

Column selectivity in reversed-phase liquid chromatography VIII. Phenylalkyl and fluoro-substituted columns

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Abstract

As reported previously, five solute–column interactions (hydrophobicity, steric resistance, hydrogen-bond acidity and basicity, ionic interaction) quantitatively describe column selectivity for 163 alkyl-silica, polar-group and cyano columns. In the present study, solute retention and column selectivity for 11 phenyl and 5 fluoro-substituted columns were compared with alkyl-silica columns of similar ligand length. It is concluded that two additional solute–column interactions may be significant in affecting retention and selectivity for the latter columns: (a) dispersion interactions of varying strength as a result of significant differences in bonded-phase polarizability or refractive index and (b) π – π interactions in the case of phenyl columns and aromatic solutes. These 16 phenyl and fluoro columns were also characterized in terms of hydrophobicity, steric resistance, hydrogen-bond acidity and basicity, and ionic interaction.

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1. Introduction

The preceding paper [1] has characterized the selectivity of various cyanopropyl columns by means of Eq. (1).

$$\log \left(\frac{k}{k_{EB}} \right) \equiv \log \alpha = \eta' \mathbf{H} - \sigma' \mathbf{S}^* + \beta' \mathbf{A} + \alpha' \mathbf{B} + \kappa' \mathbf{C} \quad (1)$$

Column selectivity is determined by hydrophobicity \mathbf{H} , steric resistance \mathbf{S}^* , hydrogen-bond acidity \mathbf{A} and basicity \mathbf{B} , and cation exchange capacity \mathbf{C} . For the definition of other symbols in Eq. (1), see Section 5 of [1]. For a detailed review of previous work that confirms both the validity of Eq. (1) and the identification of each of its five terms

($\alpha' \mathbf{H}$, $\sigma' \mathbf{S}^*$, etc.) with specific solute–column interactions, see [2].

In the present study, Eq. (1) was applied to retention data for 11 phenylalkyl (“phenyl”) columns and five columns that involve ligands which are heavily substituted by fluorine atoms (“fluoro columns”). The properties of these 16 columns are summarized in Tables 1 and 2, with values of the column parameters \mathbf{H} , \mathbf{S}^* , etc. that were determined via Eq. (1) as in [1]. Additional retention measurements were carried out in order to further test the applicability of Eq. (1) to phenyl and fluoro columns, especially as regards the possibility of solute–column interactions other than those recognized by Eq. (1). Evidence for such additional interactions is presented here, but the overall complexity of retention on fluoro and phenyl columns renders our conclusions preliminary at best. Further work will be needed before drawing any final conclusions for these columns.

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Table 1
Fluoro-substituted columns of the present study; properties and selectivity parameters

Column	Pore ^a	Ligand ^b	H	S*	A	B	C(2.8)	C(7.0)	log <i>k</i> _{EB}	S.D.
Fluoro-alkyl column										
1a. Fluophase RP ^c	10	3.5	0.698	0.028	0.103	0.039	1.034	1.417	0.532	0.065
Fluoro-phenyl columns										
2a. Discovery HS F5 ^d	12	4.0	0.631	−0.166	−0.325	0.023	0.709	0.940	0.603	0.107
3a. Ultra PFP ^e	10	2.7	0.501	−0.089	−0.228	−0.003	−0.033	0.588	0.289	0.009
4a. Allure PFP Propyl ^f	6	3.9	0.732	−0.157	−0.179	−0.037	0.710	1.485	0.833	0.047
5a. Fluophase PFP ^g	10	4.5	0.675	−0.129	−0.311	0.065	0.817	1.375	0.653	0.069

^a Pore diameter in nm.

^b Ligand coverage in $\mu\text{moles}/\text{m}^2$.

^c Thermo, propyl-linked perfluorohexyl (end-capped, type-B silica).

^d Supelco, propyl-linked perfluorophenyl (end-capped, type-B silica).

^e Restek, propyl-linked perfluorophenyl (end-capped, type-B silica).

^f Restek, propyl-linked perfluorophenyl (end-capped, type-B silica).

^g Thermo, propyl-linked perfluorophenyl (end-capped, type-B silica).

2. Background and theory

The selectivity of phenyl [3–14] and fluoro [9,10,15–22] columns has been the subject of previous experimental studies, some of which suggest that solute–column interactions are present *other* than those described by Eq. (1); e.g., (a) π – π interactions between unsaturated solutes and phenyl-ligands, (b) greatly different dispersion interactions between solutes and columns as a result of large differences in ligand polarizability, and (c) strong dipole interactions for fluoro-substituted ligands. Each of the latter proposed solute–column interactions are plausible in terms of present theory. One aim of the present study is to confirm any additional solute–column interactions (other than

those represented in Eq. (1)) so as to more completely describe the selectivity of fluoro and phenyl columns. The following re-examination of some published data (Sections 2.1–2.3) provides a starting point for our later interpretation of new experimental data for these columns in Section 4.

Before proceeding to Sections 2.1–2.3, some comment on the interpretation of literature data in terms of Eq. (1) is appropriate. With the exception of phenols, the solutes discussed are neutral compounds with minimal hydrogen-bond acidity or basicity, and molecular lengths that do not vary greatly. This means that values of the solute parameters α' , β' and κ' should be approximately zero, and values of σ' should be roughly constant [1,2]. For such solutes, Eq. (1)

Table 2
Phenyl columns of the present study; properties and selectivity parameters

Column	Pore ^a	Ligand ^b	H	S*	A	B	C(2.8)	C(7.0)	log <i>k</i> _{EB}	S.D.
Phenylhexyl columns										
1b. Luna Phenyl-Hexyl ^c	10	4	0.775	−0.124	−0.284	−0.001	0.001	0.383	0.718	0.024
2b. Betasil Phenyl-Hexyl ^d	10	2.6	0.693	−0.054	−0.323	0.021	0.038	0.341	0.637	0.027
Phenylpropyl columns										
3b. Inertsil Ph-3 ^{e,f}	10	1.4	0.526	−0.179	−0.133	0.040	0.121	0.735	0.409	0.025
4b. ProntoSIL 120-5-Phenyl ^g	12	3	0.557	−0.163	−0.217	0.022	0.167	0.706	0.387	0.022
5b. BetaBasic Phenyl ^d	15	2.7	0.571	−0.167	−0.422	0.054	0.099	0.753	0.234	0.032
6b. ProntoSIL 60-5-Phenyl ^g	6	2.6	0.703	−0.196	−0.005	−0.009	0.410	1.509	0.649	0.037
7b. XTerra Phenyl ^h	12.5	2.4	0.690	−0.076	−0.374	−0.003	0.102	−0.033	0.409	0.018
8b. Precision Phenyl ⁱ	12	3.2	0.587	−0.142	−0.304	0.030	0.094	0.504	0.420	0.022
9b. Ace Phenyl ^j	10	2.8	0.638	−0.145	−0.305	0.031	0.128	0.461	0.445	0.021
10b. Genesis Phenyl ^k			0.600	−0.147	−0.378	0.035	0.128	0.584	0.459	0.025
11b. Prodigy Phenyl-3 ^c	10	4.1	0.525	−0.198	0.051	0.024	0.228	1.465	0.358	0.016

^a Pore diameter (nm).

^b Ligand concentration (moles/m^2).

^c Phenomenex.

^d Thermo; column #5 is bonded separately with both phenyl and hexyl groups.

^e GL science.

^f Not end-capped.

^g Bischoff.

^h Waters.

ⁱ MacMod/Higgins.

^j Hichrom/ACT.

^k Jones chromatography.

reduces to

$$\log \left(\frac{k}{k_{EB}} \right) \equiv \log \alpha = \eta' \mathbf{H} - (\text{constant}) \mathbf{S}^* \quad (2)$$

or

$$\log k = \eta' \mathbf{H} + (\text{constant for a given column}) \quad (2a)$$

Plots of $\log k$ for one column versus another should therefore yield straight-line plots with little scatter. Significant deviations from such plots for the latter solutes (except phenols) suggest the presence of solute–column interactions other than those described by Eq. (1).

2.1. Fluoro columns

Fluoro-substituted columns are not widely used, but their unusual selectivity has been noted by several authors. For example, fluoro-substituted solutes are preferentially retained on fluoro columns, compared to retention on corresponding alkyl or phenyl columns [15,16,18]. This behavior has variously been attributed to the large dipole moment [21,22] and/or small polarizability of the fluoro group [19,21]. Preferential retention of other classes of compounds on fluoro columns has also been reported [16,19,22], but in some cases these comparisons involved the use of different mobile phases for the two columns. As previously noted (Fig. 4 of [1]), differences in *mobile phase* selectivity in such cases can outweigh the contribution of the stationary phase to separation selectivity.

The discussion of a previous paper [19] suggests that the selectivity of fluoro columns compared to alkyl-silica columns can be explained (at least in part) as a consequence of the very low polarizability of fluorinated ligands, as measured by their lower refractive index (RI) values compared to hydrocarbons. Similarly, the previous paper [1] suggests that dipole-dipole interactions of solute and stationary phase are unlikely to be significant in RP-LC separations. Lower polarizabilities signify reduced interaction via dispersion forces, with consequences for retention (i.e., deviations from Eq. (1)) that can be interpreted qualitatively in terms of the solubility parameter model [23,24]. In its simplest form (considering only dispersion forces), the latter can be translated as “like dissolves like”, which in turn means – other factors equal – that solutes and stationary phases with similar RI values will exhibit preferential interaction and retention. Refractive index increases in the order: fluoro-substituted compounds (least) < aliphatic compounds < aromatic compounds < polyaromatic compounds (most), suggesting that relative retention on alkyl-silica columns (higher RI) versus fluoro-alkyl columns (lower RI) should tend to increase in the same order, *apart* from the general effect of hydrophobic interactions (the $\eta' \mathbf{H}$ term of Eq. (1)). Hydrophobic interaction (as defined by Eq. (1)) involves column-solute, column-solvent and solute-solvent interactions apart from hydrogen-bonding and ionic interaction, but does *not* recognize the effect of varying stationary phase polarizability.

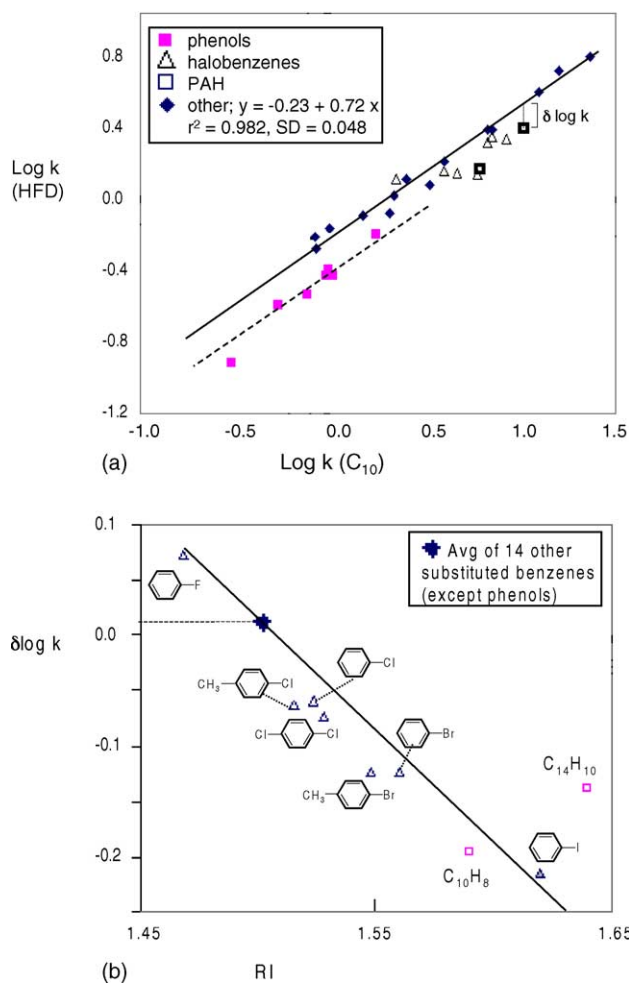


Fig. 1. Comparison of retention on a perfluorodecyl (PDF) vs. a C_{10} column for different solute groups. (a) Plot of $\log k$ values; (b) correlation of deviations $\delta \log k$ from the solid curve of (a) with solute refractive index RI. Data of [19]; see text for details.

Data reported in [19] for a heptadecafluorodecyl (HFD) and an *n*-decyl (C_{10}) column appear to support the above interpretation. Fig. 1a is a previously unreported plot of values of $\log k$ for the HFD versus C_{10} columns. The solid curve is a best fit for several neutral, substituted benzenes (solid diamonds) with similar RI values ($1.49 < RI < 1.55$), excluding halogenated derivatives and polycyclic aromatic hydrocarbons (whose RI values cover a wider range). A single solute–column interaction (hydrophobicity) appears to account largely for the retention of the substituted benzenes on both columns, as shown by their close adherence to the best-fit line (S.D. = 0.05). Otherwise, if more than one such interaction were important, it would require that the contribution to $\log k$ of each additional interaction be proportional to that for the primary interaction (which seems unlikely). In previous papers [1,2], it has been argued that hydrophobic interactions ($\eta' \mathbf{H}$ term of Eq. (1)) are almost exclusively responsible for the differential retention of neutral, substituted benzenes on various alkyl-silica columns. For unknown reasons, phenols

(solid squares) are less retained on the HFD column, by an average of -0.16 log units (dashed curve of Fig. 1a). Data for halogenated benzenes (Δ) and polycyclic aromatic hydrocarbons (PAH) deviate from the solid line to a significantly greater extent (avg. S.D. = 0.10), suggesting a further examination of these deviations. The deviations ($\delta \log k$) of the latter solutes from the solid line of Fig. 1a are a measure of the selective retention of a given solute on the HFD column versus the C_{10} column; note the example of a calculation of $\delta \log k$ in Fig. 1a.

Values of $\delta \log k$ are plotted versus the RI values of the deviating solutes in Fig. 1b, and an obvious trend is observed. Fluorobenzene is seen to be preferentially retained on the HFD column, because its value of RI is lower than the average value for the other substituted benzenes of Fig. 1a and closer to the RI value of the HFD stationary phase. Likewise, the RI values for iodobenzene and the two PAHs are closer to that of the C_{10} phase, resulting in negative values of $\delta \log k$ (or preferential retention on the C_{10} column). The plot of Fig. 1b thus suggests that the relative retention of these compounds on the HFD versus C_{10} columns may be due to significant differences in the dispersive solute–column interactions between alkyl phases and highly fluorinated phases. The small values of $\delta \log k$ for the “other substituted benzenes” of Fig. 1a, together with large differences in dipole moment for these compounds, imply that dipole orientation does not contribute significantly to solute retention on fluoro columns; i.e., fluoro columns are essentially nonpolar. Other studies confirm that perfluorinated species are generally nonpolar [24–27].

2.2. Phenyl columns

Tanaka et al. have compared retention on a phenylethyl versus a C_8 column [7], finding preferential retention on the phenylethyl column of aromatic solutes versus alkanes and alicyclics. Likewise, Hanai and Hubert [8] compared the retention of several aliphatic and aromatic solutes on a C_{18} and a phenyl column, using mobile phases composed of various mixtures of either acetonitrile (ACN) or tetrahydrofuran (THF) with water. Fig. 2 summarizes (previously unreported) comparisons of retention on the latter two columns using either (a) 70% ACN or (b) 60% THF as mobile phase. For 70% ACN as mobile phase, Fig. 2a shows that retention on the phenyl column relative to the C_{18} column increases in the order: aliphatics (least) < benzene derivatives < polycyclic aromatic hydrocarbons (most). The preferred retention of aromatic solutes on phenyl (versus alkyl) columns has been attributed to either (a) π – π interaction or (b) the greater polarizability of aromatic versus aliphatic groups (the inverse of the above case of fluoro-column selectivity; Section 2.1). The data of Fig. 2 can be interpreted in terms of either of these two solute–column interactions, whereas the interactions which give rise to the five terms of Eq. (1) (η^*H , σ^*S^* , etc.) are inconsistent with the correlations of Fig. 2.

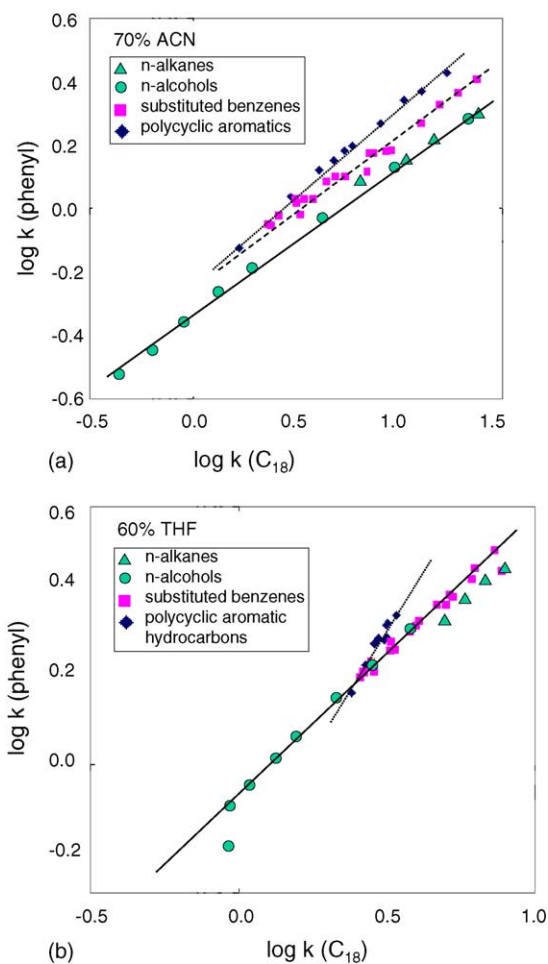


Fig. 2. Comparison of retention on a phenyl vs. a C_{10} column for different solute groups. (a) 70% acetonitrile/water as mobile phase; (b) 60% tetrahydrofuran as mobile phase. Data of [8]; see text for details.

As seen in Fig. 2b, the use of THF as organic solvent largely eliminates the preferential retention of aromatic solutes on the phenyl column. Later papers reported a preferential retention of (a) unsaturated molecules on phenyl columns [13] and (b) aromatic solutes on polystyrene columns [14], also attributed to π – π interactions.

2.3. Dispersion, dipole and π – π interactions

A substantial literature exists concerning the nature and relative importance of each of these proposed solute–column interactions. Dispersion and various dipole interactions can be discussed qualitatively in terms of solubility parameter theory [28–30]. The solubility parameter δ , which reflects the total self-interaction energy of a compound in the pure liquid (per unit volume), was originally derived assuming that only geometric mean (i.e., dispersion) interactions are important. When only dispersion interactions vary, maximum sample retention should result when the RI values of solute and stationary phase ligand are similar. Thus, other factors equal, retention on a lower-RI fluoro column versus a C_8 or C_{18}

column should increase in the order: polycyclic aromatic hydrocarbons (least) < aromatic hydrocarbons < saturated hydrocarbons \approx fluoro-substituted compounds (most). Retention on a higher-RI column such as phenyl should *decrease* in this order; i.e., the opposite trend as for a fluoro column.

Later [28–30], the solubility parameter concept was broadened to recognize other interactions, by defining contributions to δ for each interaction: e.g., dispersion, δ_d ; dipole induction, δ_{in} ; dipole orientation, δ_o . Values of δ_d increase with compound refractive index RI, and values of δ_{in} and δ_o increase with compound dipole moment. Columns with only alkyl ligands (e.g., C₈, C₁₈) have a zero dipole moment and similar polarizability (i.e., similar refractive index), so that dipole induction and orientation are not expected to contribute significantly to *relative* column selectivity as measured by **H**, **S**^{*}, etc.

π – π Interactions typically involve the charge-transfer of electrons from electron-rich (π –base) to electron-poor (π –acid) aromatic rings [31], e.g., the complexation of polycyclic aromatic compounds (π –bases) by a (π –acid) 2,4-dinitrophenyl group in chiral chromatography [32]. π – π Interaction can also involve a simple overlap of π -orbitals in two interacting molecules. Preferential π – π interactions of the solute with a phenyl column versus an alkyl-silica column can therefore be recognized by increased relative retention on the phenyl column of aromatics versus aliphatics, polycyclic aromatics versus benzene derivatives, and (especially) stronger π –acids (e.g., nitro-substituted aromatics).

The relative contributions of dispersion, dipole and π – π interactions to column selectivity can be difficult to sort out, because different interactions sometimes yield similar results for the selective retention of some solutes or sample types. For example, the relative retention on phenyl versus alkylsilica columns of aliphatic solutes < substituted benzenes < polycyclic aromatic hydrocarbons in Fig. 2a could be explained equally well by either (a) the greater polarizability of phenyl columns or (b) the increased π -basicity of phenyl columns. As background for the discussion in Sec-

tion 4 below, Table 3 provides a guide to the interpretation of relative retention for phenyl or fluoro columns versus alkyl-silica columns, as a function of solute type and specific solute–column interactions.

3. Experimental

3.1. Equipment, materials and procedures

These were described in the preceding paper [1], except for the equipment used. Most measurements were carried out on the Model 1090 system of [1]. Selected additional experiments (for solutes #13–17, 36–51 of Table 4) were carried out with an HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, in-line degasser, DAD detector, autosampler and HP Chemstation software (version A.08.03) for data analysis; either UV or RI detection were used as appropriate. For all experiments, the temperature was 35 °C and the mobile phase was 50% acetonitrile/buffer; the buffer was 30 mM potassium phosphate with a pH of 2.8 (except for berberine as solute, where the pH was either 2.8 or 7.0).

3.2. Columns

The 16 columns of Tables 1 and 2 were each the generous gift of the manufacturer. The nature of the silica (type-A or -B, see discussion of [33]) and whether the columns are end-capped or not was not known for all columns, but in most cases it can be assumed that type-B silica was used and the columns are end-capped. In any case, the nature of the silica and whether or not the column is end-capped will not play a significant role in the following discussion.

Additionally, retention measurements were also carried out on four type-B C₈ columns described in [34]: #8, Eclipse XDB-C8; #24, Acclaim C₈; #39, Precision C₈; #58, Restek Ultra C₈. All columns had dimensions of 15 cm \times 0.46 cm,

Table 3
Effects on sample retention of “additional” solute–column interactions among alkyl, phenyl and fluoro-substituted columns

Interaction	Effect on retention
Varying dispersion	Compared to alkyl-silica column b, column a will have ligands with a different RI (e.g., phenyl ligands of higher RI or fluoro-substituted ligands of lower RI) Solute of higher RI and similar dipole moment or π -acidity will be preferentially retained on the higher-RI column; solutes of lower RI will be preferentially retained on the lower-RI column
π – π	Column a will have an aromatic ring and b will not (e.g., phenyl or perfluorophenyl ligands in column a) Solute of increasing aromaticity (e.g., fused-ring polycyclic aromatics) will be preferentially retained on column a Aromatic rings more highly substituted by –NO ₂ groups (similar RI) will be preferentially retained on column a
Dipole induction	Column a will have an unsubstituted aromatic ring and b will not (e.g., phenyl ligands in column a) Solute with larger dipole moments will be preferentially retained on column a
Dipole orientation	Column a will have ligands with significant local (i.e., substituent) dipole moments, and b will not (e.g., fluoro-substituted ligands in column a) Solute with larger local dipole moments will be preferentially retained on column a

In the examples below, column b is assumed to be an alkyl-silica column with ligands of similar carbon number as for column a.

Table 4
Additional test of phenyl column selectivity

Solute	log α			$\delta \log \alpha^a$	
	C ₈ (#58) ^b	Phenylhexyl (#1b)	Phenylpropyl (#11b)	Phenylhexyl (#1b)	Phenylpropyl (#11b)
13. Fluorobenzene	-0.354	-0.310	-0.213	-0.01	-0.02
33. Chlorobenzene	-0.177	-0.127	-0.111	0.02	-0.02
34. Bromobenzene	-0.127	-0.067	-0.068	0.04	0.00
35. Iodobenzene	-0.035	0.052	0.007	0.08	0.03
36. Fluorohexane	0.079	0.040	0.025	-0.03	-0.02
37. Chlorohexane	0.303	0.270	0.176	0.02	0.02
38. Bromohexane	0.379	0.351	0.229	0.03	0.03
39. Iodohexane	0.531	0.500	0.312	0.05	0.03
25. nitrobenzene	-0.495	-0.389	-0.239	0.03	0.02
40. 1,3-Dinitrobenzene	-0.508	-0.307	-0.213	0.12	0.06
41. 1,4-Dinitrobenzene	-0.494	-0.290	-0.211	0.12	0.05
42. 1,3,5-Trinitrobenzene	-0.478	-0.150	-0.121	0.25	0.13
26. 1-Nitronaphthalene	-0.168	-0.050	0.022	0.09	0.11
27. 1-Nitrobutane	-0.500	-0.432	-0.293	-0.01	-0.03
28. 1-Nitropentane	-0.299	-0.255	-0.161	0.00	0.00
29. 1-Nitrohexane	-0.100	-0.077	-0.036	0.01	0.02
30. <i>n</i> -Propyl acetate	-0.663	-0.654	-0.457	-0.10	-0.11
31. <i>n</i> -Butyl acetate	-0.450	-0.467	-0.316	-0.09	-0.08
32. <i>n</i> -Pentyl acetate	-0.244	-0.286	-0.182	-0.08	-0.05
43. di- <i>n</i> -Butylether	0.169	0.052	0.022	-0.09	-0.07
44. di- <i>n</i> -Propyl ether	-0.298	-0.346	-0.255	-0.10	-0.10
45. Benzyl alcohol	-1.039	-0.968	-0.710	-0.10	-0.16
46. 2-Phenylethanol	-0.928	-0.864	-0.613	-0.09	-0.12
47. 3-Phenylpropanol	-0.778	-0.725	-0.491	-0.07	-0.08
10. 5-Phenylpentanol	-0.419	-0.402	-0.221	-0.05	0.00
49. 1-Pentanol	-0.900	-0.895	-0.662	-0.14	-0.19
50. 1-Hexanol	-0.680	-0.712	-0.509	-0.14	-0.15
51. 1-Heptanol	-0.464	-0.537	-0.364	-0.15	-0.12
18. 5,5-Diphenylhydantoin	-0.823	-0.694	-0.436	0.00	0.00
19. Toluene	-0.199	-0.161	-0.117	0.01	-0.01
20. Ethylbenzene	0.000	0.000	0.000	0.00	0.00
21. Acetophenone	-0.674	-0.575	-0.363	-0.01	-0.01
22. Anisole	-0.421	-0.336	-0.223	0.02	0.00
23. Benzotrile	-0.623	-0.513	-0.324	0.01	0.01
24. Ethyl benzoate	-0.268	-0.243	-0.134	-0.02	0.01
13. Fluorobenzene	-0.354	-0.310	-0.213	-0.013	-0.024
14. α,α,α -Trifluorotoluene	-0.078	-0.102	-0.053	-0.037	-0.010
15. 2,3,4-Trifluorotoluene	-0.066	-0.069	-0.043	-0.014	-0.007
16. 1,4-Difluorobenzene	-0.341	-0.299	-0.203	-0.013	-0.022
17. 1,3,5-Trifluorobenzene	-0.247	-0.240	-0.164	-0.033	-0.033

Retention data for a C₈, phenylhexyl, and phenylpropyl column. Data are grouped for the comparisons discussed in the text and summarized in Table 7.

^a value of $\delta \log \alpha$ calculated as in Fig. 6 for difference in retention on a phenyl column vs. a C₈ column.

^b Restek Ultra C₈.

with 5- μ m diameter particles (except for column #10b, which has 4- μ m particles).

3.3. Samples

The same 16 solutes used in the preceding paper [1] to characterize column selectivity by means of Eq. (1) were used in the present study for the characterization of the columns of Tables 1 and 2. These solutes are listed in Table 5 (nos. 1–5, 8–12, 18–23). Several additional solutes were also selected, in order to test different theories for possible deviations from Eq. (1) in the case of fluoro and phenyl columns. The latter solutes were selected either for their different polarizability or π -basicity (see Table 3).

3.4. Calculations

Some of these are described in [1]. Values of k for various solutes and columns are not reported here but are available from the authors.

4. Results and discussion

4.1. Fluoro columns

4.1.1. Fit of experimental data to Eq. (1)

The fit of Eq. (1) (by multiple linear regression as in [1]) to experimental values of $\log \alpha$ for fluoro columns #1a–5a of

Table 5
Initial solutes examined in the present study (data of DHM, KC)

1. Amitriptyline
2. Nortriptyline
3. <i>n</i> -Butylbenzoic acid
4. Mefenamic acid
5. <i>p</i> -Nitrophenol
6. 2,3,5,6-Tetrafluorophenol
7. 2-Naphthol
8. <i>N,N</i> -Dimethylacetamide
9. <i>N,N</i> -Diethylacetamide
10. 5-Phenylpentanol
11. <i>cis</i> -Chalcone
12. <i>trans</i> -Chalcone
13. Fluorobenzene
14. α,α,α -Trifluorotoluene
15. 2,3,4-Trifluorotoluene
16. 1,4-Difluorobenzene
17. 1,3,5-Trifluorobenzene
18. 5,5-Diphenylhydantoin
19. Toluene
20. Ethylbenzene
21. Acetophenone
22. Anisole
23. Benzonitrile
24. Ethyl benzoate
25. Nitrobenzene
26. 1-Nitronaphthalene
27. 1-Nitro- <i>n</i> -butane
28. 1-Nitro- <i>n</i> -pentane
29. 1-Nitro- <i>n</i> -hexane
30. <i>n</i> -Propyl acetate
31. <i>n</i> -Butyl acetate
32. <i>n</i> -Pentyl acetate

"Standard" solutes for the measurement of values of \mathbf{H} , \mathbf{S}^* , etc. are #1–5, 8–12, 18–23.

Table 1 and the 16 standard test solutes of Table 5 (#1–5, 8–12, 18–23) resulted in values of \mathbf{H} , \mathbf{S}^* , etc. for each column (Table 1). The average standard deviation of the fit to Eq. (1) was S.D. = 0.059 ($\pm 15\%$ in $\log \alpha$). This is poorer agreement than was found for other column types (except for polar-group columns [35]), suggesting that some new solute–column interactions *may* be involved in retention on fluoro columns. As a test for the latter possibility, we have previously used repeated regression to obtain best-fit values of the solute parameters η' , σ' , etc. [1,2,33–35]. However, this approach requires a larger number of fluoro columns than are represented in the present study and therefore could not be employed.

4.1.1.1. Interpretation of the relative fit (value of S.D.) to Eq. (1) for a given column. Another test for additional solute–column interactions [33] is provided by a comparison of values of the average S.D. over all solutes for each fluoro column with the values computed from Eq. (3):

$$\begin{aligned} \text{S.D.} = & -0.006 - 0.001|\mathbf{H} - \mathbf{H}_b| + 0.030|\mathbf{S}^* - \mathbf{S}_b^*| \\ & + 0.041|\mathbf{A} - \mathbf{A}_b| + 0.311|\mathbf{B} - \mathbf{B}_b| + 0.010|\mathbf{C} \\ & - \mathbf{C}_b| \end{aligned} \quad (3)$$

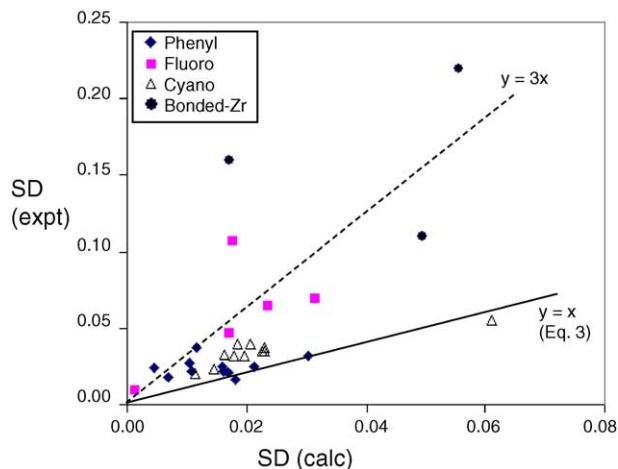


Fig. 3. Comparison of S.D. values (from fit of Eq. (1) to experimental α values) for various columns with values calculated from Eq. (2). High values of experimental S.D. values suggest a failure of Eq. (1) because of unaccounted for solute–column interactions. See text for details.

In Eq. (3), \mathbf{H}_b , \mathbf{S}_b^* , etc. refer to average values of \mathbf{H} , \mathbf{S}^* , etc. for the type-B alkyl-silica columns that were used to derive values of the solute parameters η' , σ' etc. Eq. (3) was obtained by fitting results for 130 type-A and -B columns plus 21 embedded polar group columns. For these columns Eq. (3) gave $r^2 = 0.92$ with an accuracy of ± 0.008 S.D. units. Agreement of experimental S.D. values with calculated S.D. values from Eq. (3) implies that (a) values of S.D. are primarily determined by the approximate nature of Eq. (1), which becomes less reliable for columns that are more different than the type-B alkyl-silica columns used to derive values of η' , σ' , etc. and (b) no additional solute–column interactions (other than those of Eq. (1)) contribute to column selectivity.

If values of S.D. for fluoro columns are consistently larger than predicted by Eq. (3), this would suggest that some previously unrecognized solute–column interaction is involved. Fig. 3 shows a comparison of experimental versus calculated S.D. values for four additional column types: phenyl, fluoro, cyano and bonded-zirconia. Experimental S.D. values for the fluoro and bonded-zirconia columns are seen to lie well above the $y = x$ line from Eq. (3), suggesting additional solute–column interactions for these two column types. This is also seen from the average values of [S.D. (expt) – S.D. (Eq. (3))] for these different columns: phenyl, 0.01 ± 0.01 ; cyano, 0.01 ± 0.01 ; fluoro, 0.04 ± 0.03 ; bonded-zirconia, 0.12 ± 0.06 (note that Eq. (3) itself has an uncertainty of S.D. = 0.01). The data of Fig. 3 provide further (albeit indirect) support for the failure of Eq. (1) for both fluoro and bonded-zirconia columns (apart from differences in \mathbf{H} , \mathbf{S}^* , etc. as predicted by Eq. (3)) and suggest that this failure is due either to (a) one or more new solute–column interactions or (b) a profound change in one of the five interactions recognized by Eq. (1).

4.1.1.2. Comparisons of relative column selectivity. A further test of the applicability of Eq. (1) for fluoro columns can

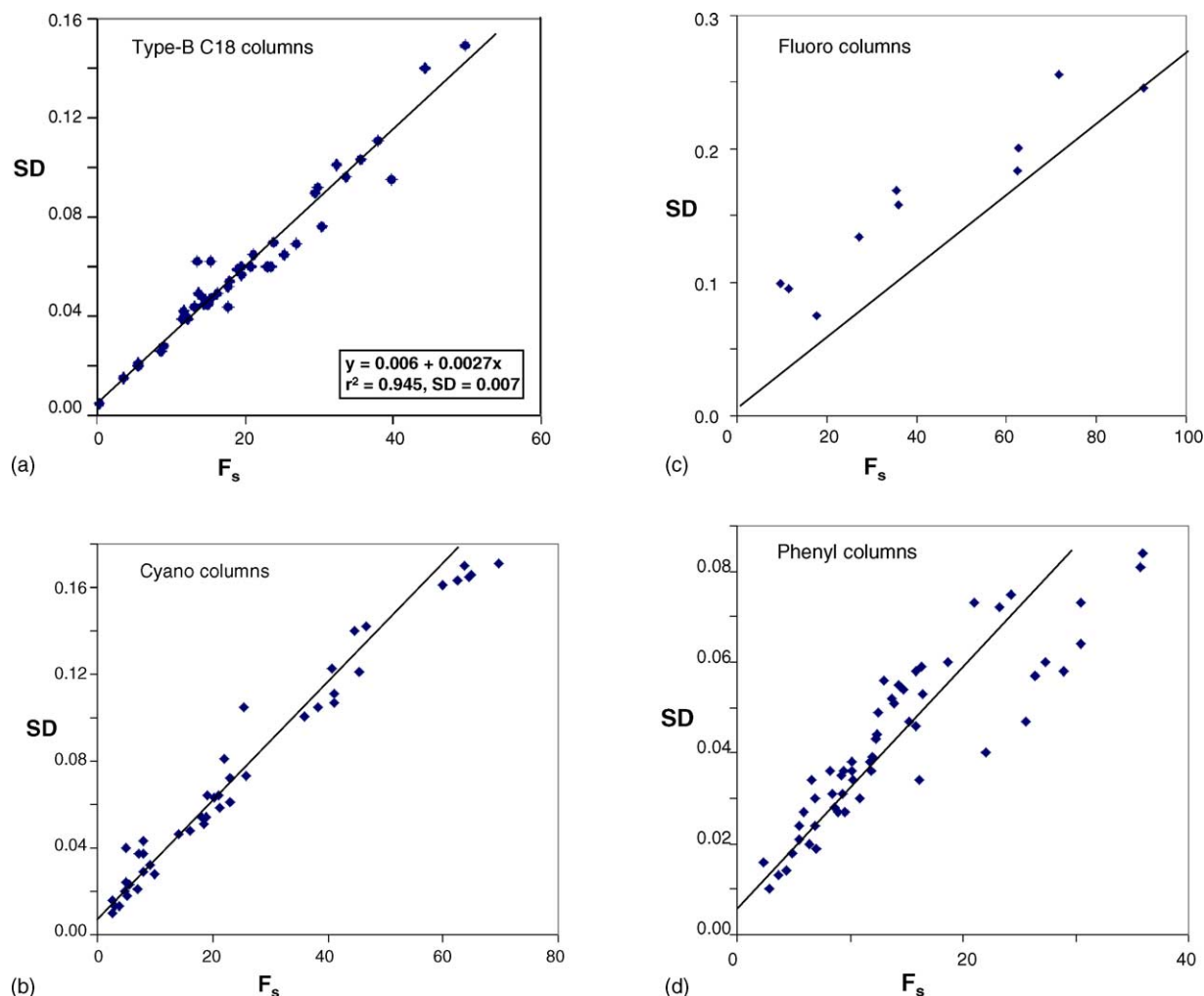


Fig. 4. Comparison of column selectivity as measured by (i) the standard deviation (S.D.) of the correlation of $\log k$ values for two columns with (ii) the column comparison function F_s (see Eq. (3) of preceding paper [1]) which is a function of values of \mathbf{H} , \mathbf{S}^* , etc. for each column. Comparisons for (a) type-B alkyl-silica columns [34], (b) cyano columns [1], (c) fluoro columns and (d) phenyl columns. The 16 standard test solutes of Section 3.3 were used for the comparisons of (b)–(d). See text for details.

be made as follows. The relative similarity of two columns can be measured either by their values of \mathbf{H} , \mathbf{S}^* , etc. or by the S.D. value for plots of $\log k$ for one column versus the other [34]. Column comparisons based on values of \mathbf{H} , \mathbf{S}^* , etc. can be made by means of the function F_s (Eq. (3) of preceding paper [1]), which generally correlates well with values of S.D. This is illustrated in Fig. 4a for several different type-B alkyl-silica columns, where the value of S.D. for $\log k$ – $\log k$ plots for various pairs of columns is plotted versus their F_s values. A good correlation of F_s and S.D. values is also seen for the cyano columns of the preceding paper (Fig. 4b), with the correlation line of Fig. 4a superimposed on these data. However, in Fig. 4c for the five fluoro columns of the present study, a much poorer agreement is observed of S.D. and F_s values with the correlation of Fig. 4a. The much larger values of S.D. in Fig. 4c than predicted by the solid line of Fig. 4a also suggests that (a) retention on fluoro columns is not fully accounted for by Eq. (1) and (b) values of \mathbf{H} , \mathbf{S}^* , etc. for fluoro columns are less reliable for the purpose of comparing

column selectivity (in contrast to the case for other column types).

Note also that if n columns are compared as in each plot of Fig. 4a–d, there are $(n^2 - n)/2$ individual comparisons (or data points in each of the plots of Fig. 4). For example if $n = 5$ (as for the fluoro columns of Fig. 4c), column #1 is compared with columns #2–5, column #2 is compared with columns #3–5, and so on for a total of 10 column comparisons.

4.1.2. Additional solute–column interactions for fluoro-alkyl columns

We have measured values of $\log k$ for all the solutes of Table 4 on fluoro columns #1a and 5a, as well as four type-B C_8 columns (#8, 24, 39, 58 from [34]; the names of these columns are provided in Section 3.2). In Fig. 5a we compare values of $\log k$ for fluoro-alkyl column #1a of Table 1 with average values for the four C_8 columns (as in Fig. 1). The solutes of Fig. 5 exclude compounds with significant values of σ' , β' , α' or κ' (#1–12 of Table 5), so that in the absence of any

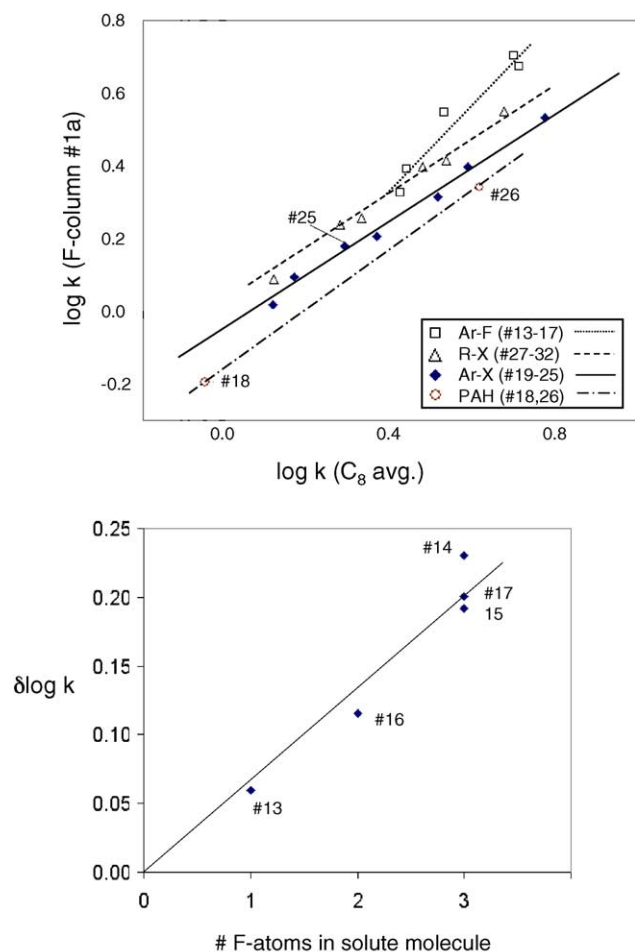


Fig. 5. Comparison of retention on fluoro column #1a vs. that of an average C_8 column for different solute groups. (a) Plot of $\log k$ values, (b) correlation of deviations $\delta \log k$ from the solid curve of (a) with the number of F-atoms in the solute molecule. See text for details.

“special” solute–column interactions for fluoro columns, hydrophobic interactions should predominate, and all the data points should fall close to a single straight line (see discussion of Section 2). This is the case for the substituted-benzene solutes labeled “Ar–X” (solid diamonds), which comprise solutes #19–25 of Table 5. It is observed that fluoro-substituted benzenes (0, #13–17 of Table 5) and substituted alkanes (Δ , #27–32) are relatively more retained on the fluoro column, while polycyclic aromatics (0, #18 and 26) are less retained. This behavior is consistent with our earlier conclusions from Fig. 1, concerning a significant differential contribution of solute–column dispersion interactions to retention on the fluoro-alkyl versus C_8 columns.

Fig. 5b plots retention deviations for the fluorobenzenes versus the number of F-atoms in the solute molecule. Retention is seen to increase regularly with the number of F-atoms, each F-atom contributing ≈ 0.07 units to $\log k$ for the fluoro column versus the average C_8 column. Thus, the data of Fig. 5 in combination with the results of Fig. 1 support our view (Section 2.1) that varying dispersion interactions largely account for the unusual selectivity of fluoro columns, as well as

their deviation from Eq. (1). Previous applications of Eq. (1) [1,33–35] have involved columns with ligands which have similar RI values, which explains why varying dispersion interactions (due to differences in ligand polarizability) were not observed in our earlier comparisons of column selectivity. Strong dipole interactions between the solute and a fluoro column do not appear to contribute to fluoro-column selectivity, as the solutes of Fig. 5a vary widely in dipole moment, but there is no apparent correlation between deviations from the solid curve of Fig. 5a (values of $\delta \log k$) and solute dipole moment. Also ruled out in the comparison of Fig. 5 are π – π interactions, since no unsaturated column-ligands are present in fluoro column #1a.

In summary, it appears that the selectivity of fluoroalkyl columns (apart from that described by values of H , S^* , etc.) includes a preferential retention of less polarizable solutes such as aliphatic derivatives, as well as solutes substituted by fluoro groups. By extrapolation from the results of Fig. 5, other more polarizable compounds (e.g., polyaromatics, or compounds substituted by higher-atomic-weight atoms such as S, Br, Se, etc.) should be preferentially retained on C_8 versus fluoro-alkyl columns—other factors equal.

4.1.3. Comparison of the selectivity of fluoro columns with corresponding C_8 or phenyl columns

Table 6 compares the selectivity of fluoroalkyl and fluorophenyl columns with the corresponding non-fluoro columns (C_8 and phenyl) in terms of Eq. (1). Because of the importance of dispersion interactions in affecting the selectivity of fluoro columns, and because such interactions are ignored in Eq. (1), attempts at a physical–chemical interpretation of their values of H , S^* , etc. may be of limited value. The fluoro-alkyl columns have significantly lower values of H (-0.13) compared to a C_8 column, which is reasonably ascribed to differences in dispersion interactions. There is less difference in H for fluorophenyl versus phenyl ($+0.03$) columns. Values of S^* are similar for fluoroalkyl and C_8 columns (-0.02 difference), but fluorophenyl columns have *much* larger values of S^* ($+0.30$) compared to phenyl columns. Other workers [22] have noted that fluorophenyl columns also possess a much greater “shape selectivity” compared to fluoroalkyl columns, as measured by values of $\alpha_{T/O}$

Table 6
Comparison of fluoro vs. corresponding non-fluoro columns; average values of each column parameter

Column type	H	S^*	A	B	C(2.8)	C(7.0)
Fluoroalkyl ^a	0.70	-0.03	0.10	0.04	1.03	1.42
Type-B (C_8) ^b	0.83	-0.01	-0.16	0.02	0.02	0.31
Difference	-0.13	-0.02	0.26	0.02	1.01	1.11
Perfluorophenyl ^c	0.63	0.14	-0.26	0.01	0.55	1.1
Phenyl ^d	0.60	-0.16	-0.23	0.02	0.16	0.74
Difference	0.03	0.30	-0.03	-0.01	0.39	0.36

^a Column #1a of Table 1.

^b Data of [34].

^c Columns #2a–5a of Table 1.

^d Data of Table 9.

(ratio of k -values for triphenylene/*o*-terphenyl). Larger values of S^* for the fluorophenyl versus fluoroalkyl columns are therefore consistent with a previously noted [35] correlation between values of S^* and $\alpha_{T/O}$:

$$S^* = 0.04 + 0.39 \log \alpha_{T/O} (r^2 = 0.40) \quad (4)$$

That is, column steric resistance (S^*) and shape selectivity ($\alpha_{T/O}$) are each much greater for fluorophenyl columns versus fluoroalkyl, phenyl or alkyl columns. In the case of values of S^* for fluorophenyl columns, this might be the result of the greater bulk of the ligands and/or a less ordered arrangement of these ligands in the stationary phase.

4.2. Phenyl columns

4.2.1. Fit of experimental data to Eq. (1)

The fit of Eq. (1) (by multiple linear regression) to experimental values of α for phenyl columns #1b–11b of Table 2 and the 16 standard test solutes of Table 5 resulted in values of H , S^* , etc. and a standard deviation of the fit (S.D.) for each column (Table 2). The average standard deviation (S.D.) for the correlation of these 11 columns and 16 solutes was S.D. = 0.025 ($\pm 6\%$ in $\log \alpha$). This is poorer agreement than was found for type-B alkyl-silica columns [34], but is close to that found for type-A alkyl-silica columns [33] and cyano columns [1].

Repeated regression as in [1,2,33–35] with a resulting change in the values of η' , σ' , etc. (but little change in H , S^* , etc.) improved the overall correlation to S.D. = 0.005 ($\pm 1\%$ in $\log \alpha$), suggesting no significant new solute–column interactions other than those described by Eq. (1). Data for phenyl columns summarized in Figs. 3 and 4d also agree with Eq. (1) within expected limits, further confirming no new solute–column interactions. However, the 16 test solutes used to evaluate the applicability of Eq. (1) for phenyl columns do not include any strong π -acceptors, and therefore do not exclude the possibility of π – π interactions for phenyl columns. Similarly, many of these 16 test solutes have similar RI values, hence also precluding a test of varying dispersion interactions.

4.2.2. Additional solute–column interactions for phenyl columns

Table 4 summarizes values of $\log \alpha$ for a large number of different solutes on C_8 column #58, phenylhexyl column #1b, and phenylpropyl column #11b. Fig. 6 compares the retention of substituted benzenes (#19–24, solid triangles), halogen-substituted benzenes (#33–35, solid diamonds), fluoro-substituted benzenes (#13–17, solid squares), aliphatic alcohols (#49–51, open circles), and a polycyclic aromatic hydrocarbon (#26, solid circle) for phenyl column #1b versus C_8 column #58. The plots of Fig. 6 (selected for comparison to Fig. 2a) appear qualitatively similar to those observed in Fig. 2a for the comparison of a phenyl versus a C_{18} column, which in each case might be explained

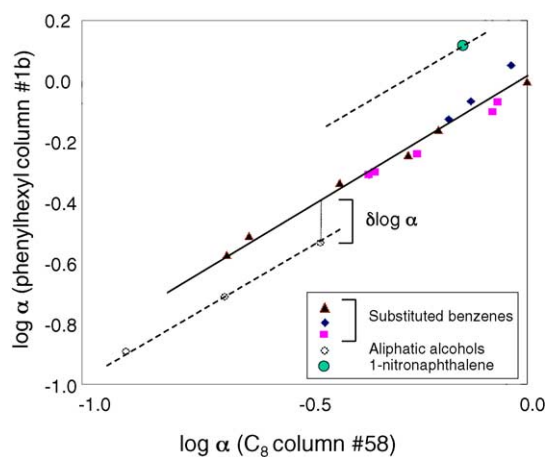


Fig. 6. Comparison of retention on phenyl column #1b vs. C_8 column #58 for different solute groups: substituted benzenes #19–24 (solid triangles), halogen-substituted benzenes #33–35 (solid diamonds), fluoro-substituted benzenes #13–17 (solid squares), *n*-alkanols (open circles) and 1-nitronaphthalene (closed circle). See text for details.

either by differential column RI values or by π – π interactions (Section 2.3). However, we will see that this simple interpretation becomes less compelling when the data of Table 4 are examined more closely. Note that values of $\delta \log \alpha$ (defined in Fig. 6) are equivalent to values of $\delta \log k$, because values of $\delta \log k$ for ethylbenzene cancel in the calculation of values of $\delta \log \alpha$. The use of values of $\log \alpha$ in Table 4, rather than $\log k$, eliminates the effect of possible differences in extra-column volume that can arise when different laboratories carry out retention measurements [33], as was true for the data of Table 4.

In Fig. 6, data for the substituted benzenes fall close to a straight line (as in the case of the plot of Fig. 5a for a fluorohexyl versus C_8 column), allowing the calculation of values of $\delta \log \alpha$ for other solutes (last two columns of Table 4 for column #1b versus C_8 and #11b versus C_8). These values of $\delta \log \alpha$ are similar for the two columns,

$$\delta \log \alpha[\text{column \#1b}] = 0.01 + 1.07 \delta \log \alpha[\text{column \#11b}] \quad (5)$$

$$(r^2 = 0.84)$$

so we will restrict our discussion to data for phenylhexyl column #1b. Because columns #1b and 11b represent near-extreme differences in the selectivity of different phenyl columns ($F_s = 34$), Eq. (4) suggests that values of $\delta \log \alpha$ (and related contributions to column selectivity) for different type-B phenyl columns will be relatively constant. This in turn means that values of H , S^* , etc. can reliably compare the selectivity of different phenyl columns (see the discussion of Section 4.3).

4.2.2.1. Contributions of differential column refractive index to the selectivity of phenyl columns. Table 7 summarizes the data of Table 4 in the form of average values of $\delta \log \alpha$ for different compounds or compound groups. The average value of $\delta \log \alpha$ for substituted benzenes (0.00) is more posi-

Table 7
Comparison of average values of $\delta \log \alpha$ for different solute groups on a phenylhexyl vs. a fluoroalkyl column

Solute		$\delta \log \alpha^a$	
		Phenyl column #1b	Fluoro column #1a
Substituted benzenes	#19–24	0.00 ± 0.01	0
Fluorobenzenes	#13–17	−0.02 ± 0.01	0.16
Halobenzenes	#33–35	0.03 ± 0.04	
Phenylalcohols	#10, 45–47	−0.08 ± 0.02	−0.1
Aliphatic alcohols	#49–51	−0.14 ± 0.01	
Alkylethers	#43–44	−0.10 ± 0.01	
Alkylacetates	#30–32	−0.09 ± 0.01	0.06
Halohexanes	#36–39	0.02 ± 0.03	
Nitroalkanes	#27–29	0.00 ± 0.01	0.09
Nitrobenzene	#25	0.03	0.02
1,3-Dinitrobenzene	#40	0.12	
1,4-Dinitrobenzene	#41	0.12	
1,3,5-Trinitrobenzene	#42	0.25	
1-Nitronaphthalene	#26	0.09	−0.07
5,5-Diphenylhydantoin	#18	0.00	−0.11

^a Value of $\delta \log \alpha$ calculated as in Fig. 6 for difference in retention on a phenyl or fluoro column vs. a C₈ column.

tive than for aliphatic alcohols (−0.14), ethers (−0.10) or acetates (−0.09), and more negative than for 1-nitronaphthalene (0.09), in agreement with differences in column RI values. However, differences in values of $\delta \log \alpha$ for other aliphatic compounds and the phenyl and C₈ column are less consistent: halogen-substituted hexanes (0.02), and nitroalkanes (0.00). We can compare these average $\delta \log \alpha$ values in Table 7 for the phenyl column with corresponding values for the fluoroalkyl column (#1a), whose unusual selectivity was attributed mainly to varying dispersion interactions (Section 4.1.2). If differential dispersion interactions play a significant role for phenyl column selectivity, comparable values of $\delta \log \alpha$ (of opposite sign) should be observed for both the phenyl and fluoroalkyl columns. However, fluoro-substituted benzenes show much larger absolute values of $\delta \log \alpha$ on the fluoro column (0.16) than on the phenyl column (0.02). Similarly, the polyaromatic solute 5,5-diphenylhydantoin has $\delta \log \alpha$ equal −0.11 on the fluoro column, but 0.00 on the phenyl column. Finally, nitroalkanes have $\delta \log \alpha$ equal 0.09 on the fluoro column, and 0.00 on the phenyl column. These comparisons suggest that differential dispersion interactions are a less important (or more complex) contributor to column selectivity for phenyl versus fluoroalkyl columns.

4.2.2.2. Contributions of π – π interactions to the selectivity of phenyl columns. Relatively large values of $\delta \log \alpha$ (0.03–0.25) are observed in Table 7 for nitro-substituted benzenes and naphthalene. Furthermore, $\delta \log \alpha$ increases regularly with increasing substitution of the benzene ring by nitro groups: one nitro (0.03), two nitros (0.12), three nitros (0.25). Similarly, $\delta \log \alpha$ for nitronaphthalene exceeds that of nitrobenzene by 0.06 units. These values of $\delta \log \alpha$ are consistent with an increase in solute retention with increasing π – π interactions. The value of $\log \alpha = 0$ for 5,5-diphenylhydantoin (#18) is also reasonable, because of the likely inability of this solute to simultaneously interact by π – π interactions with two phenyl ligands in the stationary phase.

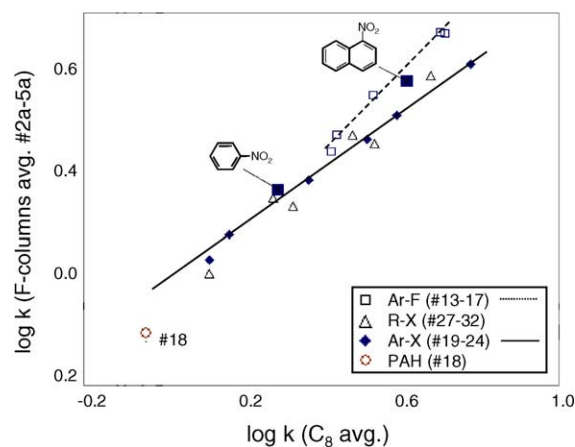


Fig. 7. Comparison of average retention on fluoro-phenyl columns #2a–5a with C₈ columns #8, 24, 39 and 58. See text for details.

The data of Table 7 are not, however, fully explained by π – π interaction alone (note the different values of $\delta \log \alpha$ for the different aliphatic solutes). It may well be that π – π interaction, differential ligand refractive index, and (possibly) dipole induction all contribute to some extent to phenyl column selectivity, and these interactions may be further altered by the severe steric constraints of the stationary phase (compared to comparable interactions in the liquid phase [37]). Any attempt to further clarify the relative importance of these various solute–column interactions will require additional experiments.

4.2.3. Additional solute–column interactions for perfluorophenyl columns

Selective retention on perfluorophenyl versus C₈ columns should be intermediate between that of fluoroalkyl and phenyl columns, and this appears to be the case. In Fig. 7, plots of average retention for various solutes on perfluorophenyl (#2a–5a) versus C₈ columns (#8, 24, 39, 58) are shown. For

the latter column comparison, aliphatic and aromatic solutes now fall close to a single curve, signifying little preferential retention of either compound type. Fluoro-substituted benzenes are still preferentially held on the fluoro column, however, and polyaromatic solute #18 is relatively less retained on the perfluorophenyl column. The two π -acids, nitrobenzene and 1-nitronaphthalene are slightly more retained on the perfluorophenyl column, but to a lesser extent than on the phenyl column—as expected for a weakening of the π -basicity of the phenyl ring by fluoro-substitution.

4.2.4. Phenyl column selectivity as a function of the mobile phase

Previous studies have established that column selectivity as measured by values of **H**, **S***, etc. does not change significantly when separation conditions other than mobile phase pH are changed [38]. However, the data of Fig. 2b versus 2a suggest that specific interactions of the solute and phenyl column are dependent on the organic solvent B used for the mobile phase, with acetonitrile (Fig. 2a) favoring such interactions compared to tetrahydrofuran (Fig. 2b). Limited additional measurements of π - π interaction as a function of the solvent composition are summarized in Table 8. Changes in column selectivity as a result of change in the mobile phase from 50% acetonitrile/buffer to 60% methanol/buffer were calculated as follows. For each solute and mobile phase studied, a change in $\log k$ ($\delta \log k$) was calculated for phenyl column #11b versus C₈ column #58. The change in $\delta \log k$ ($\delta \delta \log k$) was then calculated as $\delta \log k$ for 60% methanol minus $\delta \log k$ for 50% acetonitrile. If the change from 50% acetonitrile to 60% methanol were to have no effect on either π - π or dispersion interactions, the value of $\delta \delta \log k$ would in each case equal zero. Larger values of $\delta \delta \log k$ for methanol as solvent mean greater retention and a greater significance of π - π or dispersion interactions.

It is seen in Table 8 that values of $\delta \delta \log k$ for both the aromatic (−0.05) and aliphatic (−0.08) solutes are small and of similar size. This suggests that column selectivity as a result of varying dispersion interactions is not much affected

Table 8
Effect of mobile phase solvent on π - π selectivity for a phenyl vs. a C₈ column

Solute group	$\delta \delta \log k$
Aromatics ^a	−0.05 ± 0.03 (1 S.D.)
Aliphatics ^b	−0.08 ± 0.07 (1 S.D.)
1,3-Dinitrobenzene	0.23
1,4-Dinitrobenzene	0.25
1,3,5-Trinitrobenzene	0.39

The quantity $\delta \delta \log k$ is defined as $(\log k[\text{phenyl column \#11b}] - \log k[\text{C}_8 \text{ column \#58}])_a - (\log k[\text{phenyl column \#11b}] - \log k[\text{C}_8 \text{ column \#58}])_b$, where a refers to data for 60% methanol/buffer as mobile phase and b refers to 50% acetonitrile/buffer as mobile phase. Other conditions as in the Section 3.

^a Fluoro-, chlor-, bromo- and iodo-benzene; benzyl alcohol, 2-phenylethanol, 3-phenylpropanol.

^b 1-Pentanol, 1-hexanol, 1-heptanol, di-*n*-propylether, di-*n*-butylether.

Table 9
Comparison of phenyl vs. type-B alkyl-silica columns; average values of each column parameter

Column type	H	S*	A	B	C(2.8)	C(7.0)
Phenyl ^a	0.60	−0.16	−0.23	0.02	0.16	0.74
Type-B (C ₈) ^b	0.83	−0.01	−0.16	0.02	0.02	0.31
Difference	−0.23	−0.15	−0.07	0.00	0.14	0.43
Phenylhexyl ^c	0.73	−0.09	−0.30	0.01	0.02	0.36
Difference vs. C ₈	−0.10	−0.08	−0.14	−0.01	0.00	0.05
Difference vs. phenyl	0.13	0.07	−0.07	−0.01	−0.12	−0.07

^a Columns #3b–11b of Table 1.

^b Data of [34].

^c Columns #1b and 2b of Table 2.

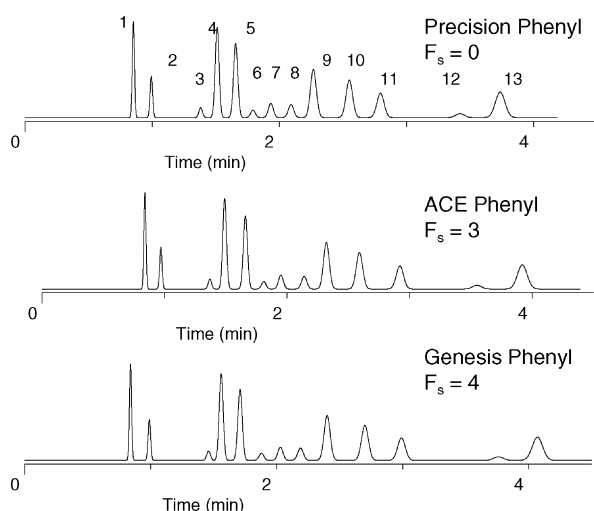
by the choice of mobile phase B-solvent. However, values of $\delta \delta \log k$ for the various nitrobenzenes of Table 8 are relatively large (0.23–0.39), which can be interpreted as an enhancement of π - π interactions by methanol versus acetonitrile as the B-solvent. Together with the data of Fig. 2, this suggests that π - π interactions (and related phenyl-column selectivity) increase for different B-solvents as tetrahydrofuran (least) < acetonitrile < methanol (most).

4.2.5. Comparison of phenyl with alkyl-silica columns of similar ligand length

Table 9 compares the difference in average value of **H**, **S***, etc. for the phenyl and phenylhexyl columns of Table 2 versus the type-B C₈ columns of [34]. On average, values of **H** for the phenyl and phenylhexyl columns are significantly lower than for the C₈ column (−0.23 to −0.10), as expected from the greater polarity (and reduced hydrophobicity) of the phenyl group when compared with a C₈ group. Values of **S*** are also lower for the phenyl and phenylhexyl groups (−0.08 to −0.15), which may be related to the generally lower ligand concentrations of columns #1–10 (2.7 $\mu\text{moles}/\text{m}^2$) compared to the corresponding type-B C₈ columns (3.7 $\mu\text{moles}/\text{m}^2$). Values of **A** are generally lower for the phenyl columns (−0.07 to −0.14), possibly due to an interaction of phenyl groups with surface silanols, similar to what was proposed for cyano columns [1]. Values of **C** are similar (0.14–0.00) for the phenyl columns, while values of **B** are essentially the same (0.00 to −0.01), in line with the modest hydrogen-bond basicity of a phenyl group in solution ($\beta_2 = 0.14$ [39]).

4.3. Likelihood of finding equivalent columns for different column types

When choosing a particular column for a routine HPLC assay, it is customary to confirm that the same separation results for columns of the same kind from different production batches. Since batch-to-batch uniformity for the separation of interest may be compromised at some future time, however, some workers prefer to identify one or more equivalent replacement columns (from a different source) before the assay procedure is released. It is often possible to replace an alkyl-silica type-B column with an equivalent column with $F_s \leq 3$ [34]. In the case of older type-A columns [33] or columns with



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Fig. 8. Comparison of separation of a representative sample on three similar phenyl columns. Conditions as in Section 3 (50% acetonitrile/buffer, pH 2.8). Sample: 1, *N,N*-dimethylacetamide; 2, *N,N*-diethylacetamide; 3, amitriptyline; 4, acetophenone; 5, *n*-butylacetate; 6, nitrobenzene; 7, fluorobenzene; 8, 1,3,5-trifluorobenzene; 9, toluene; 10, α,α,α -trifluorotoluene; 11, ethylbenzene; 12, mefenamic acid; 13, *trans*-chalcone. Values of F_s are for comparison with the Precision phenyl column in each case. Reconstructed chromatograms based on data for individual solutes from the present study are shown.

an embedded or end-capping polar group [35], however, this is less likely to be the case. The greater dissimilarity of type-A alkyl-silica columns likely reflects variable silica quality (varying metal contamination) and non-optimized manufacturing processes, while the bonding chemistry of polar-group columns varies widely [35]. Neither of the latter problems is associated with recent phenyl and cyano columns made from type-B silica, so column replacement should more often be possible for the latter columns. We have seen an example of this for cyano columns (Fig. 1 of [1]), and Fig. 8 shows some corresponding examples of similar separations involving different phenyl columns. Columns in Fig. 8 are compared with the Precision phenyl column in each case. Limited data in Fig. 4c show uniformly large values of S.D. for comparisons of separation among these five fluoro columns, suggesting that substituting one fluoro column by another may not be feasible in most cases. The above conclusions are also confirmed by the frequency of matching columns (with $F_s \leq 3$) for each column type as summarized in Table 10, which assumes that the sample is quite diverse (containing neutrals, acids and bases). The likelihood of matching a particular column increases for (a) samples which do not contain acidic or basic compounds [34], (b) a larger number of different columns available for matching, and/or (c) separations with a large resolution R_s ; for the latter situations (a–c), it is more likely to find equivalent columns based on either type-A silica or which contain a polar group.

Table 10
Frequency of “equivalent” columns for different column types (pH 2.8)

Column type	Total number of columns ^a	Avg. number equivalent columns ^b	Percentage equivalent columns ^c
Alkyl-silica type-A	38	0	0
Alkyl-silica type-B	87	1.8	2
EPG	21	0	0
Cyano	11	0.3	3
Fluoro	5	0	0
Phenyl	11	0.2	2

^a Number of columns reported in present or previous [1,33–37] papers.

^b For each column, average number of other columns with $F_s \leq 3$.

^c $100 \times (\text{avg. number of equivalent columns}) / (\text{total number of columns})$.

5. Conclusions

Previous reports [1,33–38] and review of [2] have demonstrated that the selectivity of reversed-phase columns can be described quantitatively by only five column parameters: hydrophobicity \mathbf{H} , steric resistance \mathbf{S}^* , hydrogen-bond acidity \mathbf{A} and basicity \mathbf{B} , and column cation exchange capacity \mathbf{C} .

$$\log \left(\frac{k}{k_{\text{EB}}} \right) \equiv \log \alpha = \eta' \mathbf{H} - \sigma' \mathbf{S}^* + \beta' \mathbf{A} + \alpha' \mathbf{B} + \kappa' \mathbf{C} \quad (1)$$

These five solute–column interactions appear to account for all significant contributions to the selectivity of (a) type-A and -B alkyl-silica, (b) polar embedded-group and (c) cyano columns. In the case of phenyl and fluoro-substituted (“fluoro”) columns, it appears that additional solute–column interactions contribute to retention and column selectivity. As a result, values of \mathbf{H} , \mathbf{S}^* , etc. for fluoro columns are less reliable as a means of comparing the selectivity of different fluoro columns. Phenyl columns, however, can be compared quantitatively by means of their column parameters, because any additional contributions to phenyl column selectivity appear of similar magnitude for different phenyl columns (Section 4.2.2).

The origin of these additional contributions to the selectivity of phenyl and fluoro columns was investigated by the use of several sample probes that can in principle differentiate among various solute–column interactions. The unusual selectivity of fluoroalkyl versus C_8 columns (apart from solute–column interactions described by Eq. (1)) can be rationalized by large differences in bonded-phase polarizability (as related to ligand refractive index), leading to differential dispersion interactions of solute and column. In the case of phenyl columns, π – π interactions appear to be important in contributing to the special selectivity of these columns. However, other (generally less significant) solute–column interactions are also possible. Retention on fluoro and phenyl columns is sufficiently complicated that predictions of retention and selectivity for these columns is at best no more than semi-quantitative.

π – π Interactions for phenyl columns are intensified by the use of methanol versus acetonitrile in the mobile phase, while literature data suggests that π – π interactions are weakened by the use of tetrahydrofuran. Relative to a C₈ column (other factors equal), solutes of lower refractive index are preferentially retained on a fluoro column; e.g., polycyclic aromatics < substituted benzenes < aliphatics \approx fluoro-substituted benzenes. Similarly, for phenyl columns, aliphatics < substituted benzenes < polycyclic aromatics \approx nitro-substituted benzenes.

If selectivity contributions not described by Eq. (1) are ignored, it is possible to compare fluoro and phenyl columns with corresponding alkyl-silica columns (e.g., C₈), as summarized in Tables 6 and 9. Fluoro and phenyl columns are significantly less hydrophobic (smaller values of **H**), while perfluorophenyl columns exhibit increased steric resistance (much larger values of **S**^{*}). Hydrogen-bond acidity **A** and cation-exchange capacity **C** are generally greater for fluoroalkyl columns.

With the conclusion of the present study (and present series of papers), most types of commercially available reversed-phase columns have now been studied in terms of Eq. (1). Resulting values of **H**, **S**^{*}, etc. have been reported for 182 different columns, and later unreported studies have expanded this list to more than 300 columns [2], data for which are now available in a commercial software package (Column Match[®]; Rheodyne LLC/LC Resources Group, Rohnert Park, CA 94927-1909).

6. Nomenclature

See the preceding paper [1].

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