



National Institute of Standards & Technology

Certificate

Standard Reference Material 869

Column Selectivity Test Mixture for Liquid Chromatography

(Polycyclic Aromatic Hydrocarbons)

Standard Reference Material (SRM) 869 is an acetonitrile solution of three polycyclic aromatic hydrocarbons (PAHs): benzo[a]pyrene (BaP), 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN, alternate name, dibenzo[g,p]chrysene), and phenanthro[3,4-c]phenanthrene (PhPh) (see Figure 1 for structures). A unit consists of five ampoules, each containing approximately 1.1 mL of the test solution. This solution is a new type of SRM intended for certifying the performance characteristics of reversed-phase liquid chromatography (LC) columns, rather than for providing quantitative levels of the individual constituents. SRM 869 is intended primarily for characterizing LC column selectivity for the separation of PAHs. Depending on the elution order of the three components, column selectivity can be predicted for complex PAH mixtures (particularly isomeric PAHs). Even though the primary use of this mixture in the past has been to characterize columns for PAH separations, applications to the assessment of column selectivity for other classes of compounds, such as carotene isomers, have also been demonstrated. The concentrations and relative 254 nm uv-detector responses of the components are listed in the Appendix in Table 1 as an aid to the user.

Notice and Warnings to User.

Disclaimer: Certain commercial equipment, instruments, or materials are identified in this certificate to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose. Tabulations of commercial LC columns are not intended to be all inclusive.

Storage: Sealed ampoules, as received, should be stored in the dark at temperatures between 10-30 °C.

Toxicity: This test mixture contains small amounts of polycyclic aromatic hydrocarbons, some of which have been reported to have mutagenic and/or carcinogenic properties; therefore, care should be exercised during handling and use. Use proper methods for disposal of waste.

Expiration of Certification: This SRM is valid for its intended use for three years from the date of purchase. Should the certification become invalid before then, users will be notified by NIST. Please return the attached registration form to facilitate notification.

Preparation and analytical determinations were carried out at the Center for Analytical Chemistry, Organic Analytical Research Division, by L. C. Sander and S. A. Wise.

The coordination of the technical measurements leading to certification were performed under the direction of L. C. Sander, S. A. Wise and W. E. May.

The support aspects involved in issuance of this Standard Reference Material were coordinated through the Standard Reference Materials Program by R. Alvarez.

March 28, 1990
Gaithersburg, MD 20899

William P. Reed, Acting Chief
Standard Reference Materials Program

Use of the column evaluation test mixture.

Conditions. This test mixture is intended primarily for the characterization of C₁₈ columns used in the reversed-phase liquid chromatographic separation of PAHs. To compare columns on the same basis, the user should evaluate column selectivity by chromatographing the mixture isocratically under the following conditions: mobile phase, 85% acetonitrile/water (v/v) at 25 ± 2 °C. Changes in both absolute retention and selectivity can occur with changes in temperature¹ and composition; thus these conditions should be used for all column evaluations to enable comparisons with the results reported in this certificate.

Representative separations of the test mixture are illustrated in Figures 2A-2E for several different C₁₈ columns. These chromatograms are examples of five possible cases for solute elution order, and are provided as an aid for component identification. In cases in which complete separation is achieved (Figures 2A, 2C, and 2E), BaP is the smallest peak, PhPh is intermediate in size, and TBN the largest peak (see the Appendix for peak height and peak area data).

Interpretation of results. The elution order of the three components changes with C₁₈ phase type² as shown in Figure 3. Monomerically bound C₁₈ phases (prepared using monofunctional silane reagents) have been shown to give the elution order BaP ≤ PhPh < TBN (see Figures 2A and 2B). Phases prepared using polymeric surface modification procedures (i.e., using trifunctional silanes in the presence of water) have the elution order PhPh ≤ TBN ≤ BaP (Figures 2D and 2E). Phases with intermediate properties (i.e., densely loaded monomeric or lightly loaded polymeric C₁₈ phases) are indicated by the elution order PhPh < BaP < TBN (Figure 2C).

A quantitative measure of phase selectivity can be calculated to enable relative comparisons between different C₁₈ phases. The selectivity factor $\alpha_{\text{TBN/BaP}}$ (defined as $k'_{\text{TBN}} / k'_{\text{BaP}}$, where $k' = (t_R - t_0)/t_0$, and t_R and t_0 are the retention times of the analyte and void volume marker, respectively) has been shown to correlate with PAH selectivity and phase type^{2,5}. It should be noted that while the separation of isomeric PAH mixtures is often achieved with polymeric C₁₈ phases (or other phases with component elution PhPh < TBN < BaP), this type of phase may not be the best for a given separation problem. This column evaluation test mixture enables columns to be grouped into well characterized classes to facilitate method development. A classification scheme has been proposed, based on measurement of $\alpha_{\text{TBN/BaP}}$ values for experimental and commercial C₁₈ columns⁴. Values for $\alpha_{\text{TBN/BaP}} \leq 1$ reflect polymeric C₁₈ phases, and values for $\alpha_{\text{TBN/BaP}} \geq 1.7$ reflect monomeric C₁₈ phases. For values $1 < \alpha_{\text{TBN/BaP}} < 1.7$, the synthesis scheme is less certain, and may indicate light polymerization with di- or trifunctional reagents, or densely loaded monomeric phases. A listing of commercial C₁₈ columns, grouped using this classification scheme, is provided in the Appendix, Table 2.

Relationship between retention order and PAH selectivity. Examples of changes in phase selectivity are illustrated in Figures 4 and 5. The separation of PAH isomers of molecular weight 278 (see also Figures 8 and 9) is shown in Figure 4. Selectivity factors for four commercial and experimental columns range from 0.38 to 1.80. The best separation of these isomers was achieved with the most heavily loaded polymeric phase, with $\alpha_{\text{TBN/BaP}} = 0.38$. The lowest selectivity for these isomers was found with a monomeric phase ($\alpha_{\text{TBN/BaP}} = 1.80$). Using the same set of columns, Standard Reference Material 1647a (SRM 1647a, Priority Pollutant Polycyclic Aromatic Hydrocarbons in Acetonitrile) was separated using gradient elution conditions (see Figure 5). Separation of this mixture (required by EPA Method 610) should be possible with C₁₈ columns having $\alpha_{\text{TBN/BaP}}$ values in the range ~ 0.65 $\alpha_{\text{TBN/BaP}} < \sim 0.9$. Separation can also be achieved for $\alpha_{\text{TBN/BaP}} < \sim 0.4$. Complete separation of all 16 components was possible with the two polymeric C₁₈ columns ($\alpha_{\text{TBN/BaP}} = 0.65$ and 0.38), but with a reversal in the elution of dibenz[a,h]anthracene and benzo[ghi]perylene on the heavily loaded polymeric phase. Separation of the components occurred over the widest time interval, with the heavily loaded polymeric C₁₈ phase ($\alpha_{\text{TBN/BaP}} = 0.38$). Separation of all components is generally not possible for $\alpha_{\text{TBN/BaP}} > \sim 0.9$; however by modifying column selectivity by changing chromatographic conditions (e.g., reducing column temperature, see below) baseline separation of all 16 components can be achieved.

A plot of α_{cp} (i.e., the selectivity coefficient for several critical solute pairs) vs. column selectivity ($\alpha_{\text{TBN/BaP}}$) is shown in Figure 6. SRM 1647a was separated under gradient conditions (40-100% ACN, over 30 minutes) on several commercial columns. Selectivity coefficients (α_{cp}) were calculated for three critical solute pairs, and these values were plotted as a function of $\alpha_{\text{TBN/BaP}}$. Other components in SRM 1647a were resolved regardless of

column type. It was determined empirically that a selectivity coefficient of approximately 1.02 (or 0.98) was required for baseline separation. Thus, the shaded regions in Figure 6 denote incomplete separation of two or more solutes. Two windows exist for complete resolution of all components: $\sim 0.65 < \alpha_{\text{TBN/BaP}} < \sim 0.9$, and $\alpha_{\text{TBN/BaP}} < \sim 0.4$.

Discussion.

The selectivity factor $\alpha_{\text{TBN/BaP}}$ has been shown to vary with phase type², substrate properties⁶, alkyl chain length of bonded ligand⁷, phase density³, and column temperature¹. In general, column selectivity for complex PAH mixtures (such as PAH isomers) increases with decreasing $\alpha_{\text{TBN/BaP}}$. Furthermore, separations often can be reproduced under different conditions (i.e., different combinations of phase type, alkyl chain length, column temperature, and mobile phase combination) as long as $\alpha_{\text{TBN/BaP}}$ is held constant. Thus by adjusting these chromatographic variables, differences in column selectivity among columns from different manufacturers may be compensated.

In practice, phase type, alkyl chain length, and pore size are most easily varied by changing columns. As column manufacturers begin to provide $\alpha_{\text{TBN/BaP}}$ data on their C₁₈ columns, column selection and method development will be facilitated. A few trends, however, can be summarized.

Phase type. The single most important parameter affecting column selectivity is bonded phase chemistry². As described above, phases prepared using polymeric surface modification chemistry will usually have $\alpha_{\text{TBN/BaP}}$ values less than 1, and will exhibit increased selectivity for PAH isomers compared to monomeric C₁₈ phases ($\alpha_{\text{TBN/BaP}} > 1.7$, Figure 3). Phases prepared using limited polymeric syntheses, or prepared using highly reactive monomeric syntheses may result in intermediate properties, i.e., $1 < \alpha_{\text{TBN/BaP}} < 1.7$.

Pore size. Phase selectivity is also dependent on pore size of the silica substrate. For polymeric C₁₈ phases, the ability to separate PAH isomers (shape selectivity) increases with increasing pore size⁶. For monomeric C₁₈ phases, little dependence is observed between column selectivity and pore size. For polymeric C₁₈ phases, however, phase selectivity shifts with changes in pore diameter, becoming more shape selective (more polymeric-like) with increasing pore size. Benz[a]anthracene and chrysene coelute on 60 Å polymeric columns, but are fully resolved on 300 Å polymeric columns. Similarly, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene are unresolved on the narrow pore polymeric columns, but are resolved on the wide pore polymeric columns. For both monomeric and polymeric C₁₈ phases, these trends (or lack of trends) are reflected in the retention behavior of the column selectivity test mixture.

Phase length. Changes in column selectivity are also observed for phases with different alkyl chain lengths⁷. In general, shape selectivity increases with increasing alkyl chain length. The elution order for the selectivity test mixture is as expected for the monomeric and polymeric C₁₈ phases. Benzo[a]pyrene elutes earlier than normal on phases shorter than C₁₈, and longer than normal on phases longer than C₁₈. This trend is summarized in Figure 7 as a plot of selectivity ($\alpha_{\text{TBN/BaP}}$) vs phase length. It is interesting to note that phase selectivity of monomeric and polymeric phases merge at short and long alkyl chain lengths. Monomeric and polymeric C₈ phases have similar selectivity toward PAH, as do monomeric and polymeric C₃₀ phases. Again, the selectivity factor $\alpha_{\text{TBN/BaP}}$ is indicative of overall phase selectivity for more complex PAH mixtures⁷.

Phase coverage. Among polymeric C₁₈ phases, selectivity has been shown to vary with the density or coverage of the polymeric layer³. A similar but more subtle trend has been demonstrated for monomeric C₁₈ phases⁸. Shape selectivity for PAH isomers increases with increasing phase density. This trend is illustrated for a series of PAH isomers (MW 278) in Figures 8 and 9. Baseline resolution of the isomers was achieved on a heavily loaded polymeric phase, but only partial resolution was possible on lower loaded polymeric phases. Very little resolution of the isomers was achieved on monomeric C₁₈ columns.

Temperature. Temperature can have a significant effect on phase selectivity¹. PAH shape recognition increases dramatically with decreasing temperature. Conversely, shape recognition decreases with increasing temperature. These trends are reflected in the retention behavior of the selectivity test mixture as illustrated in Figure 10. The relative retention of BaP increases with decreasing temperature, indicating an increase in shape selectivity. This behavior has been verified for several complex PAH mixtures, including SRM 1647a and the six methylchrysene

isomers¹. Although Figure 10 illustrates temperature effects for a polymeric C₁₈ column, the change in selectivity with temperature is a general phenomenon that is not dependent on phase type or column manufacturer. The dependence of selectivity on temperature for a typical monomerically bonded and polymerically bonded C₁₈ column is summarized in a plot of $\alpha_{\text{TBN/BaP}}$ vs. temperature in Figure 11. Changes in selectivity are relatively uniform for the polymeric C₁₈ phase. In contrast, changes in selectivity for the monomeric C₁₈ phase are most significant at subambient temperatures; selectivity is nearly constant above 25 °C. Two possibilities are suggested by this plot. It should be possible to duplicate "polymeric phase selectivity" with a monomeric column, by performing the separation at reduced temperature. This possibility has been demonstrated for the separation of SRM 1647a¹. Furthermore, it should be possible to achieve column selectivity characteristics not possible with available columns, i.e., by cooling polymeric C₁₈ columns. This possibility has been demonstrated for a separation of methylchrysene isomers which were previously not separable by liquid chromatography¹. Thus, column selectivity may be adjusted as required by altering column temperature. Although the temperature dependence of $\alpha_{\text{TBN/BaP}}$ will vary for individual columns, the plots in Figure 11 provide a basis for tentative predictions. For example, suppose a polymeric C₁₈ column has an $\alpha_{\text{TBN/BaP}} = 0.65$ at 25 °C, but an $\alpha_{\text{TBN/BaP}}$ value of about 0.35 is needed for the separation. By cooling the column to about 5 °C, the desired selectivity should be obtained. Similarly, a monomeric C₁₈ column with an $\alpha_{\text{TBN/BaP}} = 1.7$ at 25 °C will have an $\alpha_{\text{TBN/BaP}}$ value of about 0.8 at 0 °C (See Figure 11).

References

1. Sander, L.C. and Wise, S.A., Subambient Temperature Modification of Selectivity in Reversed-Phase Liquid Chromatography, *Anal. Chem.*, **61** (1989) 1749-1754.
2. Sander, L.C. and Wise, S.A., Synthesis and Characterization of Polymeric C₁₈ Stationary Phases For Liquid Chromatography, *Anal. Chem.*, **56** (1984) 504-510.
3. Wise, S.A. and Sander, L.C., Factors Affecting the Reversed-Phase Liquid Chromatographic Separation of Polycyclic Aromatic Hydrocarbon Isomers, *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.*, **8** (1985) 248-255.
4. Sander, L.C. and Wise, S.A., Determination of Column Selectivity Toward Polycyclic Aromatic Hydrocarbons, *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.*, **11** (1988) 383-387.
5. Sander, L.C. and Wise, S.A., Investigations of Selectivity in Reversed-Phase Liquid Chromatography of Polycyclic Aromatic Hydrocarbons. In: *Advances in Chromatography*, edited by Giddings, J.C., Grushka, E., Cazes, J. and Brown, P.R. New York: Marcel Dekker, vol 25, 1986, p. 139-218.
6. Sander, L.C. and Wise, S.A., Influence of Substrate Parameters on Column Selectivity With Alkyl Bonded Phase Sorbents, *J. Chromatogr.*, **316** (1984) 163-181.
7. Sander, L.C. and Wise, S.A., Effect of Phase Length On Column Selectivity for the Separation of Polycyclic Aromatic Hydrocarbons by Reversed-Phase Liquid Chromatography, *Anal. Chem.*, **59** (1987) 2309-2313.
8. Sentell, K.B. and Dorsey, J.G., Retention Mechanisms in Reversed-Phase Liquid Chromatography. Stationary-Phase Bonding Density and Partitioning, *Anal. Chem.*, **61** (1989) 930-934.
9. Wise, S.A., Bonnett, W.J., Guenther, F.R., and May, W.E., A Relationship Between Reversed Phase C₁₈ Liquid Chromatographic Retention and the Shape of Polycyclic Aromatic Hydrocarbons, *J. Chromatogr. Sci.*, **19** (1981) 457-465.

Appendix

The following supplementary information is supplied for the convenience of the user of this material. This information does not meet the requirements for certification by the National Institute of Standards and Technology.

Descriptive Information

This information is provided as an aid for peak identification. The values listed are not intended for quantitative comparisons.

Table 1. Gravimetric concentrations and relative responses at 254 nm.

Compound	Conc. ($\mu\text{g/g}$)	Relative Response ^a				
		A	B	C	D	E
BaP	2.1	1 [1]	1 [1]	1 [1]	1 [1]	1 [1]
PhPh	3.2	1.46 [1.65]	1 [1]	1.91 [1.46]	0.52 [0.34]	3.00 [1.68]
TBN	11.2	2.30 [4.01]	1.08 [1.42]	3.09 [3.66]	1 [1]	6.11 [4.05]

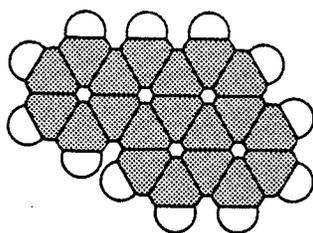
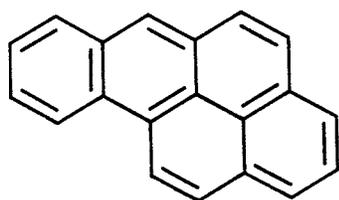
^aThese measurements refer to the separations in Figures 2A-2E. Values represent peak heights and peak areas (areas are shown in brackets) relative to BaP, and are listed only as an aid for peak identification.

Table 2. Selectivity coefficient ranges ($\alpha_{\text{TBN/BaP}}$) for various commercial C₁₈ columns.

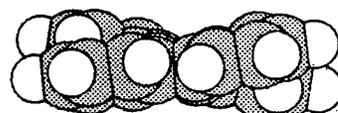
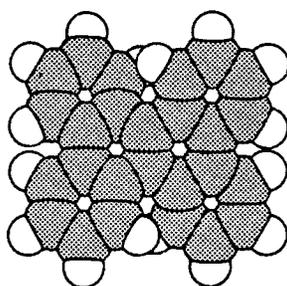
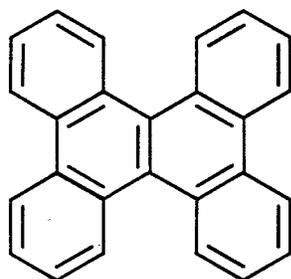
Column	$\alpha_{\text{TBN/BaP}}$
Bakerbond C18 Wide-Pore Chromspher PAH Bio Rad RP-318 Supelcosil LC-PAH Vydac 201TP Spherisorb PAH Erbasil C18 H	0.5 -- 0.9
ES Industries BF-C18 LiChrospher 100 RP-18 Bakerbond C18 Erbasil C18 M LiChrospher 60 RP-select B Partisil 5 ODS-2 Partisil 5 ODS Spherisorb ODS-1 Brownlee ODS 5A Sepralyte C18 Spherisorb ODS-2	1.0 -- 1.7
Erbasil C18 L Pecospher 5 Cr C18 Partisphere C18 Zorbax ODS Serva C18 Partisil 5 ODS-3 Hypersil ODS (HP) Microsorb C18 J&W Accuphase ODS-2 Novapak C18 Ultrasphere ODS Capcell C18 SG 120 Å Supelcosil LC-18 IBM ODS Brownlee Spheri S RP-18 ODS Hypersil Cosmosil C18-P J&W Accuphase ODS YMC 120 Å "A" Adsorbosphere C18 HS Supelcosil LC-18-DB	1.7 -- 2.2

Comments:

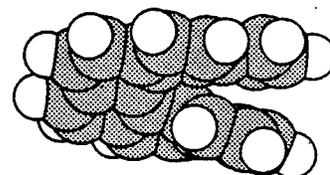
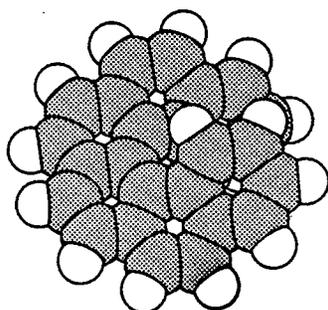
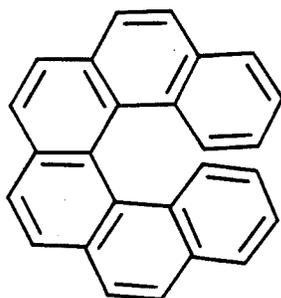
Selectivity coefficients were determined using 85% acetonitrile in water mobile phase at 2 mL/min, at ambient temperature (25 ± 2 °C). Columns are listed in order of increasing $\alpha_{\text{TBN/BaP}}$ values; however, because these values may vary with different column lots, individual $\alpha_{\text{TBN/BaP}}$ values are not listed. A tabulation of these selectivity factors will be provided upon request. The ranges shown above should not be construed to reflect lot-to-lot variability.



BENZO[a]PYRENE



TETRABENZONAPHTHALENE



PHENANTHRO[3,4-c]PHENANTHRENE

Figure 1. Structures and space filling models for compounds in the SRM 869.

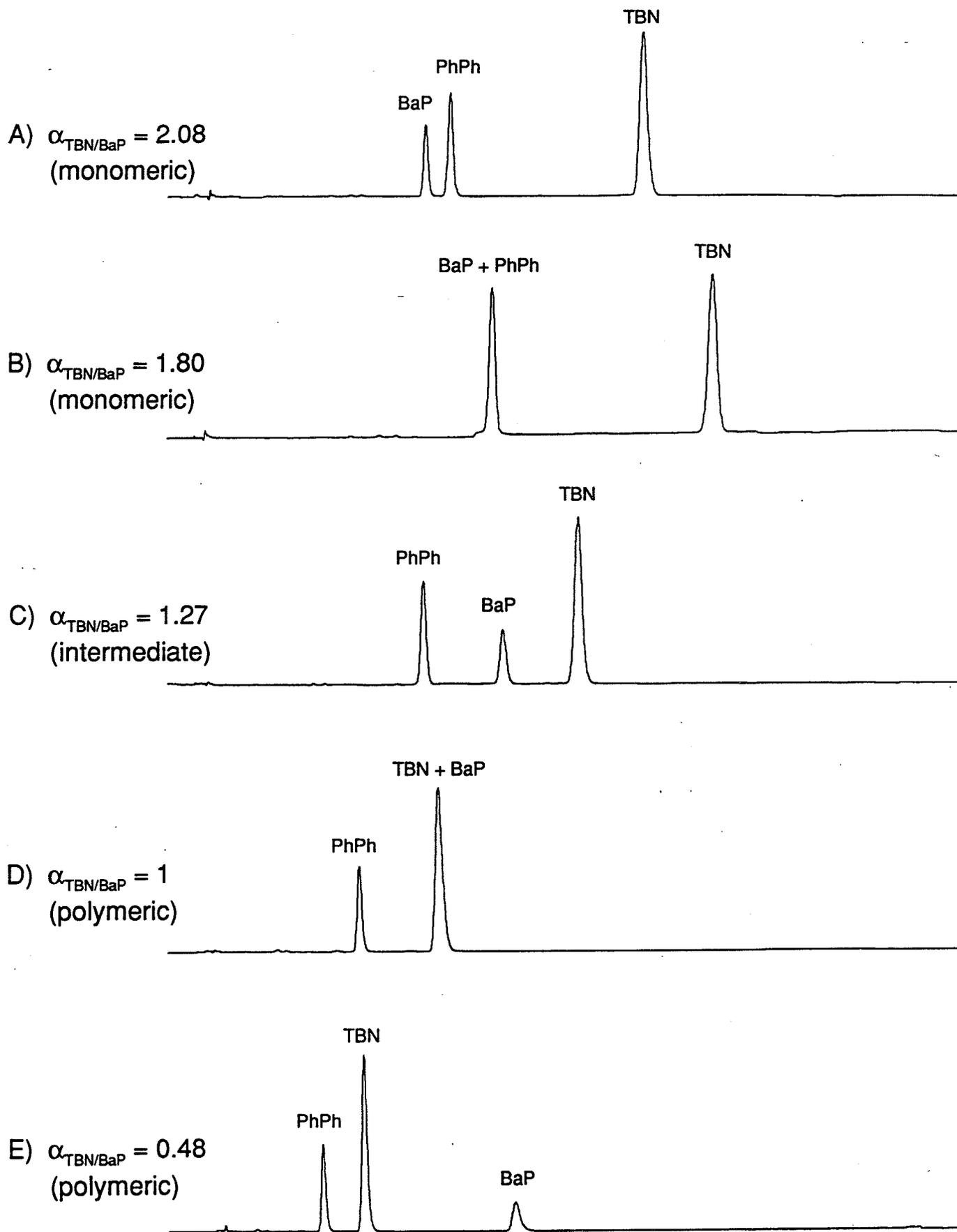


Figure 2. Separation of SRM 869 on dissimilar LC columns. Monomeric and polymeric designations refer to phases prepared with monomeric or polymeric surface modification chemistry; intermediate designation refers to a phase (possibly monomeric or polymeric) with intermediate properties. These chromatograms are provided to aid peak identification.

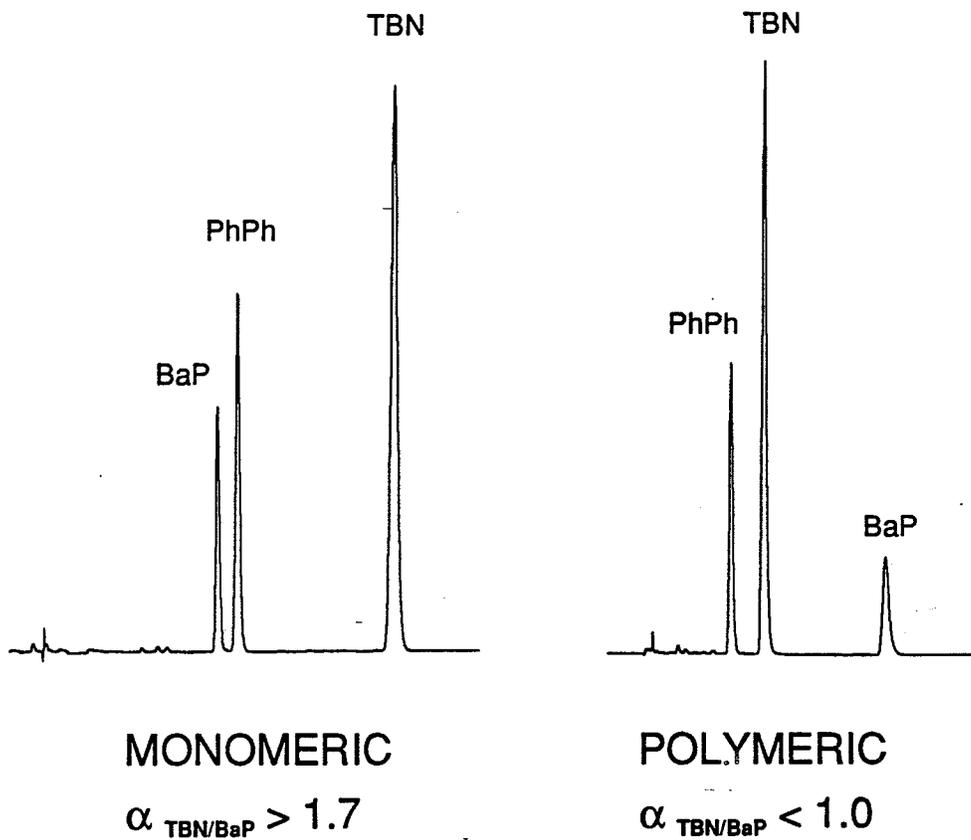


Figure 3. Separation of SRM 869 on C₁₈ columns prepared with monomeric and polymeric surface modification chemistry.

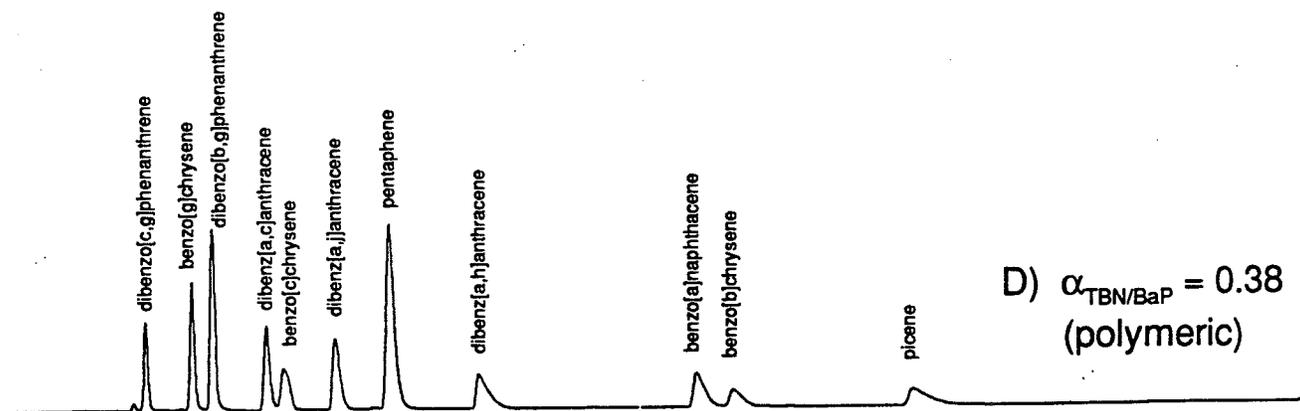
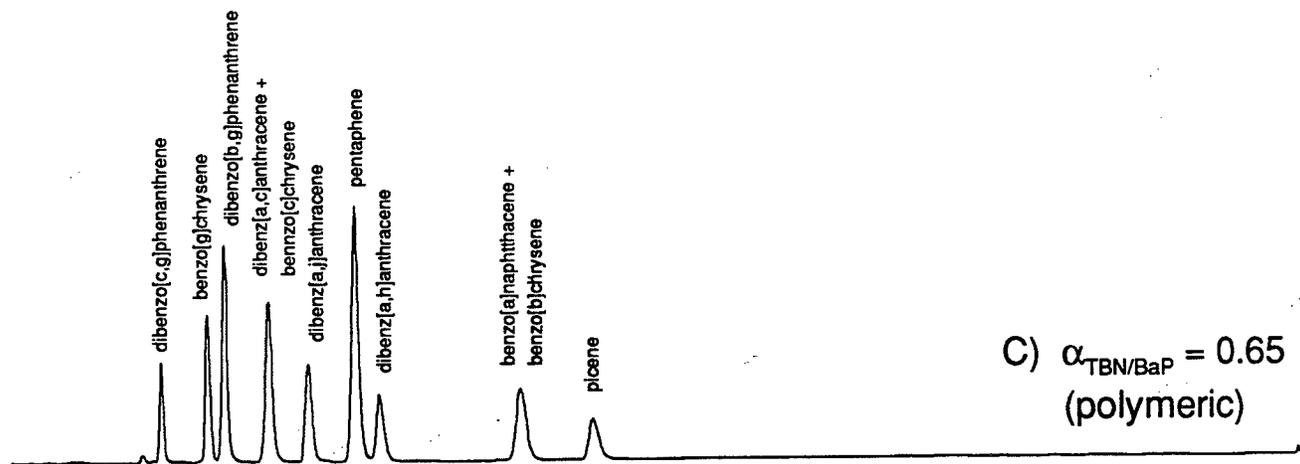
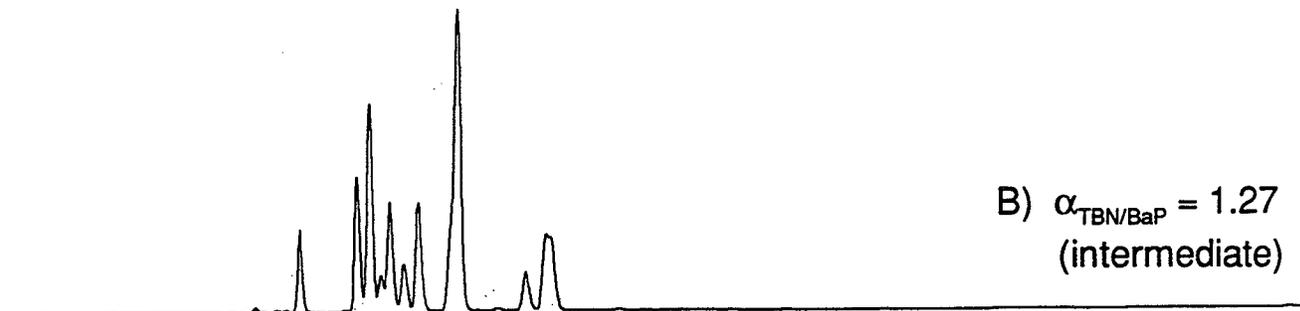
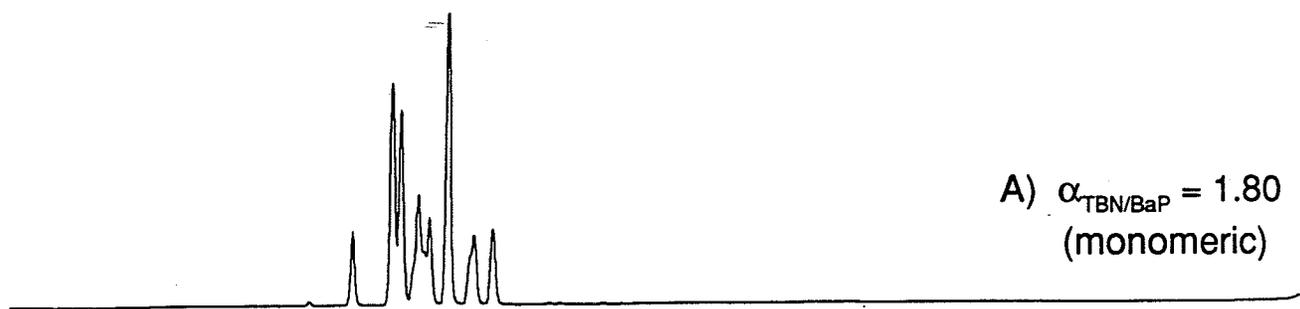


Figure 4. Separation of 11 PAH isomers of molecular weight 278 on C₁₈ columns prepared using monomeric ($\alpha_{\text{TBN/BaP}} = 1.80$) and polymeric surface modification chemistry ($\alpha_{\text{TBN/BaP}} = 0.38$).

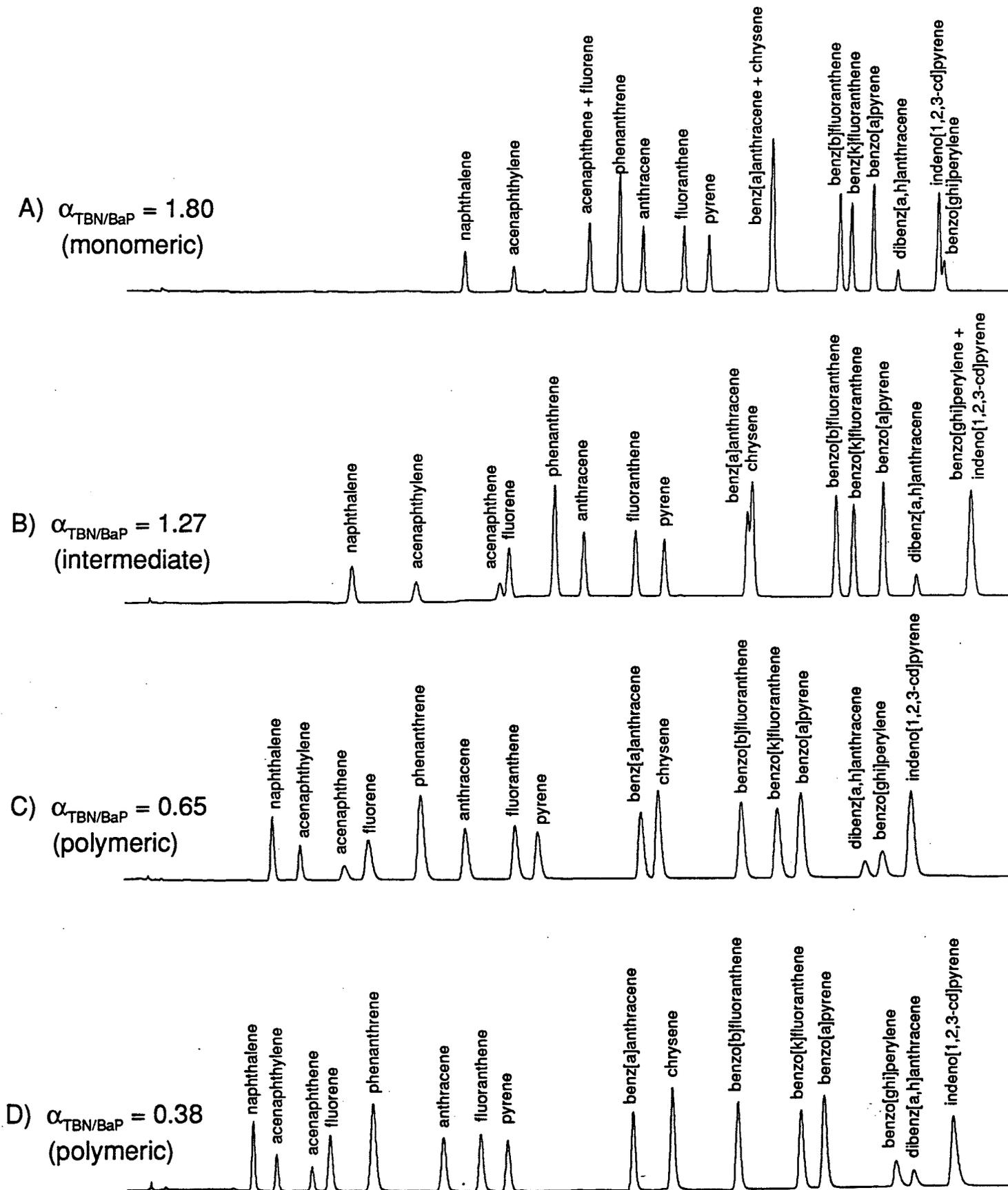


Figure 5. Separation of SRM 1647a on the same C₁₈ columns as in Figure 4. Complete separation of all 16 components was only possible with the polymeric C₁₈ columns prepared using polymeric surface modification chemistry ($\alpha_{\text{TBN/BaP}} = 0.65$ and 0.38).

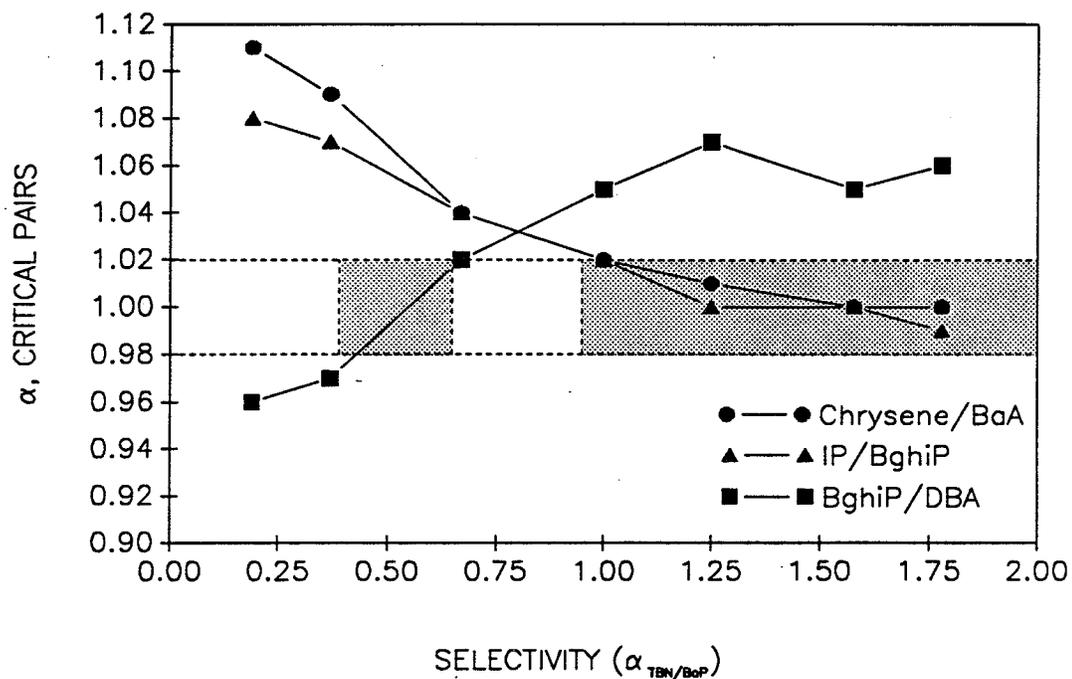


Figure 6. Plot of selectivity factors for selected critical pairs in SRM 1647a (α_{cp}) vs. column selectivity ($\alpha_{TBN/BaP}$). The mixture was separated using a linear gradient, 40-100% acetonitrile in water, over 30 minutes. Complete resolution of the critical pairs was possible for $\alpha_{cp} > \sim 1.02$ (or $\alpha_{cp} < \sim 0.98$). Shaded regions indicate coelution of components.

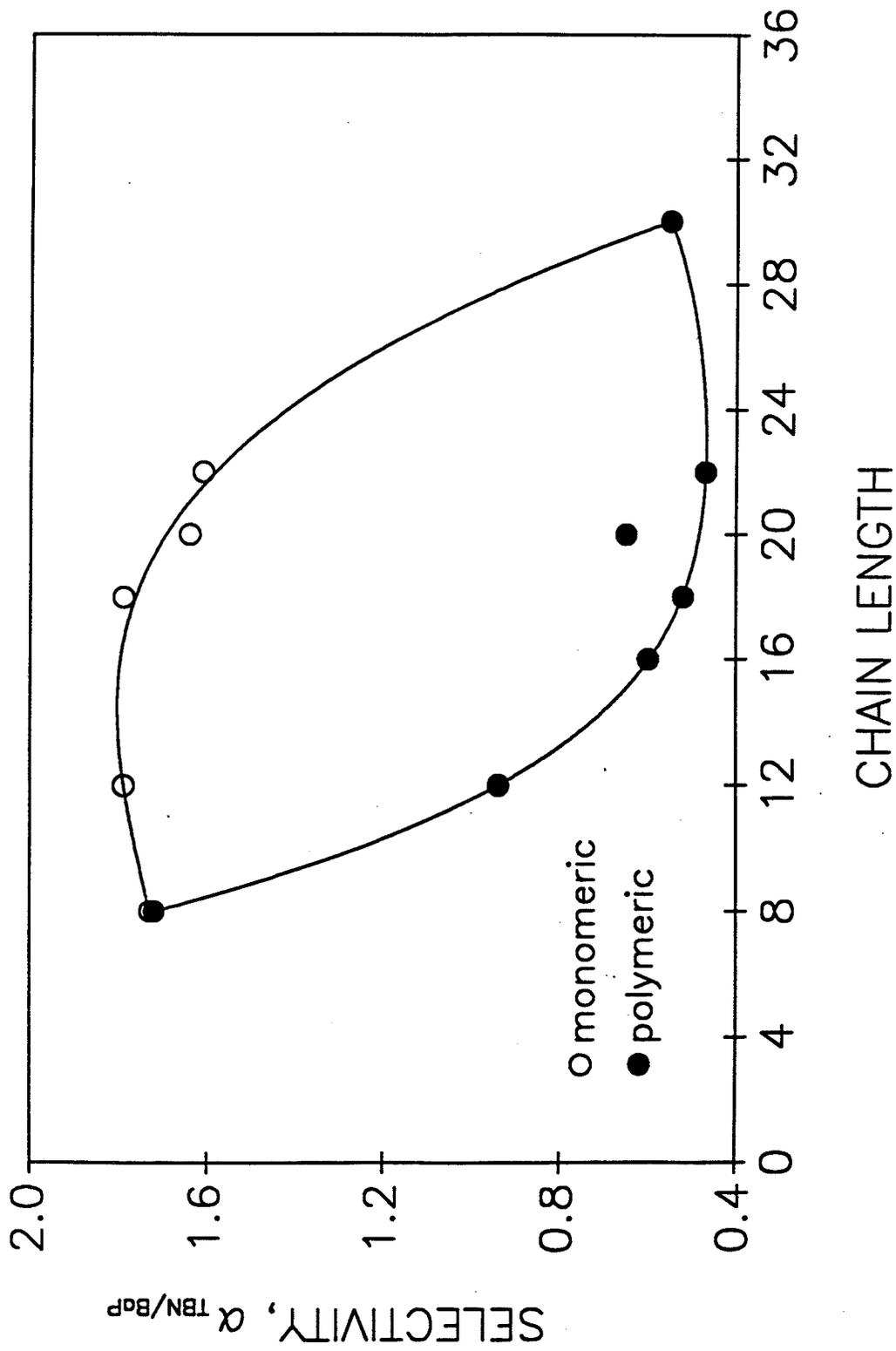


Figure 7. Plot of column selectivity ($\alpha_{TBN/BaP}$) vs alkyl chain length for alkyl phases prepared using monomeric and polymeric surface modification chemistry.

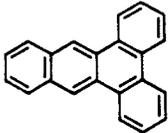
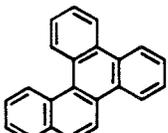
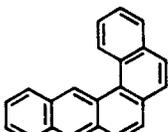
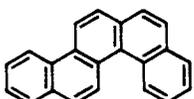
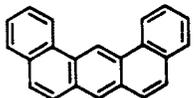
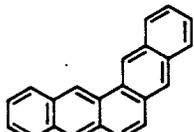
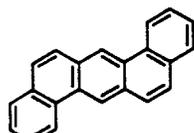
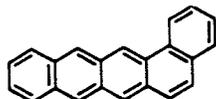
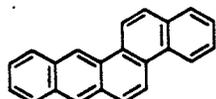
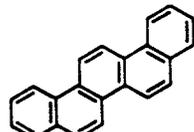
			L/B
1)		Dibenzo[c,g]phenanthrene	1.12
2)		Dibenz[a,c]anthracene	1.24
3)		Benzo[g]chrysene	1.32
4)		Dibenzo[b,g]phenanthrene	1.33
5)		Benzo[c]chrysene	1.47
6)		Dibenz[a,j]anthracene	1.47
7)		Pentaphene	1.73
8)		Dibenz[a,h]anthracene	1.79
9)		Benzo[a]naphthacene	1.77
10)		Benzo[b]chrysene	1.84
11)		Picene	1.99

Figure 8. Structures and associated length-to-breadth ratios⁹ for PAH isomers of molecular weight 278.

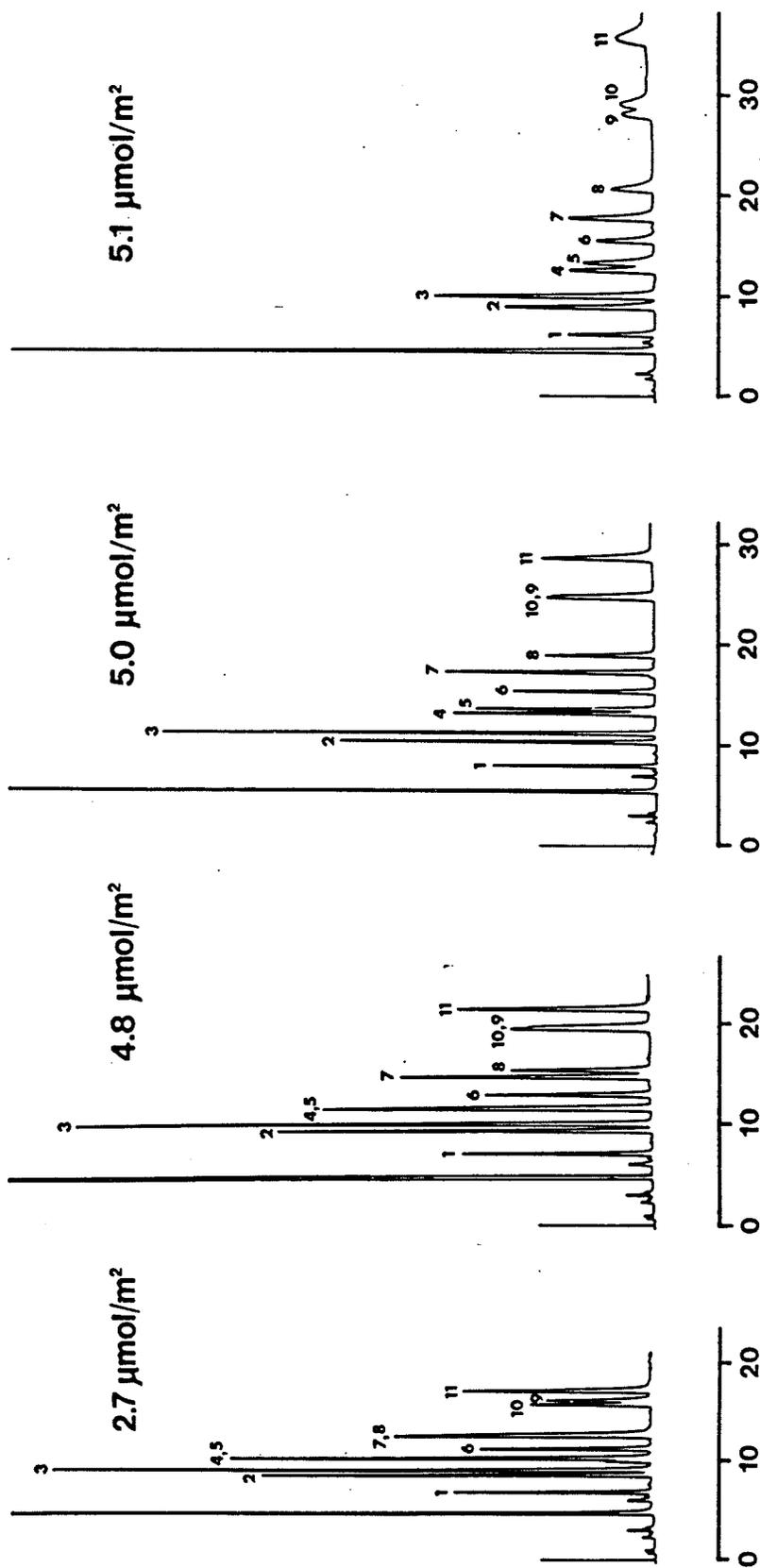


Figure 9. Separation of molecular weight 278 isomers on polymeric C18 columns with different phase loadings. Loadings are indicated in terms of surface coverage values, $\mu\text{mol}/\text{m}^2$.

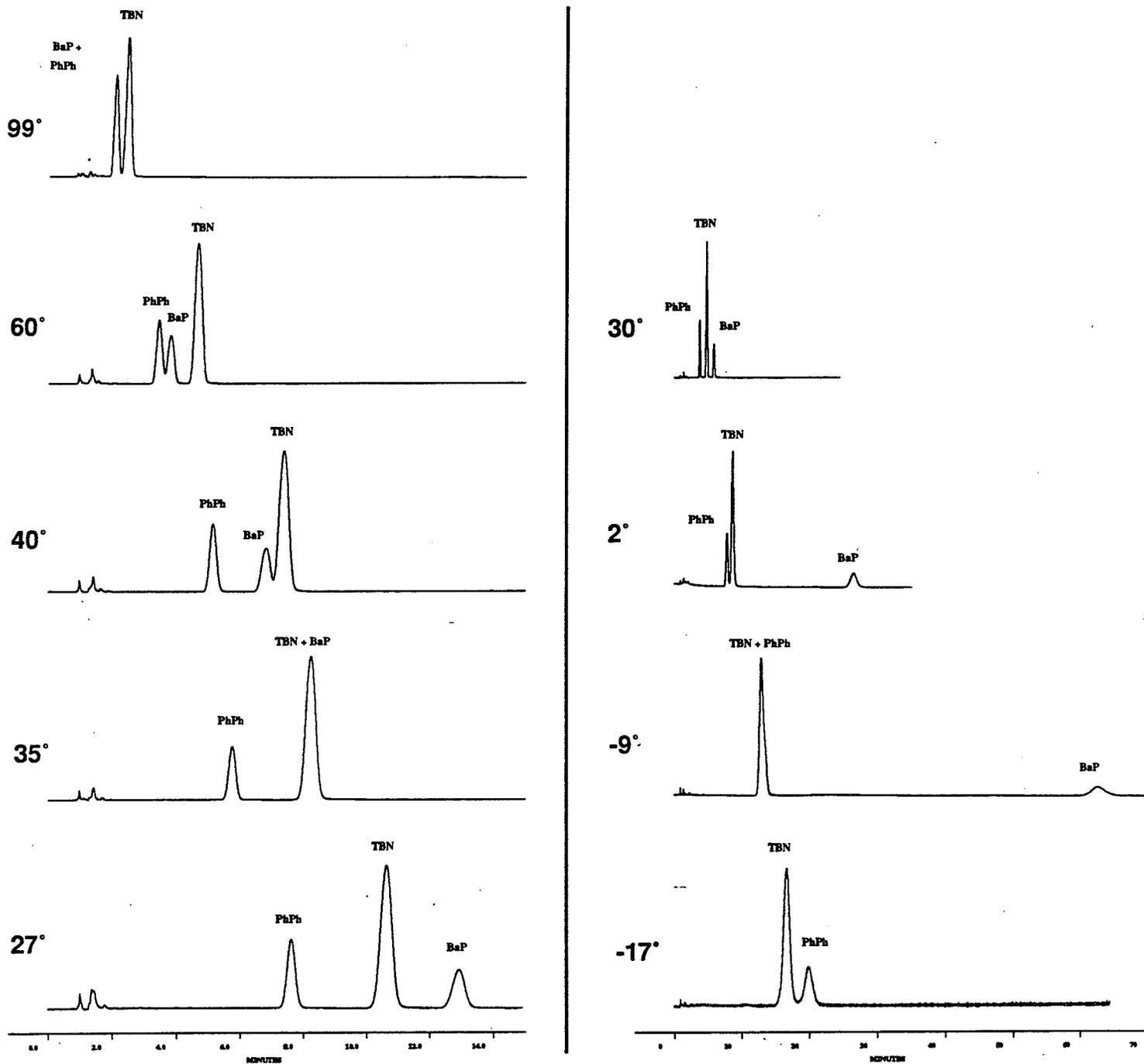


Figure 10. Separation of SRM 869 on a polymeric C₁₈ column at different temperatures. Subambient separations were carried out using 85% acetonitrile/water; separations at elevated temperatures were carried out using 75% acetonitrile.

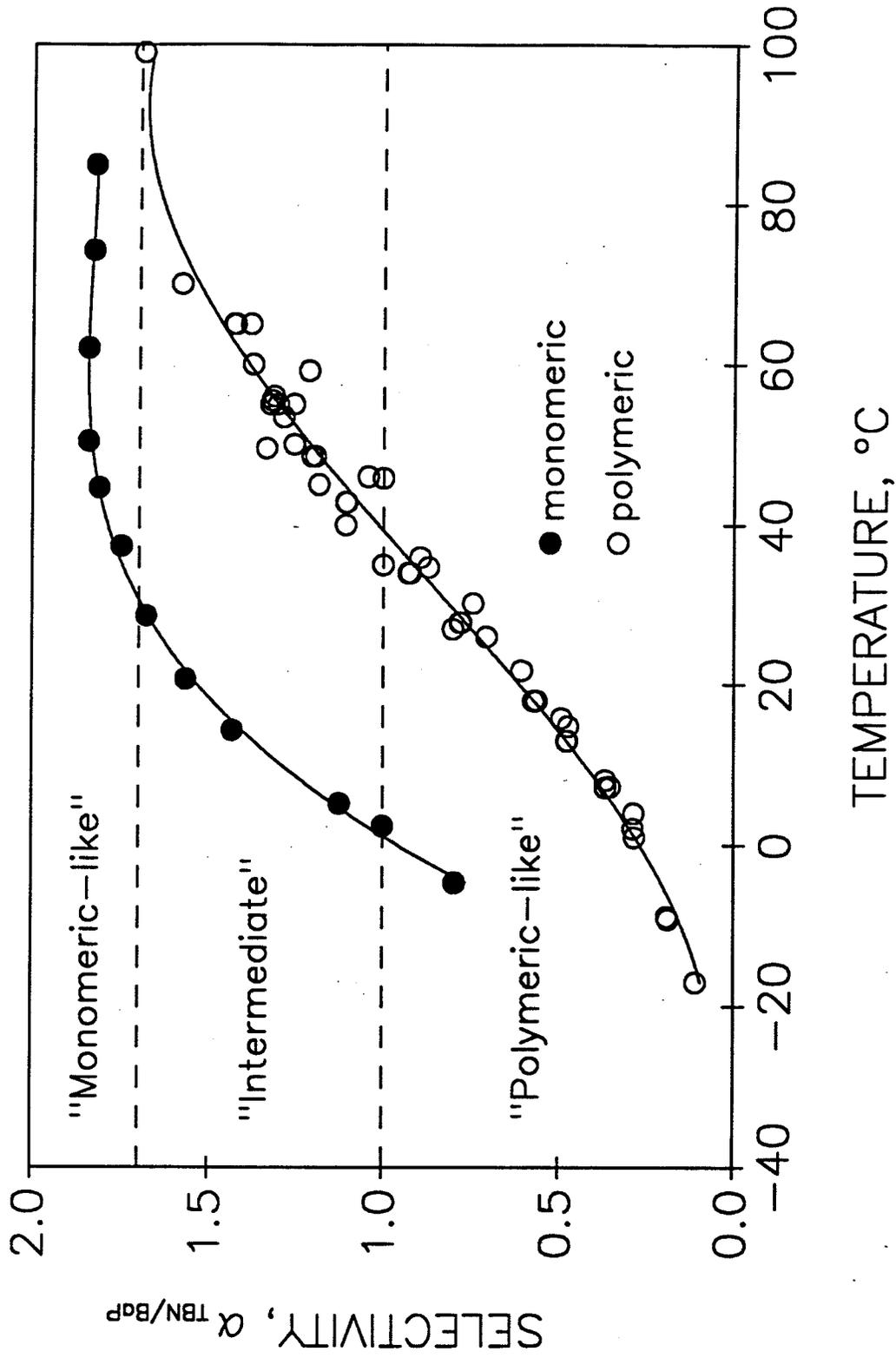


Figure 11. Phase selectivity ($\alpha_{TBN/BaP}$) plotted as a function of temperature for monomeric and polymeric C18 columns: Mobile phase, Zorbax C18, 85:15 acetonitrile/water; Vydac 201TP C18, various acetonitrile/water compositions. "Monomeric-like" and "Polymeric-like" designations refer to selectivity ranges ($\alpha_{TBN/BaP}$) typical for phases prepared by monomeric or polymeric C18 surface modification procedures; "Intermediate" refers to intermediate phase selectivity.