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Column selectivity in reversed-phase liquid chromatography VI. Columns with embedded or end-capping polar groups

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Abstract

A previous model of column selectivity for reversed-phase liquid chromatography (RP-LC) has been applied to an additional 21 columns with embedded or end-capping polar groups (EPGs). Embedded-polar-group columns exhibit a significantly different selectivity *vs*. non-EPG, type-B columns, generally showing preferential retention of hydrogen-bond donors, as well as decreased retention for hydrogen-bond acceptors or ionized bases. EPG-columns are also generally less hydrophobic (more polar) than are non-EPG-columns. Interestingly, columns with polar end-capping tend to more closely resemble non-EPG columns, suggesting that the polar group has less effect on column selectivity when used to end-cap the column versus the case of an embedded polar group. Column selectivity data reported here for EPG-columns can be combined with previously reported values for non-EPG columns to provide a database of 154 different columns. This enables a comparison of any two of these columns in terms of selectivity. However, comparisons that involve EPG columns are more approximate.

Keywords: Column selectivity; Stationary phases, LC; Embedded-polar-group columns; End-capping polar group columns; Selectivity

1. Introduction

Previous papers [1–5] have described the development of an empirical model that can be used to characterize reversed-phase liquid chromatography (RP-LC) column selectivity by means of five column-dependent (solute independent) parameters: **H** (hydrophobicity), **S*** (steric selectivity), **A** (hydrogen-bond acidity), **B** (hydrogen-bond basicity), and **C** (cation-exchange/ion interaction behavior). Given values of **H**, **S***, etc. for different alkyl-silica columns, it is possible to compare these columns quantitatively in terms of selectivity [4,5]. Previous papers in this series have described the measurement of values of **H**, **S***, etc. for alkyl-silica columns of type-A (*A*cidic; high metal content) [5] and type-B (*B*asic; low metal content) [4].

For a number of reasons, the use of RP-LC columns with an embedded or end-capping polar group (EPG) is becoming more popular [6–14]; commonly used embedded polar groups include hydrogen-bond acceptors such as amide, urea and carbamate (polar groups used for end-capping are usually not defined by the manufacturer). EPG-columns exhibit significant differences in selectivity versus non-EPG columns [13,15–19], thereby providing a further test of the ability of the present model to describe RP-LC separation and column selectivity. In this study, we have investigated the application of our previous model to retention data for 21 EPG-columns. We also compare column selectivity for representative EPG versus non-EPG alkyl-silica columns.

2. Background and theory

For the case of alkyl-silica (non-EPG) columns [5], solute retention and column selectivity can be described by:

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

Separation factors α are given as a function of k for the solute of interest and a reference compound ethylbenzene

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(k_{ref}). Values of α are in turn related to properties of the solute (η', σ', β', α', κ') and the column (**H**, **S***, **A**, **B**, **C**). Terms (i)–(v) describe the contributions to column selectivity from hydrophobicity (i), steric hindrance to retention (ii), hydrogen bonding of acceptor solutes with stationary-phase donor groups (iii) or of donor solutes with column acceptor groups (iv), and ionic interaction of anionic or (especially) cationic solutes with a negatively charged stationary phase (v). See previous papers in this series [1–5] for a discussion of the significance and use of Eq. (1); symbols in Eq. (1) and elsewhere in this paper are defined in the 'nomenclature' section of the immediately preceding paper [5].

Given experimental values of α for appropriate test solutes, as well as values of the corresponding solute parameters η', σ', etc., values of **H**, **S***, etc. can be calculated for any column by multiple linear regression of Eq. (1). The required test solutes and their values of η', σ', etc. are given in Table 2 of the preceding paper (Part V [5]), along with a further description of the application of Eq. (1).

2.1. Electrostatic repulsion of cations from some EPG-columns

Depending on the method of manufacture, some EPG-columns contain residual amino groups which at low-pH are present in the ionized form ($-NH_3^+$) [13,14]. The positively charged column can then function as an anion-exchanger, leading to increased retention and/or band tailing for acidic solutes. Conversely, a positively charged column can lead to near-zero or even "negative" retention of fully ionized sample cations, because of cation exclusion. This appears to be the case for columns 1b, 7b and (possibly) 8b of Table 1 (see Table 2). At the same time, the two acidic solutes (16 and 17 of Table 2) tail markedly on columns 1b and 7b (asymmetry function values As > 2). Ion-exclusion of partially or fully ionized *acidic* solutes also occurs for many non-EPG columns, due to the negative charge on the column for pH $\geq$ 3 [3,5]; however, at low pH this effect is less evident because of the net positive retention of the non-ionized molecule. In any case, negative values of k for the cationic solutes of Table 2 can lead to

Table 1
Properties of columns used in the present study

Column	Ligand length[a] (n_c)	Polar group	Pore (nm) (d_{pore})	C_L (μmol/m^2)
1b: Prism C18 RP[b]	C_{12}	Urea group[c]	10	3.1
2b: Prism C18 RPN[b,c]	C_{12}	Urea group	10	2.4
3b: Xterra C_8 RP[d] (13,14,16)	C_8	Carbamate group	12.4	2.75
4b: Xterra C_{18} RP[d] (13,14,16)	C_{12}	Carbamate group	12.5	2.75
5b: Symmetry Shield C8[d] (13,14,16)	C_8	Carbamate group	8.8	3.29
6b: Symmetry Shield C18[d] (13,14,16)	C_{12}	Carbamate group	9.0	3.21
7b: Zorbax Bonus RP[e] (17)	C_{14}	Amide group	8	2.1
8b: HyPurity Advance[b]	C_8	Amide group		
9b: COSMOSIL 5-C18-PAQ[f]	C_{18}	"Polar" end-capping	12	2.0
10b: ProntoSIL 120-5-C8 ace-EPS[g]	C_8	"Polar" group	12	3.1
11b: ProntoSIL 120-5-C18 ace-EPS[g]	C_{18}	"Polar" group	12	2.8
12b: ProntoSIL 200-5-C18 ace-EPS[g]	C_{18}	"Polar" group	20	3.2
13b: ProntoSIL 300-5-C18 ace-EPS[g]	C_{18}	"Polar" group	30	3.2
14b: ProntoSIL 120-5-C18 Aqplus[g]	C_{18}	"Polar" end-capping	12	3.2
15b: Synergi Hydro-RP[h]	C_{18}	"Polar" end-capping	8	4.05
16b: Synergi Polar-RP[h,c]	Ether-linked phenyl	Ether "polar group" between silica and phenyl ring with polar end-capping	8	3.15
17b: Prevail amide[i]	C_{14}	Amide group	18	3.0
18b: Inertsil ODS-Ep[j]	C_{18}	Hydroxyl group	10	1.7
19b: PRECISION C18-PE[k]	C_{18}	"Polar" end-capping	12	2.5
20b: Discovery Amide C16[l]	C_{18}	Amide group	18	2.6
21b: Discovery HS PEG[l]	?	Polyethyleneglycol group	12	3.8

[a] For EPG columns, these values do not include (a) a three-carbon spacer between the silica and the embedded polar group and (b) the number of atoms in the polar group.
[b] ThermoHypersil.
[c] Non-end-capped (other than with a polar group).
[d] Waters.
[e] Agilent.
[f] Nacalai Tesque.
[g] Bischoff.
[h] Phenomenex.
[i] Alltech.
[j] GL Sciences.
[k] MacMod/Higgens.
[l] Supelco; presumably a small $-(O\text{-}CH_2\text{-}CH_2)_n-$ group is embedded.

Table 2
Unusual retention of cationic solutes on columns 1b, 7b, 8b (pH 2.80)

Solute	k		
	Column 1b	Column 7b	Column 8b
Amitriptyline	0.00	0.00	0.00
Nortriptyline	0.00	0.00	0.01
Berberine	−0.09	−0.06	−0.02
	Band asymmetry As		
4-*n*-Butylbenzoic acid	2.4	6.7	1.00
Mefenamic acid	2.3	13.8	0.80

Experimental conditions as in Section 3.

values of the column parameter **C** that are indeterminate, while for $k = 0$, $\mathbf{C} = -\infty$. Thus, cation exclusion can represent an obvious complication in the application of Eq. (1) to the columns of Table 3. Cation exclusion for columns 1, 7 and 8 was treated here by dropping term (v) of Eq. (1) and values of α for the three cationic solutes (amitriptyline, nortriptyline, berberine). Consequently, values of **C** for columns 1, 7 and 8 in Table 1 were not determined.

3. Experimental

All procedures, equipment and materials were essentially the same as in the preceding paper, Part V [5]. The 21 columns reported here are described in Table 1 and were the generous gift of the manufacturer. For each of these 21 columns, values of the retention factor k were determined for the 19 solutes listed in Table 3. Conditions were the same as in [5]: 50% (v/v) acetonitrile/buffer, where the buffer was 60 mM potassium phosphate at pH 2.8 or 7.0; 35 °C; 2.0 ml/min; UV-detection at 205 nm; 500 ng injections of each solute.

3.1. Calculations

For each column, values of $\log\alpha$ were calculated for 15 of the 16 test solutes used in previous papers [4,5]; i.e., the solutes of Table 3, excluding compounds 8, 9, 14 and 15. Based on Eq. (1), a multiple linear regression of these $\log\alpha$ values was then carried out *vs.* values of the solute parameters η', σ', etc. reported in Table 2 of [5]; so-called "type-B solute parameters". The latter regression yielded values of the column parameters **H**, **S***, etc. for each column, as well as a standard deviation (S.D.) of the fit.

4. Results and discussion

4.1. Agreement of retention data for EPG-columns with Eq. (1)

Experimental values of α for 15 solutes and 21 columns (Tables 1 and 3) were first fit to Eq. (1), using the solute parameters previously derived from retention data for type-B columns [4]. The resulting agreement of values of $\log\alpha$ with Eq. (1) was relatively poor (average S.D. in $\log\alpha$ equals 0.057, or +14% in α, excluding data for columns 1, 7 and 8 which exhibit cation exclusion (Section 2.1)). Values of the column parameters so obtained are given in Table 4. A possible contributing factor in the poor agreement of retention data for EPG-columns with Eq. (1) is less accurate values of α for solute 10 (*N*,*N*-dimethylacetamide, because of small values of k for this solute; $0.006 \leq k \leq 0.24$ (average $k =$

Table 3
Best-fit solute parameter values for the test compounds of the present study and the EPG columns of Table 1

Solute	η'	σ'	β'	α'	κ'
1. Acetophenone	−0.751	0.302	0.065	−0.286	−0.022
2. Benzonitrile	−0.729	0.543	0.032	−0.224	−0.053
3. Anisole	−0.481	0.328	0.008	−0.216	−0.041
4. Toluene	−0.207	0.001	0.004	−0.115	−0.015
5. Ethylbenzene	0.000	0.000	0.000	0.000	0.000
6. *cis*-Chalcone	−0.067	0.971	−0.023	0.164	−0.028
7. *trans*-Chalcone	0.044	0.831	−0.032	0.268	−0.036
8. *cis*-4-Nitrochalcone[a]	−0.118	1.392	−0.040	0.221	−0.048
9. *trans*-4-Nitrochalcone[a]	0.029	1.201	−0.058	0.314	−0.031
10. *N*,*N*-Dimethylacetamide	−1.926	0.257	0.984	0.154	−0.003
11. *N*,*N*-Diethylacetamide	−1.328	−0.503	0.388	−0.610	0.063
12. 5-Phenylpentanol	−0.486	0.112	0.076	0.202	0.011
13. 4-Nitrophenol	−0.980	0.073	−0.051	0.825	0.019
14. 1,3-Dihydoxynaphthalene[a]	−1.067	−0.159	−0.098	1.044	0.007
15. *p*-Chlorophenol[a]	−0.776	0.012	−0.068	0.668	−0.044
16. 4-*n*-Butylbenzoic acid	−0.248	−0.309	0.040	1.090	0.004
17. Mefenamic acid	0.056	0.228	−0.093	1.302	0.002
18. Nortriptyline	−1.167	−0.107	−0.062	0.471	0.873
19. Amitriptyline	−1.097	0.320	−0.006	0.128	0.790

Experimental conditions as in Section 3.
[a] Additional solutes beyond the 16 solutes of [4]; 5,5-diphenylhydantoin was used as test solute in [4,5], but not in the present study.

Table 4
Selectivity of columns used in the present study. Experimental conditions as in Section 3. Values of **H**, **S***, etc. derived from application of Eq. (1) using type-B solute parameters of Table 2 of [5]. See text for details

Column	Selectivity parameters							S.D.
	H	**S***	**A**	**B**	**C**(2.8)	**C**(7.0)	$\log k_{ref}$	
1b: Prism C18 RP	0.64	−0.02	−0.52	0.28	[a]	[a]	0.683	0.11
2b: Prism C18 RPN	0.678	−0.001	−0.068	0.230	−0.544	0.625	0.530	0.077
3b: Xterra C_8 RP	0.657	−0.049	−0.604	0.099	−0.187	−0.198	0.486	0.050
4b: Xterra C_{18} RP	0.757	−0.043	−0.483	0.097	−0.170	−0.173	0.632	0.049
5b: Symmetry Shield C8	0.730	−0.006	−0.550	0.103	−0.623	0.138	0.756	0.052
6b: Symmetry Shield C18	0.850	0.027	−0.411	0.093	−0.728	0.136	0.863	0.050
7b: Zorbax Bonus RP	0.65	0.01	−1.12	0.35	[a]	[a]	0.649	0.14
8b: HyPurity Advance	0.40	−0.04	−0.14	0.24	[a]	[a]	0.210	0.10
9b: COSMOSIL 5-C18-PAQ	0.822	−0.027	−0.342	0.053	−0.353	0.047	0.744	0.023
10b: ProntoSIL 120-5-C8 ace-EPS	0.532	−0.007	−0.852	0.213	−0.282	0.094	0.567	0.089
11b: ProntoSIL 120-5-C18 ace-EPS	0.772	0.042	−0.590	0.228	−0.304	0.041	0.919	0.101
12b: ProntoSIL 200-5-C18 ace-EPS	0.765	0.021	−0.566	0.214	0.026	0.143	0.669	0.092
13b: ProntoSIL 300-5-C18 ace-EPS	0.762	0.025	−0.579	0.211	−0.054	0.136	0.457	0.087
14b: ProntoSIL 120-5-C18 Aqplus	0.947	−0.017	0.214	0.041	−0.133	0.605	0.960	0.026
15b: Synergi Hydro-RP	1.022	−0.006	0.169	−0.042	−0.077	0.260	1.053	0.018
16b: Synergi Polar-RP	0.654	−0.148	−0.257	−0.007	0.057	0.778	0.592	0.028
17b: Prevail amide	0.862	−0.063	0.251	0.033	0.058	1.209	0.982	0.037
18b: Inertsil ODS-EP	0.807	0.064	−1.525	0.050	−0.626	−0.075	0.860	0.067
19b: PRECISION C18-PE	0.976	−0.018	−0.085	−0.001	0.005	0.168	0.933	0.005
20b: Discovery Amide C16	0.720	0.013	−0.625	0.218	−0.092	−0.025	0.600	0.092
21b: Discovery HS PEG[c]	0.318	0.027	−0.713	0.128	−0.531	0.387	−0.131	0.078
Average								0.057[b]

[a] Values of **C** not determined; see text.
[b] average value, excluding columns 1, 7 and 8.
[c] Presumably a small $–(O–CH_2–CH_2)_n$-group is embedded.

0.07)). However, removal of this solute from the regression of Eq. (1) versus the remaining 14 solutes did not improve the correlation.

As in the preceding paper concerning type-A columns [5], multiple linear regression was then repeated several times to obtain the best fit of the data to Eq. (1), with a corresponding change in values of the solute parameters (but little change in column parameter values). At the same time, four additional solutes in Table 2 (8, 9, 14 and 15) were added. The best-fit solute parameters obtained in this way are given in Table 3. With the exception of columns (1b, 7b and 8b), the average (best-fit) S.D. for the remaining 18 columns is marginally acceptable (S.D. = 0.013; +3% in α), but not as good as that found for non-EPG-columns (S.D. = 0.005–0.008 [4,5]). Considering the diversity of the columns of Table 1 (see Section 4.2), as well as the limitations of Eq. (1) discussed in Appendix A of the preceding paper [5], this result represents agreement with Eq. (1) that may be as good as can be expected, without major change in Eq. (1). The quality of the observed fit (+3% in α) suggests that no major additional solute-column interactions are involved in retention on EPG-columns, other than those represented in Eq. (1).

4.2. Selectivity comparison of EPG- and non-EPG columns

As noted previously [3–5], RP-LC column selectivity varies with several properties of the column: ligand length and concentration, pore diameter, silica acidity and whether the column is end-capped or not. Additionally, the selectivity of EPG-columns varies with the nature of the polar group and whether that group is embedded or used to end-cap the column. Because of the limited number of EPG-columns included in Table 1, and their marked diversity, only a rough overall comparison of selectivity can be made for EPG-packing versus non-EPG-packing. A similar comparison as in the preceding paper [5] is shown in Table 5, assuming that each of the EPG-columns of Table 1 can be considered as “end-capped”. For most of these EPG-columns, it is not known whether they have been end-capped (other than with a polar group), but the

Table 5
Comparison of average selectivity of EPG- and type-B columns. Values for type-B columns are from [4], excluding non-end-capped columns and including only C_8 and C_{18} columns. Experimental conditions as in Section 3

Average column	**H**	**S***	**A**	**B**	**C**(2.8)	**C**(7.0)
Type-B	0.94	0.01	−0.11	0	0.04	0.22
EPG (embedded)[a]	0.68	0.00	−0.54	0.17	−0.65	0.13
Diff (EPG—type-B)	−0.26	−0.01	−0.43	0.17	−0.69	−0.09
EPG (end-capped)[b]	0.94	−0.02	−0.01	0.01	−0.14	0.27
Diff (EPG—type-B)	0.00	−0.03	0.10	0.01	−0.18	0.05

[a] All columns in Table 4, except 9b, 14b, 15b, 19b; values of **C**(2.8) do not include columns 1b, 7b and 8b, which have very low values of **C**.
[b] Polar end-capping group; columns 9b, 14b, 15b, 19b.

suppression of silanol activity by the polar group might be regarded as roughly equivalent to the effects of end-capping of non-EPG columns in the comparison of Table 5.

Columns with an embedded polar group (Table 5) are generally less hydrophobic (smaller **H**) than non-EPG (type-B) columns, less acidic toward hydrogen-bond acceptors (smaller **A**), and more basic toward hydrogen-bond donors (larger **B**). These characteristics of EPG-columns have been noted by others [13,14,18] and are expected, based on the nature of the basic, polar groups present in these columns. The cation-exchange behavior of these EPG-columns at low pH is generally lower than non-EPG columns (smaller **C**(2.8)), but values of **C** at high pH are much closer to those of non-EPG columns (less change in **C**(7.0)). Presumably the small negative charge on the silica at low pH is suppressed by the (basic) polar group in some way, while the much greater column charge at high pH largely outweighs any such effect. Polar-end-capped columns more closely resemble type-B columns (Table 5) than embedded-polar-group columns.

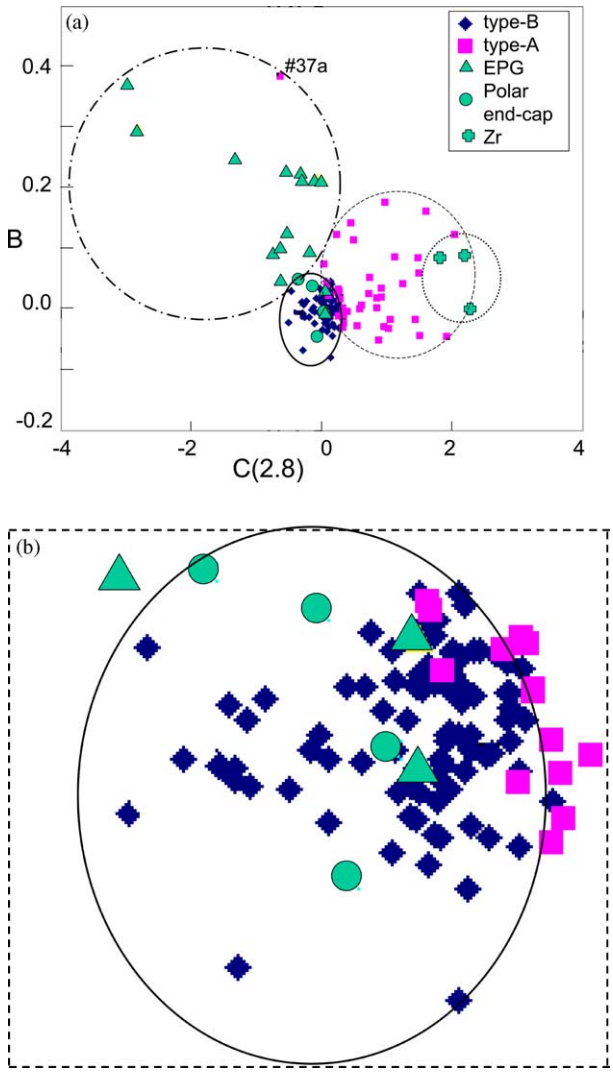

Fig. 1. Classifying different column types by means of their acidity **C** and basicity **B**: (a) data for all columns; (b) expansion of data for type-B columns. See text for details.

The various kinds of alkyl-silica columns so far studied (type-A, type-B, EPG, bonded-zirconia)) can be roughly classified according to their acidity and basicity. This is shown in Fig. 1a, where column hydrogen-bond basicity **B** is plotted versus column Bronsted acidity (approximated by **C**) for each of the 154 columns so far studied. In Fig. 1a, EPG-columns are subdivided according to whether the polar group is embedded or end-capped. These five column types fall in distinct regions of the diagram, but with some overlap (note an extreme outlier, type-A column 37a from Part V [5]). Column overlap is better shown in Fig. 1b, which is an expansion of the region in Fig. 1a that is occupied by type-B columns. The circular region in Fig. 1b (solid curve) encompasses 98% of the type-B columns, 12% of the type-A columns, and 12% of the embedded-polar-group columns. Columns with polar end-capping fall entirely within the circle that surrounds the type-B columns, as implied by Table 5. The extent of overlap in Fig. 1 for each column type reflects the fact that neither the presence of a polar group in the stationary phase, nor the absence of contaminating metals in the silica, results in a clear distinction in terms of column selectivity. The latter finding is unsurprising, since the concentration and basicity of the polar group can vary among different EPG columns, as can metal content and silica acidity in type-A columns, while these various columns also differ in other respects (end-capping, pore diameter, ligand concentration, etc.).

Fig. 2 further illustrates the selectivity properties of EPG-columns by comparing the separation of a model sample on three different columns: (a) an acidic, type-A column (Allsphere ODS1), (b) a less acidic, type-B column (Ace C18), and (c) a basic, EPG-column (7b, Bonus RP). Note the relative sample retention on each column:

Type-A column	Type-B column	EPG-column
Nitrophenol	Diethylacetamide	*Amitriptyline*
Diethylacetamide	*Nitrophenol*	Diethylacetamide
Acetophenone	*Amitriptyline*	Acetophenone
Butylbenzoic acid	Acetophenone	*Nitrophenol*
Amitriptyline	*Butylbenzoic acid*	Chalcone
Chalcone	Chalcone	*Butylbenzoic acid*

As the acidity of the column decreases and basicity increases from (a) to (c), the relative retention of the acidic solutes *nitrophenol* and *butylbenzoic acid* increases, while that of the ionized base *amitriptyline* decreases.

4.2.1. Differences in selectivity among different EPG-columns

Polar-end-capped columns 9b, 14b, 15b, and 19b show little effect of the polar group on column selectivity, except for values of **C**(2.8) (Table 5). These columns therefore tend to resemble non-EPG-columns in terms of selectivity.

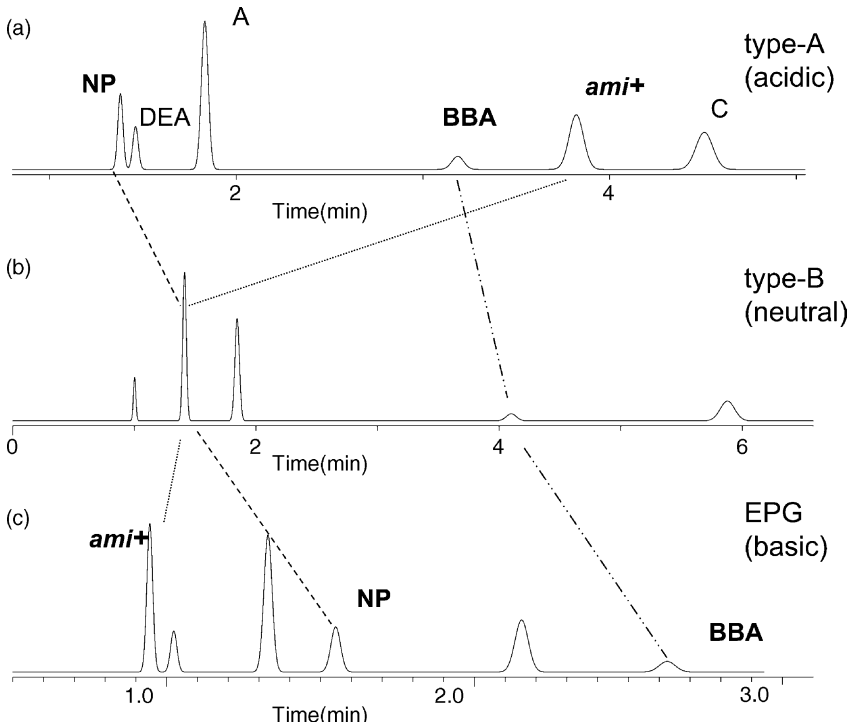

Fig. 2. Relative retention as a function of column type: (a) Allsphere ODS1 (type-A); (b) Ace C18 (type-B); (c) Bonus RP (EPG). NP, 4-nitrophenol; DEA, *N*,*N*-diethylacetamide; A, acetophenone; BBA, 4-*n*-butylbenzoic acid; ami$^+$, amitriptyline; C, *cis*-chalcone. Experimental conditions as in Section 3.

The reason for the small effect of an EPG on column selectivity is unclear, although the relative concentration of polar-end-capping groups compared to embedded polar groups may be small. Thus, when conventional alkyl-silica columns are end-capped, there is typically no more than 3–5% increase in total carbon [20,21]. Allowing for the six-fold greater carbon content of a C_{18} group versus a trimethylsilyl end-capping group, this represents no more than a 20–30% increase in the total moles of ligand bonded to the silica. Another factor may be the polar end-capping group used, which is not stated for any of the polar-end-capped columns of Table 1. As discussed below, different polar groups vary in their basicity and resulting effect on column selectivity. The combination of a less basic end-capping group with a lower concentration of that group could explain the lower values of **B** for polar-end-capped columns 9b, 14b, 15b and 19b.

For polar embedded columns, the **B**-values of Table 4 suggest that different polar groups increase in basicity as

ether ($\mathbf{B} = -0.01$) < hydroxy ($\mathbf{B} = 0.05$)
< carbamate ($0.09 \leq \mathbf{B} \leq 0.10$)
< PEG (glycol) ($\mathbf{B} = 0.15$)
< urea ($0.23 \leq \mathbf{B} \leq 0.30$) ≈ amide ($0.22 \leq \mathbf{B} \leq 0.37$)

Column 17b (amide phase, $\mathbf{B} = 0.02$) is only weakly basic and an exception to the latter series, presumably because of differences in the synthesis of this stationary phase. Likewise, these relative polar-group basicities are provisional, because of possible differences in stationary phase concentrations of the polar group, as well as the small number of columns of each type that were studied.

4.2.2. "Phenol selectivity" for EPG-columns

A few studies of EPG columns [13,18,19] have commented on the selective retention of phenols, relative to retention on non-EPG columns. Because of the pronounced hydrogen-bond basicity of EPG-columns, this is not unexpected. Table 6 summarizes solute hydrogen-bond basicity (α' values) for several donor-solutes from the present study. Additional solutes apart from those in Table 3 are represented in Table 6, for which values of α' were derived from values of k for columns 2b–6b only (and are therefore more approximate). Relatively large values of α' for phenols (average $\alpha' = 0.7$) combine with larger values of **B** for EPG-columns to yield larger values of $\alpha'\mathbf{B}$ for phenols and their selective retention on EPG versus non-EPG-columns (Fig. 2). However, the selective retention of carboxylic acids (as measured by an average $\alpha' = 1.2$) on EPG-columns is even more pronounced. Therefore, what has been referred to as "phenol selectivity" may need to be broadened to include carboxylic acids as well, as indeed observed in an earlier study [13].

Average values of α' for alcohols, benzoic acids and phenols in Table 6 can be compared with Abraham's solute hydrogen-bond acidity parameter, α_2^H, for prototypical compounds [22]: benzyl alcohol, 0.39; benzoic acid, 0.59; phenol, 0.60. Values of α' for substituted alcohols, benzoic acids and phenols are all positive, as expected, but correlate poorly

Table 6
Comparison of solute hydrogen-bond acidity (α') for EPG-column vs. non-EPG column. Experimental conditions as in Section 3; approximate values from data for columns 1b–6b only

Solute	EPG columns		Type-B columns[a]	
	α'	Average	α'	Average
Alcohols		0.3		0.1
Benzyl alcohol[b]	−0.1		−0.10	
5-Phenylpentanol	0.4		0.37	
Prednisone[b]	0.5		0.02	
Benzoic acids		1.2		0.8
4-*n*-Butyl benzoic acid	1.1		1.02	
4-*n*-Pentyl benzoic acid[b]	1.3		1.18	
2,6-Dimethyl benzoic acid[b]	1.4		0.46	
Ketoprofen[b]	0.8		0.55	
Mefenamic acid	1.3		0.92	
Phenols		0.7		0.2
Phenol[b]	0.1		−0.03	
4-Chlorophenol	0.7		0.15	
3-Nitrophenol[b]	0.7		0.16	
4-Nitrophenol	0.8		0.22	
1,3-Dihydroxy Naphthalene	1.0		0.20	
Eugenol[b]	0.6		0.15	
Danthron[b]	0.7		0.28	
Average: α'(phenol)/ α'(benzoic acid)		0.6		0.2

[a] Values from [1,3].
[b] Values derived from data for columns 1b–7b.

with values of α_2^H, presumably because of steric hindrance effects in RP-LC retention (see the preceding paper [5]), as well as the intramolecular electronic effects of various substituents on values of α_2^H for the solutes of Table 6. If we compare values of α' for the donor-solutes of Table 6 for EPG-column versus non-EPG-column, we see that the relative retention of phenols to benzoic acids appears to increase for EPG-columns (α'-ratios of 0.6 and 0.2, respectively). This difference in α' values for phenols versus carboxylic acids may be due to differences in the interaction of these solutes with absorbed water in the case of non-EPG-columns [4,5] versus the various polar groups in EPG-columns (see Section 4.2.3).

4.2.3. Further examination of the column B-parameter

For non-EPG type-B columns, it has been suggested [3,4] that column hydrogen-bond basicity **B** may be due to the presence of sorbed water in the stationary phase. This conclusion is based on several observations, one of which is the inverse correlation of values of **B** and **H**. That is, more hydrophobic columns (larger **H**) should tend to sorb less water, thereby leading to a lower hydrogen-bond basicity and lower values of **B**. With **B**-values now available for 154 columns which include type-A, type-B and EPG phases, it is possible to further examine the latter conclusion. Fig. 3a is a plot of values of **B** versus **H** for the type-B columns described in [4]. Values of **B** and **H** are correlated moderately:

$$\mathbf{B} = 0.131 - 0.141\mathbf{H} \quad (r^2 = 0.61;\ \text{S.D.} = 0.015) \qquad (2)$$

That r^2 is so much less than unity indicates that other factors besides column hydrophobicity likely contribute to both water sorption by the stationary phase and the H-bond basicity of sorbed water. The dashed lines in Fig. 3a bracket a range of +2.5S.D., which for a normal distribution will include 99% of the data points. Only one of the type-B columns (20a) falls outside this range, as expected. Sorbed water as a cause for hydrogen-bond basicity in the case of type-B column should be regarded as an unproved hypothesis, but for purposes of discussion we will assume its validity here.

In Fig. 3b, a similar plot of **B** versus **H** is shown for the type-A columns of the previous paper (5), with superimposed correlation line and error limits for type-B columns from Fig. 3a. Thirteen type-A columns (enclosed in the dashed circle of Fig. 3b) deviate from Eq. (2) by more than +2.5S.D., and *all* of these deviations are positive. This suggests some additional contribution to **B** for these particular type-A columns, possibly related to the contaminating metals associated with type-A silica. The latter possibility is strengthened by the fact that all seven columns (half of the circled outliers in Fig. 3b) from two manufacturers (12a–14a, 21a–24a) are included among the deviating type-A columns. This is consistent with the likely use of the same or similar silica by individual manufacturers. Note also the extreme deviation of column 37a, which has a very large value of **B** for a type-A column. Since we have pointed out [5] that type-A columns are more acidic than type-B columns, a reasonable question is: how can these columns be both more and less acidic at the same time? One answer is that both acidic and basic sites can be present; thus contaminating metals M^{2+} can serve to both activate adjacent silanols so as to increase their acidity, while simultaneously being available for direct interaction with an acidic solute HA; e.g., by forming a complex of the form $M^{2+}A^-$. In any case, the experimental values of **B** for type-A columns speak for themselves.

In Fig. 3c for EPG-columns, a plot similar to that shown in Fig. 3b is presented. Polar-end-capped columns (circles in Fig. 3c) fall within the error limits for type-B columns, suggesting that the basicity of these columns predominantly results from sorbed water—not the polar end-capping group. All but three of the embedded-polar-group columns have values of **B** that fall above the error limits for type-B columns. We infer from this relationship that in most cases values of **B** for EPG-columns arise from a source other than sorbed water, in agreement with our belief that the generally greater basicity of EPG-columns is due to the embedded basic polar group.

Fig. 3d shows a similar plot as in Fig. 3b and c for bonded-zirconia columns. Values of **B** fall within the error limits, suggesting that sorbed water is responsible for the hydrogen-bond basicity of these columns, Given the small data set, however, this conclusion must be regarded as tentative.

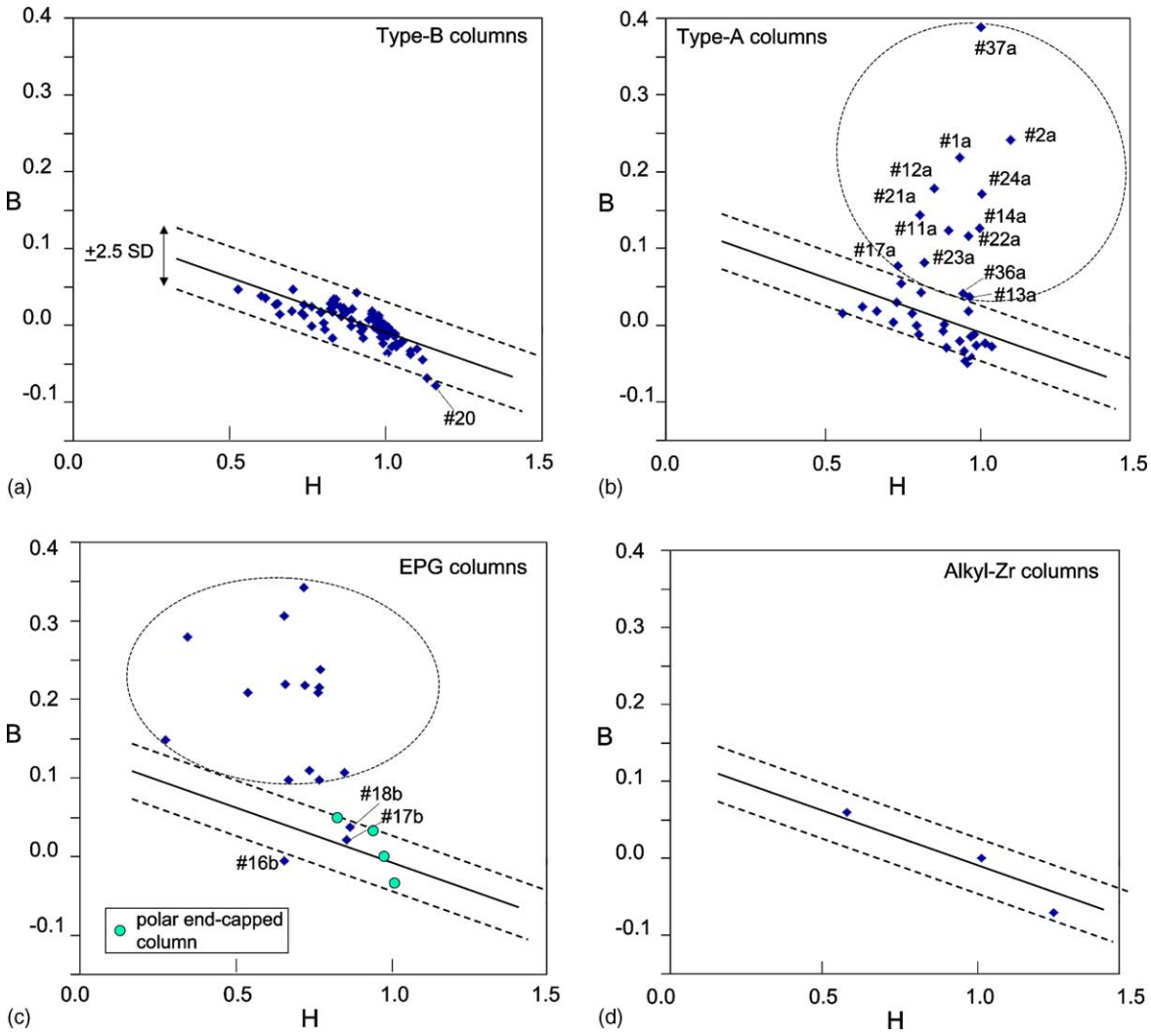

Fig. 3. Column hydrogen-bond basicity **B** as a function of column hydrophobicity **H**: (a) type-B columns; (b) type-A columns; (c) EPG-columns; (d) alkyl-zirconia columns. Curves in (b–d) taken from (a). See text for details.

4.2.4. Comparisons of C_8 vs. C_{18} columns from the same manufacturer

Previous papers in this series [3–5] have examined the dependence of values of **H**, **S***, etc. on column properties such as ligand length and concentration, pore diameter, and end-capping. For the most part, the observed relationships for a given column parameter can be rationalized in terms of the proposed solute-column interactions for each term of Eq. (1). The limited number of EPG-columns in Table 4, as well as their diversity, precludes a similar analysis for the columns of Table 4. However, we can compare changes in **H**, **S***, etc. for C_8 versus C_{18} columns of the same type from the same manufacturer; e.g., Symmetry C_8 versus C_{18}. In most such cases, it is reasonable to assume that the silica used for the C_8 and C_{18} versions of the column is similar in terms of pore diameter and silica "acidity", and that any difference in ligand concentration for a given pair of C_8 and C_{18} columns will also be similar (i.e., if the manufacturing process achieves a higher or lower ligand concentration for the C_8 column, the same is likely to be true for the C_{18} column). If this is the case, then we should see consistent changes in **H**, **S***, etc. for C_{18} versus C_8 columns. This hypothesis is examined in Table 7, where average changes in **H**, **S***, etc. for C_{18} versus C_8 columns are summarized for type-B [4], EPG-column (Table 4), and type-A column [5].

For an increase in ligand length from C_8 to C_{18}, we see in Table 7, a sizable and consistent increase in **H** (+0.14 average) for all three column types (B, EPG and A). This increase in column hydrophobicity with ligand length is expected and has been noted previously [3,4]. No significant change in **S*** (+0.01 average) is seen for C_{18} columns. Values of **A** are significantly larger (+0.12) for C_{18} columns, which has been noted before but not explained [4]. The effect of an increase in ligand length from C_8 to C_{18} on **B** is both small (−0.02 average) and variable. Changes in **C** with ligand length are similar for type-B and EPG-columns, but not for type-A columns. This is likely the result of poorer control of silica acidity in the case of type-A columns.

Table 7
Average change in **H**, **S***, etc. for C_{18} vs. C_8 columns of the same type from the same manufacturer; e.g., Symmetry C_{18} and C_8

Column type	No. of column pairs	Average change in **H**, **S***, etc. for C_{18} vs. C_8 columns[a]					
		H	**S***	**A**	**B**	**C**(2.8)	**C**(7.0)
Type-B	19	0.15 ± 0.05	0.01 ± 0.02	0.11 ± 0.06	−0.02 ± 0.01	0.04 ± 0.08	−0.02 ± 0.09
EPG	3	0.15 ± 0.08	0.03 ± 0.02	0.17 ± 0.08	0.00 ± 0.02	−0.04 ± 0.06	−0.01 ± 0.04
Type-A	8	0.13 ± 0.12	−0.01 ± 0.07	0.07 ± 0.22	−0.03 ± 0.06	0.14 ± 0.26	0.25 ± 0.41
Average[a]		0.14	0.01	0.12	−0.02	−0.02	0.08

[a] For example, the change in **H**=**H**(C_{18})–**H**(C_8).

4.3. Comparing columns in terms of selectivity

We can compare any two columns 1 and 2 in terms of selectivity by means of the relationship:

$$F_s = \{[12.5(\mathbf{H}_2 - \mathbf{H}_1)]^2 + [100(\mathbf{S}^*_2 - \mathbf{S}^*_1)]^2 + [30(\mathbf{A}_2 - \mathbf{A}_1)]^2 + [143(\mathbf{B}_2 - \mathbf{B}_1)]^2 + [83(\mathbf{C}_2 - \mathbf{C}_1)]^2\}^{1/2} \quad (3)$$

defined previously [4] ($\mathbf{H}_1$ and $\mathbf{H}_2$ refer to values of **H** for columns 1 and 2, and similarly for **S***, **A**, **B** and **C**). If two columns have $F_s \leq 3$, it is likely that the two columns will give equivalent separations for the same experimental conditions and sample. For the majority of type-B columns, it is likely that one or more "equivalent" columns can be found. Because of the greater diversity of type-A columns, finding an equivalent column is much less likely, although this also depends on the nature of the sample [5]. The even greater diversity of EPG-columns makes it still less likely that equivalent EPG-columns can be found. When the 21 EPG-columns of Table 4 were compared with each other, and with the 87 type-B columns of [4] or the 43 type-A columns of [5], only the Prevail amide column (17b) could be matched with another column (Prevail C18), with $F_s = 2.2$. A comparison of column parameters for these two columns showed only small differences for the EPG *vs.* non-EPG column (**H**, −0.01; **S***, 0.00; **A**, −0.03; **B**, 0.00; **C**(2.8), −0.0.2; **C**(7.0), +0.03), suggesting a very limited incorporation of the amide group into column 17b.

5. Conclusions

The present paper extends our evaluation of RP-LC column selectivity to include columns with an embedded or EPG, by means of the relationship:

$$\log\left(\frac{k}{k_{ref}}\right) \equiv \log\alpha = \eta'\mathbf{H} - \sigma'\mathbf{S}^* + \beta'\mathbf{A} + \alpha'\mathbf{B} + \kappa'\mathbf{C} \quad (4)$$

The agreement of experimental data with Eq. (4) (assuming the same values of η', σ', etc. as for alkyl-silica columns) was relatively poor: +14% in α for 18 or 21 columns. The three excluded columns each exhibited cation exclusion, which prevented the measurement of values of **C**. Through the use of solute parameters (η', σ', etc.) derived from retention data for EPG-columns, the agreement of experimental and calculated values (*via* Eq. (4)) of $\log\alpha$ improved to +3% in α (1S.D.). The latter agreement of experimental data with Eq. (4) suggests no additional major contributions to selectivity for EPG-columns, other than those already represented in Eq. (4).

When column selectivity parameters (**H**, **S***, etc.) for EPG-columns are compared with values of **H**, **S***, etc. for non-EPG type-B columns, columns with an embedded polar group are found to be generally less acidic (smaller values of **A** and **C**) and more basic (larger values of **B**). This results in the preferential retention of phenols and carboxylic acids on EPG-columns, and reduced retention of hydrogen-bond acceptors and ionized solutes such as protonated amines. EPG-columns are also more polar (smaller **H**), which reduces the retention of more hydrophobic solutes. Interestingly, columns which have been polar-end-capped, as opposed to possessing polar-embedded groups, tend to resemble non-EPG columns in terms of selectivity. That is, polar-end-capping appears to have relatively little effect on column selectivity, at least for the columns studied here.

Column hydrogen-bond basicity as measured by the column parameter **B** appears to arise from three different column acceptor groups, although the evidence for this finding must be considered as preliminary. For type-B columns, sorbed water in the stationary phase appears largely responsible for values of **B**. For one out of three type-A columns, contaminating metals in the silica significantly increase values of B, while the remaining type-A columns likely derive their (slight) basicity from sorbed water. For EPG-columns, four out of five columns with embedded polar groups have **B** values that appear to be determined by the polar goup, rather than water. However, the basicity of EPG-columns with end-capping polar groups is likely due to sorbed water.

EPG-columns as a group can be quite diverse in terms of properties and chemical composition. As a result, finding a different column which can provide the same selectivity and separation as a given EPG-column is unlikely in the general case. However, for samples of limited compositional range (e.g., free of acidic and basic solutes), all columns become more similar in terms of selectivity.

We have so far studied three distinct column types: type-A and type-B alkyl-silica columns, and EPG-columns. In each

case, solute parameters η', σ', etc. derived from data for type-B columns can be used to derive values of the column parameters **H**, **S***, etc. *via* Eq. (4). While the resulting accuracy of predicted retention values is +1–2% in α for type-B columns, the accuracy is poorer for type-A columns (+7%), and poorer still for EPG-columns (+14%). The use of best-fit solute parameters (derived for a particular column type) results in a marked improvement in predictive accuracy (+1–3% in α) for all column types. Derived values of **H**, **S***, etc. do not change significantly for a different set of solute parameters (i.e., "best-fit" versus values derived for type-B columns) used to derive values of **H**, **S***, etc. Therefore, when comparing columns for the purpose of matching column selectivity (i.e., obtaining different columns with "equivalent" selectivity), accurate comparisons can be made for columns of a given type (type-A, type-B, or EPG) on the basis of reported values of **H**, **S***, etc. for each column. However, corresponding comparisons of columns of *different* type (e.g., type-A versus type-B) will be less accurate (+7–14% in α), whereas acceptable comparisons require no more than +3% in α. Since it is less likely that one type of column can be matched with a column of different type, however, the reduced accuracy of such comparisons is of little practical significance.

The present study of EPG-columns is an extension of our application of Eq. (4) to non-EPG-columns (both type-A and type-B) in Parts I–V [1–5]. Overall, our results for 154 columns and 150 different solutes show reasonable agreement with Eq. (4). Comparisons of solute parameters (η', σ', etc.) with molecular structure [3] and column parameters (**H**, **S***, etc.) with the properties of the stationary phase [3–5] are generally consistent with the interactions which are believed to be the basis of each of the five terms of Eq. (1). As a result, Eq. (1) appears to represent a valid and reasonably complete description of RP-LC column selectivity for the kinds of columns so far studied. The data from this and preceding papers [4,5] allow column selectivity to be compared for any two of the 154 columns so far studied, for any sample and for any separation conditions. Similar studies for phenyl, cyano and fluoro-substituted columns are in progress, as well as a continuing addition to our column database of other type-A, type-B and EPG-columns. Recently released software (column Match®, Rheodyne LLC/LC Resources Group) provides selectivity data (values of **H**, **S***, etc.) for about 250 columns, with means for the convenient comparison of any two columns in terms of selectivity.

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