

Relevance of π – π and dipole–dipole interactions for retention on cyano and phenyl columns in reversed-phase liquid chromatography

K. Croes^a, A. Steffens^a, D.H. Marchand^a, L.R. Snyder^{b,*}

^a Department of Chemistry, University Wisconsin-River Falls, River Falls, WI 54022, USA

^b LC Resources, 26 Silverwood Court, Orinda, CA 94563, USA

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Abstract

Previous work suggests that π – π interactions between certain solutes and both phenyl and cyano columns can contribute to sample retention and the selectivity of these two column types versus alkylsilica columns. Recent studies also suggest that dipole–dipole interactions are generally unimportant for retention on cyano columns. The present study presents data for 44 solutes, three columns and two different mobile phases that were selected to further test these conclusions. We find that π – π interactions can contribute to retention on both cyano and phenyl columns, while dipole–dipole interactions are likely to be significant for the retention of polar aliphatic solutes on cyano columns. When acetonitrile/water mobile phases are used, both π – π and dipole–dipole interactions are suppressed, compared to the use of methanol/water.
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1. Introduction

Differences in relative retention or selectivity for one column versus another are determined by the various interactions between different solutes and the stationary phase. Five such interactions have been characterized and measured for more than 300 reversed-phase (RP-LC) columns [1], based on the following equation:

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

The retention factor k for a solute can be related to various properties of the column: H , hydrophobicity; S^* , steric resistance to penetration of the solute into the stationary phase; A , hydrogen-bond acidity; B , hydrogen-bond basicity; C , cation-exchange capacity of the column. The parameters η' , σ' , etc. represent complementary properties of the solute (see Nomenclature, Section 6); k_{EB} refers to the retention factor k for ethylbenzene (reference compound). Values of the column parameters H , S^* , etc. can be used to characterize column selectivity; e.g., for the selection of (a) columns of equivalent selectivity that are inter-

changeable in a routine RP-LC method [2], or (b) columns of quite different selectivity for the development of an orthogonal separation [3].

An additional possible contribution to solute retention for phenyl columns is π – π interaction [4]; phenyl groups in the stationary phase can interact strongly with nitro-substituted aromatics and polycyclic aromatic hydrocarbons (PAH), and less strongly with other aromatics. Several prior reports have proposed that π – π interactions between solute and column are also significant for retention on cyanopropyl (“cyano”) columns [5–7]. The application of Eq. (1) to several commercial cyano columns has been reported [8], but no test of the relative importance of π – π interactions was carried out in that study. Dipole–dipole interactions between solute and column also seem possible for cyano columns, because of the large dipole moment of the cyano group. However, limited studies with acetonitrile/buffer mobile phases and aromatic solutes appeared to rule out significant contributions of this kind [1].

The identification of various solute–column interactions and an assessment of their relative importance in affecting retention on different columns can be important to the practicing chromatographer, inasmuch as these interactions affect column selectivity and the ability of various columns to achieve the separation of different samples. A further study of retention

* Corresponding author. Tel.: +1 925 254 6334; fax: +1 925 254 2386.
E-mail address: snyder0036@comcast.net (L.R. Snyder).

on a C₈, phenyl and cyano column was therefore carried out, in order to further clarify the relative importance of π – π and dipole–dipole interactions for both cyano and phenyl columns (these interactions can be assumed absent for retention on alkyl-silica columns).

2. Background and theory

2.1. Cyano columns

The prior conclusion that π – π interactions contribute to the retention of aromatic and other unsaturated solutes on cyano columns [5–7] is based on (a) the preferential retention of aromatic versus aliphatic solutes on cyano columns versus C₁₈ columns, and (b) a decrease in this preferential retention when using mobile phases that contain increasing amounts of acetonitrile (ACN), compared to mobile phase containing methanol (MeOH). The suppressed retention of aromatics with ACN as B-solvent can be attributed to competitive π – π interactions between ACN and the solute molecule in the mobile phase (a similar interaction of ACN with stationary phase cyano groups would also reduce π – π interaction between solute and column). On the basis of previously reported experimental data, we find this interpretation for retention on cyano columns to be suggestive, but not compelling. Also, these (and other) past studies of π – π interaction in reversed-phase liquid chromatography (RP-LC) have usually involved mobile phases which are rich in the organic solvent (e.g., 70–100% B). Since most RP-LC separations carried out today employ mobile phases with lower concentrations of the B-solvent, conclusions based on higher %B may not be generally applicable.

2.2. Phenyl columns

Evidence for the importance of π – π interactions between phenyl columns and aromatic solutes has been summarized in [4], based on comparisons of retention for different solutes on phenyl versus C₈ columns. It was found that retention on phenyl columns (relative to that on alkylsilica columns) tends to increase in the order aliphatics < substituted benzenes < PAHs \approx nitro-substituted aromatics, which is the order of increasing π -activity of the solute. The preferential retention of PAH's and nitro-substituted aromatics on phenyl columns is also significantly greater when MeOH is used as B-solvent, compared to the use of ACN. It is plausible to attribute the reduced retention of aromatics with ACN as B-solvent to π – π interactions of ACN with either aromatic solutes in the mobile phase or phenyl groups in the stationary phase (just as for retention on cyano columns; see above).

3. Experimental

3.1. Equipment, materials and procedures

These were as described previously [4,8]: model LC-10 (Shimadzu); 40% (v/v) acetonitrile/water or 60% methanol/water

as mobile phase (except where noted otherwise); 35 °C; 2.0 mL/min; 500-ng injection of each solute; UV detection at 205 nm.

3.2. Columns

The columns used in this study were described previously [4,8]: (a) Restek Ultra C8 (“C₈”); (b) Kromasil KR-60-5CN (“cyano”); (c) Jones Genesis cyano (“cyano-2”); (d) Phenomenex Prodigy phenyl-3 (“phenyl”). Each column had dimensions of 15 cm \times 0.46 cm; columns *a*, *b* and *d* were packed with 5- μ m-diameter particles, while column *c* contains 4- μ m-diameter particles. The Kromasil cyano column was used for all of the following comparisons of retention versus column type, except for the experiments summarized in Fig. 3.

3.3. Samples

The 44 solutes used in the present study are listed in Table 1. These can be classified as “substituted benzenes” (#1–18), “ π -active” solutes (#19–32), and “aliphatics” (#33–44).

3.4. Calculations

Values of the retention factor *k* were determined as $k = (t_R - t_0)/t_0$, where *t*₀ equals the retention time for thiourea. The extra-column volume of the HPLC system was not corrected for, as the latter quantity cancels out when values of *k* are compared for different columns (as in the present study).

4. Results and discussion

The approach followed here for the interpretation of the data of Table 1 is similar to that described in [1,4]. Values of log *k* for a given mobile phase and two different columns are plotted versus each other, with deviations from a best-fit line through the data being interpreted as the result of differences in solute–column interaction. The larger the deviations or more scattered the plot, the less similar are the two columns in terms of solute retention. Fig. 1 illustrates this for comparisons of retention on (a) the cyano versus the C₈ column, and (b) the cyano versus the phenyl column, with 60% MeOH as mobile phase in each case. In each plot of Fig. 1, a roughly linear correlation of retention times is observed, which is primarily the result of hydrophobic interactions between solute and column (the usually-dominant η/H term of Eq. (1)). The scatter of data around this correlation line can be attributed to other solute interactions: π – π , dipole–dipole, etc.

Table 2 summarizes the results of similar plots for all three columns (C₈, cyano, phenyl) and both mobile phases (40% ACN, 60% MeOH). For each mobile phase, it is seen that there is a better correlation for the cyano versus phenyl column ($r^2 = 0.99, 0.87$), than for either of these columns versus the C₈ column ($r^2 = 0.93–0.95, 0.71–0.82$). These results suggest that the phenyl and cyano columns are more similar in their solute–column interactions than are the cyano and C₈ or phenyl

Table 1
Values of $\log k$ for C₈, cyano and phenyl columns with acetonitrile (ACN) or methanol (MeOH) as B-solvent

| Solute | 40% ACN mobile phase | | | 60% MeOH mobile phase | | |
|--|----------------------|--------|--------|-----------------------|--------|--------|
| | C ₈ | Phenyl | Cyano | C ₈ | Cyano | Phenyl |
| Substituted benzenes (excluding nitro-substituted derivatives) | | | | | | |
| 1. Dimethoxybenzene ^a | 0.597 | 0.227 | 0.315 | 0.193 | -0.312 | -0.005 |
| 2. Ethylbenzene ^a | 1.179 | 0.520 | 0.623 | 0.799 | -0.123 | 0.168 |
| 3. Anisole ^a | 0.682 | 0.265 | 0.334 | 0.292 | -0.300 | -0.053 |
| 4. Benzonitrile ^a | 0.449 | 0.153 | 0.217 | -0.058 | -0.417 | -0.116 |
| 5. Ethyl benzoate | 0.873 | 0.336 | 0.469 | 0.504 | -0.249 | 0.172 |
| 6. Toluene ^a | 0.948 | 0.399 | 0.467 | 0.576 | -0.219 | 0.022 |
| 7. <i>p</i> -Xylene ^a | 1.180 | 0.519 | 0.608 | 0.815 | -0.115 | 0.161 |
| 8. Acetophenone ^a | 0.392 | 0.093 | 0.074 | 0.008 | -0.442 | -0.065 |
| 9. Butyl phenyl ether | 1.456 | 0.673 | 0.829 | 1.030 | 0.002 | 0.395 |
| 10. Benzene | 0.713 | 0.262 | 0.318 | 0.331 | -0.329 | -0.128 |
| 11. Acetanilide | -0.056 | -0.160 | -0.116 | -0.262 | -0.583 | -0.371 |
| 12. Methyl benzoate ^a | 0.614 | 0.196 | 0.310 | 0.275 | -0.351 | 0.043 |
| 13. Chlorobenzene ^a | 0.940 | 0.409 | 0.493 | 0.570 | -0.174 | 0.055 |
| 14. Benzaldehyde ^a | 0.346 | 0.060 | 0.137 | -0.060 | -0.475 | -0.148 |
| 15. Fluorobenzene | 0.734 | 0.293 | 0.349 | 0.345 | -0.297 | -0.148 |
| 16. Bromobenzene | 1.000 | 0.446 | 0.546 | 0.632 | -0.133 | 0.121 |
| 17. Phenyl Acetate ^a | 0.518 | 0.166 | 0.248 | -0.161 | -0.540 | -0.129 |
| 18. Iodobenzene | 1.110 | 0.513 | 0.639 | 0.734 | -0.076 | 0.225 |
| Nitro-substituted aromatics, PAH's ("π-active") | | | | | | |
| 19. Nitrobenzene | 0.591 | 0.265 | 0.323 | 0.133 | -0.235 | -0.017 |
| 20. <i>p</i> -Nitrotoluene | 0.810 | 0.385 | 0.467 | 0.352 | -0.137 | 0.125 |
| 21. 1-Chloro-4-nitrobenzene | 0.844 | 0.420 | 0.500 | 0.380 | -0.134 | 0.147 |
| 22. <i>m</i> -Dinitrobenzene | 0.577 | 0.326 | 0.390 | 0.078 | -0.112 | 0.088 |
| 23. 1,3,5-Trinitrobenzene | 0.605 | 0.454 | 0.488 | -0.045 | -0.053 | 0.080 |
| 24. 1-Nitronaphthalene | 1.028 | 0.564 | 0.679 | 0.591 | 0.080 | 0.378 |
| 25. Naphthalene | 1.139 | 0.561 | 0.669 | 0.743 | 0.014 | 0.246 |
| 26. 1-Methylnaphthalene | 1.336 | 0.653 | 0.792 | 0.956 | 0.108 | 0.394 |
| 27. 1-Methoxynaphthalene | 1.220 | 0.618 | 0.754 | 0.825 | 0.108 | 0.372 |
| 28. Biphenyl | 1.011 | 0.541 | 0.675 | 0.584 | 0.067 | 0.378 |
| 29. Phenanthrene | 1.509 | 0.828 | 0.977 | 1.119 | 0.325 | 0.599 |
| 30. Anthracene | 1.558 | 0.838 | 1.008 | 1.164 | 0.345 | 0.627 |
| 31. <i>p</i> -Terphenyl | 1.375 | 0.692 | 0.870 | 0.984 | 0.106 | 0.458 |
| 32. Naphthacene | 2.011 | 1.106 | 1.381 | 1.617 | 0.561 | 1.010 |
| Aliphatics | | | | | | |
| 33. Ethyl acetate | 0.072 | -0.221 | -0.149 | -0.224 | -0.835 | -0.409 |
| 34. <i>n</i> -Propyl acetate | 0.356 | -0.036 | 0.048 | 0.056 | -0.667 | -0.234 |
| 35. <i>n</i> -Butyl acetate | 0.626 | 0.126 | 0.231 | 0.325 | -0.500 | -0.066 |
| 36. <i>n</i> -Pentyl acetate | 0.890 | 0.276 | 0.407 | 0.584 | -0.367 | 0.100 |
| 37. 1-Nitropropane | 0.308 | 0.050 | 0.063 | -0.240 | -0.478 | -0.377 |
| 38. 1-Nitrobutane | 0.571 | 0.212 | 0.248 | 0.035 | -0.349 | -0.203 |
| 39. 1-Nitropentane | 0.831 | 0.359 | 0.424 | 0.299 | -0.227 | -0.035 |
| 40. 1-Nitrohexane | 1.088 | 0.504 | 0.592 | 0.557 | -0.111 | 0.125 |
| 41. 1-Bromopropane | 0.785 | 0.283 | 0.347 | 0.419 | -0.291 | -0.085 |
| 42. 1-Bromobutane | 1.052 | 0.432 | 0.523 | 0.678 | -0.174 | 0.083 |
| 43. 1-Bromopentane | 1.311 | 0.572 | 0.694 | 0.928 | -0.066 | 0.248 |
| 44. 1-Bromohexane | 1.567 | 0.705 | 0.859 | 1.175 | 0.039 | 0.411 |

See Section 3 for further descriptions of these columns.

^a Solutes used to determine value of a and b in Eq. (3) for different mobile phases and column combination.

and C₈ columns. Table 2 also shows that the correlation improves markedly for 40% ACN as mobile phase ($r^2 = 0.93$ – 0.99), compared to 60% MeOH ($r^2 = 0.71$ – 0.87). The better correlation with 40% ACN is consistent with the suppression by ACN of π – π and/or dipole–dipole interactions between solute and column. Thus, the correlations of Table 2 appear to support the possible importance of π – π and/or dipole–dipole interactions on both the cyano and phenyl columns.

4.1. Interpretation of deviations from plots as in Fig. 1 (aromatic solutes)

For retention data using the solutes and columns of the present study, it is argued in Appendix A that the last four terms of Eq. (1) can be ignored to a first approximation. Eq. (1) then reduces to

$$\log k \approx \log k_{EB} + \eta' H \quad (2)$$

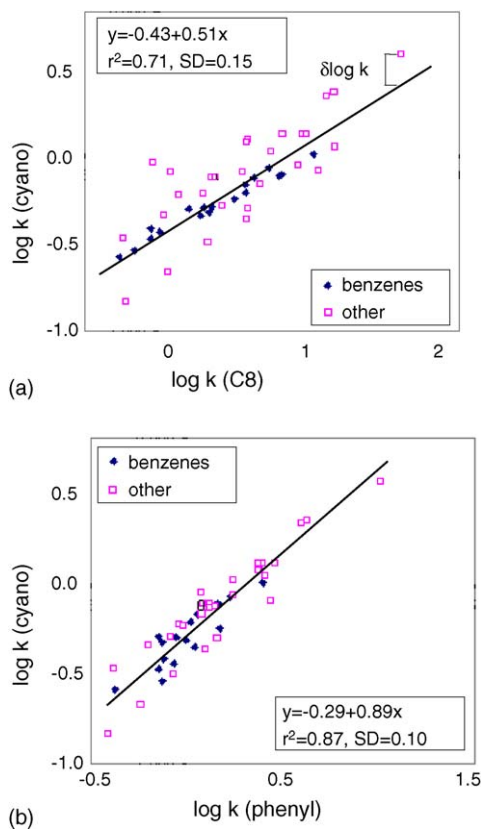


Fig. 1. Plots of retention for different pairs of columns with 60% methanol/water as mobile phase; solutes of Table 1, conditions given in Section 3. (a) cyano vs. C8 column, (b) cyano vs. phenyl column.

where only hydrophobic interactions between solute and column contribute to relative retention and column selectivity. When data are plotted as in Fig. 1, values of k_1 and k_2 for a given solute and columns 1 and 2, respectively, will be related by Eq. (2) as

$$\begin{aligned} \log k_1 &= [\log k_{EB,1} - (H_1/H_2)(\log k_{EB,2})] + (H_1/H_2) \log k_2 \\ &= a + b \log k_2 \end{aligned} \quad (3)$$

i.e., a linear plot where a and b are constants for a given pair of columns and the same mobile phase. Were Eqs. (2) and (3) to apply exactly, plots as in Fig. 1 should yield perfect correlations ($r^2 = 1.000$; $SD = 0$); the presence of other interactions represented in Eq. (1) is likely to introduce only relatively small

Table 2
Correlation of plots of $\log k$ for one column vs. another and for different mobile phases

| Mobile phase | Columns | Correlation results | | |
|--------------|---------------------------|---------------------|-------|-----------------------|
| | | r^2 | SD | Fitting equation |
| 40% ACN | Cyano vs. C ₈ | 0.932 | 0.069 | $y = -0.144 + 0.594x$ |
| | Phenyl vs. C ₈ | 0.953 | 0.066 | $y = -0.133 + 0.693x$ |
| | Cyano vs. phenyl | 0.987 | 0.031 | $y = -0.032 + 0.860x$ |
| 60% MeOH | Cyano vs. C ₈ | 0.709 | 0.146 | $y = -0.435 + 0.515x$ |
| | Phenyl vs. C ₈ | 0.824 | 0.119 | $y = -0.168 + 0.582x$ |
| | Cyano vs. phenyl | 0.870 | 0.096 | $y = -0.287 + 0.891x$ |

Table 3

Average deviations $\delta \log k$ from “ideal” retention (Eq. (3), based on solutes #1–4, 6–8, 12–14 and 17; see Table A.1 and related discussion) for the cyano and phenyl columns and for different mobile phases.

| Mobile phase | Column | Solute group | Average $\delta \log k$ | SD |
|--------------|--------|---------------|-------------------------|------|
| 40% ACN | Cyano | Benzenes | 0.00 | 0.02 |
| | | π -Active | 0.10 | 0.05 |
| | | Aliphatics | -0.05 | 0.05 |
| | Phenyl | Benzenes | 0.01 | 0.03 |
| | | π -Active | 0.13 | 0.06 |
| | | Aliphatics | -0.03 | 0.04 |
| 60% MeOH | Cyano | Benzenes | 0.02 | 0.03 |
| | | π -Active | 0.24 | 0.09 |
| | | Aliphatics | -0.06 | 0.13 |
| | Phenyl | Benzenes | 0.00 | 0.09 |
| | | π -Active | 0.24 | 0.15 |
| | | Aliphatics | -0.03 | 0.14 |
| Average ACN | | Benzenes | 0.00 | 0.03 |
| | | π -Active | 0.11 | 0.06 |
| | | Aliphatics | -0.04 | 0.05 |
| Average MeOH | | Benzenes | 0.00 | 0.07 |
| | | π -Active | 0.24 | 0.12 |
| | | Aliphatics | -0.04 | 0.13 |
| Average all | | Benzenes | 0.00 | 0.05 |
| | | π -Active | 0.18 | 0.11 |
| | | Aliphatics | -0.04 | 0.10 |

values of $\delta \log k$ (± 0.02 – 0.04 units, Appendix A). The quantity $\delta \log k$ is defined in Fig. 1a. Since π - π and dipole–dipole interactions are not recognized in Eq. (1) or (2), larger deviations from Eq. (2) (values of $\delta \log k$) can be attributed to the latter solute–column interactions. In the following discussion, we will attempt to relate these deviations $\delta \log k$ from “ideal” retention (Eq. (3)) to π - π and dipole–dipole interactions.

The application of Eq. (3) for the calculation of deviations $\delta \log k$ requires that the slope b and intercept a be determined from solutes for which Eq. (2) applies as closely as possible. Appendix A suggests the use of compounds #1–4, 6–8, 12–14 and 17 for this purpose, and summarizes the fit of Eq. (3) for these solutes and various column/mobile phase combinations used for the determination of values of $\delta \log k$. It is of interest next to examine resulting values of $\delta \log k$ for the three solute groups of Table 1, the two mobile phases (40% ACN, 60% MeOH), and the cyano and phenyl columns (compared to the C₈ column; Table 3). For all columns and mobile phases, the average value of $\delta \log k$ for substituted benzenes (#1–18), π -active compounds (#19–32) and aliphatics (#33–44) are summarized at the bottom of Table 3: benzenes, 0.00; π -active compounds, +0.18; aliphatics, -0.04. These results are consistent with π - π interactions for the cyano and phenyl columns. For 40% ACN versus 60% MeOH, the average values of $\delta \log k$ do not change for the substituted benzenes (0.00, 0.00) or aliphatics (-0.04, -0.04), but there is a marked difference for the π -active solutes (0.11 [ACN], 0.24 [MeOH]). This further supports the relative importance of π - π and (possibly) dipole–dipole interactions for the cyano and phenyl columns; i.e., ACN in the mobile phase should suppress solute–column π - π and dipole–dipole interactions.

Table 4

Correlation with Eq. (4) for values of $\delta \log k$ for π -active solutes (#19–32) as a function of solute molecular structure (number of aromatic rings or nitro-substituents in the molecule)

| Mobile phase | Column | r^2 | SD | Fit to Eq. (4) ^a |
|---|--------|-------|------|--|
| 40% ACN | Cyano | 0.797 | 0.02 | $y = 0.036 (\#rings) + 0.073(\#nitro)$ |
| | Phenyl | 0.827 | 0.03 | $y = 0.060 (\#rings) + 0.066(\#nitro)$ |
| 60% MeOH | Cyano | 0.496 | 0.06 | $y = 0.094 (\#rings) + 0.142(\#nitro)$ |
| | Phenyl | 0.793 | 0.07 | $y = 0.135 (\#rings) + 0.077(\#nitro)$ |
| Average for all columns and mobile phases | | 0.733 | 0.04 | $y = 0.081 (\#rings) + 0.077(\#nitro)$ |

See text for details.

^a (#rings) refers to the number of aromatic rings in the molecule minus one; (#nitro) refers to the number of nitro groups in an aromatic molecule.

If we consider only solutes in the π -active group (#19–32), it is interesting to compare values of $\delta \log k$ for each solute as a function of (a) the number of “additional” aromatic rings in the molecule (total number of aromatic rings minus one, since values of $\delta \log k$ are relative to substituted benzenes which each have one aromatic ring), and (b) the total number of nitro-substituent groups. Molecules with a more extended π -electron ring system (or a greater number of aromatic rings) should be stronger π -bases and therefore interact more strongly with either a cyano or phenyl column. Similarly, aromatic molecules substituted by nitro groups are stronger π -acids, also resulting in stronger π - π interaction. Thus, a rough correlation of values of $\delta \log k$ with the number of rings and nitro groups in the molecule is expected for π - π interaction. Table 4 summarizes correlations of values of $\delta \log k$ for all of the π -active solutes in terms of the equation

$$\delta \log k = a(\#rings) + b(\#nitro) \quad (4)$$

Here, “(#rings)” refers to the number of aromatic rings in the molecule minus one, and “(#nitro)” refers to the number of nitro-substituents in the molecule. Reasonable correlations are observed with Eq. (4) ($r^2 = 0.5$ – 0.83), considering that this is a greatly over-simplified representation of the complex dependence of “ π -activity” on molecular structure.

Table 6

Corrected values of $\delta \log k$ for aliphatic functional groups of Table 1 (#33–44)

| Functional group | 40% ACN | | 60% MeOH | | Cyano–phenyl | | Dipole moment ^b |
|---|---------|--------|----------|--------|--------------|----------|----------------------------|
| | Cyano | Phenyl | Cyano | Phenyl | 40% ACN | 60% MeOH | |
| Ester (–CO ₂ –) (average for #33–36) | –0.158 | –0.175 | –0.336 | –0.521 | 0.017 | 0.185 | 1.7 |
| SD | 0.013 | 0.005 | 0.033 | 0.001 | | | |
| Nitro (NO ₂) (average for #37–40) | –0.029 | –0.104 | –0.026 | –0.484 | 0.075 | 0.458 | 3.5 |
| SD | 0.004 | 0.004 | 0.011 | 0.003 | | | |
| Bromo (Br–) (average for #41–44) | –0.071 | –0.113 | –0.116 | –0.384 | 0.042 | 0.268 | 2 |
| SD | 0.013 | 0.006 | 0.020 | 0.001 | | | |
| Correction ^a | 0.010 | 0.021 | 0.023 | 0.090 | | | |

See text for details.

^a This quantity was subtracted from $\delta \log k$ for each additional CH₃– or –CH₂– group in the molecule.

^b Ref. [9].

Table 5

Selectivity parameters for columns used in the present study [1]

| Column | Column selectivity parameter values | | | | | |
|-----------------------------|-------------------------------------|-----------------------|----------|----------|-------------------|-------------------|
| | <i>H</i> | <i>S</i> [*] | <i>A</i> | <i>B</i> | <i>C</i> (pH 2.8) | <i>C</i> (pH 7.0) |
| C ₈ ^a | 0.876 | 0.031 | –0.23 | 0.016 | 0.043 | 0.012 |
| Cyano ^b | 0.44 | –0.135 | –0.578 | –0.014 | 0.216 | 1.036 |
| Cyano-2 ^c | 0.424 | –0.114 | –0.681 | –0.013 | –0.001 | 0.573 |
| Phenyl ^d | 0.525 | –0.198 | 0.051 | 0.024 | 0.228 | 1.465 |

^a Restek Ultra C8 (“C₈”).

^b Kromasil KR-60-5CN.

^c Jones Genesis cyano.

^d Phenomenex Prodigy phenyl-3.

4.2. Relative importance of dipole–dipole interactions for the cyano column

Aliphatic solutes are unlikely to exhibit π - π interactions, so to a first approximation we can assume that their $\delta \log k$ values are the result of dipole–dipole interactions only. For a given group of aliphatic homologs (e.g., esters, #33–36), values of $\delta \log k$ change regularly with increasing carbon number or alkyl substitution, because cyano and phenyl columns have much lower values of S^* than the C₈ column (Table 5), and the addition of a CH₃– or –CH₂– group to a molecule increases σ' by about 0.15 units. Table 6 summarizes contributions to $\delta \log k$ for the aliphatic

solutes (#33–44), and corrections for varying alkyl substitution. The average change x in $\delta \log k$ for the addition of a CH_3 - or $-\text{CH}_2$ - group is summarized in the last row of Table 6. To correct for this molecular weight contribution to $\delta \log k$, the number of CH_3 - or $-\text{CH}_2$ - groups in the molecule is multiplied by x and subtracted from $\delta \log k$ for each compound (for a given column and mobile phase). Values of $\delta \log k$ corrected in this way were averaged for each group of compounds as summarized in Table 6 (with their std. deviations). For example, the average value of $\delta \log k$ (corrected) for an ester group (cyano column, 40% ACN) is -0.158 ± 0.013 .

We can next assess the relative importance of dipole–dipole interactions for the cyano column and aliphatic solutes. First, we note that the corrected $\delta \log k$ values of Table 6 are all negative. This reflects the fact that these values for aliphatic solutes are relative to substituted benzenes, and the latter solutes are retained on both the cyano and phenyl columns by π – π interaction. Therefore, we must correct for the increased π – π interaction of the reference solutes relative to the aliphatic solutes of Table 6. In Table 3, we note that the average values of $\delta \log k$ for π -active solutes are similar for both cyano and phenyl columns, suggesting that the contribution of π – π interaction to retention on both columns is approximately the same. Therefore we can subtract the average value of $\delta \log k$ (corrected) for a phenyl column from the value for a cyano column; see the values in Table 6 labeled “cyano–phenyl”. As expected, much larger values of the latter are found for 60% MeOH compared to 40% ACN, due to interaction of the ACN molecule with both the solute and the stationary phase. There is also a strong correlation of cyano–phenyl values for these three aliphatic functional groups with their dipole moments:

$$(60\% \text{ MeOH}) \quad (\text{cyano} - \text{phenyl}) = 0.129 \text{ (dipole moment)} \\ r^2 = 0.967, \text{ SD} = 0.026 \quad (5)$$

$$(40\% \text{ ACN}) \quad (\text{cyano} - \text{phenyl}) = 0.020 \text{ (dipole moment)} \\ r^2 = 0.814, \text{ SD} = 0.013 \quad (6)$$

A similar approach was followed for the substituted benzenes (#1–18), but no significant correlation with dipole moment was observed (as previously reported for a cyano column with 50% ACN as mobile phase [8]). This is likely due to the greater difficulty of aligning dipoles for solute and column when the solute dipole is sterically hindered by the attached phenyl ring. A similar difference between aliphatic and phenyl derivatives was noted for the hydrogen-bond interaction of acceptor-solutes with silanol groups in the stationary phase of an RP-LC column [1].

Illustrative separations on the three columns with 50% MeOH as mobile phase are presented in Fig. 2. Samples are more retained on the C_8 column relative to the cyano or phenyl columns, as pointed out previously [4,8]. Relative retention is seen to be significantly different—primarily as a result of π – π interactions for the cyano and phenyl columns, and dipole–dipole interactions for the cyano column. However, the

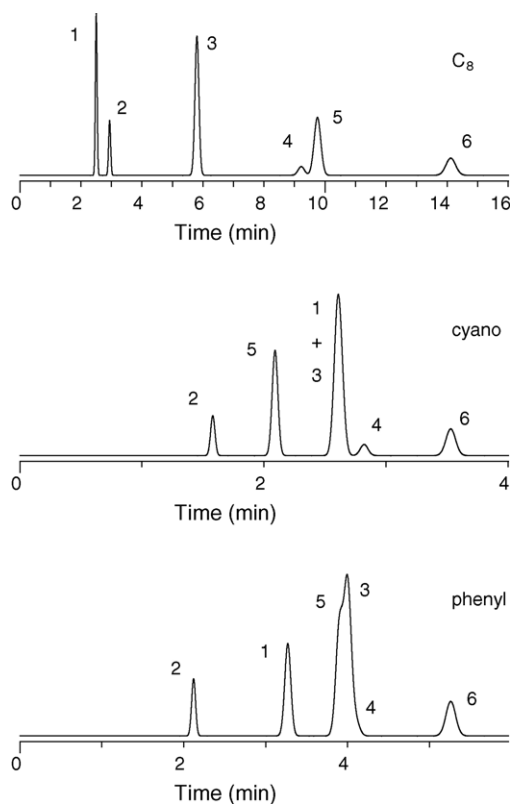


Fig. 2. Separations of the same sample on the C_8 (a), cyano (b), and phenyl column (c); 50% methanol/water as mobile phase, other conditions in Section 3. Sample is 1,3,5-trinitrobenzene (1), 1-*n*-propylacetate (2), 1-chloro-4-nitrobenzene (3), 1-nitro-*n*-hexane (4), 1-*n*-pentylacetate (5), naphthalene (6). Chromatograms reconstructed from individual injections.

solutes chosen for Fig. 2 represent extremes in their ability to participate in the latter two interactions. The selectivity of cyano and phenyl columns versus alkylsilica columns (such as the C_8 column of the present study) is significantly increased if mobile phase strength (%B) is adjusted to give comparable retention (or if gradient elution is used), due to the combined effects of solvent-strength and column selectivity [8].

4.3. Modification of Eq. (1) for phenyl and cyano columns

It is now clear that Eq. (1) does not account completely for retention on cyano and phenyl columns. The addition of terms for π – π interaction ($\pi'P$) and dipole–dipole interaction ($\mu'D$) might be useful in this regard:

$$\log k \equiv \log k_{\text{EB}} + \eta'H - \sigma'S^* + \beta'A + \alpha'B + \kappa'C \\ + \pi'P + \mu'D \quad (7)$$

where it is understood that values of $P > 0$ only occur for cyano and phenyl columns, and values of $D > 0$ are found only for cyano columns (P and $D = 0$ for all other columns). One might speculate on how much variation in values of P and D can occur for different cyano and phenyl columns, but there is little hard evidence to support such conjectures. A previous paper [4] suggests that values of P will be larger for a phenylhexyl column

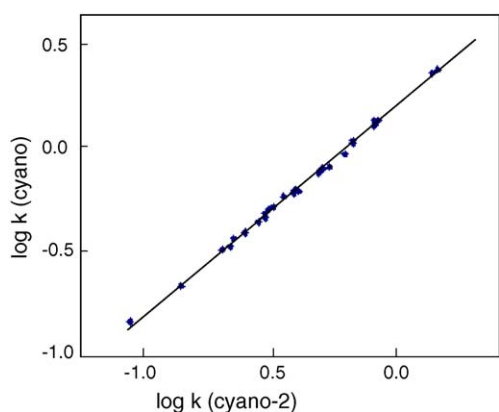


Fig. 3. Plots of retention for the Kromasil cyano column vs. the Jones cyano column ("cyano-2") using 60% MeOH/water as mobile phase and the following solutes: #1–10, 19–27, 29, 30, 33–40.

compared to a phenylpropyl column. It should also be noted that both the $\pi'P$ and $\mu'D$ terms are solvent-specific; acetonitrile as B-solvent leads to values for each term that are about half as large as when using methanol. Thus, values of P and D in Eq. (7) are no longer mobile-phase independent, and Eq. (7) is only intended as a conceptual extension of previously developed Eq. (1); i.e., not a validated relationship for quantitative predictions.

Limited data were obtained for a second cyano column (Jones Genesis cyano, ["cyano-2"]), whose column parameters differ somewhat from those of the Kromasil cyano column (Table 5). Fig. 3 shows a plot of values of $\log k$ for the Kromasil versus Jones column (60% MeOH mobile phase), with the following correlation

$$y = 0.164 + 1.013x \quad r^2 = 0.999, \text{ SD} = 0.014 \quad (8)$$

The excellent correlation of values of $\log k$ for these two columns suggest that contributions to retention from π – π and dipole–dipole interactions are likely to be similar for different cyano columns. This is not unexpected, as we are dealing with the same stationary-phase ligand ($-\text{C}_3-\text{C}\equiv\text{N}$) for all cyanopropyl columns.

5. Conclusions

The present study has analyzed retention data for 44 solutes, using four columns and two different mobile phases (292 measurements of k). Comparisons of retention were made for a cyano and a phenyl column versus a C_8 column, in order to assess the relative importance of π – π and dipole–dipole interactions for the cyano and phenyl columns. As found previously, π – π interactions can contribute to the retention of " π -active" solutes (e.g., PAH's, nitro-substituted aromatics) on a phenyl column. On the basis of the present study, it appears that π – π interactions are of comparable importance in affecting retention on the cyano column as well. The use of acetonitrile-containing mobile phases suppresses these π – π interactions, thereby reducing the preferential retention of aromatic solutes on phenyl and cyano columns by about 50% compared to methanol as solvent. Consequently, a change from one solvent to the

other will result in shifts in the relative retention of π -active solutes.

It was observed previously [1] for 50% acetonitrile-water as mobile phase that the dipole moment of aromatic solutes appears not to affect their retention on cyano columns. That is, dipole–dipole interactions appeared to be unimportant for these separations. The present study, however, suggests that dipole–dipole interactions can be important for cyano columns and aliphatic solutes substituted by functional groups (e.g., nitro) with large dipole moments. The contribution to retention ($\delta \log k$) from dipole–dipole interaction is about 1/6 as great for acetonitrile/water as for methanol/water as mobile phase, for reasons similar to the suppression of π – π interactions by acetonitrile in the mobile phase. Column selectivity as defined by the column parameters H , S^* , etc. of Eq. (1) can be expanded for cyano and phenyl columns along the lines of Eq. (7) above.

6. Nomenclature

| | |
|----------------------|--|
| a, b | constants for a given pair of columns and the same mobile phase (Eqs. (3) and (4)) |
| A | column hydrogen-bond acidity (relative to an average type-B alkyl-silica column), related to number and accessibility of silanol groups in the stationary phase; also "type-A" column based on metal-containing silica |
| ACN | acetonitrile |
| B | column hydrogen-bond basicity (relative to an average type-B alkyl-silica column) |
| 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 |
| D | a measure of column dipolarity (Eq. (7)); the tendency of a column to interact with solutes substituted by groups with large dipole moments (especially aliphatic compounds) |
| H | column hydrophobicity (relative to an average type-B alkyl-silica column) |
| H_1, H_2 | values of H for columns 1 and 2, respectively |
| k | retention factor, equal to $(t_R - t_0)/t_0$ |
| k_1, k_2 | values of k for columns 1 and 2, respectively |
| $\log k_{\text{EB}}$ | value of k for ethylbenzene as solute |
| MeOH | methanol |
| P | column π -activity (Eq. (7)); tendency of a column to interact with PAH's and nitro-substituted aromatics |
| PAH | polycyclic aromatic compound |
| r | correlation coefficient |
| RP-LC | reversed-phase liquid chromatography |
| S^* | steric resistance to insertion of bulky solute molecules into the stationary phase |
| SD | standard deviation |
| t_0 | column dead time (min) |
| t_R | retention time (min) |
| α' | relative solute hydrogen-bond acidity (Eq. (1)) |
| β' | relative solute hydrogen-bond basicity (Eq. (1)) |
| $\delta \log k$ | contribution to $\log k$ other than hydrophobicity; see Fig. 1a and related text |

Table A.1
Fitting equations for the calculation of values of $\delta \log k$

| Mobile phase | Column | Fitting equation and statistics | | |
|--------------|---------------------------|---------------------------------|-------|-------|
| | | Equation | r^2 | SD |
| 40% ACN | Cyano vs. C ₈ | $y = -0.112 + 0.540x$ | 0.993 | 0.015 |
| | Phenyl vs. C ₈ | $y = -0.073 + 0.590x$ | 0.968 | 0.034 |
| 60% MeOH | Cyano vs. C ₈ | $y = -0.435 + 0.405x$ | 0.961 | 0.030 |
| | Phenyl vs. C ₈ | $y = -0.093 + 0.293x$ | 0.894 | 0.037 |

Each column is compared with the C₈ column, based on k -values for solutes #1–4, 6–8, 12–14 and 17.

| | |
|-----------|--|
| η' | relative solute hydrophobicity (Eq. (1)) |
| κ' | relative charge on solute molecule (positive for cations, negative for anions) |
| μ' | solute dipole moment (mainly for aliphatic compounds) (Eq. (7)) |
| π' | tendency of a solute to undergo π – π interactions with a column (Eq. (7)) |
| σ' | relative steric resistance of solute molecule to penetration into stationary phase (σ' is larger for more bulky molecules) (Eq. (1)) |

Appendix A. Significance of last four terms of Eq. (1) for solutes of Table 1 and columns of Table 2

The 44 solutes of Table 1 do not include any ionizable compounds; therefore, the last two terms of Eq. (1) ($\alpha'B$, $\kappa'C$) are equal to zero. Likewise none of these compounds have significant hydrogen-bond-acceptor activity, so the $\beta'A$ term can also be ignored. Values of σ' are also known [10] or can be estimated; avg. $\sigma' = 0.16 \pm 0.26$ (solutes #1–44). The average difference in

value of S^* in Table 5 is 0.15, suggesting that the average value of the $\sigma'S^*$ term of Eq. (1) is 0.02 ± 0.04 . The average variation of values of $\delta \log k$ as a result of the $\sigma'S^*$ term will therefore be ± 0.04 units; i.e., also a relatively small contribution to values of $\log k$.

Solutes used for the determination of values of a and b in Eq. (3) should have similar values of σ' , so as to minimize contributions of the related solute–column interactions to $\delta \log k$ values. Certain solutes can be rejected for either larger or smaller values of σ' , leading to the selection of the solutes noted in Table 1 (solutes #1–4, 6–8, 12–14 and 17), marked by footnote “a”. For these solutes, we have the fitting equations of Table A.1, used in Table 3 for the calculation of values of $\delta \log k$.

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