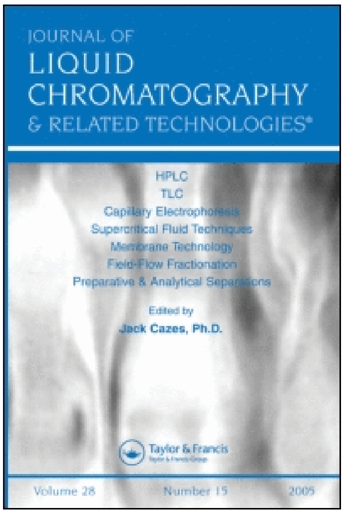

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MODIFICATION OF SELECTIVITY IN REVERSED-PHASE LIQUID CHROMATOGRAPHY OF POLYCYCLIC AROMATIC HYDROCARBONS USING MIXED STATIONARY PHASES

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ABSTRACT

Monomeric and polymeric C_{18} materials provide significantly different selectivities for polycyclic aromatic hydrocarbons (PAH) in reversed-phase liquid chromatography. Selectivity factors vary in a regular manner with respect to surface concentration of C_{18} groups on different C_{18} columns. In this study, we investigated the feasibility of "customizing" a C_{18} column to provide an intermediate selectivity by mixing 5-μm polymeric C_{18} material from two different lots with high and low C_{18} surface concentrations. Polymeric C_{18} materials from different production lots were mixed in ratios of 30/70, 50/50, and 70/30 (w/w). Selectivity factors for these columns were found to be similar to those predicted by the linear addition of the selectivities of the two individual phases. The PAH selectivities on these mixed columns were also found to be comparable to data obtained from coupled short columns of appropriate lengths each containing one of these different C_{18} materials. These studies indicate that columns of specific selectivity can be prepared by either mixing two different C_{18} materials or by coupling columns containing each of these different phases. The use of mixed phase columns is illustrated for the analysis of a fraction containing five condensed ring PAH isomers (molecular weight 278) isolated from an air particulate sample.

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INTRODUCTION

Monomeric and polymeric C_{18} materials provide significantly different selectivities for polycyclic aromatic hydrocarbons (PAH) in reversed-phase liquid chromatography (1,2). Polymeric C_{18} phases generally provide larger selectivity factors (α) than monomeric C_{18} phases for most planar PAH solutes. One polymeric C_{18} material investigated has a very low surface area (∿90 m^2/g for the underivatized silica) which provides a high C_{18} surface coverage and a unique selectivity for PAH separations when compared to monomeric C_{18} phases and to other polymeric C_{18} phases prepared on higher surface area silica (300-400 m^2/g). Recently, we reported that selectivity differences for PAH on different C_{18} columns from various manufacturers were related to the monomeric or polymeric nature of the chemically bonded layer and to the surface concentration of the C_{18} layer (2). In addition, several different lots of a polymeric C_{18} phase from one manufacturer were studied with respect to C_{18} surface coverage and selectivity factors for PAH. In this study (2) the selectivity factors for selected PAH (relative to benzo[*a*]pyrene) on the different polymeric columns were found to vary linearly as the surface concentration of the C_{18} groups varied. As an extention of these studies, we investigated the feasibility of "customizing" a C_{18} column to provide an intermediate selectivity by physically mixing two polymeric C_{18} materials from different production lots (i.e., lots with high and low C_{18} surface coverage). The selectivities of these "mixed" phase columns were compared to that obtained by coupling two short columns (in appropriate lengths) each containing one of these same C_{18} materials.

EXPERIMENTAL

A liquid chromatograph with a fixed wavelength UV detector at 254 nm and an autosampler were used for all liquid chromatographic measurements. HPLC grade acetonitrile and water were used as the mobile phase. PAH standards were obtained from several sources as previously reported (2). Columns and bulk packing material (∿2g) from two different bonding production lots (lots 11 and 15) of polymeric C_{18} material (5-μm) and one lot (lot 17) of monomeric C_{18} material (5-μm) were obtained from the manufacturer. The lot numbers were arbitrarily assigned by the authors and correspond to those in the previous study (2). The manufacturer indicated that lot 11 had a low carbon loading, whereas lot 15 had a high carbon loading. Both the polymeric and monomeric C_{18} materials used in this study utilized the same low surface area silica support (90 m^2/g).

The columns (25 cm x 4.6 mm i.d.) containing the mixtures of lots 11 and 15 [70/30, 50/50, and 30/70 (w/w) lot 11/lot 15] were prepared by the manufacturer. Short columns (12.5 cm x 4.6 mm i.d.) containing packing from lots 11 and 15 were also prepared by the manufacturer.

Specific surface area and percent carbon measurements on the chemically modified C_{18} materials were determined and used to calculate the surface concentrations of the C_{18} ligands on the silica as described previously (2).

The mobile phase mixtures were pre-mixed and allowed to equilibrate to room temperature prior to use. All retention data were obtained under isocratic and isothermal conditions (27 °C). Retention times were obtained from injections of acetonitrile solutions of each compound. Capacity factors, k', were determined from $k' = \frac{(t-t_o)}{t_o}$, where t is the retention time of the solute and t_o is the unretained time which was determined from an injection of acetone in a mobile phase of acetonitrile. Selectivity factors (α) for each compound were calculated from $\alpha = k'/k'_{BaP}$, where k'_{BaP} is the capacity factor for benzo[*a*]pyrene.

RESULTS AND DISCUSSION

In gas chromatography Laub *et al.* (3) and Kong *et al.* (4) have reported the use of mixed liquid phases to optimize the separation of selected PAH solutes. In liquid chromatography Glajch and

Kirkland (5) recently reported mixing LC packing materials with different chemically bonded functionalities (i.e., C_8, CN, and phenyl) to optimize the separation of phenylthiohydantoin derivatives of 20 amino acids. Ogan and Katz (6) evaluated the retention and selectivity characteristics of several PAH on columns with different proportions of C_2 bonded material mixed with C_{18} materials and found the contribution of the individual phases to be additive.

In a recent study (2) seven different production lots of a polymeric 5-μm C_{18} material were found to have C_{18} surface concentrations varying from 4.3 — 8.2 μmoles/m^2. The selectivity factors for PAH solutes on these columns varied linearly as the C_{18} surface concentration increased. Two lots of material from this previous study were selected to evaluate the feasibility of mixing C_{18} materials with high and low surface concentrations to obtain columns with intermediate selectivities. The physical characteristics of these two lots of polymeric C_{18} material are summarized in Table 1. The materials from lots 11 and 15 were rejected by the manufacturer's normal criteria for selectivity in the separation of PAH, i.e., insufficient resolution of benz[*a*]anthracene and chrysene on the low coverage material and insufficient resolution of dibenz-

TABLE 1. Physical Characteristics of Different C_{18} Materials

Column Lot[a]	Surface Area[b] (m^2/g)	Percent Carbon[c] (wt% ± 1s)	Surface Concentration[d] (μmol/m^2)	k'_{BaP}[e]
11 (polymeric)	61.0	7.5 ± 0.2	5.7 ± 0.2	3.1
15 (polymeric)	52.4	9.3 ± 0.1	8.2 ± 0.1	4.9
17 (monomeric)	53.4	4.0 ± 0.1	3.1 ± 0.1	0.9

[a] Lot numbers were arbitrarily assigned by authors as in ref. 2.

[b] Determined by BET.

[c] Four samples, uncertainty is ±1σ.

[d] Calculated from equation 2 in ref. 2.

[e] 85/15 acetonitrile/water (v/v) as mobile phase.

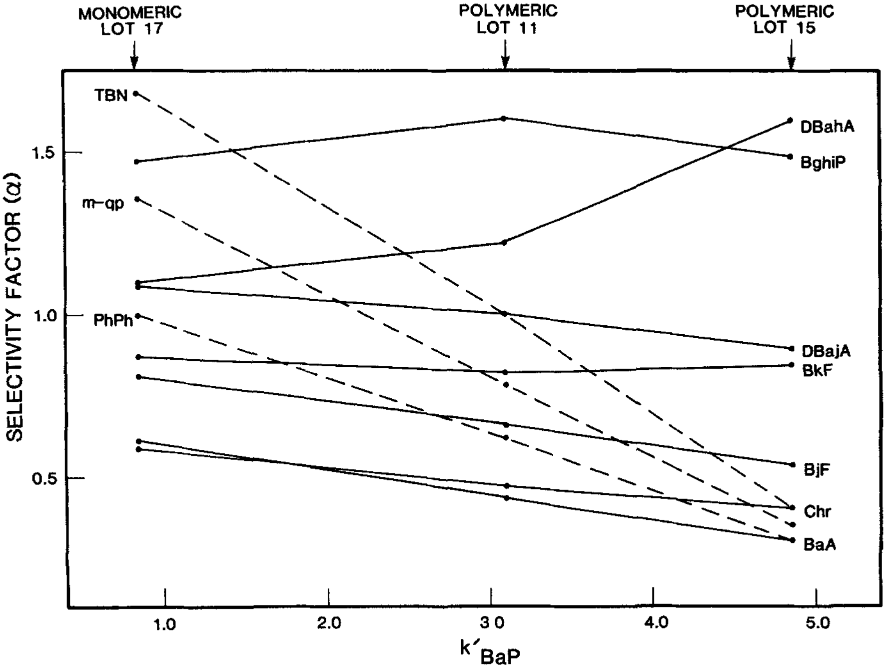

FIGURE 1: Selectivity factors (α), relative to benzo[*a*]pyrene, for selected PAH on three columns containing a monomeric C_{18} material (lot 17) and two polymeric C_{18} materials (lots 11 and 15). BaA = benz[*a*]anthracene, Chr = chrysene, BjF = benzo[*j*]fluoranthene, BkF = benzo[*k*]fluoranthene, DBajA = dibenz[*a,j*]anthracene, BghiP = benzo[*ghi*]-perylene, DBahA = dibenz[*a,h*]anthracene, TBN = 1,2:3,4:5,6:7,8-tetrabenzonaphthalene, PhPh = phenanthro-[3,4-*c*]phenanthrene, and m-qp = *m*-quinquephenyl.

[*a,h*]anthracene and benzo[*ghi*]perylene on the high coverage material. Data are also included in Table 1 for a monomeric C_{18} phase prepared on the same low surface area silica (90 m^2/g) as the two polymeric C_{18} materials.

Selectivity factors for several PAH solutes on these three C_{18} columns are plotted in Figure 1 as a function of k'_{BaP}. As noted previously (2) and as shown in Table 1, k'_{BaP} values are related to the C_{18} surface concentrations. This linear relationship of k'_{BaP} and surface coverage is valid, however, only when C_{18} phases prepared on silica of the same surface area are compared. In the previous study (2) data for columns from five different lots of the polymeric material and from a monomeric column were plotted in a similar manner as in Figure 1. However, the monomeric column in the previous study was prepared on a high surface area silica (∿300 m^2/g) with 13% carbon resulting in a k'_{BaP} value of ∿5.5. The monomeric column in the present study was prepared on a low surface area silica (90 m^2/g) with a 4% carbon loading resulting in a k'_{BaP} value of 0.9. As shown in Figure 1, when the selectivity factors are plotted as a function of k'_{BaP}, the linear trends observed for the polymeric columns can generally be extrapolated back to the monomeric column. This behavior suggests that for some types of PAH solutes (particularly nonplanar solutes such as TBN and PhPh in Figure 1) selectivity is dependent only on the extent of C_{18} surface coverage (when phases prepared on silicas with similar surface areas are compared) rather than the monomeric or polymeric nature of the C_{18} phase.

Mixed phase columns were prepared by combining material from lots 11 and 15 in proportions of 70/30, 50/50, and 30/70 (w/w) lot 11/lot 15. The selectivity factors (α) for several PAH are plotted in Figure 2 as a function of k'_{BaP} values obtained for columns from lots 11 and 15. The values on the abscissa for the mixed phase columns (70/30, 50/50, and 30/70 lots 11/15) were determined from the α values of the PAH. The data in Figure 2 indicate that columns of intermediate selectivities can be prepared by mixing the two phases of different selectivity. The selectivities of these mixed phase columns were found, however, to be slightly different from those predicted by the actual mixture proportions, i.e., predicted k'_{BaP} values of 3.62, 3.97, and 4.32 compared to actual values of 3.84, 4.10, and 4.38 for the 70/30, 50/50, 30/70 lot 11/15 mixtures, respectively. The actual and predicted values are in better agreement as the proportion of lot 15 (high coverage) increases.

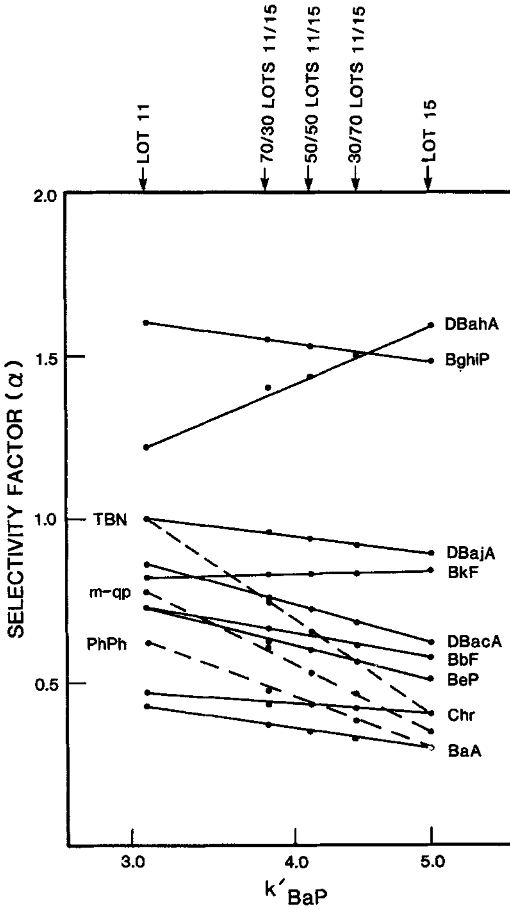

FIGURE 2: Selectivity factors (α), relative to benzo[*a*]pyrene, for selected PAH on polymeric C_{18} columns from mixtures of two different lots. BeP = benzo[*e*]pyrene, BbF = benzo[*b*]fluoranthene, DBacA = dibenz[*a*,*c*]anthracene. See Figure 1 for additional compound identification.

Differences in column selectivity for seven PAH solutes are illustrated in Figure 3. Six columns were compared: the monomeric phase, the high and low coverage polymeric phases, and three columns consisting of mixtures of the two polymeric phases. Phenanthro[3,4-*c*]phenanthrene (no. 2) and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (no. 4) are nonplanar solutes for which the selectivity factors vary significantly as the C_{18} surface concentration varies (see Figure 2). These differences in selectivity are discussed in detail elsewhere (2). On the monomeric column the phenanthro[3,4-*c*]phenanthrene and the 1,2:3,4:5,6:7,8-tetrabenzonaphthalene have longer retention times relative to the planar PAH than on the polymeric columns. Since the selectivity factors for these two solutes are very sensitive to changes in the C_{18} surface coverage, they have been used as an empirical test to evaluate the monomeric or polymeric characteristics of C_{18} columns by comparing their selectivity factors relative to a planar PAH solute (7). For the selected solutes, the columns from lots 11 and 15 did not separate all seven solutes. However, a mixed phase column of 70% lot 11 and 30% lot 15 provided the appropriate selectivity to achieve separation of all of these solutes. These studies indicate that columns of specific selectivity can be prepared by mixing C_{18} phases of different selectivities.

The selectivity factors obtained for the 50/50 lot 11/lot 15 mixed column were compared with data obtained by coupling two short columns (12.5 cm each), one containing material from lot 11 and one containing material from lot 15. The results shown in Table 2 indicate that short columns of appropriate lengths packed with materials from different lots can be coupled to achieve selectivities similar to those obtained by physically mixing the different packing materials. Thus, the chromatographer could have a collection of short C_{18} columns of different selectivities which could be coupled together in various combinations to achieve the necessary selectivity for a particular separation.

To illustrate the potential use of mixed phase columns of different selectivities, a fraction containing five condensed ring

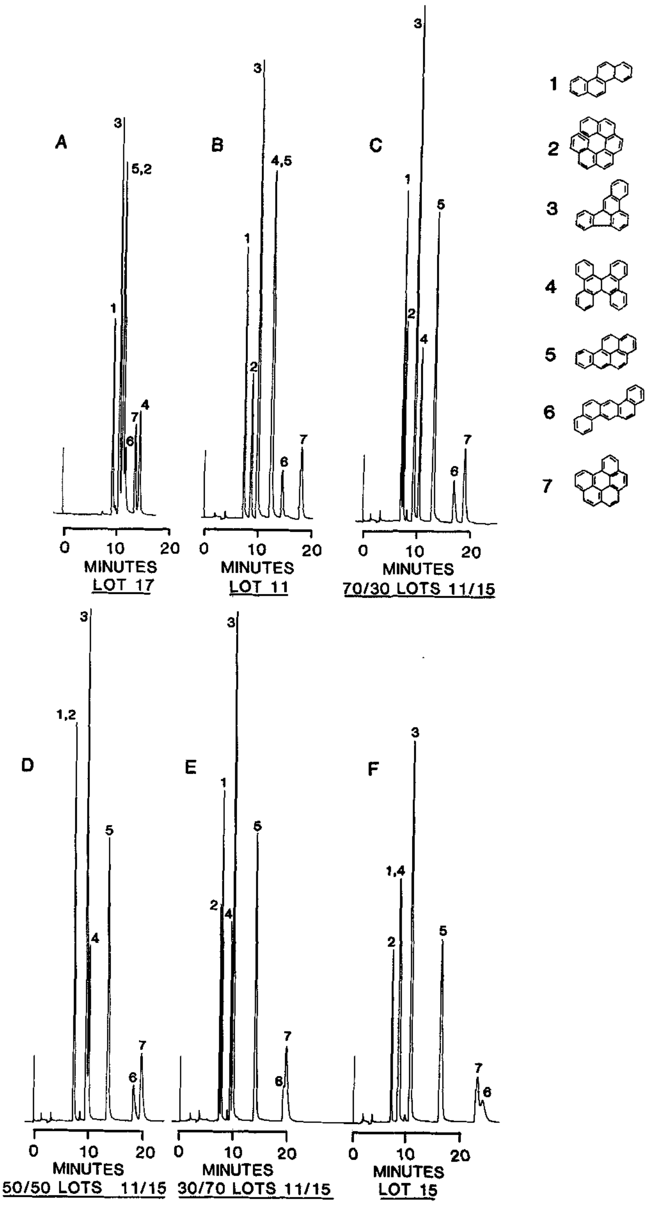

FIGURE 3: Reversed-phase LC separation of selected PAH on a monomeric C_{18} column from lot 17 (A) and on polymeric C_{18} columns from lot 11 (B) and lot 15 (F) and mixtures of these two lots [70/30 (C), 50/50 (D), and 30/70 lot 11/15 (E)]. Compound identification: (1) chrysene, (2) phenanthro[3,4-*c*]phenanthrene, (3) benzo[*b*]fluoranthene, (4) 1,2:3,4:5,6:7,8-tetrabenzonaphthalene, (5) benzo[*a*]pyrene, (6) dibenz[*a,h*]anthracene, and (7) benzo[*ghi*]perylene.

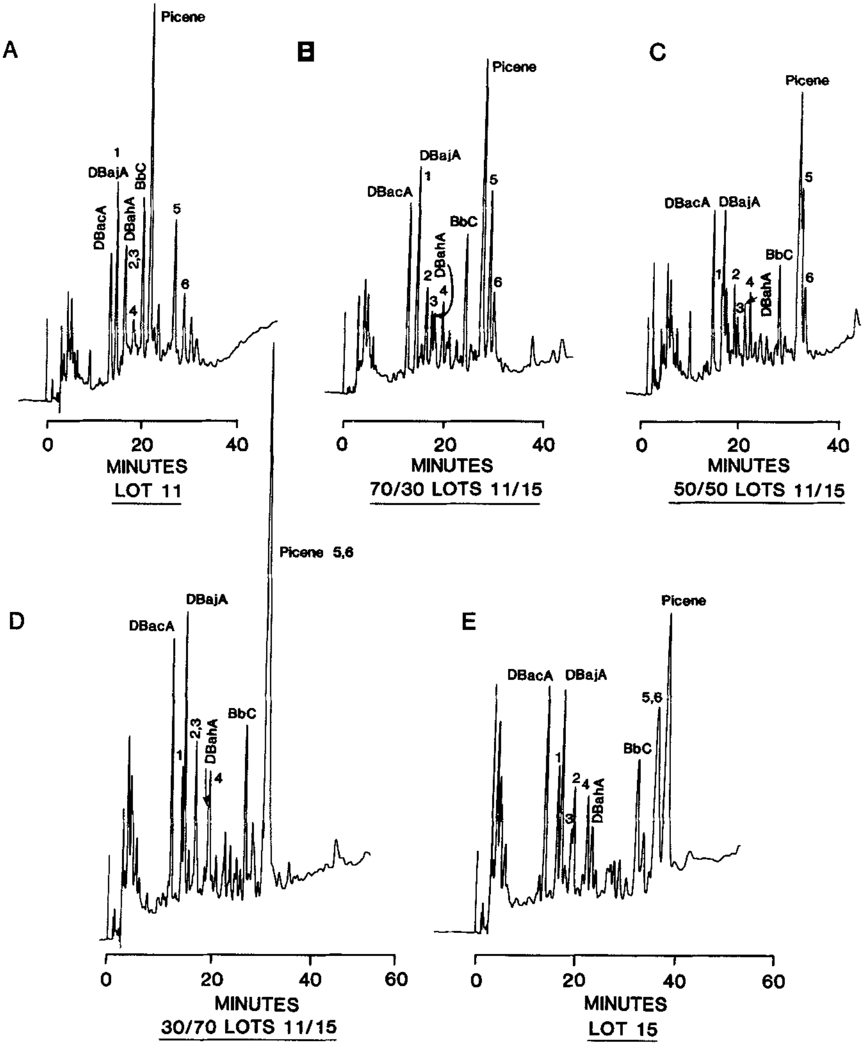

FIGURE 4: Reversed-phase LC separation of five condensed ring PAH fraction (isolated from an air particulate extract) on C_{18} columns of different selectivities (A) lot 11, (B) 70/30 lot 11/15, (C) 50/50 lot 11/15, (D) 30/70 lot 11/15, and (E) lot 15. Peak identification: DBacA = dibenz[*a,c*]anthracene, DBajA = dibenz[*a,j*]anthracene, DBahA = dibenz[*a,h*]anthracene, BbC = benzo[*b*]chrysene, and no. 6 = coronene. Peaks 1-5 are unknown.

TABLE 2. Selectivity Factors (α) for Selected PAH on Mixed vs. Coupled Columns

	50/50 Lot 11/15	
	Mixed	Coupled
m-Tetraphenyl	0.288	0.291
Phenanthro[3,4-*c*]phenanthrene	0.436	0.438
9,10-Diphenylanthracene	0.454	0.459
m-Quinquephenyl	0.557	0.564
Tetrabenzonaphthalene	0.676	0.676
Dibenz[*a,c*]anthracene	0.728	0.730
Dibenz[*a,j*]anthracene	0.939	0.941
Dibenz[*a,h*]anthracene	1.42	1.43

PAH (molecular weight of 278) and coronene, which was isolated from an air particulate sample as described previously (8), was separated on columns from lots 11 and 15 and on the three mixed phase columns. This fraction contains coronene and five PAH isomers of molecular weight 278 which were identified by gas chromatography-mass spectrometry (GC-MS) and LC with fluorescence detection as dibenz[*a,c*]anthracene, dibenz[*a,j*]anthracene, dibenz[*a,h*]anthracene, benzo[*b*]chrysene, and picene. In addition, at least one unidentified PAH isomer of molecular weight 278 was confirmed by GC-MS.

The LC analyses of this fraction on the five different columns are shown in Figure 4. All of these chromatograms were obtained under the same chromatographic conditions. The five identified PAH isomers of molecular weight 278 were separated on each of the five columns. However, the unknown peaks (nos. 1-5) and coronene coelute with each other and/or with the identified peaks on some of the columns. These chromatograms illustrate the advantage of having several columns of differing selectivities for the analysis of complex environmental PAH mixtures.

CONCLUSIONS

The complexity of PAH mixtures often necessitates the modification of column selectivity to obtain the separation of particular constitutents. Two methods for achieving a particular selectivity for PAH in reversed-phase LC have been described in this paper, i.e., physically mixed C_{18} sorbents of different selectivities and coupled columns each containing a different C_{18} sorbent. Both of these methods provide a selectivity which is intermediate to that of the individual C_{18} materials and which is related to the proportions of each material. These two approaches allow the chromatographer to "customize" a column with an optimized selectivity for the separation of the components of interest. In practice the coupled column approach provides the most flexibility for preparing columns of a specific selectivity. With the availability of several short columns containing C_{18} materials with greatly differing selectivities, a particular intermediate selectivity can be achieved readily by coupling the appropriate columns.

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