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Column selectivity in reversed-phase liquid chromatography VII. Cyanopropyl columns

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Abstract

Eleven cyanopropyl ("cyano") columns were characterized by means of a relationship developed originally for alkyl-silica columns. Compared to type-B alkyl-silica columns (i.e., made from pure silica), cyano columns are much less hydrophobic (smaller **H**), less sterically restricted (smaller $\mathbf{S}^*$), and have lower hydrogen-bond acidity (smaller **A**). Because sample retention is generally much weaker on cyano versus other columns (e.g., C_8, C_{18}), a change to a cyano column usually requires a significantly weaker mobile phase in order to maintain comparable values of *k* for both columns. For this reason, practical comparisons of selectivity between cyano and other columns (i.e., involving different mobile phases for each column) must take into account possible changes in separation due to the change in mobile phase, as well as change in the column.

Keywords: Column selectivity; Reversed-phase liquid chromatography; Cyanopropyl columns

1. Introduction

Previous reports [1–6] have described reversed-phase column selectivity in terms of a general relationship:

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

Here, *k* is the retention factor of any solute, k_{EB} the value of *k* for a non-polar reference solute (ethylbenzene), and the remaining selectivity-related symbols represent empirical, eluent- and temperature-dependent properties of the solute (η', σ', β', α', κ') or eluent- and temperature-independent properties of the column (**H**, $\mathbf{S}^*$, **A**, **B**, **C**). The five terms of Eq. (1) represent contributions to solute retention and column selectivity from various solute-column interactions. Thus, the various column parameters measure the following column properties (relative to a hypothetical average type-B C_{18} column): **H**, hydrophobicity; $\mathbf{S}^*$, steric resistance to insertion of bulky solute molecules into the stationary phase (similar to, but not the same as, "shape selectivity" [3,4]); **A**, column hydrogen-bond acidity; **B**, column hydrogen-bond basicity; **C**, column cation-exchange activity (which varies with mobile phase pH). The parameters η', σ', etc., denote complementary properties of the solute (Section 5); see [3–6] for (a) the dependence of solute parameters η', σ', etc., on solute molecular structure and (b) column parameters as a function of column properties (pore diameter, ligand length and coverage, etc.).

Values of the column parameters **H**, $\mathbf{S}^*$, etc., quantitatively describe column selectivity and have been reported for about 150 different columns [4–6]. It is, therefore, easy and convenient to select columns of either similar or very different selectivity [4]; similar columns are required when a column already in use requires replacement. Very different columns are useful in method development in order to create deliberate changes in selectivity.

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The application of Eq. (1) has been described for C_3–C_{30} alkyl-silica columns [4,5], which differ in ligand coverage, pore diameter, end-capping and silica purity ("type-A" versus "type-B"), as well as columns [6], containing polar-groups (e.g., amide, urea, carbamate) which have been either inserted into an alkyl ligand or used to end-cap the column. The similar use of Eq. (1) to interpret retention data for a small number of bonded-zirconia columns has also been reported [5]. The present paper extends our study of column selectivity (based on Eq. (1)) to cyanopropyl ("cyano") columns.

Compared to C_8 or C_{18} columns, cyano columns are less commonly used—in part because of concerns about their stability [7,8] and reproducibility [9]. Nevertheless, pronounced differences in sample retention and selectivity are often noted for cyano versus alkyl-silica columns, as addressed in several studies [10–16]. A common observation is that cyano columns are less retentive (i.e., more polar) versus C_8 or C_{18} columns. In order to achieve comparable retention (values of k) on a cyano versus a C_8 or C_{18} column, (e.g., for a desirable range of $0.5 \leq k \leq 20$ [17]), a decrease in mobile phase strength (%B) is usually necessary. Typically, a decrease of 10–20% B will be required for the cyano column versus a C_8 or C_{18} column [12,13]; e.g., 30% acetonitrile/buffer (cyano) versus 50% acetonitrile/buffer (C_8). Consequently, when "practical" separations on a cyano versus a C_8 or C_{18} column are compared, differences in separation selectivity can result from changes in both the column (i.e., cyano versus C_8) and the mobile phase (e.g., 30% B versus 50% B). A previous study [13] suggests that resulting differences in separation selectivity for a cyano versus a C_8 or C_{18} column (with change in the mobile phase to maintain the same average retention) are primarily a result of the change in mobile phase, rather than the change in column.

2. Experimental

2.1. Equipment, materials and procedures

These were as described previously [4], except for the use of a model 1090 HPLC system (Hewlett-Packard). The mobile phase was either 30% (v/v) or 50% (v/v) acetonitrile/buffer, and the final mobile phase contained 30 mM potassium phosphate. The mobile phase pH was either 2.80 or (for berberine as solute only) 7.00. Other conditions were 35 °C, 2.00 mL/min flow rate, 500-ng injection of each solute, and UV detection at 205 nm. In every experiment, columns were equilibrated prior to sample injection as described in [4].

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

S. no.	Column	Properties			Selectivity parameters							S.D.[c]
		d_{pore}[a]	C_L[b]	End-cap?	**H**	**S***	**A**	**B**	**C**(2.8)	C(7.0)	log k_{ref}	
1	Discovery CN[d]	18	3.5	Yes	0.397	−0.110	−0.615	−0.002	−0.035	0.513	−0.198	0.033
2	Thermo CN[e]	15			0.404	−0.111	−0.709	−0.009	−0.029	0.491	−0.088	0.035
3	ProntoSil CN[f]	12	3.2	No	0.370	−0.114	−0.414	−0.028	0.168	0.668	−0.041	0.040
4	Luna CN[g]	10	3.8	Yes	0.452	−0.112	−0.323	−0.024	0.439	1.321	0.104	0.023
5	Inertsil CN[h]	10	2.8	No	0.369	0.049	−0.808	0.083	−2.607	−1.297	0.050	0.055
6	Ace 5CN[i]	10	2.9	Yes	0.409	−0.107	−0.729	−0.008	−0.086	0.441	−0.019	0.035
7	Kromasil KR60-5CN[j]	6			0.440	−0.135	−0.578	−0.014	0.216	1.036	0.306	0.040
8	Precision CN[k]	12		Yes	0.431	−0.114	−0.485	0.019	−0.041	0.606	0.111	0.032
9	Genesis CN 120A[l]	12			0.424	−0.114	−0.681	−0.013	−0.001	0.573	0.134	0.037
10	Genesis CN 300A[l]	30			0.397	−0.108	−0.645	−0.009	0.025	0.397	−0.340	0.032
11	Nova-Pak CN HP 60A[m]	6	2.0	No	0.362	−0.165	0.100	0.000	0.691	1.175	−0.413	0.020
	Average[n]				0.41	−0.11	−0.58	−0.01	0.07	0.67	−0.01	0.03
	S.D.[o]				0.03	0.01	0.14	0.01	0.17	0.31	0.20	0.01

Measurements at 50% acetonitrile/buffer, 35 °C; see Section 2 for other conditions.

[a] Pore diameter (nm).
[b] Ligand concentration (μmoles/m^2).
[c] Standard deviation of fit of Eq. 1 to data for solutes of Table 2.
[d] Supelco.
[e] Thermo-Hypersil.
[f] Bischoff.
[g] Phenomenex.
[h] GL Sciences.
[i] Hichrom/ACT.
[j] Akzo-Nobel.
[k] Higgins Analytical.
[l] Argonaut/Jones Chromatography.
[m] Waters (type-A silica).
[n] Average values of **H**, **S***, etc., for type-B columns nos. 1–4 and 6–10.
[o] Standard deviation of average values of **H**, **S***, etc., for columns nos. 1–4 and 6–10.

2.2. Columns

The cyano columns used in the present study are described in Table 1. These 11 columns were each the generous gift of the manufacturer. Columns nos. 1–10 are manufactured from high-purity (type-B) silica [18], while column no. 11 is made from lower-purity (type-A) silica. The column properties in Table 1 were provided by the manufacturer; all but one column had dimensions of 15 cm × 0.46 cm and were packed with 5-μm-diameter particles. Column no. 11 (Novapak CN) had a diameter of 0.39 cm and a particle size of 4 μm.

2.3. Samples

The same 16 solutes (plus berberine) used in a preceding study [4] to characterize the selectivity of 87 type-B columns were used in the present study to characterize the columns of Table 1. These solutes are listed in Table 2 with values of η' σ', etc., from [4].

2.4. Calculations

Values of the retention factor k were determined as $k = (t_R - t_0)/t_0$, where t_0 equals the retention time for thiourea. Resulting values of k for different solutes and columns are not reported here but are available from the authors. Given values of k for each of the 16 test solutes of Table 2 and 11 columns of Table 1, corresponding values of α were calculated, equal to the ratio of k-values for the compound in question and ethylbenzene, respectively. Resulting values of α were then fit (multiple linear regression) to Eq. (1) using values of the solute parameters (η', σ', etc.) that were reported previously for type-B columns [4] (Table 2). This application of Eq. (2) leads to the values of **H**, $\mathbf{S}^*$, etc., shown in Table 1 for columns nos. 1–11; see [4–6] for details.

Values of **C** at pH 7.0 were determined [2] from:

$$\mathbf{C}(7.0) = \mathbf{C}(2.8) + \log\left(\frac{k_{7.0}}{k_{2.8}}\right) \quad (2)$$

where $k_{7.0}$ and $k_{2.8}$ refer to values of k for berberine (a quaternary ammonium salt) at pH 7.00 and 2.80, respectively. Whereas **H**, $\mathbf{S}^*$, **A**, **B** and **C**(2.8) were determined for a mobile phase of 50% acetonitrile/buffer, $\log(k_{7.0}/k_{2.8})$ in Eq. (2) was measured for 30% acetonitrile/buffer.

3. Results and discussion

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

The fit of Eq. (1) (by multiple linear regression) to experimental values of α for each column and the 16 solutes of Table 2 resulted in values of **H**, $\mathbf{S}^*$, etc., and a standard deviation (S.D.) of the fit (Table 1). The average S.D. value for these 11 columns is 0.034 log units, equivalent to ±8% in α. This agreement of data for cyano columns with Eq. (1) is poorer than found previously for 87 type-B alkyl-silica columns (S.D. = 0.005 or ±1% in α [4]), but is no worse than the agreement with Eq. (1) for other column types; e.g., type-A alkyl-silica columns, S.D. equal ±0.032 log units or ±8% in α [5]; columns with an embedded or end-capping polar-group, S.D. equal 0.073 or ±18% in α [6]. A reviewer has suggested that the generally smaller values of k for cyano columns might result in larger errors in values of log k, which could also reduce the accuracy of Eq. (1).

The poorer agreement of Eq. (1) for type-A alkyl-silica and polar-group columns has been discussed [5,6]. It is believed that no major, new solute-column interactions are responsible for the larger values of S.D. found for these two column types; rather, Eq. (1) is an approximate relationship which becomes less accurate for columns that are more different (in terms of values of **H**, $\mathbf{S}^*$, etc.) when compared with an average type-B column (from which the values of η', σ',

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

S. no.	Solute	η'	σ'	β'	α'	κ'
1	Acetophenone	−0.744	0.133	0.059	−0.152	−0.009
2	Benzonitrile	−0.703	0.317	0.003	0.080	−0.030
3	Anisole	−0.467	0.062	0.006	−0.156	−0.009
4	Toluene	−0.205	−0.095	0.011	−0.214	0.005
5	Ethylbenzene	0	0	0	0	0
6	4-Nitrophenol	−0.968	0.040	0.009	0.098	−0.021
7	5-Phenylpentanol	−0.495	0.136	0.030	0.610	0.013
8	5,5-Diphenylhydantoin	−0.940	0.026	0.003	0.568	0.007
9	*Cis*-chalcone	−0.048	0.821	−0.030	0.466	−0.045
10	*Trans*-chalcone	0.029	0.918	−0.021	−0.292	−0.017
11	*N*,*N*-dimethylacetamide	−1.903	0.001	0.994	−0.012	0.001
12	*N*,*N*-diethylacetamide	−1.390	0.214	0.369	−0.215	0.047
13	4-*n*-butylbenzoic acid	−0.266	−0.223	0.013	0.838	0.045
14	Mefenamic acid	0.049	0.333	−0.049	1.123	−0.008
15	Nortriptyline	−1.163	−0.018	−0.024	0.289	0.845
16	Amitriptyline	−1.094	0.163	−0.041	0.300	0.817

etc., in Table 2 are derived). When the multiple regression of log α values for the latter columns was repeated so as to derive "best-fit" values of η', σ', etc., for these columns (as described in [5,6]), the agreement with Eq. (2) was much improved (S.D. = ±0.012–0.013), with only minor changes in the column parameters. When a similar treatment of the data for cyano columns was carried out in the present study (repeated multiple regression to obtain a best-fit to Eq. (1)), the final value of S.D. was 0.006 (±1.4% in α; i.e., very good agreement),) and again only minor changes in the column parameters were observed. We therefore, conclude that no new solute-column interactions (other than the five interactions described by Eq. (1)) are indicated for reversed-phase retention on cyano columns. However, in the following paper [19] it is pointed out that π–π interactions contribute to the retention of some solutes on phenyl columns, yet Eq. (1) (which does not recognize π–π interactions) provides a good fit for phenyl columns and the test solutes of Table 2 (because no strong π-acids are included). Others have suggested [20–22] that cyano columns are also capable of π–π interaction, but the present study provides no information on the relative importance of π–π interaction in determining the selectivity of cyano columns. Judging from the related behavior of phenyl columns (see discussion of Table 6 of [19]), the effects of π–π interaction (if significant) are likely to be similar for different cyano columns, in which case relative selectivity among cyano columns will not be affected by π–π interaction.

3.2. Selectivity of cyano versus alkyl-silica columns

Table 3 compares average values of **H**, **S***, etc., for (a) the cyano columns of Table 1 (excepting atypical columns no. 5, Inertsil CN and no. 11 Nova-Pak CN) and (b) several type-B C_4 and C_5 columns from [4]; the length of a cyanopropyl ligand is roughly the same as $C_{4.5}$ (the average of C_4 and C_5 ligands). Atypical column no. 5 may differ from the other cyano columns studied by us in the nature of the silane used to produce the stationary phase. Thus, Inertsil ODS-3 from the same manufacturer is made from a difunctional silane, whereas most alkyl-silica columns are produced from a monofunctional silane.

Values of **H** for the cyano columns are much lower (0.41 versus 0.69, or a difference of −0.28) relative to a $C_{4.5}$ column, reflecting the greater polarity of a $-C_3-C{\equiv}N$ group versus a $C_{4.5}$ group. Values of **S*** for the cyano columns are also smaller (−0.12), possibly due to an ordering of the cyanopropyl groups. Thus, the large dipole moment of a $-C{\equiv}N$ group should lead to a repulsion of adjacent groups (which will be aligned in the same direction), which in turn should lead to a more regular spacing between the cyanopropyl ligands. This in turn might allow an easier access of solute molecules into the stationary phase, with a reduction in values of **S***. Values of **A** for cyano columns are considerably lower (−0.22) versus an average $C_{4.5}$ column, possibly because of cyano–silanol interaction and a resulting neutralization of the hydrogen-bond acidity of column silanols (which are responsible for column **A** values). Values of **B** and **C**(2.8) are each similar in value for cyano and alkyl-silica columns, while values of **C**(7.0) are higher (+0.47) for cyano columns. Values of **A** and **C** vary significantly among these nine cyano columns (S.D. = 0.14–0.31; last row of Table 1), so conclusions based on average values of **A** and **C** should be treated with caution.

3.3. Quantitative comparisons of selectivity among different columns

We have previously developed a quantitative means of comparing the selectivity of two columns 1 and 2 in terms of the function F_s [4], where,

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

Here, $\mathbf{H}_1$ and $\mathbf{H}_2$ refer to values of **H** for columns 1 and 2, respectively (and similarly for values of **S***, **A**, etc.). It was found [4] that if $F_s \leq 3$ for two columns 1 and 2, the two columns are likely to provide equivalent selectivity and separation for different samples and conditions. The application of Eq. (3) to separations on cyano columns is illustrated in Fig. 1, where the same sample and separation conditions are used with four different columns. Values of F_s for the comparison of each column with the Discovery CN column of Fig. 1a are shown in the figure. Very similar separations result for all four columns of Fig. 1, despite the fact that the column of Fig. 1d has $F_s = 5$, which somewhat exceeds the maximum value of $F_s = 3$ for "equivalent" columns. Differences in run time (or values of k) are observed for these four columns, but such differences in absolute retention can be minimized by a change in flow rate (which has no effect on selectivity). For

Table 3
Comparison of Cyano and alkyl-silica columns of similar ligand length in terms of selectivity

Column	Selectivity parameters (average)					
	H	**S***	**A**	**B**	**C** (2.8)	**C** (7.0)
Cyano[a]	0.41	−0.11	−0.58	−0.01	0.07	0.67
$C_{4.5}$ (type-B)[b]	0.69	0.01	−0.36	0.02	0.05	0.20
Cyano − $C_{4.5}$	−0.28	−0.12	−0.22	−0.03	0.02	0.47

[a] Average value for cyano columns nos. 1–4 and 6–10 of Table 1.
[b] Average values for C_4 and C_5 columns from [4].

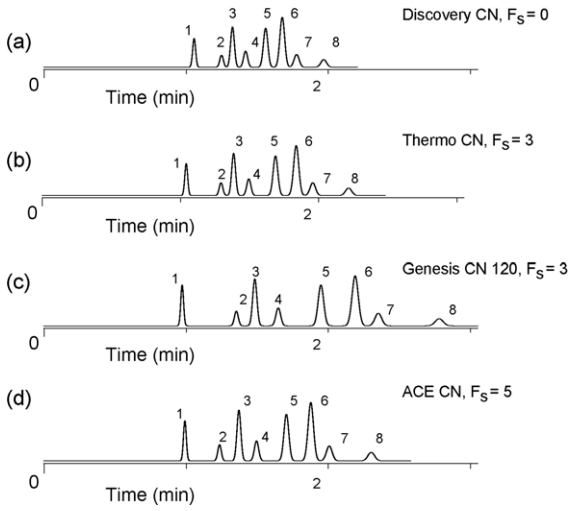

Fig. 1. Comparative separation of a model 8-component sample with similar cyano columns and a mobile phase of 50% ACN/buffer (other conditions as in Section 2); columns identified in the figure. The sample is composed of *N*,*N*-dimethylacetamide (1); nortriptyline (2); acetophenone (3); 5-phenylpentanol (4); toluene (5); ethylbenzene (6); mefenamic acid (7); *trans*-chalcone (8). Reconstructed chromatograms based on data for individual solutes from the present study are shown.

columns of very different selectivity (as measured by values of F_S), large changes in separation are expected; this is illustrated in the two separations of Fig. 2, for which $F_S = 41$. Note the rearrangement of band nos. 2–4 and partial coalescence of band nos. 6 and 7 in the separation of Fig. 2b versus that of Fig. 2a.

The separations of Figs. 1 and 2 were carried out with the same experimental conditions (i.e., 50% acetonitrile/pH 2.8 buffer; 35 °C) used to measure the values of **H**, $\mathbf{S}^*$, etc., of Table 1 (which were used in Eq. (3) to calculate the values of F_S shown in Fig. 1). However, values of **H**, $\mathbf{S}^*$, etc., determined as in Table 1 have been found to be approximately applicable for different mobile phase compositions, temperatures or samples [2]—as long as only the column is changed, the columns are not too dissimilar (e.g., $F_S < 30$), and extreme changes in conditions are avoided. Thus, values of F_S based on the values of Table 1 can predict relative col-

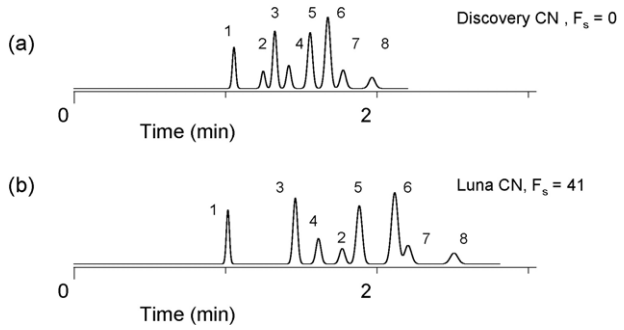

Fig. 2. Comparative separation on dissimilar cyano columns. Conditions and sample as in Fig. 1 with columns identified in the figure. Reconstructed chromatograms based on data for individual solutes from the present study are shown.

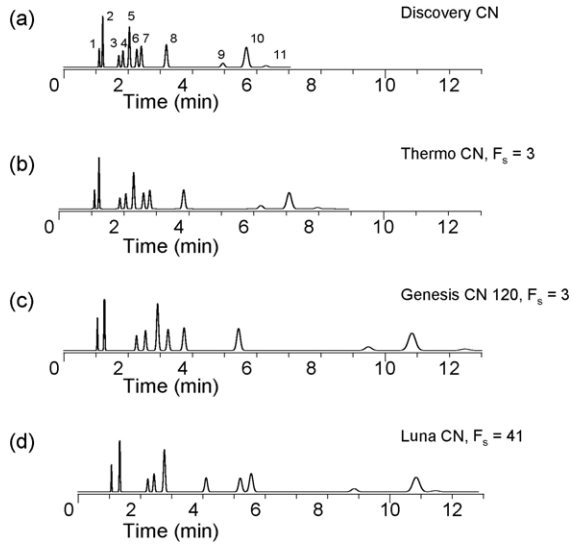

Fig. 3. Comparison of separation on cyano columns with mobile phase of 30% ACN/buffer. Columns identified in the figure and values of F_S determined from the column parameters of Table 1 (obtained with 50% ACN/buffer). Conditions as in Fig. 1 (unless noted otherwise), with columns identified in the figure. Sample is composed of *N*,*N*-dimethylacetamide (1); *N*,*N*-diethylacetamide (2); acetophenone (3); benzonitrile (4); 5,5-diphenylhydantoin (5); 4-*n*-hexylaniline (6); amitriptyline (7); ethylbenzene (8); *cis*-chalcone (9); mefenamic acid (10); and *trans*-chalcone (11). Reconstructed chromatograms based on data for individual solutes from the present study are shown.

umn similarity when either the mobile phase or temperature is changed (but the same) for both columns. This is illustrated in Fig. 3 for the separation of a different sample with a mobile phase of 30% ACN/pH 2.8 buffer; i.e., change in mobile phase from 50 to 30% ACN/buffer. For the three columns of Fig. 3a–c, $F_S \leq 3$, and the separations are virtually identical except for differences in absolute retention (which again can be adjusted by a change in flow rate). For the separation in Fig. 3d (with $F_S = 41$), there are obvious differences in selectivity (i.e., changes in values of the separation factor α for adjacent bands), but no change in separation order.

3.4. "Practical" selectivity of cyano versus C_8 columns

As noted in the Introduction, the generally weaker retention of a sample on cyano columns (versus C_8 or C_{18} columns) typically requires the use of a weaker mobile phase. When a weaker mobile phase is used for the cyano column, the selectivity of the cyano versus C_8 column (for the same sample) is further altered—due to the added effect of a change in mobile phase on selectivity. This is illustrated in Fig. 4, where the separation of a representative sample is shown for three different conditions: (a) 50%-ACN, C_8 column; (b) 50%-ACN, cyano column; (c) 30%-ACN cyano column. Relative to the initial separation on the C_8 column ("a", with 50% ACN), the separation on the cyano column ("b", also with 50% ACN; $F_S = 18$) is moderately different: band nos. 1–6 retain their same separation order, while band nos. 7–9 show a change

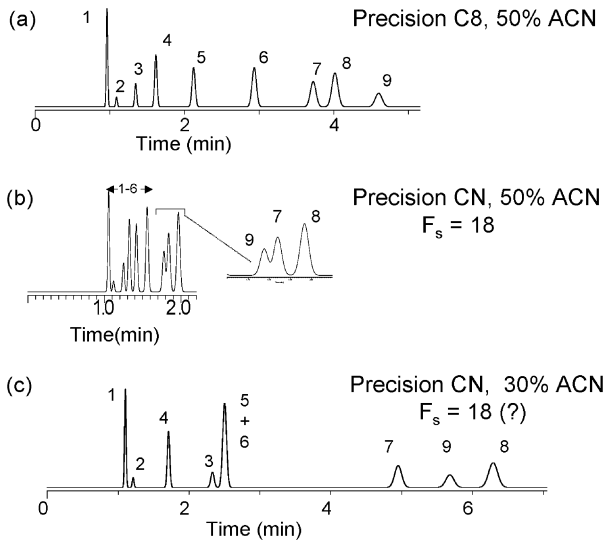

Fig. 4. Comparative separations of a model 9-component mixture on (a) a Precision C8 column with a mobile phase of 50% ACN-buffer (pH 2.8); (b) same separation and conditions, using a Precision CN column; (c) separation as in (b), except mobile phase is changed to 30% ACN-buffer. Other conditions as in Section 2. Sample components are *N*,*N*-dimethylacetamide (1); *N*,*N*-diethylacetamide (2); nortriptyline (3); acetophenone (4); 5-phenylpentanol (5); toluene (6); *cis*-chalcone (7); *trans*-chalcone (8); and mefenamic acid (9). The "?" in (c) refers to the fact that F_s values are not valid when the mobile phase is not the same for two columns. Reconstructed chromatograms based on data for individual solutes from the present study are shown.

in the relative retention of band no. 9. However, when the mobile phase for the cyano column is changed from 50% to 30% ACN ("c"), much greater changes in separation selectivity result: a reversal of the positions of band nos. 3 and 4, a coalescence of band nos. 5 and 6, and (again) a change in relative retention of band no. 9. Because of this change in mobile phase for one column but not the other, the separation of Fig. 4c can no longer be described by $F_s = 18$ for these two columns. The example of Fig. 4 confirms that a comparison of selectivity between a cyano and a C_8 (or other) column in terms of values of **H**, $\mathbf{S}^*$, etc., will be misleading, if a weaker mobile phase is used for the cyano column only. Fig. 4 also suggests that a change from a C_8 or C_{18} column to a cyano column—with the required change in mobile phase %*B*—will generally lead to a large change in separation selectivity (as noted previously [12]).

4. Conclusions

The characterization of column selectivity by means of a reversed-phase selectivity relationship (Eq. (1); see also the review of [23]):

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

has been extended to 11 cyanopropyl (cyano) columns. The fit of Eq. (1) to experimental data for these columns resulted in an average standard deviation S.D. = 0.034 (±8% in values of α). While this is relatively poor agreement compared to the similar fit of Eq. (1) to 87 type-B alkyl-silica columns (±1% in values of α), it appears that no new solute-column interactions (other than those defined by Eq. (1)) are suggested for cyano columns. Rather, the greater error of the fit of cyano-column retention data arises from the approximate nature of Eq. (1). Thus, the use of best-fit values of η', σ', etc., for cyano columns markedly improves the fit to Eq. (1) (±1% in α).

Compared to type-B alkyl-silica columns of similar ligand length (average of C_4 and C_5 columns), cyano columns are (a) much less hydrophobic (average decrease in **H** of 0.28 units, due to the greater polarity of a cyano column), (b) less restrictive to the penetration of "bulky" solute molecules into the stationary phase (average decrease in $\mathbf{S}^*$ of 0.12 units), and (c) have weaker hydrogen-bond acidity (average decrease in **A** of 0.22 units). The differences in selectivity of cyano versus alkyl-silica columns can be rationalized in terms of the physico-chemical properties of cyanoalkyl ligands.

Comparisons of column selectivity are useful for various reasons. Eq. (1) allows a quantitative approach to selecting either equivalent or very different columns in terms of selectivity. Several of the cyano columns studied were found to have "near-equivalent" selectivity, allowing the replacement of one cyano column by another in a typical separation. Quantitative comparisons of cyano columns with other column types (C_{18}) in terms of "column selectivity" are usually not practical, because cyano columns generally require a different (weaker) mobile phase, with consequent changes in separation selectivity due to the mobile phase per se. This also suggests that the replacement of a C_8 or C_{18} column by an "equivalent" cyano column will not usually be possible. On the other hand, the replacement of an alkyl-silica column by a cyano column (or vice versa)—combined with a change in %*B* that maintains the same average retention of the sample—will often result in a large change in separation selectivity, which can prove useful in method development or for so-called orthogonal separations.

5. Nomenclature

The following list contains all symbols defined in the present and immediately following papers (Parts VII and VIII). Reference to a defining equation (e.g., Eq. VII-2) indicates both the paper (e.g., Part VII) and equation number (e.g., 2).

A	"type-A" column based on metal-contaminated silica
A	column hydrogen-bond acidity (relative to an average type-B alkyl-silica column), related to number, accessibility and acidity of silanol groups in the stationary phase; Eq. VII-1
$\mathbf{A}_b$	average value of **A** for type-B columns; Eq. VIII-3

$\mathbf{A}_1$, $\mathbf{A}_2$ value of **A** for columns 1 and 2; Eq. VII-3
ACN acetonitrile
B "type-B" column based on pure silica
B column hydrogen-bond basicity (relative to an average type-B alkyl-silica column); Eq. VII-1
$\mathbf{B}_b$ average value of **B** for type-B columns; Eq. VIII-3
$\mathbf{B}_1$, $\mathbf{B}_2$ value of **B** for columns 1 and 2; Eq. VII-3
C column cation-exchange activity (relative to an average type-B alkyl-silica column); related to number and accessibility of ionized silanols in stationary phase; Eq. VII-1
$\mathbf{C_b}$ average value of **C** for type-B columns; Eq. VIII-3
$\mathbf{C}_1$, $\mathbf{C}_2$ value of **C** for columns 1 and 2; Eq. VII-3
CN cyanoalkyl (cyano) column
C(2.8) value of **C** for pH 2.8 (Eq. VII-2)
C(7.0) value of **C** for pH 7.0 (Eq. VII-2)
EPG column containing an embedded polar-group
F_s column selectivity comparison function, based on differences in **H**, $\mathbf{S}^*$, **A**, **B** and **C** for two columns (Eq. VII-3); assumes a sample that contains acidic and basic solutes
H column hydrophobicity (relative to an average type-B alkyl-silica column); Eq. VII-1
$\mathbf{H}_1$, $\mathbf{H}_2$ value of **H** for columns 1 and 2; Eq. VII-3
$\mathbf{H}_b$ average value of **H** for type-B columns; Eq. VIII-3
HFD heptadecafluorodecyl
k retention factor, equal to $(t_R - t_0)/t_0$
k_{ref} value of k for ethylbenzene
$k_{2.8}$, $k_{7.0}$ values of k for berberine at pH 2.8 and 7.0, respectively (Eq. VII-2)
MeOH methanol
PAH polycyclic aromatic hydrocarbon
r correlation coefficient
RI refractive index
$\mathbf{S}^*$ steric resistance to insertion of bulky solute molecules into the stationary phase (relative to an average type-B alkyl-silica column) (Eq. VII-1); as $\mathbf{S}^*$ increases, bulky solute molecules experience greater difficulty in penetrating the stationary phase and being retained; Eq. VII-1
$\mathbf{S}^*_b$ average value of $\mathbf{S}^*$ for type-B columns; Eq. VIII-3
$\mathbf{S}^*_1$, $\mathbf{S}^*_2$ value of $\mathbf{S}^*$ for columns 1 and 2; Eq. VII-3
S.D. standard deviation
t_0 column dead time (min)
t_R retention time (min)
THF tetrahydrofuran
type-A column based on (impure) metal-contaminated silica
type-B column based on (pure) metal-free silica
Zr alkyl-zirconia column

Greek letters

α separation factor for two solutes; here, α equals k for a solute divided by k for ethylbenzene
α' solute relative hydrogen-bond acidity, measured for a given mobile phase and temperature
β' solute relative hydrogen-bond basicity, measured for a given mobile phase and temperature; also, difference in an experimental value of $\log\alpha$ versus a predicted value
$\delta\delta \log k$ change in $\delta \log k$ due to a change in mobile phase organic solvent (Table 8 of Part VIII)
$\delta\delta \log \alpha$ change in $\delta \log \alpha$ due to a change in mobile phase organic solvent
η' solute relative hydrophobicity, measured for a given mobile phase and temperature
κ' relative charge on solute molecule (positive for cations, negative for anions), measured for a given mobile phase and temperature
σ' relative steric resistance of solute molecule to penetration into stationary phase (σ' is larger for more bulky molecules), measured for a given mobile phase and temperature

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