarity dC_{18}''.
[f] Berberine not eluted from column at pH 7.0.

Table 2
Samples used in present study

Mixture #1	Mixture #2a
Thiourea	Nortriptyline
Amitriptyline	Acetophenone
n-Butylbenzoic acid	Mefenamic acid
Mixture #1a	Mixture #3
N,*N*-Diethylacetamide	*p*-Nitrophenol
5-Phenylpentanol	Anisole
Ethylbenzene	
Mixture #2	Mixture #3a
N,*N*-Dimethylacetamide	Benzonitrile
5,5-Diphenylhydantoin	*cis*-Chalcone
Toluene	*trans*-Chalcone
	Mixture #4
	Berberine

''static'' equilibration. The ratio of k values for the 90- and 20-min injections for all columns was found equal to 1.002±0.007; i.e., essentially constant within the experimental error of such measurements (±0.5%) as determined in the present study. For studies such as the present which rely on precise, repeatable retention measurements, the problem of retention drift as in Fig. 1a represents an important reproducibility issue and is currently the subject of further study in our laboratory.

Retention drift at pH 7.0 was not observed to be a problem.

3.6. Calculations

Retention factors, k, were determined for each solute of Table 2 and each column of Table 1 as

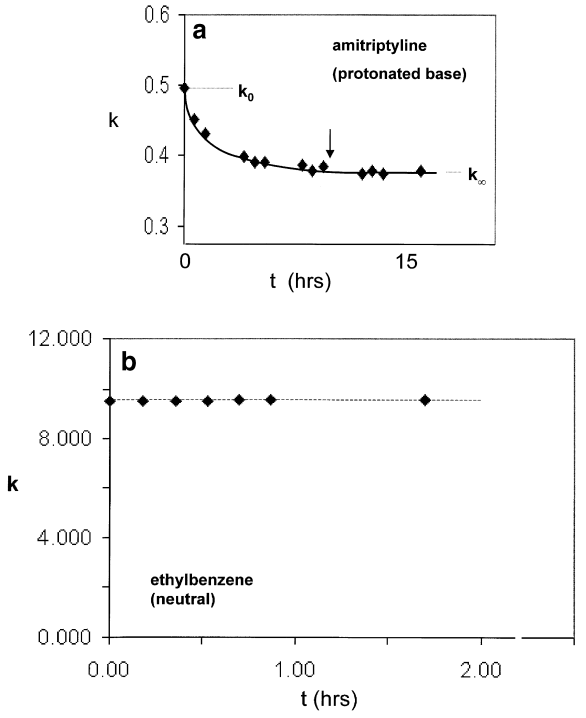


Fig. 1. Equilibration of Symmetry C_{18} column during flow of pH 2.8 mobile phase through column. Retention factor k for amitriptyline (a) and ethylbenzene (b) plotted versus time. Arrow in (a) indicates completion of column equilibration. Experimental conditions were a mobile phase of 50% acetonitrile–buffer, 35 °C, and 1.5 ml/min.

described above; $k = (t_R - t_0)/t_0$, where t_0 is the retention time of thiourea (values of k can be obtained from the authors). Values of the column parameters **H**, **S**, etc., were determined from Eq. (1) by multiple regression of values of $\log \alpha$ for each column versus values of the solute parameters listed in Table 3 (see Appendix A for the derivation of these values). Table 1 summarizes resulting values of the column parameters and the standard deviation of the fit of Eq. (1) to data for each column. Values of **C** at pH 7.0 were determined [2] from:

$$\mathbf{C}(7.0) = \mathbf{C}(2.8) + \log(k_{7.0}/k_{2.8}), \quad (4)$$

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. Applicability of Eq. (1) for the alkyl-silica columns of Table 1

For 10, previously studied C_{18} columns [1,3], we concluded that the accurate prediction of solute retention via Eq. (1) (±1% in α for 90 solutes) is evidence that all significant contributions to column selectivity are accounted for by terms (i)–(v) of this

Table 3
Revised solute parameter values for the compounds of Table 2 (see Appendix A)

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

relationship. In the present study, a wider range in stationary phase compositions was investigated: 91 monomeric and one polymeric type-B alkyl-silica columns with C_3, C_4, C_5, C_8, C_{18} and C_{30} ligands, pore diameters ranging from 6 to 30 nm, varying ligand concentration (0.9–7.9 $\mu mol/m^2$), and with or without end-capping (unverified information supplied by the manufacturer; see Table 1). Also included in Table 1 are a monolithic column (#42) and two hybrid-particle columns (#76,77; XTerra MS C_8 and C_{18}).

For experimental convenience, only the 16 test solutes of Table 3 were used with Eq. (1), versus the 90 solutes used previously [1,3]. However, the solutes of Table 3 include two or more compounds whose retention is primarily determined by each of terms (ii)–(v) of Eq. (1); i.e., the solutes of Table 3 should allow a reasonable test of Eq. (1) for the columns studied.

4.1.1. Monomeric type-B columns

As discussed in Appendix A, solute parameter values were first obtained for solutes #1–16 of Table 3, using columns #1–82c of Table 1. The application of Eq. (1) to retention data for these solutes and columns allowed the calculation of values of **H**, **S**, etc., for each column, and the prediction of experimental values of log α for these solutes and columns. Table 1 lists values of **H**, **S**, etc., and SD (standard deviation) for the fit of values of log α for each column to Eq. (1); the average SD for columns #1–82c was 0.005 log units, or ±1.2% in α. Three columns in Table 1 (#18, 20, 48) have significantly larger SD values (0.015–0.021), corresponding to errors in α of 4–5%. That is, three out of these 85 columns exhibit marginal agreement with Eq. (1). The smaller number (16) of test solutes used in the present study versus the 90 solutes of Refs. [1,3] represents a less stringent test of the validity of Eq. (1) for the columns of Table 1, with less assurance that Eq. (1) has captured all significant contributions to column selectivity. The reader must weigh our results accordingly.

Values of η', σ', etc., reported in Table 3 differ somewhat from values reported in Refs. [1,3], for reasons discussed in Section 2.3 and Appendix A. A comparison of these two sets of solute-parameter values shows reasonable agreement ($0.81 \leq r^2 \leq 1.00$) between old and new values. Differences in values of log α from Eq. (1) which can arise from these differences in values of η', σ', etc., were estimated from the range in values of each column parameter; the resulting change in values of log α is only 0.002–0.004 (1 SD); i.e., not much greater than the experimental repeatability of values of log α (±0.002 units), and well within our target of ±0.012 units, corresponding to ±3% in α.

4.1.2. "Special" columns

In addition to the monomeric type-B columns of Table 1, five alkyl-silica columns of "special" design were also included: (a) a polymeric phase #87, (b) two (intentionally) severely under-bonded packings #83,84, with ligand concentrations of 1.2–1.4 $\mu mol/m^2$, and (c) two proprietary packings #85, 86 described as "... (having) a 15% carbon load leading to a relatively retentive, hydrophobic surface, (which) allows use of 100% aqueous mobile phases without the 'phase collapse' seen on other C_{18} phases." Results for columns #83–87 are summarized in Table 1; the fit of retention data to Eq. (1) ranges from marginal to poor: $0.015 \leq SD \leq 0.039$, corresponding to ±4–9% errors in predicted values of α. The accuracy of Eq. (1) for some of these "special" columns (#83–87) may therefore prove inadequate for the purpose of selecting closely equivalent columns whose values of α for a give sample should agree within ±3%. However, there should be no problem in selecting columns of very different selectivity. The reason for greater errors in the application of Eq. (1) to these "special" columns is now believed due to their greater acidity (large values of **A** and **C**). A fuller discussion will be presented in the following paper of this series (Part V), which deals with type-A alkyl-silica columns.

4.2. Values of *H*, *S*, *A*, *B*, and *C* as a function of column properties

It has been shown [3] that values of **H**, **S**, etc., vary with such properties of the column as ligand length n_C (C_8 versus C_{18}) and concentration C_L ($\mu mol/m^2$), pore diameter d_{pore} (nm), and whether or not the column is end-capped. The most obvious difference in the present alkyl-silica columns is in

the length of the alkyl chain (C_3–C_{30}). Other workers have noted differences in selectivity for C_8 versus C_{18} columns [10], so it is useful to compare values of **H**, **S**, etc., for different ligand lengths; see Fig. 2. For each of these column parameters except **C**, there are apparent trends in parameter values with ligand length, hence justifying an approximate column selectivity classification according to ligand size. However, there is also extensive overlap of these values of **H**, **S**, etc., for different column chain lengths, and in many cases a C_{18} column can appear more similar to a C_8 or even a C_5 column than to another C_{18} column.

The present study provides further information on the relationship of each column selectivity parameter to column properties (Table 1). A simple test of the dependence of values of **H**, **S**, etc., on column properties (n_C, C_L, d_{pore}) is afforded by multiple regression; for example, for column parameter **H**:

$$\mathbf{H} = a + b \log n_C + c \log d_{pore} + d \log C_L + e \text{ (end-capped?)}, \quad (5)$$

where a is a constant and b–d are coefficients which denote the relative effects of n_C, d_{pore}, and C_L on **H**; e is the response of **H** to end-capping—"(end-capped?)" has a value of 0 for non-end-capped columns and 1 for end-capped-columns**.** Similar relationships as for Eq. (5) can be assumed for **S**, **A**, **B** and **C**. The log functions of n_C, d_{pore}, and C_L in

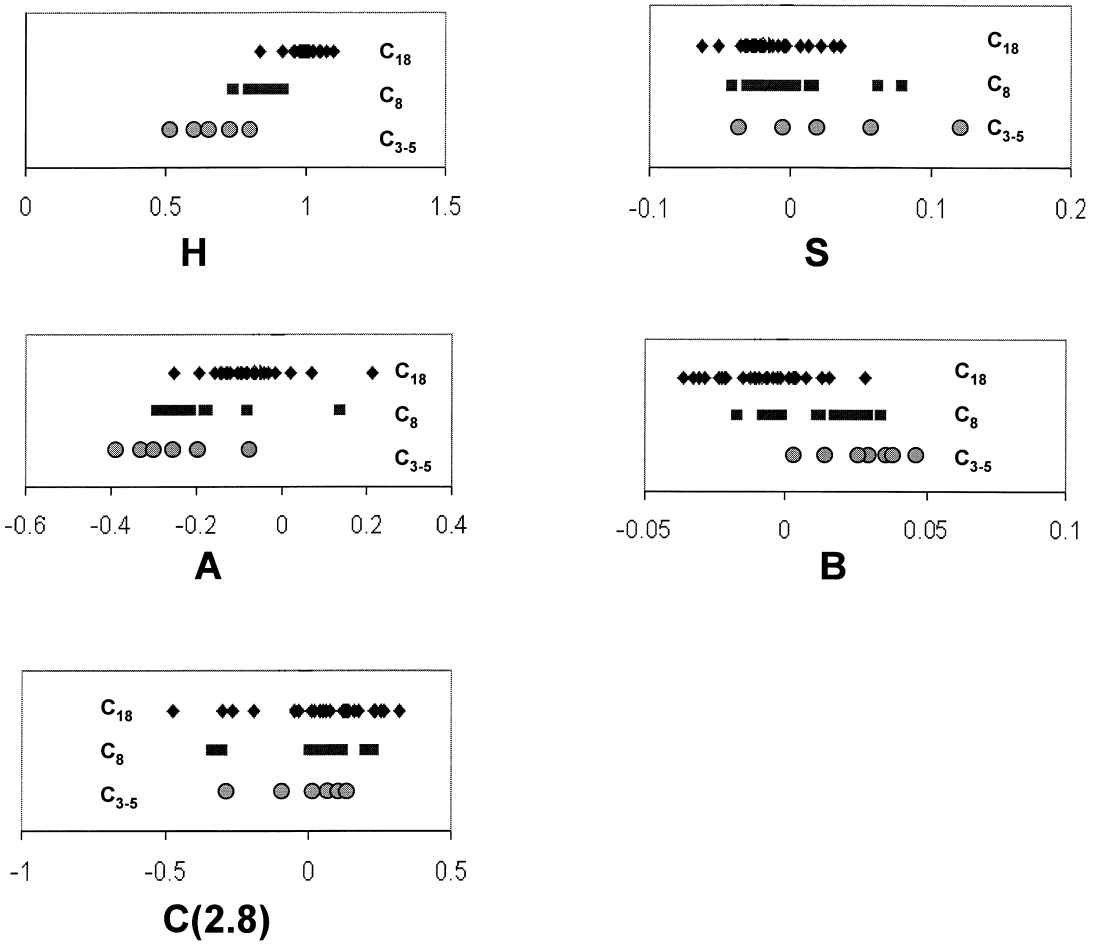


Fig. 2. Column selectivity parameters as a function of ligand length. See text for details.

Eq. (5) were chosen in view of the logarithmic nature of the column parameters **H**, **S**, etc.

Eq. (5) does not take into account differences in the starting silica or bonding process used to make the various columns of Table 1, which can further affect values of **A** and **C**. One means of minimizing the impact on selectivity of differences in silica or bonding process is to compare columns from the same manufacturer. In Table 1, four, three-column sets (each set from the same manufacturer) can be identified in which only one or two column properties vary within each set. In Table 4a,c–e, Eq. (5) is applied to these four column sets. Table 4f groups these 12 columns together, and Table 4b and g present data for column pairs which are identical except for a change in a single column property. In comparing the effects of different column properties on values of **H**, **S**, etc. (Table 4), note that values of *b* (ligand length), *c* (pore diameter), and *d* (ligand concentration) correspond to the effect of a 10-fold change in the property on **H**, **S**, etc. For a (more typical) 2-fold change in the latter column properties, values of *b*–*d* should each be multiplied by 0.3.

The results of Table 4 can be summarized as follows:

(1) **H** (column hydrophobicity) increases with increasing ligand length n_C and concentration C_L, and decreases for larger pore diameters d_{pore}. Similar changes were reported in Ref. [3] for a smaller number of columns and are consistent with other studies, as well as the nature of hydrophobic interaction between solute and column.

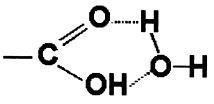
(2) **–S** (increased resistance to penetration of the solute into the stationary phase) increases with increasing ligand length n_C and concentration C_L, and decreases for larger pore diameters d_{pore} (the opposite behavior versus that of **H**). Similar changes were reported in [3] and are consistent with increased resistance to penetration (smaller **S**) for greater ‘‘crowding’’ of ligands in the stationary phase.

(3) **A** (column hydrogen-bond acidity) decreases for end-capped columns, as expected. End-capping removes and/or obstructs silanols (–SiOH), which are responsible for the hydrogen-bond acidity of the column. Other changes in **A** with column properties are discussed in Appendix B.

(4) **B** is not significantly affected by end-capping, which rules out silanols or siloxane groups as a cause of column hydrogen-bond acidity. Changes in **B** with other column properties are in the opposite direction as changes in **H**, which is further confirmed by an inverse correlation of **B** and **H**:

$$\mathbf{B} = 0.13–0.14\mathbf{H} \quad (r^2 = 0.62, \mathrm{SE} = 0.014) \qquad (6)$$

If water molecules serve as stationary-phase acceptor sites, the preferential (and unexpected [3]) hydrogen-bond retention of carboxylic acids versus phenols might be the result of a twofold (therefore stronger) hydrogen-bond interaction of water molecules with a –COOH group:



(I)

(5) **C** (a measure of the negative charge on the column) decreases for end-capped columns, as expected for the removal and/or obstruction of ionized silanol groups. Other changes in **C** with column properties are discussed in Appendix B.

*4.2.1. Comparison of values of **C**(7.0) versus **C**(2.8)*

Because silanol ionization must increase as mobile phase pH increases, the value of **C**(7.0) for a given column should always be greater than **C**(2.8) (recall that the quaternary ammonium compound berberine is used to measure **C**(7.0)); Eq. (4)). In general this is true; the average value of **C**(7.0)–**C**(2.8) for the columns of Table 1 is 0.15. However, several columns have *smaller* values of **C**(7.0), in some cases by as much as 0.08 units (columns #12, 25, 77). The probable reason for this anomaly is the greater buffer cation concentration (K^+) in the pH 7.0 mobile phase versus the pH 2.8 mobile phase. Phosphate concentration was held constant (30 m*M*), which means that K^+ concentration is greater at pH 7.0 versus pH 2.8. Other studies [11] have shown that cationic solutes exhibit decreased retention at pH 7.0 as buffer cation concentration increases, the normal consequence of an ion-exchange retention process. Values of **C**(7.0) reported in Table 1 should therefore be considered *relative* values.

Table 4
Column selectivity parameters as a function of column properties. Correlation of values of **H**, **S**, etc., with Eq. (5) and equivalents (where **S**, **A**, etc., replace **H**). (a) Columns #82a–c; only C_L varies, $\mathbf{H} = a + d \log C_L$ (and similarly for **S**, **A**, etc.); (b) StableBond C_{18}; only C_L varies [1]; (c) Columns #3–7; n_c and d_{pore} vary, $\mathbf{H} = a + b \log n_c + c \log d_{pore}$ (and similarly for **S**, **A**, etc.); (d) Columns #54–56; n_c varies, C_L approximately constant; $\mathbf{H} = a + b \log n_c$ (and similarly for **S**, **A**, etc.); (e) Columns #10–12; n_c varies, C_L approximately constant; $\mathbf{H} = a + b \log n_c$ (and similarly for **S**, **A**, etc.); (f) Columns #3–7, 10–12, 54–5, 82a–c; n_c, d_{pore}, C_L and end-capping vary; $\mathbf{H} = a + b \log n_C + c \log d_{pore} + d \log C_L + e$ (end-capped?) (and similarly for **S**, **A**, etc.); (g) Symmetry C_{18}, end-capped and non-end-capped; only end-capping varies

	H	**S**	**A**	**B**	**C**(2.8)	**C**(7.0)
(a) Columns #82a–c						
r^2	0.766	0.838	1.000	0.789	0.959	0.991
SE	0.077	0.030	0.002	0.025	0.023	0.035
a	0.748	0.040	−0.199	0.003	−0.376	0.314
d (C_L)	0.386	−0.189	0.379	−0.132	0.310	−0.997
(b) StableBond C_{18}						
2.08 mol/m^2	0.998	0.021	0.271	0.006	0.085	
1.79 mol/m^2	0.967	0.042	0.264	0.009	0.05	
Change	0.031	−0.021	0.007	−0.003	0.035	
Approximate *d* (C_L)	0.76	−0.51	0.17	−0.07	0.85	
(c) Columns #3–7						
r^2	0.991	0.960	0.947	0.831	0.991	0.448
SE	0.022	0.010	0.044	0.011	0.014	0.110
a	0.501	0.156	0.005	−0.001	−0.434	1.093
b (n_c)	0.503	−0.112	0.348	−0.028	0.229	0.014
c (d_{pore})	−0.157	0.019	−0.244	0.048	0.261	−0.244
(d) Columns #54–56						
r^2	0.999	0.802	0.995	0.983	0.877	0.001
SE	0.004	0.007	0.011	0.001	0.027	0.134
a	0.465	0.005	−0.600	0.028	0.047	0.225
b (n_c)	0.382	−0.029	0.301	−0.016	0.146	0.007
(e) Columns #10–12						
r^2	0.997	0.979	1.000	0.712	0.384	0.762
SE	0.013	0.003	0.000	0.017	0.025	0.022
a	0.435	0.030	−0.577	0.058	−0.006	0.058
b (n_c)	0.487	−0.051	0.404	−0.058	0.043	−0.085
(f) Columns #3–7, 10–12, 54–5, 82a–c						
r^2	0.933	0.935	0.971	0.731	0.906	0.864
SE	0.046	0.017	0.037	0.018	0.069	0.169
a	0.437	0.163	−0.012	0.012	−0.550	0.792
b (n_c)	0.465	−0.087	0.365	−0.042	0.142	−0.063
c (d_{pore})	−0.195	0.032	−0.298	0.058	0.255	0.127
d (C_L)	0.476	−0.149	0.221	−0.041	0.660	−0.244
e (end-capping)	−0.092	−0.048	−0.353	−0.017	−0.255	−0.643
(g) Symmetry C_{18}						
Non-end-capped	1.03	−0.029	0.388	−0.023	0.038	0.812
End-capped	1.048	−0.057	0.007	−0.004	−0.179	0.151
Change	0.02	−0.03	−0.38	0.02	−0.22	−0.66

Protonated bases often tail at neutral pH, and this has been attributed to the interaction of cationic solutes with ionized silanols [12]. Larger values of **C**(7.0), corresponding to increased ion interaction of ionized bases with the column, would therefore be expected to correlate with increased tailing of cationic solutes at pH 7.0. As summarized in Appendix C, a published ranking of columns according to

''silanol activity'' as measured by peak tailing and resulting lower values of the plate number N at pH 6.0 correlates with values of **C**(6.0) as follows: ''very low'' silanol activity, $\mathbf{C} = -0.02 \pm 0.19$; ''low'' activity, $\mathbf{C} = 0.05 \pm 0.12$; ''moderate'' activity, $\mathbf{C} = 0.46 \pm 0.16$; ''high'' activity, $\mathbf{C} = 1.15 \pm 0.05$. The latter results appear to confirm a relationship at near-neutral pH of band tailing with increased retention as a result of the ionic interaction of protonated bases and ionized silanols.

4.3. Practical comparisons of column selectivity

Given values of **H**, **S**, etc., as in Table 1, any two columns can be compared in terms of selectivity. That is, ''equivalent'' columns should have similar values of **H**, **S**, etc., while columns with very different values of **H**, **S**, etc., will have very different selectivities. For reasons to be discussed, however, we need to know quantitatively how changes in **H**, **S**, etc., affect values of $\log \alpha$ (Section 4.3.1), and it would be convenient if some function of **H**, **S**, etc., can be derived that provides a *single* measure of relative column selectivity (Section 4.3.2).

4.3.1. Dependence of values of log α on *H, S, etc.*

The quantitative dependence of separation factors, α, on values of **H**, **S**, etc., is primarily of interest when we are comparing columns of similar selectivity. In this case, we need to know how large a difference in **H**, **S**, etc., is allowable for some maximum permitted difference in values of α. This is discussed in Appendix D and summarized in Table 5. The second row of Table 5 lists the allowable change in values of each column parameter for an average change in α equal to 1%. This allowed difference in each column parameter varies widely; e.g., from 0.080 units for values of **H**, to 0.007 units for **B**. Differences in values of **H** have a smaller effect on α, because solute hydrophobicity and values of η' correlate with retention; see the discussion of Fig. 4b,c in Ref. [1]. The last row in Table 5 gives the allowable change in values of **H**, **S**, etc., for a maximum allowable variation ($\pm 3\%$) in values of α.

4.3.2. Comparing the selectivity of two columns by means of a single measure

Given values of **H**, **S**, etc., for a large number of commercially available columns, we need a simple procedure for comparing the relative selectivity of any two of these columns. An obvious approach is to plot values of $\log k$ for one column versus another, as in Fig. 3a. From such a plot, relative selectivity can be defined by the standard deviation (SD) of the best fit; for the Inertsil C_8 and Discovery C_8 columns of Fig. 3a, SD = 0.13. The larger is SD, the more different are the columns. Likewise, an SD ≤ 0.012 suggests that changes in α for one column versus the other will be less than 3%; i.e., such columns can be regarded as ''equivalent''. However, the latter approach requires retention data for a large enough number of ''appropriate'' solutes to yield a representative value of SD.

Assuming that values of **H**, **S**, etc., are available for columns under consideration, a more convenient procedure for comparing column selectivity is to visualize columns of different selectivity in terms of a five-dimensional plot in space, the data point for each column being represented by its coordinates (values of **H**, **S**, **A**, **B**, and **C**). We can then define a column selectivity function, F'_s, as the *distance* between two columns (1) and (2) in this five-dimensional plot:

Table 5
Effect of a change in column parameters **H**, **S**, etc., on separation: see text and Appendix D for details

	Absolute change in $\log \alpha_{12}$ for a change in **H**, **S**, etc., by 0.01 unit				
	H	**S**	**A**	**B**	**C**
SD	0.0005	0.0041	0.0012	0.0061	0.00326
Δ(allowed) = allowed change in **H**, **S**, etc.[a]					
For 1% change in α	0.080	0.010	0.033	0.007	0.012
For 3% change in α	0.240	0.029	0.100	0.020	0.037

[a] For a maximum change in $\log \alpha$ by 0.004 (equal to 1% in α); $\Delta(\text{allowed}) = (0.004 \times 0.01)/\text{SD}$.

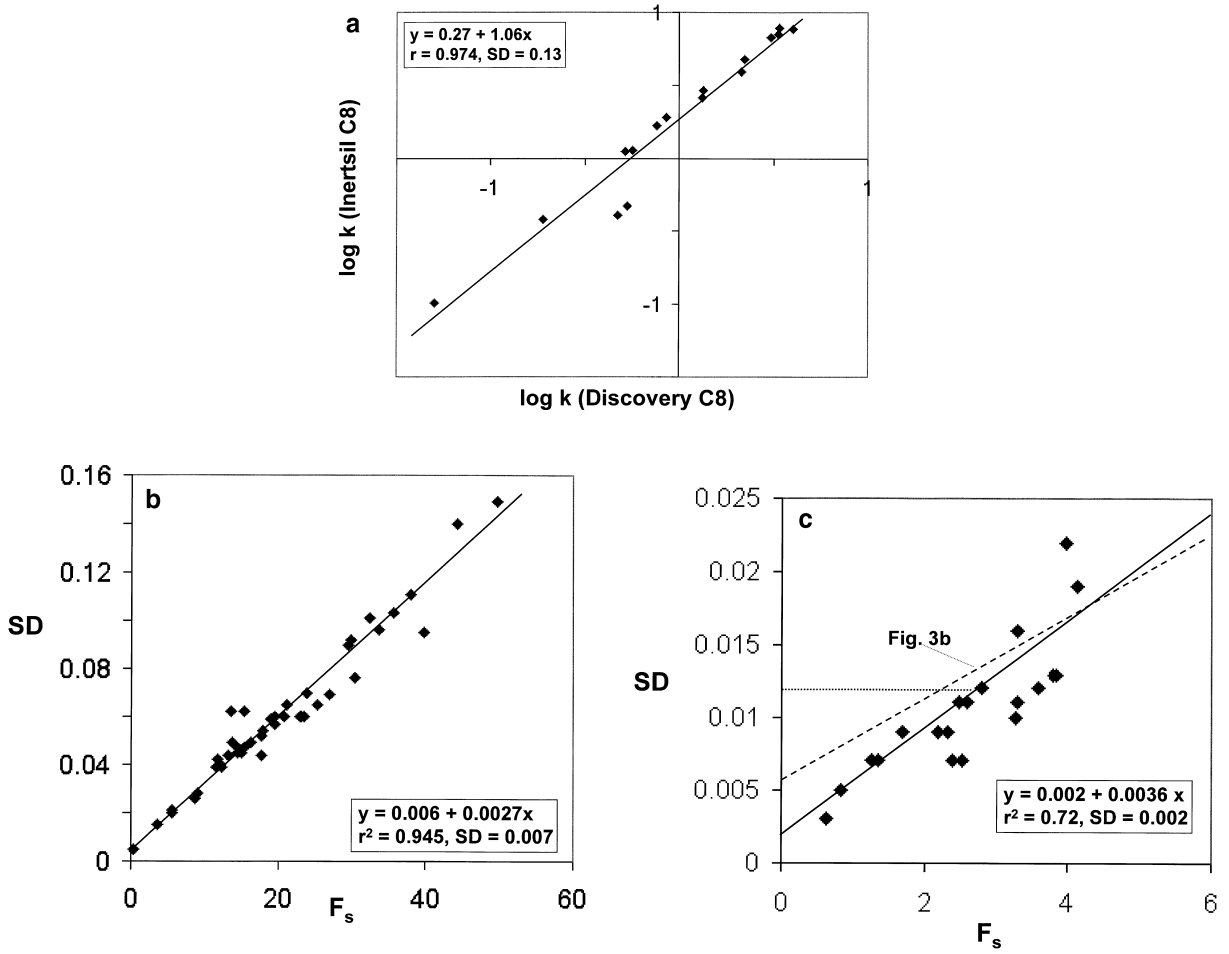


Fig. 3. The selectivity of two columns compared. (a), plots of log k for Inertsil C_8 and Discovery C_8 columns (compounds of Table 2); (b) plot of SD versus F_s for 67 solutes and 10 columns of [1]; (c) plot as in (b) for compounds of Table 2 and selected column pairs from Table 1. See text for details.

$$F'_s = \{(\mathbf{H}_2-\mathbf{H}_1)^2 + (\mathbf{S}_2-\mathbf{S}_1)^2 + (\mathbf{A}_2-\mathbf{A}_1)^2 + (\mathbf{B}_2-\mathbf{B}_1)^2 + (\mathbf{C}_2-\mathbf{C}_1)^2\}^{1/2} \quad (7)$$

Eq. (7) represents a straightforward extension of the Pythagorean theorem. Because the column parameters **H**, **S**, etc., vary in their relative contribution to selectivity (Table 5), the different terms of Eq. (7) must be weighted accordingly:

$$F_s = \{[f_{ch}(\mathbf{H}_2-\mathbf{H}_1)]^2 + [f_{cs}(\mathbf{S}_2-\mathbf{S}_1)]^2 + [f_{ca}(\mathbf{A}_2-\mathbf{A}_1)]^2 + [f_{cb}(\mathbf{B}_2-\mathbf{B}_1)]^2 + [f_{cc}(\mathbf{C}_2-\mathbf{C}_1)]^2\}^{1/2} \quad (8)$$

The individual weighting factors f_{ch}, f_{cs}, etc., are equal to the reciprocal of values of ''$1/\Delta$(allowed)'' from the next-to-last last row of Table 5. Columns of similar selectivity will have small values of F_s, and vice versa for columns of very different selectivity.

A verification of Eq. (8) is shown in Fig. 3b, by means of a plot of SD versus F_s. Data for 67 solutes and 10 C_{18} columns from [1] were used to calculate values of SD from plots of log k for one column versus another, while corresponding values of F_s were determined from values of **H**, **S**, etc., reported in Ref. [1]. A reasonable correlation is noted ($r^2 =$ 0.945). The ability of values of F_s to accurately

measure *small* differences in column selectivity is of special interest (see discussion of Section 2.2). Several columns from Table 1 are similar in terms of values of F_s, and it is of interest to compare SD values for these column pairs with values of $F_{s;}$ see Fig. 3c. The correlation equations of SD versus F_s in Fig. 3b,c differ slightly (dashed versus solid curves in Fig. 3c), which likely reflects experimental uncertainty and the different samples involved in Fig. 3b versus c.

Fig. 3c allows us to estimate the maximum allowable value of F_s for two columns, if they are to provide ''equivalent'' separation; i.e., values of SD≤ 0.012 log units, measured as in Fig. 3a. It appears from Fig. 3c that two columns with $F_s \leq 3$ can be considered ''equivalent''. We can illustrate the significance of values of F_s by some representative separations. Retention data collected in the present study allow us to reconstruct chromatograms of various mixtures of the compounds of Table 3. In Fig. 4a, we take the Discovery C_8 column as example of a starting column. In this case, we have

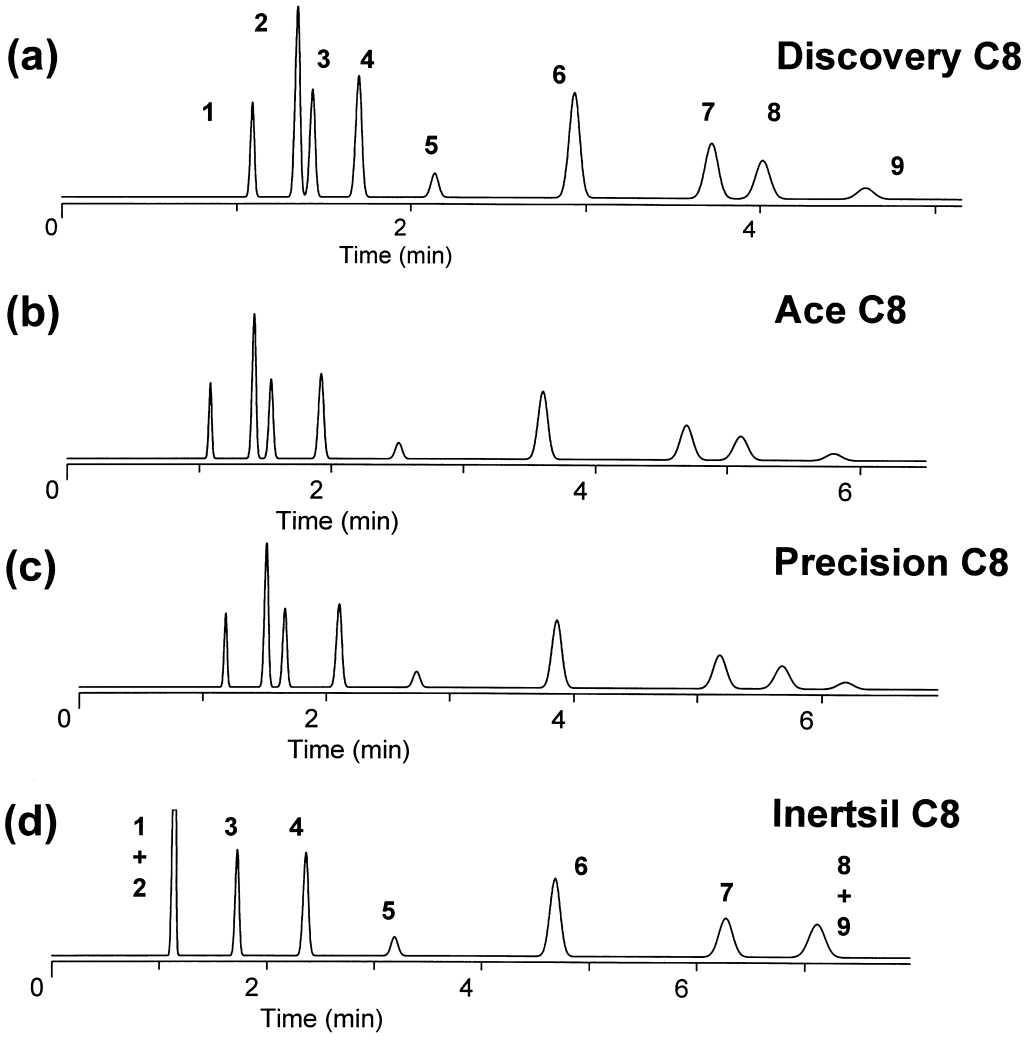


Fig. 4. Comparisons of column selectivity. Samples: (1) *N*,*N*-diethylacetamide; (2) nortriptyline; (3) 5,5-diphenylhydantoin; (4) benzonitrile; (5) anisole; (6) toluene; (7) *cis*-chalcone; (8) *trans*-chalcone; (9) mefenamic acid. See also Table 6. (a–d) Columns identified in the figure. Experimental conditions as in Section 3.

selected a maximum number of sample components that still allow baseline separation of all bands with the Discovery C_8 column. Similar chromatograms (same sample and conditions) are shown in Figs. 4b–d for three other columns. Values of F_s and SD for plots of $\log k$ for each column versus the Discovery C_8 column are given in Table 6. Most people would regard the separations of Figs. 4a–c as ''near equivalent'', despite marginal values of SD equal 0.016 (±4% in α), and F_s equal 4 for the Precision C_8 column. The Inertsil C_8 column of Fig. 4d provides a very different separation from that with the Discovery C_8 column, as expected from its values of $F_s = 38$ and SD = 0.132.

4.3.3. Column selectivity as a function of the sample

The column comparison function F_s assumes that the sample is sufficiently diverse so that all five contributions to column selectivity will be important (hydrophobicity, steric interaction, hydrogen bonding of acids and bases, ion interaction). This will often *not* be the case. For example, if no significantly ionized compounds are present in the sample, the column parameter **C** will likely be unimportant. Similarly, if acidic solutes are absent, the parameter **B** can be ignored. For samples which do not include acids and/or bases, the column comparison function can be modified for a better description of relative column selectivity:

$$\text{(no bases present) } F_s(-\mathbf{C}) = \{[f_{ch}(\mathbf{H}_2-\mathbf{H}_1)]^2 + [f_{cs}(\mathbf{S}_2-\mathbf{S}_1)]^2 + [f_{ca}(\mathbf{A}_2-\mathbf{A}_1)]^2 + [f_{cb}(\mathbf{B}_2-\mathbf{B}_1)]^2\}^{1/2} \quad (9a)$$

Table 6
Evaluation of column selectivity for the separations of Fig. 4

	Column compared with Discovery C_8		
	Ace C_8	Precision C_8	Inertsil C_8
SD	0.008	0.016	0.132
F_s	1	4	38

Comparisons of values of F_s and the standard deviation SD of plots of $\log k$ for one column versus another. In each case, the reference column is Discovery C_8.

$$\text{(no acids present) } F_s(-\mathbf{B}) = \{[f_{ch}(\mathbf{H}_2-\mathbf{H}_1)]^2 + [f_{cs}(\mathbf{S}_2-\mathbf{S}_1)]^2 + [f_{ca}(\mathbf{A}_2-\mathbf{A}_1)]^2 + [f_{cc}(\mathbf{C}_2-\mathbf{C}_1)^2]\}^{1/2} \quad (9b)$$

$$\text{(neither acids nor bases present) } F_s(-\mathbf{B},\mathbf{C}) = \{[f_{ch}(\mathbf{H}_2-\mathbf{H}_1)]^2 + [f_{cs}(\mathbf{S}_2-\mathbf{S}_1)]^2 + [f_{ca}(\mathbf{A}_2-\mathbf{A}_1)]^2\}^{1/2} \quad (9c)$$

Values of the above functions defined by Eqs. (9a)–(9c) will be smaller than F_s, meaning that columns which are judged to be non-equivalent by Eq. (8), because $F_s \gg 3$, may prove to be equivalent ($F_s < 3$) for samples which are free of acids or bases.

4.3.4. Relative importance of different column parameters in controlling selectivity

Given five different contributions to column selectivity (**H**, **S**, etc.), which of these parameters has the greatest potential for creating changes in selectivity and separation? The range in values for each parameter (difference between largest and smallest values) defines the maximum possible change in that parameter. If this range is divided by the Δ(allowed) value from Table 5, we have the maximum change in α from a maximum change in a given column parameter. Thus, relative to the maximum change from a change in **H**, we have the following changes in α for a maximum change in each column parameter:

$$\mathbf{H}\,(1.0) < \mathbf{B}\,(1.9) < \mathbf{S}\,(2.5) < \mathbf{A}\,(3.3) \ll \mathbf{C}(2.8)\,(11.1) \ll \mathbf{C}(7.0)\,(19.9)$$

The contribution of silanols (**A** and **C**) to varying column selectivity is seen to be greatest (3.3–19.9-fold larger change in α versus a change in **H**), which is commonly accepted to be the case. Likewise, ionized silanols (**C**) play the most important role in determining variations in column selectivity, especially for pH > 6 where more silanols are ionized. On the other hand, the ionization of basic solutes decreases at higher pH, which can greatly decrease the importance of **C** in affecting separation; i.e., unless a solute is *completely* ionized, the effect of **C** on the retention of that solute at *any* pH is markedly reduced, because values of κ' decrease sharply with only partial loss of solute ionization [3].

4.4. Comparisons of present and previous measurements of column selectivity

Various means for the measurement of column selectivity have been reported previously [8], based on (a) the solvation parameter model [13], (b) principal component analysis (PCA) [14], and (c) retention data for test solutes believed to measure specific solute–column interactions [15–17]. We have previously compared Eq. (1) with the conceptually similar solvation parameter model [1,3]. Because the solute parameters of Eq. (1) are derived empirically, and because Eq. (1) recognizes two additional contributions to column selectivity ($\sigma'\mathbf{S}$ and $\kappa'\mathbf{C}$), Eq. (1) provides a more accurate and complete description of column selectivity versus the solvation parameter model. PCA can provide a description of column selectivity that is equally detailed and reliable as Eq. (1) [14], but resulting column selectivity parameters cannot be related to the known interactions between solute and column. PCA has also not been extended to allow quantitative comparisons of column selectivity as in Section 4.3. Test solutes deemed to be indicative of various solute–column interactions are commonly used to describe column selectivity, but with the exception of Eq. (1) no attempt has so far been made to show that such measurements can provide a complete characterization of column selectivity.

4.4.1. Previously used test solutes

Values of k or (more commonly) α are commonly used as measures of the various solute–column interactions described by Eq. (1) (terms (i)–(v)). A summary of such measurements for several RP-LC columns was reported by Euerby et. al. [17]. We can compare results for the test solutes reported in [17] with the column parameters reported here for 19 columns which were examined in both studies (#2,3,3a,4,9,12,29,31–33,41,47,51,54,61,70,74,77, 82 of Table 1).

4.4.1.1. Column hydrophobicity (*H*)

Column hydrophobicity is measured in [17] by values of *methylene selectivity* α_{CH2}; α_{CH2} is the ratio of k values for *n*-pentyl- versus *n*-butylbenzene, using methanol–water (80:20%, v/v). Because of the logarithmic nature of values of **H**, we expect that values of **H** will correlate linearly with values of $\log \alpha_{CH2}$. Such a correlation is observed for the present study (columns #1–87 of Table 1) for values of α_{CH2} calculated from the ratio of k values for ethylbenzene and toluene: $\mathbf{H} = -0.27 + 6.28 \log \alpha_{CH2}$; $r^2 = 0.96$, SD = 0.03. The corresponding correlation for the 19 columns reported both here and in Ref. [17] is somewhat poorer: $\mathbf{H} = 0.09 + 5.34 \log \alpha_{CH2}$; $r^2 = 0.77$, SD = 0.06. The latter correlation is likely adversely affected by (a) the use of columns from different lots here and in Ref. [17], (b) the use of a different mobile phase in the two studies (50% ACN–buffer versus 80% methanol–water), and (most important) (c) a wider range in column properties and values of **H** for all 92 columns of Table 1.

4.4.1.2. Other solute–column interactions (*S*, *A*, *B*, *C*)

Retention data for several other test solutes are reported in [17] for columns in Table 1. Shape selectivity is believed to correlate with values of $\alpha_{T/O}$ (the k-ratio for triphenylene versus *o*-terphenyl; 80% methanol–water mobile phase). Silanol hydrogen-bond activity is measured by $\alpha_{C/P}$ for caffeine–phenol (30% methanol–water). Ion-exchange capacity is measured by $\alpha_{A/P}$ for benzylamine–phenol at pH 2.7 and 7.6 (30% methanol–buffer). These four measurements correspond, respectively, to values of **S**, **A**, **C**(2.8) and **C**(7.0). The corresponding correlations between the measurements of [17] and the latter column parameters are summarized in Table 7.

The correlation of **S** with $\log \alpha_{T/O}$ in Table 7 is marginal ($r^2 = 0.40$) but in the right direction ($b = -0.39$). That is, columns which are relatively *less* accessible to the bulky *o*-terphenyl solute (which means larger values of $\alpha_{T/O}$) should have smaller values of **S** — if **S** (''steric intraction'') and $\alpha_{T/O}$ (''shape selectivity'') both measure the same column property. A similarly poor correlation ($r = 0.29$), also in the ''right'' direction, was found [3] for the dependence of **S** on another measure of shape selectivity ($\alpha_{TBN/BaP}$, the ratio of k values for tetrabenzonaphthalene and benzo[*a*]pyrene). It has been shown [3] that ''shape selectivity'' differs in some respects from ''steric selectivity''; shape selectivity is significant for more rigid solute molecules, polymeric stationary phases, and high-organic mo-

Table 7
Correlations of test-solute measurements of Ref. [17] with values of **H**, **S**, **A**, **C**(2.8) and **C**(7.0); $y = a + bx$: see text for details

Correlation	r^2	SE	a	b
H versus log α_{CH2}	0.77	0.06	0.09	5.34
S versus log $\alpha_{T/O}$	0.40	0.04	0.04	−0.39
A versus log $\alpha^{c}_{C/P}$	0.03	0.14	−0.03	0.07
C(2.8) versus log $\alpha_{A/P}$ at pH 2.7	0.70	0.10	0.48	0.43
C(7.0) versus log $\alpha_{A/P}$ at pH 7.6	0.33	0.29	0.50	0.73

$\mathbf{H} = a + b \log \alpha_{CH2}$; $\mathbf{S} = a + b \log \alpha_{T/O}$; $\mathbf{A} = a + b \log \alpha_{C/P}$; $\mathbf{C} = a + b \log \alpha_{A/P}$.

bile phases (80–100% B); steric selectivity is important for less rigid molecules, monomeric phases, and intermediate mobile phase compositions (e.g., 50% acetonitrile–buffer). Most RP-LC separations correspond more closely to the latter conditions; i.e., steric selectivity will generally be more significant than shape selectivity.

The correlation of **A** with log $\alpha_{C/P}$ is negligible ($r^2 = 0.03$), possibly due to the low H-bond basicity of aromatic proton acceptors (such as caffeine) in RP-LC [3]. That is, despite its pronounced H-bond basicity in solution [18], caffeine appears to be a poor choice of test solute for the measurement of RP-LC silanol activity as a H-bond donor. The use of a different mobile phase (30% methanol–water versus 50% acetonitrile–buffer) may also be a factor in the poor correlation of $\alpha_{C/P}$ with **A**, but other work [2] suggests that values of **H**, **S**, **A** and **B** do not vary much with changes in the mobile phase.

Values of **C**(2.8) correlate moderately with values of log $\alpha_{A/P}$ at pH 2.7 ($r^2 = 0.70$), but there is a poorer correlation of **C**(7.0) with values of log $\alpha_{A/P}$ at pH 7.6 ($r^2 = 0.32$). This may be the result of a partial deprotonation of benzylamine at pH 7.6, i.e., the presence of even a small fraction of non-ionized benzylamine molecules would have a large effect on benzylamine retention, unrelated to the ion-exchange retention of ionized aniline and values of **C**. Differences in the retention of benzylamine at pH 7.0 versus 7.6 may also be a factor.

If we accept that values of **H**, **S**, etc. (Eq. (1)), provide an adequate characterization of column selectivity, then the results of Table 7 suggest that the test solutes of [17] provide at best only crude measures of column selectivity. We instead recommend the column parameters of Table 1 (**H**, **S**, etc.) for this purpose. Unpublished results suggest that the latter column parameters can be determined in a total time of less than 4 h per column, using only six or seven appropriate solutes.

5. Conclusions

An empirical relationship for characterizing column selectivity has been proposed [1–3]:

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

Here, the experimentally measurable parameters **H**, **S**, **A**, **B**, and **C** define column selectivity as a function, respectively, of column Hydrophobicity, Steric resistance to penetration of the solute into the stationary phase, hydrogen-bond Acidity and Basicity, or Cation-exchange activity. Values of **H**, **S**, etc., are useful for choosing columns of either similar or different selectivity; i.e., having either similar or different values of **H**, **S**, etc. Similar columns are needed for routine assays, where a backup column may be required. Different columns are useful in method development, when a change in column selectivity is needed, or for the development of ‘‘orthogonal’’ separations.

A previous application of Eq. (1) to retention data for 10 monomeric, type-B C_{18} columns gave agreement with Eq. (1) of ±1% in α, suggesting that all significant contributions to column selectivity are recognized by Eq. (1). The present study provides a further test of Eq. (1) for 92 type-B columns of varying ligand length (C_3–C_{30}), ligand concentration, pore diameter, and end-capping, including one polymeric packing. A similar agreement with Eq. (1) (±1.2% in α, 1 SD) was found for 87 monomeric

columns, suggesting that Eq. (1) is reliable for most alkyl-silica columns currently used in RP-LC. That is, no new contributions to column selectivity were found for these columns, so that the column parameters **H**, **S**, etc., are believed to completely define column selectivity. A poorer agreement with Eq. (1) ($\pm$4–9% in α, 1 SD) was found for one ''polymeric'' (as opposed to monomeric) column and four columns from one manufacturer that were intentionally ''special'' in their preparation and properties.

Values of the column parameters **H**, **S**, etc., were compared with certain column properties: ligand length n_C and concentration C_L, particle pore diameter d_{pore}, and end-capping. The dependence of **H** and **S** on column properties supports our current interpretation of the solute–column interactions which are associated with these column parameters. Values of **B** and **H** vary with column properties in opposite fashion, supporting our belief that **B** is determined largely by water molecules that are retained in the stationary phase. Because carboxylic acids can interact with water by two hydrogen bonds, versus only one for phenol solutes, this can explain the reduced retention of phenols versus acids as a result of hydrogen bonding to a proton acceptor in the stationary phase. Values of **A** and **C** decrease sharply with end-capping, in agreement with our belief that these column parameters are the result of interactions of the solute with column silanols. As predicted from the work of McCalley [12], larger values of the column parameter **C** correlate with increased peak tailing for protonated bases.

A convenient means of comparing the selectivity of any two alkyl-silica columns is presented here, by means of a simple function (F_s) of **H**, **S**, etc., for the two columns. Two columns for which $F_s \leq 3$ are expected to provide equivalent separations for most samples and conditions. Similarly, when it is desired to change to a column of very different selectivity (for the improvement of separation during method development), the largest possible value of F_s is desirable. For samples which do not contain acids and/or bases, differences in column selectivity as measured by values of F_s becomes less pronounced (because **B** and **C** become less important in Eq. (1)). For samples which are free of acids and/or bases, the likelihood of finding two columns with equivalent selectivity therefore becomes greater. The relative importance of these five column parameters in affecting column selectivity and separation increases in the order

H (least effective) < **B** < **S** < **A** ≪ **C** (most effective).

The effect of **C** on column selectivity increases with pH, due to increasing ionization of column silanols.

For a number of reasons, a procedure is needed for determining whether two RP-LC columns are equivalent in terms of selectivity. Several different ways of characterizing column selectivity have been reported [8], including principal component analysis, test solutes believed to measure different solute–column interactions, and the solvation equation. None of these past measures of column selectivity are able to guarantee that two columns are equivalent in terms of selectivity, whereas the present paper suggests that values of **H**, **S**, etc., from Eq. (1) for two columns *can* be used to determine whether or not the columns are equivalent in terms of selectivity and separation. Hence, the column selectivity data of Table 1 for 92 type-B columns, together with the column comparison procedure described here (F_s function), now allows users a convenient and reliable procedure for selecting two or more equivalent columns without the need for further experiments.

6. Nomenclature

A	column hydrogen-bond acidity, related to number and accessibility of silanol groups in the stationary phase
B	column hydrogen-bond basicity
C	column cation-exchange activity, related to number and accessibility of ionized silanols in stationary phase
C(2.8)	value of **C** for pH 2.8
C(6.0)	value of **C** for pH 6.0
C(7.0)	value of **C** for pH 7.0
C_L	ligand concentration (μmoles/m^2)
d_{pore}	pore diameter (nm)
f_{ch}, f_{cs}, etc.	weighting factors in Eq. (8); $f_{ch}=12.5$; $f_{cs}=100$; $f_{ca}=30$; $f_{cb}=143$; $f_{cc}=83$
F_s	column selectivity comparison function; a function of differences in **H**, **S**,

	A, **B** and **C** for two columns (Eq. (8)); assumes a sample that contains acidic and basic solutes
F_s(–**B**)	column selectivity comparison function for sample that does not contain acidic compounds (Eq. (9b))
F_s(–**B**,**C**)	column selectivity comparison function for sample that does not contain acids or bases (Eq. (9c))
F_s(–**C**)	column selectivity comparison function for sample that does not contain basic compounds (Eq. (9a))
H	column hydrophobicity
$\mathbf{H}_1$, $\mathbf{H}_2$	values of **H** for columns 1 and 2
k	retention factor, equal to $(t_R - t_0)/t_0$
k_{ref}	value of k for ethylbenzene
n_C	ligand length, measured as the number of $-CH_2-$ plus $-CH_3$ units in the chain
S	column steric accessibility; as **S** decreases, bulky solute molecules experience greater difficulty in penetrating the stationary phase and being retained
$\mathbf{S}_1$, $\mathbf{S}_2$	values of **S** for columns 1 and 2
SD	standard deviation
t_0	column dead time (min)
t_R	retention time (min)
α	separation factor for two solutes
α'	solute hydrogen-bond acidity
α_{CH2}	ratio of k values for *n*-pentyl- versus *n*-butylbenzene; also, ratio for ethylbenzene versus toluene
$\alpha_{T/O}$	ratio of k values for triphenylene versus *o*-terphenyl
$\alpha_{TBN/BaP}$	ratio of k values for tetrabenzonaphthalene versus benzo[*a*]pyrene
$\alpha_{C/P}$	ratio of k values for caffeine versus phenol
$\alpha_{A/P}$	ratio of k values for benzylamine versus phenol
β'	solute hydrogen-bond basicity
Δ	contribution of solute–column interactions other than hydrophobicity to retention (Eq. (3))
η'	solute hydrophobicity
κ'	relative charge on solute molecule (positive for cations, negative for anions)
σ'	steric resistance of solute molecule to penetration into stationary phase (σ' is larger for more bulky molecules)

Acknowledgements

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Appendix A. Derivation of final values of the solute parameters η', σ', etc.

Solute parameter values for the compounds of Table 2 were reported in [1,3], for a 50% ACN–water mobile phase in the case of nonionizable solutes #1–12, and a 50% buffer mobile phase for ionizable solutes #13–16. Data reported here for all solutes were determined using 50% ACN–buffer, so it is necessary to correct previous values of η', σ', etc., for the presence of buffer in the mobile phase. It was found that the change in log k ($\delta\log k$) for buffered (30 m*M* phosphate, pH 2.8) versus unbuffered mobile phase could be correlated with values of log k for the unbuffered mobile phase:

$$\delta\log k = 0.004 - 0.009 \log k \text{ (unbuffered)} \quad (r^2 = 0.64, \text{SE} = 0.004) \qquad \text{(A-1)}$$

Eq. (A-1) allows the estimation of values of log k for the various nonionizable solutes and columns of (1,2) for a buffered mobile phase, in place of values for the original unbuffered mobile phase. Given these new values of log k, it is then possible to calculate corresponding values of log α. Finally, given the original column parameters of Ref. [1], multiple regression of values of log α versus values of **H**, **S**, etc., in terms of Eq. (1) yields initial solute parameter values for the compounds of Table 2.

The latter (initial) solute parameter values were further revised by a repetitive application of Eq. (1) (multiple regression) to values of $\log \alpha$ for the columns of Table 1. In this way, a best fit of both solute and column parameters were obtained for columns #1–82c of Table 1. Resulting values of the solute parameters are summarized in Table 3, and corresponding values of the column parameters are shown in Table 1.

Appendix B. Dependence of values of A and C, etc., on column properties

A increases with ligand length, an increase in pore diameter, or an increase in ligand concentration. End-capping decreases **A**. Recalling that values of *b–d* are based on very large (10-fold) changes in each column property, *the effect of end-capping on* ***A*** *is by far most significant*. It is likely that a reduction in C_L allows a more effective end-capping, with a net *decrease* in silanol concentration; that is, the smaller end-capping group (trimethylsilyl) allows a greater reaction of silanols compared to larger C_8 or C_{18} groups. The latter observation can explain the observed *increase* in **A** with increase in C_L. Reasons for the observed increase in **A** with ligand length and decrease with pore diameter are less obvious.

The column parameter **C** is a measure of the negative charge on the column, which results from ionized silanols. Thus **C** should increase with increasing silica acidity and increasing accessibility of ionized silanols. The results of Table 4 are in general agreement with the latter prediction. Thus, end-capping removes silanols and decreases **C**. An increase in C_L increases **C**, apparently for the same reason as for **A** (see above). More speculatively, an increase in pore diameter, other considerations equal, appears to decrease the hydrogen-bonding interaction of adjacent silanols [19,20], leading to an increase in ''free'' silanols—which are believed to be more acidic [5].

Appendix C. Increased peak tailing and lower values of *N* for columns with higher values of C

Basic compounds often exhibit tailing peaks, which is usually attributed to the interaction of protonated solutes with ionized silanols [21,22]. Because values of **C** also increase with increasing silanol ionization, increased tailing of basic solutes should correlate with values of **C** for different columns. A grouping of columns according to ''silanol activity'' has been reported recently [23]. Increased ''silanol activity'' was measured by the average plate number *N* for amitriptyline and pyridine at pH 6.0; the mobile phase was either 60% methanol–buffer (pyridine) or 80% methanol–buffer (amitriptyline), and the buffer was 25 m*M* potassium phosphate (pH 6.0) (R. Moody (MacMod Analytical), personal communication). An increase in peak tailing corresponds to a decrease in *N*, and four groups of columns were reported based on average values of *N* or ''silanol activity''; i.e., ''very low'' silanol activity (larger values of *N*)>''low'' activity>''moderate'' activity and ''high'' silanol activity (small values of *N*). Each column group contained two or more columns from the present study, which allowed the estimation of values of **C** for each of these columns at pH 6.0; i.e., given values of **C** at pH 2.8 and 7.0, a value of **C** at pH 6.0 can be obtained by interpolation. The results of this comparison of ''silanol activity'' or peak tailing with values of **C** at pH 6.0 is summarized in Table 8.

Table 8
Correlation of peak tailing (''silanol activity'') with the column parameter **C**: see Appendix C for details

Columns	''Silanol activity'' [23]	**C**(6.0)[a]
#29,31,47,50,71,77	''very low''	−0.02±0.19
#9,12,45,41,46,51,74	''low''	0.05±0.12
#2,4,43	''moderate''	0.46±0.16
Type-a[b]	''high''	1.15±0.05

[a] Average of values of **C**(6.0) obtained by interpolation of **C**(2.8) and **C**(7.0) values.
[b] Unreported data for Waters Spherisorb ODS-1 and ODS-2.

Appendix D. Allowable differences in H, S, etc., for columns of equivalent selectivity

For a change in column parameters defined as δ**H**, δ**S**, etc., a change in the separation factor α for any adjacent band pair (1) and (2) is given by Eq. (1) as

$$\delta \log \alpha_{12} = (\eta'_2 - \eta'_1)\,\delta\mathbf{H} + (\sigma'_2 - \sigma'_1)\,\delta\mathbf{S} + (\beta'_2 - \beta'_1)\,\delta\mathbf{A} + (\alpha'_2 - \alpha'_1)\,\delta\mathbf{B} + (\kappa'_2 - \kappa'_1)\,\delta\mathbf{C} \quad \text{(D-1)}$$

where η'_1 and η'_2 refer to values of η' for bands (1) and (2), respectively, and similarly for the remaining solute parameters of Eq. (2) (α', β', α', κ'). If solutes ≠1–67 of Ref. [1] are arranged in order of increasing retention for column ≠3 of (1), Eq. (D-1) permits the calculation of $\delta\log\alpha_{12}$ for each adjacent band pair, as a result of some difference in values of each column parameter. The average absolute change in $\delta\log\alpha_{12}$ ($|\delta\log\alpha_{12}|$) for each band pair was first determined via Eq. (D-1) for a change in each column parameter of +0.01 units; Table 5 summarizes the results of this calculation for each of the five column parameters, in terms of SD values of $|\delta\log\alpha_{12}|$. If the allowed difference in $\log\alpha_{12}$ for two ''equivalent'' columns were ≤0.004 (±1% in α_{12}), then the allowed change in each column parameter is given in the second row of data in Table 5. These latter values are determined by the average difference in the solute parameter η', σ', etc., for adjacent bands. In the case of values of **H**, a rather large difference is allowable (Δ=0.08), because values of η' correlate strongly with solute retention; i.e., values of $(\eta'_2-\eta'_1)$ for adjacent bands are generally small, making the term $(\eta'_2-\eta'_1)\,\delta\mathbf{H}$ of Eq. (D-1) relatively less significant (cf. Fig. 5b,c of Ref. [1]). The results of Table 5 are to some extent dependent on the sample and are therefore only approximate when applied to other samples.

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