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Retention behavior of alkyl-substituted polycyclic aromatic sulfur heterocycles in reversed-phase liquid chromatography

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Abstract

Retention indices for 79 alkyl-substituted polycyclic aromatic sulfur heterocycles (PASHs) were determined by using reversed-phase liquid chromatography (LC) on a monomeric and polymeric octadecylsilane (C₁₈) stationary phase. Molecular shape parameters [length, breadth, thickness (*T*), and length-to-breadth ratio (*L/B*)] were calculated for all the compounds studied. Based on separations of isomeric methylated polycyclic aromatic hydrocarbons on polymeric C₁₈ phases, alkyl-substituted PASHs are expected to elute based on increasing *L/B* ratios. However, the correlation coefficients had a wide range of values from *r* = 0.43 to *r* = 0.93. Several structural features besides *L/B* ratios were identified to play an important role in the separation mechanism of PASHs on polymeric C₁₈ phases. First, the location of the sulfur atom in a bay-like-region results in alkylated-PASHs being more retentive than non-bay-like-region alkylated-PASHs, and they elute later than expected based on *L/B* value. Second, the placement of the alkyl group in the *k* region of the structure resulted in a later elution than predicted by *L/B*. Third, highly nonplanar methyl-PASHs (i.e., 1-Me and 11-MeBbN12T) elute prior to the parent PASH (BbN12T).

Keywords

Retention indices; Reversed-phase liquid chromatography stationary phases; Molecular descriptors; Retention behavior; Polycyclic aromatic compounds; Alkyl-substituted polycyclic aromatic sulfur heterocycles

1. Introduction

Reversed-phase liquid chromatography (LC) on octadecyl (C₁₈) stationary phases has been shown to provide excellent separations of polycyclic aromatic hydrocarbons (PAHs) [1–6] and methyl-PAHs (MePAHs) [1, 2, 7]. Previous studies have demonstrated that not all C₁₈ stationary phases provide the same selectivity for MePAHs [1, 7]. Wise *et al.* showed the

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excellent selectivity of polymeric C₁₈ phases for separation of methyl-substituted phenanthrene (MePhe), pyrene (MePyr), fluoranthene (MeFlu), chrysene (MeChry), benzo[*a*]anthracene (MeBaA), benzo[*c*]phenanthrene (MeBcPhe) and benzo[*a*]pyrene (MeBaP) [1]. The type of the C₁₈ phase, i.e., monomeric or polymeric, played an important role in the separations of the MePAHs. Typically, monomeric C₁₈ phases are prepared by reaction of monofunctional silanes [7, 8] and polymeric C₁₈ phases are prepared using trifunctional silanes in the presence of water which results in cross-linking to form silane polymers on the silica surface [8]. In general, the MePAH isomers were better resolved on the polymeric C₁₈ phases than the monomeric C₁₈ phases, and the elution order of MePAH isomers generally followed the increase of a molecular shape parameter denoted as length-to-breadth (*L/B*) ratio.

In a later study, Wise *et al.* investigated the LC separations of MeChry, methylperylene (MePer), and methylpicene (MePic) on 16 commercially available C₁₈ columns [7]. Each of the columns were characterized using a standard reference material developed at the National Institute of Standard and Technology for testing the selectivity of LC columns (SRM 869b). Selectivity values for tetrabenzonaphthalene (TBN) and BaP ($\alpha_{TBN/BaP}$) were used to classify the type of C₁₈ phases for PAH selectivity. Generally, $\alpha_{TBN/BaP} > 1$ represent polymeric C₁₈ phases; $\alpha_{TBN/BaP}$ in the range of 1.0 – 1.7 represent intermediate polymeric C₁₈ phases; and values for $\alpha_{TBN/BaP} < 1.7$ represent monomeric C₁₈ phases. For the separation of MePAHs, Wise *et al.* [7] observed that isomers with some nonplanarity and small *L/B* values would elute prior to the parent PAH as the polymeric nature increases ($\alpha_{TBN/BaP}$ decreases). The nonplanarity of these PAHs, which has since been characterized using a molecular shape parameter denoted as the thickness (*T*), is due to the presence of the methyl group in the *bay*-region of the PAH structure. Several publications have summarized the correlation between LC retention of PAHs and MePAHs on polymeric C₁₈ phases with molecular shape parameters (*L/B* and *T*) [2, 4]. While the focus of this study was to evaluate only molecular shape parameters, quantitative structure-retention relationship (QSRR) technique has been shown to be useful for predicting the GC retention behavior of saturated S-heterocyclic compounds on standard nonpolar polydimethylsiloxane siloxane stationary phases [9].

In the current study, we investigate the LC separation of alkyl-substituted polycyclic aromatic sulfur heterocycles (PASHs), (see Fig. 1) on both monomeric and polymeric C₁₈ stationary phases. In past decades, PASHs have received far less attention than PAHs despite their carcinogenic and mutagenic potential [9, 10] and their presence in air particulate matter [11, 12], sediments [13–15], coal liquids [16], diesel [15], heavy oil [17–21], crude oil [15, 18, 22–31], shale oil [16, 22, 32, 33], coal tar [22, 23, 29, 34–36], mussels [13, 14,], and fish [13, 14,]. In a previous study [37], we reported LC retention indices for 70 PASHs on both monomeric and polymeric C₁₈ phases. In this study, LC retention indices were determined for 79 alkyl-substituted PASHs, and the correlation of retention on the polymeric C₁₈ phase and PASH geometry (*L/B* and *T*) was investigated. Only two previous publications have reported the reversed-phase LC retention behavior of isomeric PASHs [2, 38], and there are no previous reports of LC retention index data for alkyl-substituted PASH.

2. Material and methods

2.1. Chemicals

The following PAHs and PASHs were purchased from commercial sources with high purity (>95%): dibenzothiophene (DBT) (Acros Organics, Springfield, NJ); 1-, 2-, and 4-MeDBT, 2,8- DiMeDBT, 1,4,7-, 3,4,7-, 2,3,7-, 2,3,8-, 2,4,7-, 2,4,8-, 2,4,6,- and 1,3,7-TriMeDBT (Astec, Munster, Germany); 2-EtDBT, 3-EtDBT, and 4-EtDBT (Chiron AS, Trondheim, Norway); and BbN12T, BbN21T, BbN23T, benzo[*a*]anthracene (BaA), benzo[*b*]chrysene (BbC), and dibenzo[*a,h*]pyrene (DBahP) (BCR, Brussels, Belgium); naphthalene (Nap) and phenanthrene (Phe) (Fluka, Buchs, Switzerland). The remaining alkyl-substituted PASHs were synthesized in the laboratories of M.L.L. at Brigham Young University (Provo, UT). Standard Reference Material (SRM) 869b, "Column Selectivity Test Mixture for Liquid Chromatography" was obtained from the Office of Standard Reference Materials at the National Institute of Standards and Technology (Gaithersburg, MD, USA). HPLC grade acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Molecular Descriptor Calculations

The molecular modeling programs and procedure for calculating the molecular shape parameters have been described in detail previously [2, 39]. Briefly, ChemDraw 3D software (PerkinElmer, Waltham, MA, USA) was used to draw the molecular structures of PASHs and converted into the mol file formats. Commercial molecular modeling programs (PC-Model and MMX, Serena Software, Bloomington, IN, USA) and algorithms were used for calculations of the molecular descriptors [length (*L*), breadth (*B*), thickness (*T*) and length-to-breadth ratio (*L/B*)].

2.3. Liquid chromatographic retention data

LC retention index values (*log I*) were calculated according to equation 1 with the following index markers: (2) Nap, (3) Phe, (4) BaA, (5) BbC, and (6) DBahP [3].

$$\log I = \frac{\log R_x - \log R_n}{\log R_{n+1} - \log R_n} \quad (1)$$

R is the corrected retention volume, *x* represents the solute, and *n* and *n + 1* represent the lower and higher eluting PAH standards. Previous studies have investigated the retention behavior of PAHs on C₁₈ stationary phase using *log I* values as their basis for retention indices [2, 38]. The *log I* values are based on three measurements obtained from reference standards. The precision (standard deviation) of the *log I* values was equal to or less than ± 0.02 *log I* units. Baseline resolution of two components is achieved with a difference of ~ 0.06 *log I* units on both the monomeric and polymeric C₁₈ phase.

2.4. Instrumentation and chromatographic conditions

LC retention measurements were recorded using the same instrumentation described in a companion publication on LC separation of parent PASHs [37]. Separations were performed

on monomeric (Agilent 5) and polymeric (Zorbax Eclipse PAH) C₁₈ columns purchased from Agilent (Avondale, PA) with the following characteristics: 25 cm length, 4.6 mm diameter, and 5 μm average particle diameters. Table 1 lists the optimal separation and detection conditions for each isomer set. PASHs were detected using selected wavelengths that correspond to a compromise among the maximum absorbance wavelengths obtained from UV-vis spectroscopic analysis of pure standards. Except for MeTeP112T isomers, LC retention index data were obtained under isocratic conditions with 85/15 (v/v) acetonitrile-water as the mobile phase with a flow-rate of 1.0 mL/min. LC retention index data were obtained for the MeTeP112T using the conditions listed in Table 1.

3. Results and Discussion

Previous studies have shown that the retention behavior of methyl-substituted PAH isomers to be significantly different on monomeric and polymeric C₁₈ phases [1, 2, 7]. In the present study, two commercially available C₁₈ columns were selected and used to investigate the selectivity of 10 isomeric sets of alkyl-substituted PASHs. Previous studies from Sander and Wise [7, 42] have demonstrated the use of $\alpha_{TBN/BaP}$ values to classify the C₁₈ phases for PAH separations. The $\alpha_{TBN/BaP}$ values for the monomeric and polymeric C₁₈ phases used in this study are 1.97 and 0.49, respectively.

The molecular structures of the seven parent PASHs investigated in this study are shown in Fig. 1 with the numbering of the positions available for substitution. The molecular shape parameters (L , B , T , and L/B ratio) and retention indices (monomeric and polymeric C₁₈) for the alkyl-substituted PASHs are summarized in Tables 2 and 3. Table 4 summarizes the regression calculations for the correlation of LC retention on the polymeric C₁₈ phase with L/B ratio. The polymeric C₁₈ phase was the only phase to demonstrate a good correlation and will be discussed in detail. Previous studies have used correlation coefficients (r) as a parameter for measuring the linear correlations for the retention of MePAHs on polymeric C₁₈ phases and L/B [2,7]. The correlation coefficient demonstrates a more significant linear trend when close to 1. Instances where the correlation coefficient is not close to 1, the calculation of a t -test value is determinations using equation 2 for accessing if the correlation coefficient represents a significant linear trend [41]:

$$t_{exp} = \frac{|r| \sqrt{n-2}}{\sqrt{1-r^2}} \quad (2)$$

where n is the number of data points. The calculated t_{exp} value is compared to the t_{crit} value at the desired significance level based on the degrees of freedom ($n - 2$). A significant correlation does exist for the correlation coefficient if the t_{exp} is greater than the t_{crit} . These concepts will be applied in the following sections for discussing the correlations between the MePASHs retention on the polymeric C₁₈ phase and L/B values. In cases where the correlation coefficients are found to not demonstrate a significant trend, data points are removed from the correlation plots to help investigate possible explanations.

3.1. MeDBT and EtDBT

Fig. 2 shows the LC separation of DBT with the four possible MeDBT isomers and with three of the four possible EtDBT isomers on both C_{18} phases. In the case of MeDBT, all four isomers were separated (only partial resolution of the 3-Me and 4-Me) on the polymeric C_{18} phase; however, on the monomeric phase the 3-MeDBT and 4-MeDBT co-eluted. From the $\log I$ values for the MeDBTs (Table 2), which were determined from individual standard measurements, 3-MeDBT elutes before the 4-MeDBT demonstrating that the elution order is the same on both C_{18} phases. A similar elution order was observed for the EtDBT isomers on both C_{18} phases. In comparison to the separation of MeDBTs, the three EtDBTs were baseline resolved on both C_{18} phases under faster separation conditions.

With the exception of 4-Et and 4-MeDBT, the retention behavior of the alkyl-substituted isomers follows the increasing L/B values. The 4th substitution position (see Fig. 1) is located on the carbon closest to the sulfur atom creating a bay-like-region (see Fig. S1) on the structure. In a companion study [37] the LC retention behavior of the parent PASHs is reported, and this study demonstrated that the three- and four-ring unsubstituted PASHs eluted later than expected based on L/B when the sulfur atom was located in a bay-region of the structure. These observations suggest that when the sulfur atom is located in a bay-like-region, the sulfur atom is protected from interacting with the stationary phase (C_{18}). The correlation of retention for the MeDBT and EtDBT isomers on the polymeric C_{18} phase vs. L/B (see Table 4, Fig. S2) resulted in correlation coefficients of $r = 0.43$ and $r = -0.67$, respectively. The negative correlation coefficient for the EtDBT isomer group is a direct reflection of the isomer with the lowest L/B value (4-EtDBT = 1.28) eluting last in the separation. The correlation coefficients for the MeDBT and EtDBT isomers do not provide a significant linear trend based on the t_{exp} values of 0.68 ($n = 4$, $\alpha = 0.05$, $t_{\text{crit}} = 4.30$) and 1.23 ($n = 3$, $\alpha = 0.05$, $t_{\text{crit}} = 12.71$), respectively [41].

3.2. DiMeDBT

The LC separations for 15 of the 16 possible DiMeDBT isomers on the polymeric C_{18} phase is shown in Fig. 3A. Retention indices for both C_{18} phases are reported in Table 2. Complete LC separation of the fifteen DiMeDBT isomers was not achieved on either C_{18} phase. A plot of retention on the polymeric C_{18} phase vs. L/B for the 15 DiMeDBT isomers is shown in Fig. 3B with a correlation coefficient of $r = 0.60$. The t_{exp} value for the correlation coefficient was 2.70 ($n = 15$, $\alpha = 0.05$, $t_{\text{crit}} = 2.13$) indicating that there is a significant linear trend between the retention on the polymeric C_{18} phase for DiMeDBT and L/B [41]. The correlation coefficient improves to $r = 0.89$ by removing the isomers containing one or two methyl groups located in substitution positions 4 or 6 next to the sulfur atom, which tend to elute later than predicted by L/B (see Section 3.1).

3.3. TriMeDBT

The LC separations of DBT with 8 of the 28 possible TriMeDBT isomers on both monomeric and polymeric C_{18} phases are shown in Fig. 4. The polymeric C_{18} phase provided the most comprehensive separation of the eight TriMeDBT isomers with the co-elution of 1,3,7- and 1,4,7-TriMeDBT. The monomeric C_{18} phase had significantly more co-elution of the isomers: i.e., 2,4,8-, 1,3,7-, and 3,4,7-TriMeDBT and 2,4,7- and 2,4,6-

TriMeDBT. The elution order for the TriMeDBT isomers is different on the two C₁₈ phases with the exception of 2,3,8-TriMeDBT, which elutes first on both columns. As shown in Fig. S3, there is a low correlation between the L/B and the retention characteristics of the TriMeDBT isomers on the polymeric C₁₈ phase with a correlation coefficient of $r = 0.43$. The t_{exp} value for the correlation coefficient was 1.17 ($n = 8$, $\alpha = 0.05$, $t_{\text{crit}} = 2.31$) indicating that there is not a significant linear trend between the retention on the polymeric C₁₈ phase for TriMeDBT and L/B [41]. The 20 missing isomers are needed to have a better understanding of the correlation between the retention of TriMeDBT and L/B .

3.4. MeN12T and MeN21T

Fig. 5 shows the LC separations of N12T with seven of eight possible MeN12T isomers (Fig. 5A) and N21T with all eight possible MeN21T isomers (Fig. 5B). The polymeric phase provided the most comprehensive separation for both isomer sets. In the case of MeN12T isomers, the separation conditions resulted in only two co-eluting isomers (4-Me and 6-Me). The LC analysis of the MeN21T isomers resulted in co-elution of two pairs of isomers: 9-Me and 5-MeN21T and 4-Me and 2-MeN21T.

The correlations of LC retention on the polymeric C₁₈ phase and L/B values for the MeN12T and MeN21T isomers is shown in Fig. S4A and S4B with correlation coefficients $r = 0.75$ and $r = 0.54$, respectively. The correlation coefficients for the MeN12T and MeN21T isomers do not provide a significant linear trend based on the t_{exp} values of 2.55 ($n = 7$, $\alpha = 0.05$, $t_{\text{crit}} = 2.36$) and 1.58 ($n = 8$, $\alpha = 0.05$, $t_{\text{crit}} = 2.31$), respectively [41]. The structures of N12T and N21T are identical except for the position of the sulfur atom in the heterocyclic ring. These similarities may account for the retention behavior of both isomer sets. In both cases, the isomers with the largest L/B values were 7-Me (1.54) and 2-MeN12T (1.54) isomers and 2-Me (1.43) and 7-MeN21T (1.52), and these two isomers elute last. However, in both cases the isomers with the smallest L/B values elute in the middle of the separation.

Phenanthrene (Phe) is the three-ring benzenoid PAH with a similar structure to both N12T and N21T. Previous work by Wise *et al.* evaluated the correlation between the retention of five MePhe isomers and their L/B values [2]. The correlation coefficient (r) and t_{exp} value was 0.88 and 3.24 ($n = 5$, $\alpha = 0.05$, $t_{\text{crit}} = 3.18$), respectively, indicating that there is a significant linear trend between the retention on the polymeric C₁₈ phase for MePhe and L/B [41]. The $\alpha_{\text{TBW/BAP}}$ value for the polymeric C₁₈ phase used during the MePhe isomer study was ~ 0.7 . With the exception of 9-MePhe, the retention of the MePhe isomers follows the L/B trend. The methyl group in the structure of 9-MePhe is the only isomer to be located in the k region of the Phe structure. Similar results were observed in the present study for 4-Me and 5-Me isomers of N12T and N21T, which are located in the k region (Fig. S1) of the structure. The isomers with the methyl group located in the bay-region for N12T (9-Me) and N21T (1-Me and 9-Me) elute later than predicted by L/B . 4-MePhe has the methyl group located in the bay-region of the Phe structure but the same retention behavior was not observed on a polymeric C₁₈ phase. This difference can be directly related to the nonplanarity of 4-MePhe ($T = 4.69$) in comparison to 9-MeN12T ($T = 4.20$), 1-MeN21T ($T = 4.23$), and 9-MeN21T ($T = 4.24$). In both isomer sets, the isomers with the methyl group closest to the sulfur atom (9-MeN12T and 4-MeN21T), simulating a *bay*-like-region, are

retained on the polymeric C_{18} phase longer than expected based on L/B . When 9-MeN12T and 4-MeN21T are removed from the plots in Fig. S4, the correlation coefficients improve to $r = 0.83$ and $r = 0.67$, respectively. The new correlation coefficient for the MeN12T does provide a significant linear trend based on the t_{exp} value of 2.96 ($n = 6$, $\alpha = 0.05$, $t_{crit} = 2.45$) [41]. However, the new correlation coefficient for the MeN21T does not provide a significant linear trend based on the t_{exp} value of 2.02 ($n = 7$, $\alpha = 0.05$, $t_{crit} = 2.36$) [41] because of the unexpected early elution of 8-MeN21T for no obvious reason.

3.5. MeBbN12T

The LC separation of BbN12T and 10 MeBbN12T isomers is shown in Fig. 6A. The polymeric C_{18} phase provided the most comprehensive separation of the MeBbN12T isomers with co-elution of only 2-Me and 10-MeBbN12T. The analysis of the 10 BbN12T isomers on the monomeric C_{18} phase only provided chromatographic separation of 3 MeBbN12T isomers (data not shown). Interestingly, 1-MeBbN12T and 11-MeBbN12T elute prior to BbN12T on the polymeric C_{18} phase. This retention behavior can be attributed to the nonplanarity of 1-Me and 11-MeBbN12T due to steric hindrance effects of the methyl substitution as indicated by the larger T values (1-Me and 11-Me; $T = 5.28$ and $T = 4.99$, respectively). The majority of the methyl-substituted PASH isomers have similar T values ($T = 4.20$, Table 2 and 3) indicating planar molecular structures. The behavior of methyl isomers eluting prior to the parent on polymeric C_{18} phases was reported for selected PAHs by Wise *et al.* [7].

Gas chromatography (GC) selectivity studies by Mössner *et al.* observed similar behavior for the MeBbN12T isomers on a liquid crystalline stationary phase, which also provides a separation based on the shape of the solute [36]. A plot of GC retention vs. L/B for the MeBbN12T isomers had a correlation coefficient of $r = 0.73$ [36]. The correlation coefficient improves to $r = 0.89$ when 1-Me and 11-MeBbN12T are removed from the plot.

Benzo[*c*]phenanthrene (BcPhe) is the fourring benzenoid PAH with a structure similar to BbN12T. As with the earlier discussion regarding Phe, Wise *et al.* evaluated the correlation between the LC retention of the six possible MeBcPhe isomers on a polymeric C_{18} phase and their L/B values [2]. In the case of MeBcPhe isomers, 1-MeBcPhe ($T = 5.94$) elutes closely to BcPhe ($T = 4.99$) on a polymeric C_{18} phase with a $\alpha_{TBN/BaP}$ value of ~ 0.7 .

Fig. 7A shows the plot of LC retention on the polymeric phase vs. L/B for the 10 MeBbN12T isomers with a correlation coefficient of $r = 0.50$. The t_{exp} value for this correlation coefficient was 1.63 ($n = 10$, $\alpha = 0.05$, $t_{crit} = 2.23$) indicating that there is not a significant linear trend between the retention on the polymeric C_{18} phase for TriMeDBT and L/B [41]. This low correlation coefficient can be partially attributed to the 1-Me and 11-MeBbN12T isomers eluting earlier than predicted by the L/B ratio. The correlation coefficient slightly improves to $r = 0.63$ and the t_{exp} value = 1.97 ($n = 8$, $\alpha = 0.05$, $t_{crit} = 2.31$) when 1-Me and 11-MeBbN12T are removed from the plot in Fig. 7A, indicating that other separation factors are contributing to deviations from the expected L/B trend. 6-Me and 8-MeBbN12T ($L/B = 1.22$ and 1.45 , respectively), with the methyl group located near the sulfur atom in the heterocyclic ring (bay-like-region), elute later than predicted by L/B . When these two isomers are also removed from the plot in Fig. 7A, correlation coefficient

improves to $r = 0.86$ and the t_{exp} value = 3.67 ($n = 6$, $\alpha = 0.05$, $t_{\text{crit}} = 2.45$) indicating a significant linear trend.

3.6. MeBbN21T

LC separation of BbN21T with 9 of 10 possible MeBbN21T isomers on the polymeric C_{18} phase is shown in Fig. 6B. The polymeric C_{18} phase provided baseline separation of the nine MeBbN21T isomers except for 7-Me and 10-MeBbN21T. The monomeric C_{18} phase only provided baseline resolution of one MeBbN21T isomer (5-MeBbN21T, data not shown). The correlation of LC retention on the polymeric phase and L/B value for the MeBbN21T isomers is shown in Fig. 7B with a correlation coefficient of $r = 0.93$, which represents the highest correlation among all of the isomer groups studied. The t_{exp} value for this correlation coefficient was 6.65 ($n = 9$, $\alpha = 0.05$, $t_{\text{crit}} = 2.36$) indicating that there is a significant linear trend between the retention on the polymeric C_{18} phase for MeBbN21T and L/B [41]. 1-MeBbN21T provides the most protection of the sulfur atom in the heterocyclic ring and 5-Me and 6-Me are located in the k region of the structure. Based on previous discussions, these structural isomers would have been expected to elute later in the chromatogram. Chrysene (Chry) is the four-ring benzenoid PAH with a structure similar to BbN21T. The separation of the six possible MeChry isomers on a polymeric phase ($\alpha_{\text{TCN/BaP}} \sim 0.7$) provided an excellent correlation between their retention and L/B ($r = 0.99$) [2]. For both the MeChry and MeBbN21T isomers, the structure isomers with the methyl group located in the k region (5-Me and 6-MeChr/BbN21T) and bay region (4-Me and 5-MeChr and 1-MeBbN21T) have similar elution order.

3.7. MeBbN23T

The LC separation of BbN23T and the 10 possible MeBbN23T isomers on the polymeric C_{18} phase is shown in Fig. 6C. Unfortunately, some of the reference compounds of the MeBbN23T isomers contained impurities (*). The polymeric phase provided a separation of MeBbN23T isomers with only two pairs of co-eluting isomers. On the monomeric phase (see Table 3, 1-MeBbN23T is the only isomer to have a significant change in elution order in comparison to the polymeric C_{18} phase. The plot of retention on the polymeric phase vs. L/B value is shown in Fig. 7C with a correlation coefficient of $r = 0.71$. The t_{exp} value for this correlation coefficient was 2.87 ($n = 10$, $\alpha = 0.05$, $t_{\text{crit}} = 2.31$) indicating that there is a significant linear trend between the retention on the polymeric C_{18} phase for MeBbN23T and L/B [41]. 1-Me (1.54), 6-Me (1.55), and 10-MeBbN23T (1.57) have the smallest L/B values of the 10 isomers but 6-MeBbN23T elutes later than expected. The late elution of 6-MeBbN23T can be explained based on previous discussions about the position of the methyl groups. This explanation is further illustrated by 4-MeBbN23T, which elutes last, even though based on L/B value (1.70) this isomer would be expected to elute before 9-Me (1.80), 8-Me (1.84), and 3-MeBbN23T (1.84).

3.8. MeTeP112T

The LC separation and retention vs. L/B plot for 5 of 10 possible MeTeP112T isomers on the polymeric C_{18} phase are shown in Fig. 8. This isomer group is the only group of *peri*-condensed PASHs. In comparison to the monomeric phase (data not shown), the polymeric phase provided the best separation with only minimal co-elution of 7-Me and 1-MeTeP112T.

The plot of retention on the polymeric phase vs. L/B value is shown in Fig. 8 with a correlation coefficient of $r = 0.85$. However, the t_{exp} value for this correlation coefficient was 2.79 ($n = 5$, $\alpha = 0.05$, $t_{\text{crit}} = 3.18$) indicating that there is not a significant linear trend between the retention on the polymeric C_{18} phase for the 5 MeTeP112T isomers and L/B [41]. The 5 missing isomers are needed to have a better understanding of the correlation between the retention of MeTeP112T and L/B . Benzo[*a*]pyrene (BaP) is the five-ring benzenoid PAH with a structure similar to TeP112T. The separation of the twelve possible MeBaP isomers on a polymeric phase ($\alpha_{\text{TeP112T/BaP}} \sim 0.7$) provided a correlation coefficient of $r = 0.81$ between their retention and L/B [2]. The t_{exp} value for this correlation coefficient was 4.48 ($n = 12$, $\alpha = 0.05$, $t_{\text{crit}} = 2.23$) indicating that there is a significant linear trend between the retention on the polymeric C_{18} phase for MeBaP and L/B [41].

4. Conclusion

The LC retention behavior of 79 alkylated PASHs on monomeric and polymeric C_{18} stationary phases is reported and represent the most extensive characterization of the LC retention behavior of alkyl-substituted PASHs ever reported. Similar to the behavior of alkylated-PAHs, the separation of alkylated PASHs was more selective on a polymeric C_{18} phase compared to a monomeric C_{18} phase. Correlation coefficients for PASH retention vs. L/B ranged from $r = 0.43$ (MeDBT) to $r = 0.93$ (MeBbN21T). Correlations between the L/B of alkyl-substituted PASHs and retention on the polymeric C_{18} phase were similar to methyl-substituted PAHs; however, other structure features were found to have an impact on the separation of alkylated-PASHs. Alkyl-substituted PASHs eluted later than predicted based on L/B if the alkyl-substituted PASH structure had the following features: (1) the sulfur atom was located in a *bay*-like-region or (2) alkyl group substitution was in the *k* region. Lastly, when the alkyl-substituted PASHs have a high degree of nonplanarity (1-Me and 11-MeBbN12T), they eluted prior to the parent PASH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Structures and substitution position numbering for the PASHs studied.

Fig. 2.
LC separations of DBT with four MeDBT isomers and three EtDBT isomers on different C₁₈ stationary phases: monomeric and polymeric.

Fig. 3. (A) LC separation of the 15 DiMeDBT isomers on the polymeric C₁₈ phase. (B) Plot of retention ($\log I$) on the polymeric C₁₈ phase versus L/B value for the 15 DiMeDBT isomers. Numbers identify the DiMeDBT isomers.

Fig. 4. LC separations of DBT with eight TriMeDBT isomers on different C₁₈ stationary phases: monomeric and polymeric. Numbers identify the TriMeDBT isomers.

Fig. 5. LC separations on the polymeric C₁₈ phase of (A) N12T and seven MeN12T isomers and (B) N21T and eight MeN21T isomers. Numbers identify the positions of the methyl groups on the parent PASHs.

Fig. 6. LC separations on the polymeric C₁₈ phase of (A) BbN12T with ten MeBbN12T isomers, (B) BbN21T with nine MeBbN21T isomers, and (C) BbN23T with ten MeBbN23T isomers. Numbers identify the positions of the methyl groups on the parent PASHs.

Fig. 7.

Plots of retention ($\log I$) on the polymeric C_{18} phase versus L/B value for (A) ten MeBbN12T, (B) nine MeBbN21T, and (C) ten MeBbN23T. Numbers identify the positions of the methyl groups on the parent PASHs.

Fig. 8. LC separations of TeP112T with five MeTeP112T isomers on a polymeric C_{18} phases and their plot of retention ($\log I$) versus L/B value for the 5 MeTeP112T isomers. Numbers identify the positions of the methyl groups on the parent PASHs.

Table 1

LC conditions for separating individual groups of PASHs.

Isomer Sets	Time (min)	ACN ^a (% v/v)	H ₂ O ^a (% v/v)	Flow Rate (mL min ⁻¹)	λ (nm)
Three-ring PASH					
MeDBT	60.0 ^b	50	50	1.5	234
EtDBT	15.0 ^b	85	15	1.0	234
DiMeDBT	20.0 ^b	85	15	1.0	234
TriMeDBT	0.0	75	25	1.0	234
	30.0	100	0		
	50	100	0		
MeN12T	20.0 ^b	75	25	1.0	261
MeN21T	35.0 ^b	85	15	0.25	261
Four-ring PASH					
MeBbN12T	50.0 ^b	85	15	0.5	262
MeBbN21T	50.0 ^b	85	15	1.0	254
MeBbN23T	85.0 ^b	85	15	0.5	274
Five-ring PASH					
MeTeP112T	0.0	85	15	1.5	254
	20.0	100	0		
	50.0	100	0		

^aVolume Fraction^bIsocratic conditions for the total run time.

Table 2

LC retention indices and molecular shape parameters for the alkyl-substituted isomers of DBT, N12T, and N21T.

PASHs	Molecular Dimensions				Log I	
	L (Å)	W (Å)	T (Å)	L/B	Monomeric C ₁₈	Polymeric C ₁₈
DBT	11.62	8.02	4.06	1.45	3.28	2.84
1-MeDBT	11.25	8.57	4.24	1.31	3.54	3.26
2-MeDBT	12.32	8.15	4.21	1.51	3.57	3.26
3-MeDBT	12.74	8.01	4.21	1.59	3.64	3.43
4-MeDBT	11.55	8.12	4.21	1.42	3.68	3.44
1-EtDBT	11.23	9.35	4.24	1.20	- <i>a</i>	- <i>a</i>
2-EtDBT	13.46	8.15	4.22	1.65	3.93	3.38
3-EtDBT	13.68	8.02	4.23	1.71	4.00	3.56
4-EtDBT	11.57	9.03	4.23	1.28	4.08	3.65
1,2-DiMeDBT	12.28	8.52	4.36	1.44	4.01	3.63
1,3-DiMeDBT	12.30	8.54	4.23	1.44	4.11	3.69
1,4-DiMeDBT	11.58	9.24	4.23	1.25	4.16	3.72
1,6-DiMeDBT	11.26	8.54	4.26	1.32	4.16	3.75
1,7-DiMeDBT	12.33	8.54	4.23	1.44	4.10	3.72
1,8-DiMeDBT	12.05	9.10	4.23	1.32	4.00	3.55
1,9-DiMeDBT	11.42	9.03	5.21	1.26	- <i>a</i>	- <i>a</i>
2,3-DiMeDBT	12.51	8.21	4.22	1.52	4.01	3.61
2,4-DiMeDBT	11.96	8.94	4.23	1.34	4.17	3.70
2,6-DiMeDBT	11.96	8.20	4.24	1.46	4.17	3.79
2,7-DiMeDBT	13.08	8.20	4.21	1.60	4.12	3.75
2,8-DiMeDBT	12.04	8.78	4.22	1.37	4.05	3.50
3,4-DiMeDBT	12.67	8.05	4.26	1.57	4.10	3.85
3,6-DiMeDBT	12.63	8.08	4.23	1.56	4.24	3.99
3,7-DiMeDBT	13.80	7.99	4.22	1.73	4.19	3.96
4,6-DiMeDBT	11.58	8.24	4.21	1.41	4.25	3.82
1,3,7-TrMeDBT	13.46	8.57	4.24	1.57	4.66	4.21
1,4,7-TrMeDBT	12.69	9.34	4.24	1.36	4.71	4.22

PASHs	Molecular Dimensions				Log I	
	L (Å)	W (Å)	T (Å)	L/B	Monomeric C ₁₈	Polymeric C ₁₈
2,3,7-TriMeDBT	13.76	8.13	4.23	1.69	4.56	4.15
2,3,8-TriMeDBT	13.17	8.65	4.22	1.52	4.49	3.85
2,4,6-TriMeDBT	12.26	8.72	4.22	1.41	4.75	4.14
2,4,7-TriMeDBT	13.39	8.72	4.21	1.54	4.73	4.26
2,4,8-TriMeDBT	12.49	8.95	4.22	1.40	4.65	3.98
3,4,7-TriMeDBT	13.79	8.11	4.22	1.70	4.66	4.45
N12T	11.28	8.07	4.16	1.40	2.84	2.90
2-MeN12T	12.40	8.05	4.33	1.54	3.59	3.44
3-MeN12T	11.99	8.08	4.21	1.48	- ^a	- ^a
4-MeN12T	11.26	9.19	4.20	1.23	3.48	3.30
5-MeN12T	11.28	9.30	4.22	1.21	3.47	3.25
6-MeN12T	11.46	8.07	4.23	1.42	3.45	3.30
7-MeN12T	12.41	8.07	4.21	1.54	3.51	3.41
8-MeN12T	11.50	8.53	4.21	1.35	3.42	3.23
9-MeN12T	11.34	9.27	4.23	1.22	4.02	3.32
N21T	11.28	8.07	4.06	1.40	2.78	2.74
1-MeN21T	11.01	8.91	4.23	1.24	3.30	3.15
2-MeN21T	11.95	8.34	4.21	1.43	3.39	3.24
4-MeN21T	11.04	8.81	4.21	1.25	3.38	3.23
5-MeN21T	11.13	9.29	4.22	1.20	3.33	3.17
6-MeN21T	11.28	8.06	4.21	1.40	3.29	3.20
7-MeN21T	12.26	8.05	4.21	1.52	3.34	3.28
8-MeN21T	11.70	8.42	4.21	1.39	3.27	3.12
9-MeN21T	10.85	8.79	4.24	1.24	3.31	3.17

^aReference standards was not available; therefore no measurements were obtained for these compounds.

Table 3

LC retention indices and molecular shape parameters for the methyl isomers of BbN12T, BbN21T, BbN23T, and TeP112T.

PASHs	Molecular Dimensions				Log I	
	L (Å)	W (Å)	T (Å)	L/B	Monomeric C ₁₈	Polymetric C ₁₈
BbN12T	12.72	9.31	4.06	1.37	4.00	3.75
1-MeBbN12T	12.53	9.48	5.28	1.32	4.23	3.61
2-MeBbN12T	12.57	9.91	4.21	1.27	4.44	3.98
3-MeBbN12T	13.86	9.31	4.21	1.49	4.54	4.37
4-MeBbN12T	13.58	9.32	4.22	1.46	4.52	4.28
5-MeBbN12T	12.70	9.78	4.22	1.30	4.56	4.25
6-MeBbN12T	12.71	10.5	4.21	1.22	4.68	4.32
8-MeBbN12T	13.47	9.31	4.21	1.45	4.70	4.51
9-MeBbN12T	13.82	9.31	4.21	1.48	4.64	4.43
10-MeBbN12T	12.51	9.63	4.21	1.30	4.49	3.98
11-MeBbN12T	12.53	9.36	4.99	1.34	4.37	3.73
BbN21T	13.66	8.13	4.06	1.68	4.13	4.22
1-MeBbN21T	13.65	9.16	4.23	1.47	4.77	4.90
2-MeBbN21T	13.62	8.75	4.21	1.56	- a	- a
3-MeBbN21T	14.79	8.12	4.21	1.82	4.79	5.39
4-MeBbN21T	13.89	8.11	4.23	1.71	4.75	5.27
5-MeBbN21T	13.66	9.30	4.22	1.47	4.68	4.63
6-MeBbN21T	13.66	9.35	4.29	1.46	4.74	4.78
7-MeBbN21T	13.52	8.57	4.23	1.58	4.74	4.96
8-MeBbN21T	14.21	8.19	4.21	1.74	4.75	5.06
9-MeBbN21T	14.79	8.12	4.21	1.82	4.82	5.45
10-MeBbN21T	13.62	8.42	4.21	1.62	4.84	4.97
BbN23T	13.90	8.17	4.06	1.70	4.05	4.05
1-MeBbN23T	13.88	9.04	4.24	1.54	4.48	4.37
2-MeBbN23T	14.33	8.75	4.21	1.64	4.75	4.36
3-MeBbN23T	15.02	8.16	4.21	1.84	4.63	5.02
4-MeBbN23T	13.89	8.17	4.20	1.70	4.71	5.22

PASHs	Molecular Dimensions				Log I	
	L (Å)	W (Å)	T (Å)	L/B	Monomeric C ₁₈	Polymeric C ₁₈
6-MeBbN23T	13.91	8.99	4.21	1.55	4.58	4.62
7-MeBbN23T	13.95	8.38	4.22	1.67	4.49	4.48
8-MeBbN23T	15.06	8.17	4.21	1.84	4.62	5.00
9-MeBbN23T	14.77	8.20	4.21	1.80	4.59	4.70
10-MeBbN23T	13.58	8.67	4.22	1.57	4.44	4.22
11-MeBbN23T	13.74	8.64	4.24	1.59	4.51	4.56
TeP112T	13.64	9.32	4.05	1.46	5.03	4.83
1-MeTeP112T	13.61	9.43	4.20	1.44	5.79	5.12
2-MeTeP112T	14.76	9.32	4.20	1.59	- ^a	- ^a
3-MeTeP112T	14.42	9.30	4.22	1.55	5.67	5.31
4-MeTeP112T	13.38	9.90	4.21	1.35	- ^a	- ^a
5-MeTeP112T	13.62	10.52	4.21	1.30	5.60	4.92
6-MeTeP112T	13.63	9.34	4.23	1.46	- ^a	- ^a
7-MeTeP112T	13.79	9.32	4.22	1.48	5.58	5.09
8-MeTeP112T	14.77	9.32	4.21	1.59	- ^a	- ^a
9-MeTeP112T	14.05	9.30	4.19	1.51	5.28	5.43
10-MeTeP112T	13.22	9.42	4.23	1.40	- ^a	- ^a

^aReference standards was not available; therefore no measurements were obtained for these compounds.

Table 4Correlation coefficients relating retention on the polymeric C₁₈ phase versus L/B .

PASHs	Number of PASHs	Equation	Correlation Coefficient	Degrees of Freedom	t -value, $\alpha = 0.05$	
					t_{crit}	t_{exp} Significant
MeDBT	4	$y = 0.36x + 2.82$	0.43	2	4.30	0.68 No
EtDBT	3	$y = -0.39x + 4.14$	-0.66	1	12.71	1.23 No
DiMeDBT	15	$y = 0.64x + 2.81$	0.60	13	2.18	2.70 Yes
TriMeDBT	8	$y = 0.60x + 3.25$	0.43	6	2.45	1.17 No
MeN12T	7	$y = 0.11x + 3.21$	0.75	5	2.57	2.55 No
MeN21T	8	$y = 0.25x + 2.87$	0.54	6	2.45	1.58 No
MeBbN12T	10	$y = 1.54x + 2.06$	0.50	8	2.31	1.62 No
MeBbN21T	9	$y = 1.74x + 2.20$	0.93	7	2.36	6.65 Yes
MeBbN23T	10	$y = 1.98x + 1.35$	0.71	8	2.31	2.87 Yes
MeTe112T	5	$y = 1.32x + 2.93$	0.85	3	3.18	2.79 No