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RETENTION CHARACTERISTICS OF SEVERAL BONDED-PHASE LIQUID CHROMATOGRAPHY COLUMNS FOR SOME POLYCYCLIC AROMATIC HYDROCARBONS

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SUMMARY

We have compared the retention of twelve polycyclic aromatic hydrocarbons (PAHs) on columns packed with octadecyl bonded phases from eight different manufacturers. The capacity ratios and selectivity factors were calculated for each compound on every column, under conditions of isocratic elution with identical mobile phase strength and similar linear flow velocities. We observed a wide range of capacity ratios and significantly different selectivity on different material. These data did not correlate with the carbon content or the surface concentration of alkyl groups for the various stationary phases. We also report the retention and selectivity characteristics for these PAHs on different manufacturing lots of the octadecyl silica used for one of the commercial columns.

INTRODUCTION

Chemically bonded stationary phases are now the most widely used column packing material for liquid chromatography (LC). These materials consist of organic functional groups chemically bonded to a base support material. Silica serves as the support material in the vast majority of chemically bonded phases, with *n*-octadecyl groups being the most common bonded organic material. While such C₁₈ bonded-phase silica is available from several manufacturers, it is not necessarily all chemically identical. There are four different synthetic routes to chemically bonded silica¹⁻⁴. Organic attachment via organosilanes is used almost universally, but the characteristics of the final material are still very much affected by parameters such as the nature of the reactants, reaction temperature, reaction time and solvents^{2,5,6}.

The purpose of analytical chromatography is the separation of compounds in a sample mixture. The quantitative description of the separation of two compounds, the resolution R_s (ref. 7),

$$R_s = \left(\frac{\sqrt{N}}{4} \right) \left(\frac{k'_1}{1 + k'_1} \right) \left(\frac{\alpha - 1}{\alpha} \right) \quad (1)$$

is governed by three factors: the efficiency of the column, N ; the retention of one of the compounds, measured as the capacity ratio, k_1' ; and the relative retention of the second compound, measured as the selectivity factor, α ,

$$\alpha = k_1'/k_2' \quad (2)$$

The column efficiency is often used as a means of judging the quality of the column packing process, although it is also a function of the nature of the hydrocarbonaceous layer on the silica support^{5,8-10}. There have been many studies of the effect of several bonded-phase characteristics, including chain length, surface coverage, and residual silanols on the retention of specific solutes^{3,5,9-16}. However, the most important factor influencing the resolution is the selectivity factor, which is a complex (and incompletely understood) function of the mobile phase composition and the nature of the organic bonded-phase^{3,5,11,13,16,18-20}.

Smillie *et al.*²¹ reported different separations of some polycyclic aromatic hydrocarbons (PAHs) on three commercial C_{18} bonded-phase columns. Nice and O'Hare²² reported very different selectivity factors for steroids on five commercial C_{18} columns. Rabel *et al.*¹⁶ made a detailed study of the selectivity factors for several aromatic compounds on two commercial C_{18} columns that differ only in the amount of organic bonded-phase. Scott and Kucera²³ compared five commercial reversed-phase columns, including three C_{18} bonded-phase columns. In the course of our work on the analysis of environmental samples for PAHs²⁴, we observed that the selectivity factor for certain PAH pairs was different on columns from different manufacturers. In the following, we describe a comparison of the retention and selectivity characteristics of several commercial C_{18} bonded-phase columns for these PAHs. We also report the selectivity factors for these PAHs for recent manufacturing lots of the C_{18} bonded-phase silica for Perkin-Elmer HC-ODS columns.

EXPERIMENTAL

Equipment

A Perkin-Elmer Series 3 liquid chromatograph, a Perkin-Elmer Model 650-10LC fluorescence detector and a Perkin-Elmer Sigma 10 chromatographic data station were used for these experiments. A Rheodyne Model 7120 injection valve fitted with a 10- μ l sample loop was used to inject the test mixture. Narrow-bore tubing of 0.007 in. (0.2 mm) I.D. was used to connect the column to the injection valve and to the fluorescence detector. A separate set of connecting tubing was made for each different type of column end fitting.

Reagents

Acetonitrile was used as purchased from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.) or from MCB Reagents (Cincinnati, Ohio, U.S.A.). Water was filtered, deionized and purified by a carbon bed system from Continental Water Conditioning (El Paso, Texas, U.S.A.). Solvents used as mobile phases were degassed with a Branson Model 185 ultrasonic probe (Danbury, Conn., U.S.A.). All solvents were filtered through a 0.47- μ m Millipore filter (Bedford, Mass., U.S.A.).

Chrysene, benzo[*a*]pyrene, benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene were

obtained in pure form from Analabs (North Haven, Conn., U.S.A.). The benzo-fluoranthene isomers were obtained as individual 100- $\mu\text{g}/\text{ml}$ solutions in toluene from Nanogen (Watsonville, Calif., U.S.A.). All other PAH standards were obtained as 100- $\mu\text{g}/\text{ml}$ solutions in hexane from Chem Services (West Chester, Pa., U.S.A.).

A stock mixture of the PAH standards was prepared from the individual solutions. The final solvent mixture was 81% hexane, 11% chloroform, 6% acetonitrile and 2% toluene. The concentrations of the individual PAHs in this mixture, given in Table I, were chosen to give fluorescence peaks of comparable magnitude in the chromatograms. Portions of this test mixture were diluted 1:10 or 1:50 with acetonitrile for injection.

TABLE I
CONCENTRATIONS OF INDIVIDUAL PAHs IN STOCK MIXTURE

No.	Compound	Concentration ($\mu\text{g}/\text{ml}$)
1	Anthracene	14.5
2	Fluoranthene	9.68
3	Pyrene	19.35
4	Benz[<i>a</i>]anthracene	14.5
5	Chrysene	14.5
6	Benzo[<i>e</i>]pyrene	9.68
7	Benzo[<i>b</i>]fluoranthene	1.45
8	Benzo[<i>k</i>]fluoranthene	0.097
9	Benzo[<i>a</i>]pyrene	1.45
10	Dibenz[<i>a,h</i>]anthracene	2.90
11	Benzo[<i>ghi</i>]perylene	2.90
12	Indeno[1,2,3- <i>cd</i>]pyrene	127

Columns

The columns are described in Table II. We purchased two pre-packed C_{18} silica columns each from DuPont Instruments (Wilmington, Del., U.S.A.), Whatman (Clifton, N.J., U.S.A.), Waters Assoc. (Milford, Mass., U.S.A.), Brownlee Labs. (Santa Clara, Calif., U.S.A.) and the Separations Group (Hesperia, Calif., U.S.A.).

Bulk packing purchased from Macherey-Nagel (Duren, G.F.R.), and bulk packing provided by Johns-Manville (Denver, Colo., U.S.A.) were packed in columns in the Perkin-Elmer column facility. In addition, three experimental columns containing mixtures of 25, 50 and 75% LiChrosorb RP-2 in LiChrosorb RP-18 were provided by Brownlee Labs. Brownlee Labs. also provided us with an experimental column packed with LiChrosphere 100 derivatized with *n*-octyl bonded-phase, a column packed with LiChrosphere 500 with *n*-octyl bonded-phase and a column packed with a 1:1 mixture of these two experimental packings.

Procedure

New columns were flushed with pure acetonitrile for at least 20 min prior to use. Acetonitrile-water mixtures were used as the mobile phase, ranging from 60 to 100% acetonitrile. The flow-rate for each column was adjusted according to the column cross-sectional area, referenced to 0.3 ml/min for 0.26-cm diameter columns. Thus, flow-rates of 0.7 and 0.9 ml/min were used for 0.39- and 0.46-cm diameter columns, respectively. The unretained volume was determined from injections of pure

TABLE II
COMMERCIAL STATIONARY PHASES

No.	Column	Column dimensions (cm)	Surface area (m^2/g) ^a	Carbon content (%)	Surface coverage σ_c ($\mu\text{mole}/m^2$)	k' (Benzol(a)pyrene) ^{†††}
1	Zorbax ODS	25 × 0.46	200	10 ^{††}	2.4	14.5
2	Chromosorb LC-7	25 × 0.26	300	15	2.9	9.41
3	Nucleosil-10 C ₁₈	25 × 0.26	300	n.d.	—	6.31
4	LiChrosorb RP-18	25 × 0.46	300	19.8 ^{**}	5.6 ^{†††}	13.2
5	HC-ODS	25 × 0.26	100	8.5	8.2	8.23
6	Vydac 201 TP	25 × 0.31	100	n.d.	—	6.74
7	μ bondapak C ₁₈	30 × 0.39	300	10 ^{††}	1.8	3.82
8	Partisil-10 ODS-2	25 × 0.46	400 [†]	16 [†]	2.3	16.3

^a Surface area of underivatized silica.

^{††} From ref. 23.

^{†††} σ_c quoted by E. M. Merck.

[†] From ref. 16.

^{††} From ref. 22.

^{†††} Acetonitrile-water (80:20), room temperature.

TABLE III
PEAK IDENTIFICATION FOR FIGS. 1-5

Compound	Peak number
Benz[<i>a</i>]anthracene	4
Chrysene	5
Benzo[<i>e</i>]pyrene	6
Benzo[<i>b</i>]fluoranthene	7
benzo[<i>k</i>]fluoranthene	8
Benzo[<i>ghi</i>]perylene	11
Indeno[1,2,3- <i>cd</i>]pyrene	12

acetonitrile with the fluorescence detector set at 260 nm excitation and 280 nm emission and 70 or 80% acetonitrile in water as the mobile phase. For injections of the PAH standard mixture, the fluorescence detector was set at 305 nm excitation and 430 nm emission. Capacity factors were calculated using the retention time data from the Sigma 10 data station. In cases of peak overlap or ambiguous peak identity, compounds were injected individually. Selectivity factors for the various PAHs were calculated relative to benzo[*a*]pyrene.

RESULTS

The seven PAHs identified in Table III are often poorly separated by reversed-phase LC. The chromatograms of these compounds run on five commercial C₁₈ bonded-phase columns, using an identical mobile phase composition in all cases, are shown in Figs. 1-5. The k' values were calculated for each of these compounds on each of these columns, as well as the three other commercial C₁₈ bonded-phase columns. Benzo[*a*]pyrene was also included in the test mixture, and the k' data sets from the different columns are presented as a function of benzo[*a*]pyrene retention in Fig. 6. These k' data were used to calculate the selectivity factors, α , for each compound relative to benzo[*a*]pyrene according to eqn. 2 (i.e., k_2' was always that of

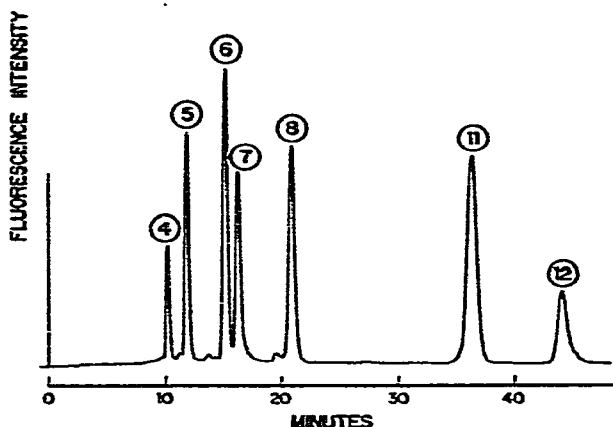


Fig. 1. Chromatogram of the PAHs in Table III on HC-ODS column. Mobile phase: acetonitrile-water (80:20). Flow-rate: 0.3 ml/min. Fluorescence detection at 305 nm excitation, 430 nm emission.

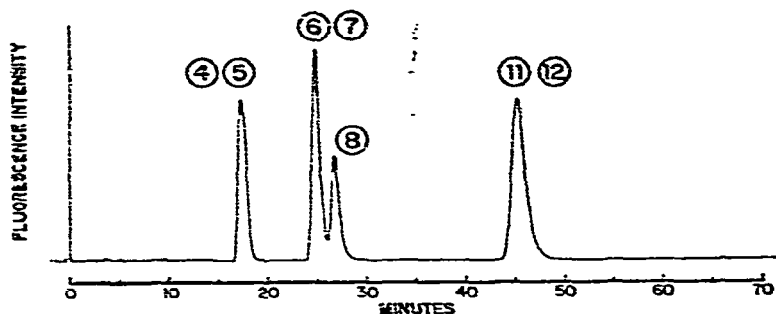


Fig. 2. Chromatogram of the PAHs in Table III on LiChrosorb RP-18 column. Conditions as in Fig. 1 except the flow-rate is 0.9 ml/min.

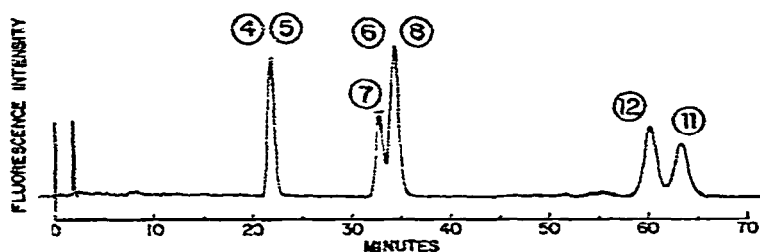


Fig. 3. Chromatogram of the PAHs in Table III on Partisil-10 ODS-2 column. Conditions as in Fig. 1 except the flow-rate is 0.9 ml/min.

benzo[*a*]pyrene). These selectivity factor data are presented graphically in Fig. 7 as a function of benzo[*a*]pyrene k' .

Experimental columns packed with various mixtures of Merck RP-2 and RP-18 bonded-phase material were also evaluated using the same test mixture and mobile phase strength. The retention time data from these chromatograms were used

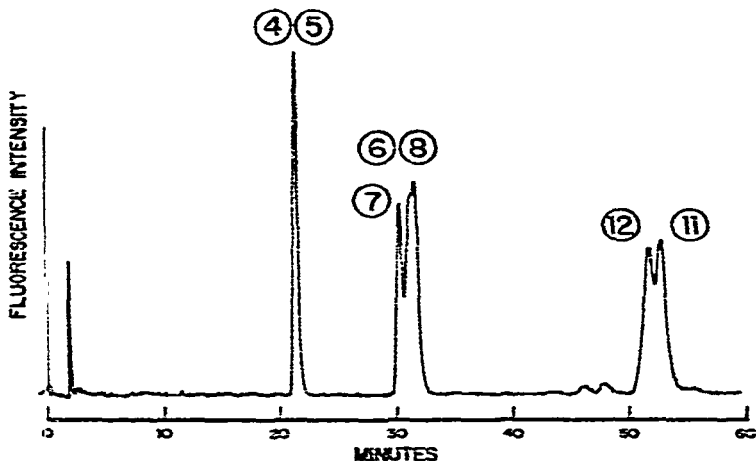


Fig. 4. Chromatogram of the PAHs in Table III on Zorbax ODS column. Conditions as in Fig. 1 except the flow-rate is 0.9 ml/min.

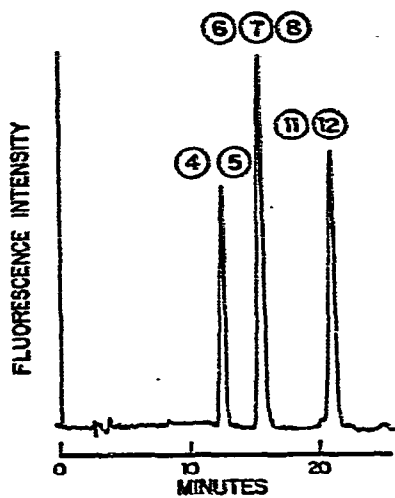


Fig. 5. Chromatogram of the PAHs in Table III on μ Bondapak C_{18} column. Conditions as in Fig. 1 except the flow-rate is 0.7 ml/min.

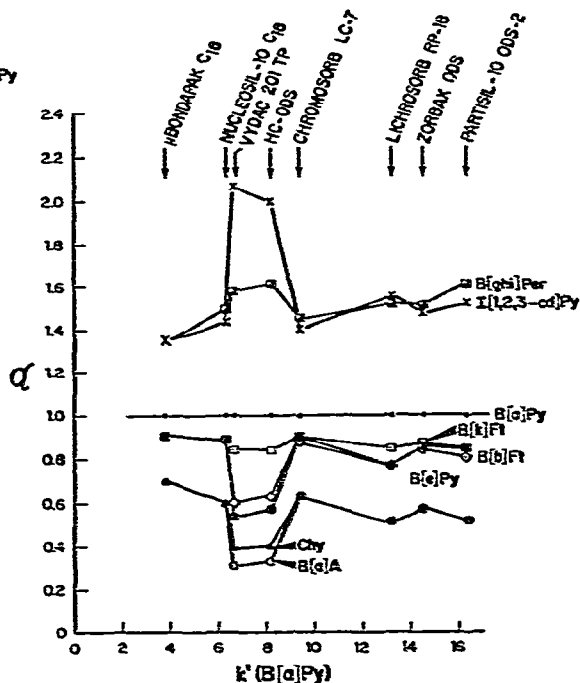
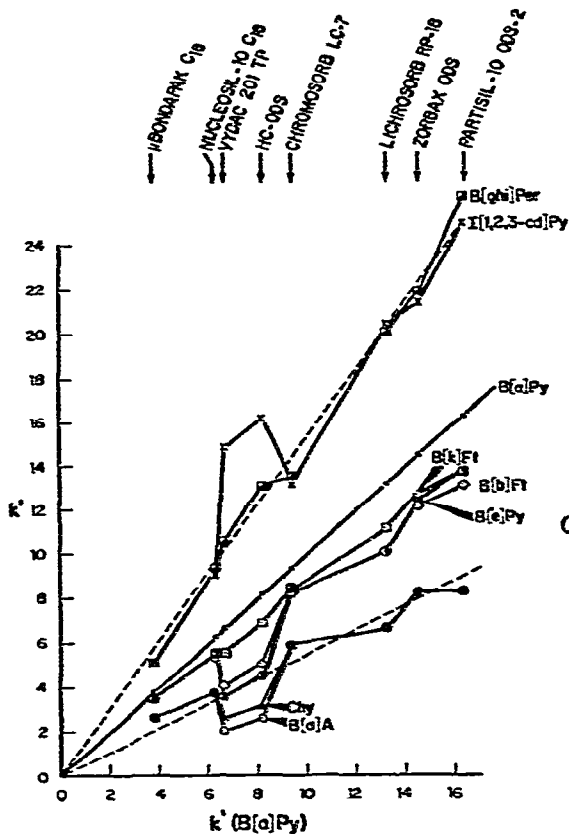


Fig. 6. Capacity ratios for the PAHs in Table III on different commercial C_{18} columns as a function of the k' of benzo[a]pyrene.

Fig. 7. Selectivity factors from the k' data in Fig. 6, plotted as a function of the k' of benzo[a]pyrene.

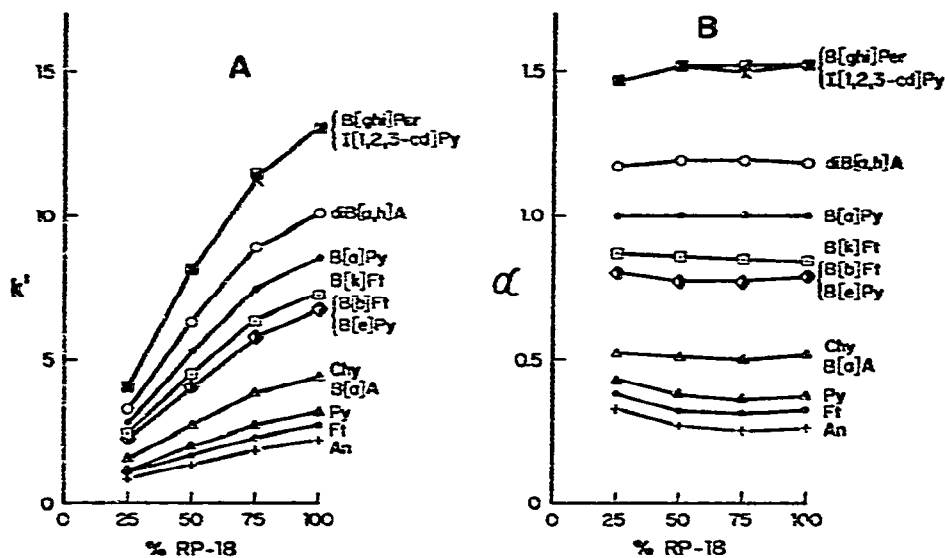


Fig. 8. (A) Capacity ratios for the PAHs in Table I on experimental columns packed with mixtures of LiChrosorb RP-2 and RP-18. Abscissa is the percentage LiChrosorb RP-18. (B) Selectivity factors for data in Part A.

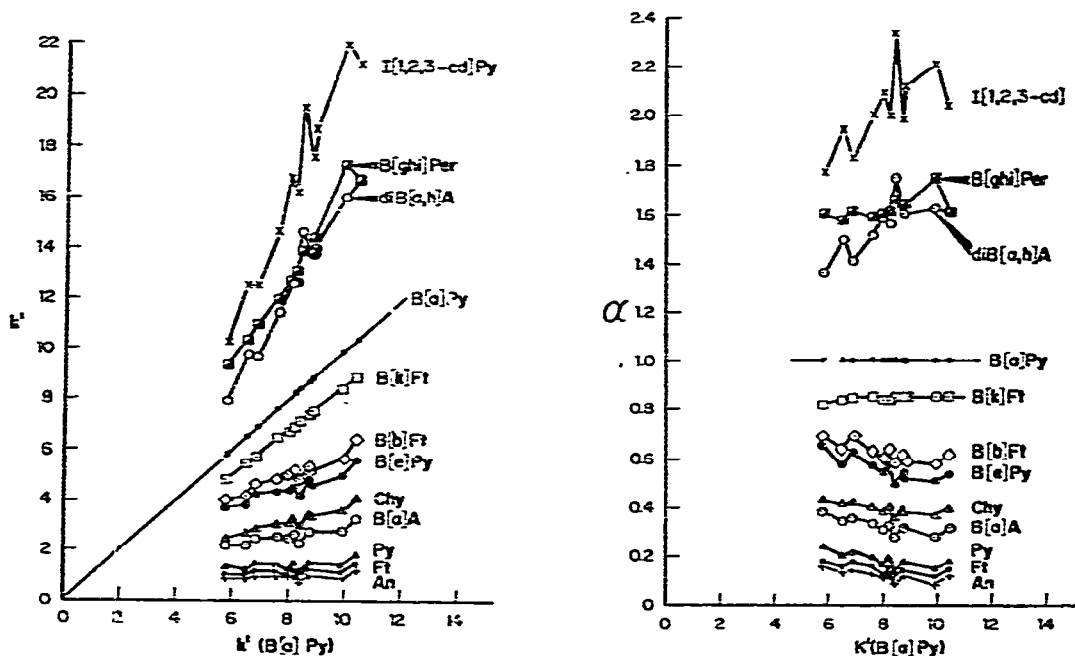


Fig. 9. Capacity ratios for the PAHs in Table I on different manufacturing lots of the C_{18} silica used in HC-ODS columns, plotted as a function of the k' of benzo[a]pyrene.

Fig. 10. Selectivity factors, relative the benzo[a]pyrene, from the k' data in Fig. 9, plotted as a function of the k' of benzo[a]pyrene.

to calculate k' and α just as with the commercial columns and these results are presented in Fig. 8 together with the data from the pure LiChrosorb RP-18 column.

Finally, there has been little published about the variations among different manufacturing lots for commercial columns. Figs. 9 and 10 give the capacity ratios and selectivity factors for the PAHs listed in Table I for different manufacturing lots of the C_{18} bonded-phase silica used in HC-ODS columns.

DISCUSSION

The chromatograms in Figs. 1–5 show large variations in the retention of seven PAHs on C_{18} bonded-phase silica columns from different manufacturers. These particular compounds are of more than just academic interest, as all but benzo[e]pyrene are on the U.S. EPA priority pollutant list. The complete separation of these compounds simplifies their identification and quantification in environmental samples. There are several parameters that affect retention which could vary with different manufacturing processes for C_{18} silica. Kikta and Grushka⁹ have shown that k' increases with the carbon content of the bonded-phase silica, while Hennion *et al.*⁵ found that k' initially increases with carbon content, but eventually reaches a plateau. Increases in the carbon content of a particular bonded-phase silica reflect an increased surface concentration of the alkyl functional group. However, carbon content data alone is inadequate for purposes of comparing columns from different origins since they can differ in surface area. Kirkland²⁵ and Unger *et al.*¹¹ have used the surface concentration of alkyl groups, σ_c ($\mu\text{mole}/\text{m}^2$), in comparisons of columns. (We have elected to use the symbol σ_c rather than α_{exp} as used by Unger *et al.*¹¹ in order to avoid confusion with the conventional use of α as the selectivity factor.) Tanaka *et al.*¹⁷ have reported that k' increases with σ_c .

We have used the carbon content and specific surface area data, where available, to calculate σ_c for the bonded-phase silicas in Table II. We found little correlation between the k' data for an individual PAH on the different columns and these σ_c values. There are several factors which may explain this observed lack of correlation between k' and σ_c . First, the calculation of σ_c as described by Unger *et al.*¹¹ and others^{13,15} uses the specific surface area of the underivatized silica with a correction factor for the contribution of the organic layer to the weight of the bonded-phase silica. But Unger *et al.* also have noted that chemical modification of porous silica can reduce the specific surface area and pore volume by as much as 54 and 61%, respectively. A similar observation was also made by Sorrell and Rowan²⁶ and Gilpin and Burke²⁷. Furthermore, Gilpin and Burke have reported that the reduction in surface area is more pronounced for silicas having high surface areas (and hence, smaller pores). Thus, the use of the specific surface area of the starting silica results in erroneously low values of σ_c , with the amount of error likely to be different for different silicas. The specific surface area of each of these bonded-phase silicas is needed to calculate the true σ_c . Second, the organic layer chemically attached to the silica surface can be monomeric or polymeric, depending on the silane and reaction conditions used in the derivatization step^{1–4,6,17}. The chromatographic consequences of this are not well characterized. Several studies have determined that the maximum monomeric coverage of silica by octadecyl groups is about $3.4 \mu\text{mole}/\text{m}^2$ (refs. 11, 13, 18, 19). The σ_c values for the LiChrosorb RP-18

and the HC-ODS materials suggest that these are polymeric. Several other of the bonded-phase silicas in Table II would have σ_c values greater than $3.4 \mu\text{mole}/\text{m}^2$ if their specific surface areas were reduced by 50% by chemical derivatization. Presumably, Zorbax ODS is monomeric since literature from DuPont Instruments clearly specifies that a monofunctional silane is used in its preparation.

Another variable in the manufacture of C_{18} silica is the use of a small silane, such as trimethylchlorosilane, to "cap" accessible hydroxyl groups remaining after the silica has been treated with octadecylsilane. Hemetsberger *et al.*¹⁹ and Tanaka *et al.*¹⁷ have shown that capping does not significantly alter carbon content or σ_c of a bonded-phase silica, but whereas Hemetsberger *et al.*¹⁹ reported little effect of capping on k' , most other authors have found that k' is increased by capping^{10,14,17}. Very little information is available about the use of capping in the preparation of commercial bonded-phase silicas. The HC-ODS material is not capped.

Since we were unable to identify a variable that clearly correlated with the different retention characteristics observed in chromatograms such as those in Figs. 1-5, we have plotted the sets of PAH k' data as a function of the retention of benzo[*a*]pyrene on the different columns, Fig. 6. Retention in reversed-phase LC is dominated by solvent-solute interactions, with stationary phase-solute interactions making secondary contributions. We have used a constant mobile phase composition throughout this series of experiments in order to bring out the chromatographic differences attributable to the stationary phases. Our test compounds are all members of the same chemical class, and to first order, we expect that differences in these octadecyl silica packings will affect them all in the same way. By plotting the data as a function of the retention of an arbitrarily chosen reference PAH, we have empirically ordered the data as a function of some pertinent characteristic(s) of these octadecyl silicas. Thus, if benzo[*a*]pyrene exhibits increased retention on one column relative to a second, we expect the other PAHs in Table I will also exhibit increased retention. This general trend is evident in Fig. 6 for all columns except the HC-ODS and Vydac 201 TP columns. The elution order is much the same on these latter two columns, but the range of k' values is much larger, suggesting that an additional retention mechanism is operating.

If changes in retention of the PAHs on the different columns were exactly proportional to the changes in retention of benzo[*a*]pyrene, *i.e.* if the retention of each of these compounds were linear functions of the same characteristic(s) of the bonded-phase material, then the data points for a given compound would fall on a straight line passing through the origin in Fig. 6. Such lines, examples of which are indicated by dotted lines in Fig. 6, represent constant selectivity factors relative to benzo[*a*]pyrene. The actual data points do not fall exactly on a line, indicating small differences in the selectivity for these compounds on these columns. This is more clearly seen in the plot of the selectivity factors relative to benzo[*a*]pyrene for these compounds, again as a function of the retention of benzo[*a*]pyrene, in Fig. 7. Here, deviations from a horizontal line represent changes in the selectivity factor, *i.e.* changes in retention that are not proportional to the changes in benzo[*a*]pyrene retention. The different retention characteristics of the HC-ODS and Vydac 201 TP columns noted earlier are very evident in this plot of selectivity factors. Ignoring the HC-ODS and Vydac columns, the selectivity factors for these PAHs on the other columns are similar, but not identical. The selectivity of benzo[*ghi*]perylene

relative to indeno[1,2,3-*cd*]pyrene varies from column to column, and benzo[*e*]pyrene overlaps one or the other of the benzofluoranthene isomers if the latter are resolved. Benz[*a*]anthracene and chrysene are not resolved on the other columns tested and exhibit a variable selectivity relative to benzo[*a*]pyrene. These last two compounds have been reported to be resolved ($R_s = 0.7$, $\alpha = 0.96$) on a Partisil-5 ODS column with methanol-water (80:20)²⁸. We were unable to duplicate this separation with the Partisil-10 ODS-2 column using this mobile phase.

Others have noted selectivity differences on commercial C_{18} bonded-phase columns. Nice and O'Hare²² reported different selectivities for six steroids on five C_{18} columns. Rabel *et al.*¹⁶ compared the selectivity of the Partisil-10 ODS and Partisil-10 ODS-2 columns for several compounds, including some aromatics. However, these last investigators altered the mobile phase composition to give $k' = 1$ for the first eluting solute on each column. Hence, the observed selectivity differences may reflect mobile phase effects as well as column differences. Scott and Kucera²³ found excellent agreement between the carbon content of Partisil-10 ODS, Partisil-10 ODS-2 and LiChrosorb RP-18 columns and the selectivity factors of 2-ethylanthraquinone relative to anthraquinone on these columns.

Since solvent-solute interactions greatly influence retention in reversed-phase chromatography, the incomplete resolution of several of the PAHs in Figs. 2-5 might simply reflect the use of an inappropriate mobile phase strength. Consequently, we have also examined the retention of these compounds on these columns for mobile phase compositions ranging from 60 to 100% acetonitrile in water. For each mobile phase composition, the selectivity factor for each compound relative to benzo[*a*]pyrene was calculated, and then these values were plotted as a function of the acetonitrile content of the mobile phase. It was clear from these plots²⁹ that adjustment of the mobile phase composition would not accomplish separation of these PAHs on columns other than the HC-ODS and Vydac TP columns.

Capping of silica derivatized with octadecylsilane replaces accessible silanols with trimethylsiloxanes. In a very simplistic way, the very short alkyl chains on LiChrosorb RP-2 might mimic the behavior of the capped sites, so we evaluated the retention and selectivity characteristics of experimental columns packed with three different proportions of LiChrosorb RP-2 mixed with LiChrosorb RP-18, as packed by Brownlee Labs. Capping has a very local effect on the nature of the bonded phase, whereas the mixtures of different alkyl bonded phases we evaluated were heterogeneous on a macroscopic scale. The data in Fig. 8 do indicate that the contribution of the individual components were simply additive.

A similar approach to the question of the effect of different pore sizes on the retention of these solutes, using columns packed with mixtures of C_{18} bonded silicas having different pore diameters, yielded inconclusive results because of very low column efficiencies.

Very little has been published on the comparison of the chromatographic characteristics of different manufacturing lots of bonded-phase silicas. Tanaka *et al.*¹⁷ compared carbon content, surface concentration and selected solute retention values for octyl bonded stationary phases prepared from three different lots of commercial silica and found that although carbon content and surface coverage were nearly identical for each bonded stationary phase, the chromatographic characteristics of each were different. We have monitored the retention and selectivity characteristics of

recent lots of the C_{18} silica used for Perkin-Elmer HC-ODS columns, and these data are presented in Figs. 9 and 10. These data span about nine months time. As before, the data sets from individual columns are plotted as a function of the benzo[*a*]pyrene k' . A wide range of k' values is evident, the range increasing with increased retention. The relative standard deviation increases from 11% for anthracene to 23% for indeno[1,2,3-*cd*]pyrene.

The variations in k' for some compounds, especially benzo[*k*]fluoranthene and benzo[*ghi*]perylene, are clearly correlated with the variations in k' for benzo[*a*]pyrene. This indicates nearly constant selectivity factors for these compounds, and Fig. 10 shows this to be the case. The relative standard deviation of the α data for benzo[*k*]fluoranthene and benzo[*ghi*]perylene on the different lots were 1.1 and 3.0%, respectively. All lots showed the same general selectivity pattern for the other compounds, but with a larger variability in the actual numerical values of α . The largest variability occurred for the first three eluting compounds, anthracene, fluoranthene and pyrene. As is seen in Fig. 9, the k' values for these compounds do not appear to be strongly correlated with those of benzo[*a*]pyrene, and this is confirmed by the large range of values. The relative standard deviations for the α data sets of the other compounds were 10% or less. Interestingly, the variations in α for dibenz[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene parallel each other (*i.e.* their relative selectivity was approximately constant), while those of benzo[*e*]pyrene and benzo[*b*]fluoranthene also varied in parallel, but in the opposite direction from the first pair. These effects suggest a common mechanism is involved for these compounds.

CONCLUSIONS

In conclusion, the variable selectivity observed on the C_{18} bonded-phase columns from different manufacturers means that a chromatographic analysis accomplished on one C_{18} column cannot always be duplicated on a C_{18} column from a different manufacturer. No one type of C_{18} column can do all separations, so it is not desirable to have all C_{18} columns identical in behavior. What is needed is the identification of the properties of C_{18} bonded-phase packings that affect the separation of various compounds, so that it would then be possible to select the column appropriate for a given sample on the basis of the specifications provided by manufacturers. For example, retention is governed in part by the product of the total surface area and the surface concentration of bonded organic groups, so the carbon content, the specific surface area, and the density of the final derivatized material are among the relevant specifications.

When developing chromatographic analyses, one also needs to know how much variation in retention and selectivity can be expected from different manufacturing lots of a given stationary phase. For this reason we have presented the retention and selectivity data on the C_{18} silica used for the HC-ODS column. While the range of k' values is somewhat large for some of these PAHs, the selectivity factors are fairly constant.

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