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

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Abstract

Retention indices for 70 polycyclic aromatic sulfur heterocycles (PASHs) were determined using reversed-phase liquid chromatography (LC) on a monomeric and a polymeric C₁₈ stationary phase. Molecular shape parameters [length, breadth, thickness (*T*), and length-to-breadth ratio (*L/B*)] were calculated for all the compounds studied. Correlations between the retention on the polymeric C₁₈ phase and PASH geometry (*L/B* and *T*) were investigated for six specific PASH isomer groups with molecular mass (MM) 184 Da, 234 Da, 258 Da, 284 Da, 334 Da, and 384 Da. Similar to previous studies for polycyclic aromatic hydrocarbons (PAHs), PASH elution order on the polymeric C₁₈ phase was generally found to follow increasing *L/B* values. Correlation coefficients for retention vs *L/B* ranged from *r* = 0.45 (MM 184 Da) to *r* = 0.89 (MM 284 Da). In the case of smaller PASHs (MM 184–234 Da), the location of the sulfur atom in the bay-region of the structure resulted in later than expected elution of these isomers based on *L/B*. In the case of the larger PASHs (MM 258–384 Da), nonplanarity had a significant influence on earlier than predicted elution based on *L/B* values.

Keywords

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

1. Introduction

Polycyclic aromatic compounds (PACs) comprise a complex class of condensed multi-ring benzenoid compounds originating from a wide variety of natural and anthropogenic sources. The parent homocyclic species, which contain only carbon and hydrogen, are the familiar polycyclic aromatic hydrocarbons (PAHs). In recent decades, PAHs are one of the most

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studied groups of environmental contaminants because of their high carcinogenic and mutagenic potential [1–4]. However, their sulfur analogues, polycyclic aromatic sulfur heterocycles (PASHs), have received far less attention although their presence has been confirmed in air particulate matter [5, 6], sediments [7–9], coal liquids [10], diesel [9], heavy oil [11], crude oil [9, 11–17], shale oil [10, 12, 15, 18], coal tar [12–15, 19–21], mussels [7, 8], and fish [7, 8]. In addition, their carcinogenic and mutagenic potential have been reported [22, 23]. PASHs exist in an even greater variety of structures compared to PAHs due to the presence of the sulfur atom. Therefore, the number of isomers and alkylated isomers can be extremely large, which increases the difficulty to quantify individual PASH isomers in complex natural mixtures.

Reversed-phase liquid chromatography (LC) on octadecylsilane (C_{18}) stationary phases has been shown to provide excellent separations of isomeric PAHs [24–31]. However, not all C_{18} stationary phases provide the same selectivity for PAHs. In past decades, several studies have compared different commercial C_{18} columns for the separation of PAHs with particular emphasis on the separation of isomeric PAHs [28, 29]. These studies demonstrated that all stationary phases were C_{18} but some provided significantly enhanced selectivity for the separation of isomeric PAHs. One of the studies focused on the importance of the synthesis of the bonded C_{18} phase. Typically, monomeric C_{18} phases are prepared by reaction of monofunctional silanes [24–26] and polymeric C_{18} phases are prepared using trifunctional silanes in the presence of water, which results in cross-linking to form silane polymers on the silica surface [24, 25].

Polymeric C_{18} phases have been demonstrated to separate PAH isomers [24, 26, 27, 33] based on shape of the PAH solute. The relationship between the PAH shape and LC retention on polymeric C_{18} phases was first reported by Wise *et al.* [24]. The shape of the PAH was defined as the length-to-breadth ratio (L/B) of the box drawn around the molecule that produces the maximum L/B value. Retention of PAH isomers was observed to increase with increasing L/B values. In later studies, the similarity of LC retention on polymeric C_{18} phases and gas chromatography (GC) retention on liquid crystalline stationary phases was demonstrated for isomeric PAHs [31–34]. In general, the nonplanar PAH isomers, as indicated by a thickness parameter (T), elute earlier than expected based on L/B values. Farkas *et al.* [35] have demonstrated the usefulness of the quantitative structure-retention relationship technique (QSRR) to predict the retention indices for saturated S-heterocyclic compounds on standard nonpolar polydimethylsiloxane siloxane stationary phases.

In the current study, the LC separation of isomeric PASHs on both a monomeric and a polymeric C_{18} stationary phase was investigated. There are few publications that have reported the reversed-phase LC separation of isomeric PASHs [36, 37]. In the current study, LC retention indices were determined for 70 PASHs on both monomeric and polymeric C_{18} phases. Correlation of PASH geometry (L/B and T) and retention on both the monomeric and polymeric C_{18} phases was investigated. The polymeric C_{18} phase was the only phase to demonstrate good correlation and will be discussed in detail. This study provides the most comprehensive investigation of reversed-phase LC retention behavior of PASH to date. A similar study for alky-substituted PASHs is published elsewhere [38].

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); benzo[*b*]naphtho[1, 2-*b*]thiophene (BbN12T), benzo[*b*]naphtho[2, 1-*b*]thiophene (BbN21T), benzo[*b*]naphtho[2, 3-*b*]thiophene (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 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) were 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 Descriptors Calculations

Molecular shape parameter calculations have been described in detail previously [36, 39]. Briefly, ChemDraw 3D software (PerkinElmer, Waltham, MA, USA) was used to draw the molecular structures of PASHs and convert them 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. UV-vis absorption spectrometry

LC detection of PASHs was achieved using selected wavelengths that corresponds to a compromise among the maximum absorbance wavelengths obtained with a commercial spectrophotometer (model Cary 100, Agilent) from measurements of pure standards. The light source was a 75 W pulsed xenon lamp with a 3000 nm min⁻¹ maximum scan rate. The Czerny-Turner monochromator had an accuracy (± 0.2 nm to 0.4 nm with < 0.189 nm resolution) and reproducibility (< 0.02 nm, *N* = 10). The monochromator 1200 lines mm⁻¹ grating (30 × 35 mm) were blazed at an angle of 8.6° at 240 nm. Detection was made with a photomultiplier tube (P928) with wavelength range from 190 to 900 nm. Measurements were made with a standard 700 μ L quartz cuvette with 1 cm path length.

2.4. 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 [28].

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

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 [34, 35]. 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 $\pm 0.02 \log I$ units. Baseline resolution of two components could be achieved with a difference of $\sim 0.06 \log I$ units on both the monomeric and polymeric C_{18} phase.

2.5. Instrumentation and chromatographic conditions

LC-UV analysis was performed using a liquid chromatograph (1200 series, Agilent, Avondale, PA) coupled to a UV-vis detector (UV2000, Thermo Scientific, Waltham, MS). The LC system was equipped with a gradient pump (G1311A), a degasser (G1322A) and an auto sampler (G1329A). The instrument was computer controlled using commercial software (Chromeleon, Thermo Scientific). Separations were carried out on a monomeric (Agilent 5) and polymeric (Zorbax Eclipse PAH) C_{18} columns purchased from Agilent (Avondale, PA) with the following characteristics: 25 cm length, 4.6 mm diameter, and 5 μm average particle diameters.

Both LC columns were characterized using the Column Selectivity Test Mixture for Liquid Chromatography (SRM 869b) using selectivity ratios ($\alpha_{TBN/BaP}$) of tetrabenzonaphthalene (TBN) and benzo[*a*]pyrene (BaP). The $\alpha_{TBN/BaP}$ values were determined with a mobile phase of 85/15 (v/v) acetonitrile (ACN) – water (H_2O) at a flow-rate of 1.5 mL min^{-1} . The optimal separation and detection conditions for each isomer set are listed in Table 1. The LC retention index data ($\log I$) were determined using these conditions.

3. Results and Discussion

In previous studies, the LC retention behavior of isomeric PAHs has been shown to be significantly different on various C_{18} phases that can be classified as monomeric or polymeric [26–28]. Sander and Wise [28] previously proposed a scheme that uses an LC column test mixture (SRM 869) for determining $\alpha_{TBN/BaP}$ values to classify the type of C_{18} phase. In general, the shape selective behavior of the stationary phase for separation of complex isomeric PAH mixtures increases with decreasing $\alpha_{TBN/BaP}$. Values for $\alpha_{TBN/BaP}$ 1.7 represent monomeric C_{18} phases, $\alpha_{TBN/BaP}$ in the range of 1.0 to 1.7 represent intermediate polymeric C_{18} phases, and values for $\alpha_{TBN/BaP} < 1$ represent polymeric C_{18} phases [29].

In the present study, two commercially prepared C_{18} columns were selected and used to investigate the LC selectivity for separating isomeric PASHs. The polymeric C_{18} phase had a $\alpha_{TBN/BaP} = 0.49$ and the monomeric C_{18} phase had a $\alpha_{TBN/BaP}$ value of 2.05. The LC retention behavior of isomeric PASHs on the two C_{18} phases was investigated and the optimized conditions for the separations are listed in Table 1. The correlation between retention on the polymeric C_{18} phase and the molecular shape parameters (L , B , T , and L/B ratio) for isomeric PASH was determined. The molecular shape parameters and retention indices ($\log I$) for the three-, four-, five-, six- and seven-ring PASHs are summarized in Tables 2, 3, and 4. The LC retention indices reported here represent the most extensive compilation of reversed-phase LC retention data for PASHs.

Table 5 summarizes the regression calculations for the correlation of L/B and LC retention on the polymeric C_{18} phase for six groups of PASH isomers. Previous studies have used correlation coefficients (r) as a parameter for measuring the linear correlations for PAH retention and L/B [36]. The unique shape selectivity for the separation of PAHs on polymeric C_{18} phases is demonstrated most dramatically for mixtures with a large numbers of isomers. Generally, the trend is best expressed when the correlation coefficient is close to 1. In cases where the correlation coefficient is not close to 1, it is necessary to use a proper statistical test to determine if there is a significant trend for the correlation coefficient [40]. The calculation of a t -test value is the simplest statistical method for these determinations using equation 2:

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

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 r value if the t_{exp} is greater than the t_{crit} . These concepts will be applied in the following sections for discussing the correlations between PASH retention on the polymeric C_{18} 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. Three- and four-ring PASH

The molecular structures of the three-ring PASHs (4 isomers) and four-ring PASHs (1 PASH with MM 208 Da and 13 PASH isomers with MM 234 Da) included in the present study are shown in Fig. 1. LC separations of the three-ring PASH isomers are shown in Fig. 2.

Baseline resolution of all four components was not achieved on either C_{18} phase; however, the best separation was obtained with the monomeric C_{18} phase. Typically, polymeric C_{18} phases provide better separation of isomeric PAHs as discussed earlier. The elution order of the three-ring PASH isomers was similar on both C_{18} phases with the exception of DBT. On the monomeric C_{18} phase, N21T and N23T are only partially resolved; however, on the polymeric C_{18} phase they are baseline resolved. The elution order of the four three-ring PASH isomers on the polymeric C_{18} phase follows the expected elution order based on L/B values with the exception of N12T, which elutes fourth on the polymeric C_{18} phase rather than second as expected based on L/B values.

The correlation of retention for the four isomers on the polymeric C_{18} phase vs. L/B (see Table 5, Fig. S1) resulted in a low correlation coefficient of $r = 0.45$, which provides a t_{exp} value of 0.71 ($t_{\text{crit}} = 4.30$, $\alpha = 0.05$, $n = 4$) indicating that there is not a significant linear trend between PASH retention and L/B values [40]. N12T and N21T have the same L/B value but N12T has the heterocyclic sulfur atom located in the *bay*-region of the structure. The correlation coefficient improves to $r = 0.93$ when N12T is removed (see Fig. S1), however; the t_{exp} value (2.51) is below the t_{crit} value (12.71) indicating there is not a significant correlation coefficient to indicate a linear trend is represented. In this case, the

number of data points is low because of the limited number of possible three ring PASH isomers (4). The influence of the sulfur in the bay-region becomes more evident with the larger PASH as discussed below.

LC separations of the four-ring PASHs on both monomeric and polymeric C_{18} phases are shown in Fig. 3. P19T is the only PASH with MM 208 Da and elutes prior to the MM 234 Da isomers as expected. The 13 MM 234 Da isomers were not baseline resolved on either C_{18} phase. The polymeric C_{18} phase provided the best separation of the 13 isomers. The monomeric C_{18} phase had significantly more co-elution between the following isomer groups: (1) P21T, P23T, P32T, and P34T and (2) P43T, P910T, and P12T.

A plot of retention on the polymeric C_{18} phase vs. L/B for the 13 MM 234 Da isomers is shown in Fig. 4 with a correlation coefficient of $r = 0.75$, which provides a t_{exp} value of 3.76 ($t_{\text{crit}} = 2.23$, $\alpha = 0.05$, $n = 13$) indicating that there is a significant linear trend between PASH retention and L/B values [40]. The correlation coefficient for the MM 234 Da is similar to the correlation of the four-ring *cata*-condensed PAHs with MM 228 Da ($r = 0.80$, $n = 4$) [36]. In general, later elution than expected based on L/B was observed for four of the five isomers (P43T, P910T, A12T, and BbN21T; numbers 6, 4, 9, and 12, respectively) with the heterocyclic sulfur atom located in a bay-region of the structure (the fifth isomer is P12T; number 10). The largest difference was with P910T (number 4), expected to elute first with an L/B of 1.13; however, it actually elutes fourth.

PASH isomers with relatively similar L/B and T values would be expected to have similar retention behaviors on a polymeric C_{18} stationary phase. The following four isomers pairs meet this criteria: (1) P12T and P21T, (2) P23T and P32T, (3) P34T and P43T, and (4) A12T and A21T. In three of the isomer pairs, the isomer with the sulfur atom located in the *bay*-region (P12T and A12T) or *fi*-region (P43T) eluted several minutes later than the isomers with the sulfur not located in either region. These observations suggest that when the sulfur atom is located in the *bay*-region or *fi*-region, the sulfur atom is protected from having interactions with the stationary phase (C_{18}). In the case of P23T and P32T, the sulfur atoms are not located in the *bay*-region of the structure and have similar retention behaviors ($\log I$ values).

3.2. Five-ring PASH

The molecular structures of the five-ring *peri*-condensed PASHs with MM 258 Da (10 isomers) and five-ring *cata*-condensed PASHs with MM 284 Da (20 isomers) are shown in Fig. 5 and 6, respectively. The LC separations of the isomers with MM of 258 Da on the monomeric and polymeric C_{18} phases are shown in Fig. 7. Baseline resolution of all components was not achieved on either C_{18} phase; however, the most complete separation was achieved with the polymeric C_{18} phase. The separation on the monomeric phase occurs over an 8 min range, whereas the separation on the polymeric phase covers a 31 min range. B45P19T and B45P91T co-elute on both C_{18} phases as shown in Fig. 7. Similar observations were noticed in the GC separation for these two PASHs on various stationary phases, however; there was a sufficient separation difference that both PASHs provided slightly different retention index values (data not shown). These two isomers have identical L/B values and differ only in position of the sulfur atom in the heterocyclic ring. In the case

of these two isomers, the location of the sulfur atom had no observed influence on the separation. However, B12P34T (position 1) and B12P43T (position 2), which only differ structurally by the location of the sulfur atom, had significantly different retention behavior on both C₁₈ phases. The separation of these two isomers is reversed on the monomeric and polymeric C₁₈ phases. B12P34T has the sulfur atom located in the bay-region and elutes 8 min after B12P43T. B45P19T and B45P91T, which co-elute, have identical *L/B* values and neither isomer has the sulfur atom located in the bay-region. Similar observations were observed and discussed in Section 3.1. for the MM 234 Da cata-condensed PASH isomers.

A plot of retention on the polymeric C₁₈ phase vs. *L/B* for the 10 MM 258 Da isomers is shown in the insert of Fig. 7 with a correlation coefficient of $r = 0.75$, which provides a t_{exp} value of 3.21 ($t_{\text{crit}} = 2.31$, $\alpha = 0.05$, $n = 10$) indicating that there is a significant linear trend between PASH retention and *L/B* values [40]. From the plot it can clearly be seen that two of the isomers, B12P43T and TeP112T (numbers 1 and 10), have significantly different elution compared to the remaining eight isomers. In the case of TeP112T, the later elution may be attributed to the sulfur atom located in the bay-region of the structure as discussed in Section 3.1 and 3.2. In comparison to the five-ring *peri*-condensed PAHs with MM 252 Da ($r = 0.36$, $n = 12$), the correlation coefficient is significantly improved for the five-ring *peri*-condensed PASHs for no obvious reason [36].

Fig. 8 shows LC separations on the monomeric and polymeric C₁₈ phases of the largest isomer set in the current study with 20 isomers with a MM of 284 Da. Unfortunately, some of the reference compounds contained minor impurities (*) which are indicated in the chromatogram. The polymeric C₁₈ phase provided the most comprehensive separation over 45 min with co-elution occurring between the following isomer pairs: (1) DiN[21:12]T and TriP21T, (2) BbP32T and DiN[21:23]T, (3) BbP23T and A23BT, and (4) BbP21T and DiN[12:23]T. The elution of all 20 isomers on the monomeric phase occurs over a span of only 8 min. As a result, significantly more co-elution occurs on the monomeric phase.

The correlation of retention on the polymeric C₁₈ phase and *L/B* value for the 20 MM 284 Da isomers is shown in Fig. 9A with a correlation coefficient of $r = 0.84$, which provides a t_{exp} value of 8.31 ($t_{\text{crit}} = 2.10$, $\alpha = 0.05$, $n = 20$) indicating that there is a significant linear trend between PASH retention and *L/B* values [40]. The correlation coefficient for the MM 284 Da is similar to the correlation of the five-ring *cata*-condensed PAHs with MM 278 Da ($r = 0.93$, $n = 11$) [36]. Unlike earlier results, the overall results show that the placement of the sulfur atom appears to have limited influence on the elution of MM 284 Da isomers. 14 of the 20 MM 284 Da isomers have planar molecular structures, as indicated by the similar thickness parameters ($T = 4.06$ Å to 4.20 Å). Early elution of nonplanar PAHs has been reported in LC separations utilizing shape-selective stationary phases [29]. BbP43T, DiN[21:12]T, TriP21T, and B34P12T are the most nonplanar isomers ($T = 4.79$ Å to 5.35 Å) included in this isomer set. BbP12T and BbP910T are slightly nonplanar with T values ranging from 4.33 Å to 4.36 Å. Fig. 9B shows the plot with correlation of the retention on the polymeric C₁₈ phase with the *L/B* value for the seven nonplanar isomers, which results in a correlation coefficient of $r = 0.94$. The calculated t_{exp} value is 6.18 ($t_{\text{crit}} = 2.57$, $\alpha = 0.05$, $n = 7$) indicating that there is a significant linear trend between the retention of the nonplanar five-ring PASH isomers and *L/B* values [40]. Fig. 9C shows the plot of the

correlation of retention on the polymeric C₁₈ phase and thickness (T) of these six MM 284 Da isomers, which results in a correlation coefficient of $r = -0.84$. The calculated t_{exp} value is 5.17 ($t_{\text{crit}} = 2.23$, $\alpha = 0.05$, $n = 13$) indicating that there is a significant linear trend between the retention of nonplanar five-ring PASH isomers and T values [40]. The results confirm that the elution of planar isomers on the polymeric C₁₈ phases follow increasing L/B ratio; non-planar isomers elute earlier than expected based on L/B and have a negative correlation with degree of non-planarity (i.e., as indicated by thickness).

3.3. Six-ring PASH

The molecular structures of the six-ring PASHs with MM 282 Da (3 isomers) and MM 334 Da (12 isomers) are shown in Fig. 10. Fig. S2 shows the LC separations of the isomers with a MM of 282 Da on the monomeric and polymeric C₁₈ phases. Baseline resolution was achieved for the three isomers on both the monomeric and polymeric C₁₈ phases over 7.5 min and 28 min, respectively. The elution order was the same on both C₁₈ phases and followed the predicted elution based on L/B values. B45TriP112T and Per112T have similar L/B values (1.13 and 1.11, respectively) but the T values (4.06 and 5.06 Å, respectively) are drastically different. As a result, Per112T eluted significantly earlier than the other two isomers.

Fig. 11 shows the LC separations of the 12 six-ring PASHs isomers of MM 334 Da on the monomeric and polymeric C₁₈ phases. Baseline resolution of all components was achieved on the polymeric C₁₈ phase over a 40 min time interval (10 to 50 min). The monomeric C₁₈ phase also provided a separation of the majority of the 12 isomers with minimal co-elution over an 8 min interval (12 to 20 min). BbC12T and N21P910T were the only isomers co-eluting on the monomeric phase. On the monomeric phase BbC23T elutes first, whereas on the polymeric phase, BbC23T elutes late in the separation (9th of 12), as expected based on the large L/B ratio ($L/B = 2.08$).

Of particular interest is the fact that 9 of the 12 isomers investigated in this study are nonplanar (Table 4; $T = 4.20$ Å, ranging from 4.56 to 6.70 Å). A plot of LC retention on the polymeric C₁₈ phase vs. L/B for the MM 334 Da isomers is shown in Fig. 12A, with a correlation coefficient of $r = 0.51$ and a t_{exp} value of 1.88 ($t_{\text{crit}} = 2.23$, $\alpha = 0.05$, $n = 12$) indicating that there is not a significant linear trend between PASH retention and L/B values [40]. Fig. 12B shows the correlation between the retention on the polymeric C₁₈ phase vs. L/B for the nine nonplanar MM 334 Da isomers with an improved correlation coefficient of $r = 0.83$. The calculated t_{exp} value is 3.94 ($t_{\text{crit}} = 2.36$, $\alpha = 0.05$, $n = 9$) indicating that there is a significant linear trend between the retention of nonplanar six-ring PASH isomers and L/B values [40]. Fig. 12C shows the plot of LC retention on the polymeric C₁₈ phase vs. the T values of the nine nonplanar isomers with a correlation coefficient of $r = -0.92$. The calculated t_{exp} value is 6.21 ($t_{\text{crit}} = 2.36$, $\alpha = 0.05$, $n = 9$) indicating that there is a significant linear trend between the retention of nonplanar six-ring PASH isomers and T values [40]. Similar to the nonplanar MM 284 Da isomers, elution on the polymeric C₁₈ phase for the MM 334 Da isomers follow the trends of increasing L/B ratios and decreasing T values.

3.4. Seven-ring PASH

The molecular structures of the seven-ring PASHs with MM 306 Da, MM 332 Da, and MM 384 Da are shown in Fig. 13. Even though only a limited number of seven-ring PASHs were available, the present study is the first to report the LC retention data ($\log I$) for these isomers. Fig. 14A and Fig. 14B shows the LC separations of three PASH isomers with MM 306 and MM 332 Da on the monomeric and polymeric C_{18} phases, respectively. Baseline resolution was achieved for the three PASHs on both C_{18} phases; however, the separation on the polymeric phase occurred over a total of 65 min while the monomeric phase achieves the separation in 7 min. N[218:345]Py12T elutes first on both C_{18} phases. B67Per112T elutes third on the monomeric phase but second on the polymeric phase. Fig. 14C and Fig. 14D show the LC separations of the four seven-ring PASH with MM 384 Da on the monomeric and polymeric phases, respectively. The elution order of the four isomers was different on the two columns. The most significant difference was observed for BbB56C21T, which elutes third within 10 min on the monomeric phase, but last on the polymeric phase at ~70 min. Correlation of retention on the polymeric C_{18} phase vs. L/B (Fig. S3 and Table 5) resulted in a correlation coefficient of $r = 0.80$ and a t_{exp} value of 1.89 ($t_{\text{crit}} = 4.30$, $\alpha = 0.05$, $n = 4$). This t_{exp} value indicates that there is not a significant linear trend between the retention of seven-ring PASH isomers and L/B values [40].

4. Conclusions

Retention indices for 70 PASHs were determined using a monomeric and polymeric C_{18} stationary phase. The results presented here represent the most extensive characterization of the LC retention behavior of PASHs ever reported. Similar to the behavior of PAHs, isomeric PASHs are generally better resolved on the polymeric C_{18} stationary phase than on the monomeric C_{18} phase. Correlation between the molecular shape parameters of PASHs (L/B and T) and retention on the polymeric C_{18} phase were similar to PAHs. Correlation coefficients for PASH retention vs. L/B ranged from $r = 0.45$ (MM 184 Da) to $r = 0.89$ (MM 284 Da). For larger PASHs (MM 284 Da), non-planarity (T) had a significant influence on the separation of PASHs. PASHs with larger T values had earlier elution than expected based on L/B values. Besides the L/B and T values, the location of the sulfur atom plays an important role in the separation mechanism of smaller PASHs (MM 258 Da). PASHs that only differ structurally by the location of the sulfur atom (similar L/B and T values) are expected to have similar retention behaviors ($\log I$ values). However, PASHs are more retentive when the sulfur atom is located in the *bay*-region or *fi*-region of the structure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Murakami M, Sato N, Anegawa A, Nakada N, Harada A, Komatsu T, Takada H, Tanaka H, Ono Y, Furumai H. Multiple evaluations of the removal of pollutants in road runoff by soil infiltration. *Water Res.* 2008; 42:2745–2755. [PubMed: 18328527]
2. Chen J-C, Huang J-S, Chen C-M, Guo J-S. Emission characteristics of PAHs, benzene and phenol group hydrocarbons in O2/RFG waste incineration processes. *Fuel.* 2008; 87:2787–2797.

3. Courter LA, Luch A, Musafia-Jeknic T, Arlt VM, Fischer K, Bildfell R, Pereira C, Phillips DH, Poirier MC, Baird WM. The influence of diesel exhaust on polycyclic aromatic hydrocarbon-induced DNA damage, gene expression, and tumor initiation in Sencar mice in vivo. *Cancer Letters*. 2008; 265:135–147. [PubMed: 18353537]
4. Kobayashi R, Okamoto RA, Maddalena RL, Kado NY. Polycyclic aromatic hydrocarbons in edible grain: A pilot study of agricultural crops as a human exposure pathway for environmental contaminants using wheat as a model crop. *Environ. Res.* 2008; 107:145–151. [PubMed: 18295196]
5. Lee ML, Novotny M, Bartle KD. Gas chromatography/mass spectrometric and nuclear magnetic resonance determination of polynuclear aromatic hydrocarbons in airborne particulates. *Anal. Chem.* 1976; 48:1566–1572. [PubMed: 962144]
6. Wise SA, Benner BA, Chesler SN, Hilpert LR, Vogt CR, May WE. Characterization of the polycyclic aromatic hydrocarbons from two standard reference material air particulate samples. *Anal. Chem.* 1986; 58:3067–3077.
7. Paasivirta J, Herzschuh R, Lahtiperä M, Pellinen J, Sinkkonen S. Oil residues in Baltic sediment, mussel and fish. I. Development of the analysis methods. *Chemosphere*. 1981; 10:919–928.
8. Paasivirta J, Kääriäinen H, Lahtiperä M, Pellinen J, Sinkkonen S. Oil residues in Baltic sediment, mussel and fish. II. Study of the Finnish Archipelago 1979–1981. *Chemosphere*. 1982; 11:811–821.
9. Yang C, Zhang G, Wang Z, Yang Z, Hollebone B, Landriault M, Shah K, Brown CE. Development of a methodology for accurate quantitation of alkylated polycyclic aromatic hydrocarbons in petroleum and oil contaminated environmental samples. *Anal. Methods*. 2014; 6:7760–7771.
10. Willey C, Iwao M, Castle RN, Lee ML. Determination of sulfur heterocycles in coal liquids and shale oils. *Anal. Chem.* 1981; 53:400–407.
11. Nolte T, Posch TN, Huhn C, Andersson JT. Desulfurized Fuels from Athabasca Bitumen and Their Polycyclic Aromatic Sulfur Heterocycles. Analysis Based on Capillary Electrophoresis Coupled with TOF MS. *Energy Fuels*. 2013; 27:97–107.
12. Schmid B, Andersson JT. Critical Examination of the Quantification of Aromatic Compounds in Three Standard Reference Materials. *Anal. Chem.* 1997; 69:3476–3481. [PubMed: 21639271]
13. Zeigler C, Wilton N, Robbat A Jr. Toward the Accurate Analysis of C1–C4 Polycyclic Aromatic Sulfur Heterocycles. *Anal. Chem.* 2012; 84:2245–2252. [PubMed: 22339202]
14. Zeigler CD, Robbat A Jr. Comprehensive Profiling of Coal Tar and Crude Oil to Obtain Mass Spectra and Retention Indices for Alkylated PAH Shows Why Current Methods Err. *Environ. Sci. Technol.* 2012; 46:3935–3942. [PubMed: 22428566]
15. Mössner SG, Wise SA. Determination of Polycyclic Aromatic Sulfur Heterocycles in Fossil Fuel-Related Samples. *Anal. Chem.* 1999; 71:58–69. [PubMed: 21662926]
16. Yang B, Hou W, Zhang K, Wang X. Applications of solid-phase microextraction to the determination of polycyclic aromatic sulfur heterocycles in Bohai Sea crude oils. *J. Sep. Sci.* 2013; 36:1636–1643. [PubMed: 23596144]
17. Zeigler C, Schantz M, Wise SA, Robbat A Jr. Mass Spectra and Retention Indexes for Polycyclic Aromatic Sulfur Heterocycles and Some Alkylated Analogs. *Poly. Arom. Compds.* 2012; 32:154–176.
18. Andersson JT, Schmid B. Polycyclic aromatic sulfur heterocycles IV. Determination of polycyclic aromatic compounds in a shale oil with the atomic emission detector. *J. Chromatogr. A.* 1995; 693:325–338.
19. Antle PM, Zeigler CD, Gankin Y, Robbat A. New Spectral Deconvolution Algorithms for the Analysis of Polycyclic Aromatic Hydrocarbons and Sulfur Heterocycles by Comprehensive Two-Dimensional Gas Chromatography-Quadrupole Mass Spectrometry. *Anal. Chem.* 2013; 85:10369–10376. [PubMed: 24063305]
20. Antle P, Zeigler C, Robbat A Jr. Retention behavior of alkylated polycyclic aromatic sulfur heterocycles on immobilized ionic liquid stationary phases. *J. Chromatogr. A.* 2014; 1361:255–264. [PubMed: 25155062]
21. Mössner SG, Lopez de Alda MJ, Sander LC, Lee ML, Wise SA. Gas chromatographic retention behavior of polycyclic aromatic sulfur heterocyclic compounds, (dibenzothiophene, naphtho[*b*]thiophenes, benzo[*b*]naphthothiophenes and alkyl-substituted derivatives) on stationary phases of different selectivity. *J. Chromatogr. A.* 1999; 841:207–228.

22. McFall T, Booth GM, Lee ML, Tominaga Y, Pratap R, Tedjamulia M, Castle RN. Mutagenic activity of methyl-substituted tri-and tetracyclic aromatic sulfur heterocycles. *Mutat. Res. Genet. Toxicol.* 1984; 135:97–103.
23. Pelroy RA, Stewart DL, Tominaga Y, Iwao M, Castle RN, Lee ML. Microbial mutagenicity of 3- and 4-ring polycyclic aromatic sulfur heterocycles. *Mutat. Res. Genet. Toxicol.* 1983; 117:31–40.
24. Wise S, Bonnett WJ, Guenther FR, May WE. A Relationship Between Reversed-Phase C18 Liquid Chromatographic Retention and the Shape of Polycyclic Aromatic Hydrocarbons. *J. Chromatogr. Sci.* 1981; 19:457–465.
25. Sander LC, Wise SA. Synthesis and characterization of polymeric C18 stationary phases for liquid chromatography. *Anal. Chem.* 1984; 56:504–510.
26. Wise SA, Sander LC. Factors affecting the reversed-phase liquid chromatographic separation of polycyclic aromatic hydrocarbon isomers. *J. High. Resolut. Chromatogr.* 1985; 8:248–255.
27. Wise SA, Benner BA, Liu H, Byrd GD, Colmsjoe A. Separation and identification of polycyclic aromatic hydrocarbon isomers of molecular weight 302 in complex mixtures. *Anal. Chem.* 1988; 60:630–637. [PubMed: 3382016]
28. Sander LC, Wise SA. Determination of column selectivity toward polycyclic aromatic hydrocarbons. *J. High. Resolut. Chromatogr.* 1988; 11:383–387.
29. Wise SA, Sander LC, Lapouyade R, Garrigues P. Anomalous behavior of selected methyl-substituted polycyclic aromatic hydrocarbons in reversed-phase liquid chromatography. *J. Chromatogr. A.* 1990; 514:111–122.
30. Wise SA, Deissler A, Sander LC. Liquid Chromatographic Determination of Polycyclic Aromatic Hydrocarbon Isomers of Molecular Weight 278 and 302 in Environmental Standard Reference Materials. *Polycycl. Aromat. Compd.* 1993; 3:169–184.
31. Wise SA, Sander LC, Chang HCK, Markides KE, Lee ML. Shape selectivity in liquid and gas chromatography: Polymeric octadecylsilane (C18) and liquid crystalline stationary phases. *Chromatographia.* 1988; 25:473–479.
32. Schubert P, Schantz MM, Sander LC, Wise SA. Determination of Polycyclic Aromatic Hydrocarbons with Molecular Weight 300 and 302 in Environmental-Matrix Standard Reference Materials by Gas Chromatography/Mass Spectrometry. *Anal. Chem.* 2003; 75:234–246. [PubMed: 12553757]
33. Poster DL, Kucklick JR, Schantz MM, Vander Pol SS, Leigh SD, Wise SA. Development of a House Dust Standard Reference Material for the Determination of Organic Contaminants. *Environ. Sci. Technol.* 2007; 41:2861–2867. [PubMed: 17533850]
34. Poster DL, Benner BA, Schantz MM, Sander LC, Wise SA, Vangel MG. Determination of Methyl-Substituted Polycyclic Aromatic Hydrocarbons in Diesel Particulate-Related Standard Reference Materials. *Polycycl. Aromat. Compd.* 2003; 23:113–139.
35. Farkas O, Héberger K, Zenkevich IG. Quantitative structure-retention relationships XIV Prediction of gas chromatographic retention indices for saturated O-, N-, and S-heterocyclic compounds. *Chemom. Intell. Lab. Syst.* 2004; 72:173–184.
36. Wise, SA.; Sander, LC. Chromatographic Separations Based on Molecular Recognition. Jinno, K., editor. Wiley-VCH; 1997. p. 1-64.
37. Wise, SA.; Campbell, RM.; May, WE.; Lee, ML.; Castle, RN. Polynuclear Aromatic Hydrocarbons, Seventh International Symposium. Cooke, M.; Dennis, AJ., editors. Battelle Press; 1983. p. 1247-1266.
38. Wilson WB, Sander LC, Lee ML, Wise SA. Retention behavior of alkyl-substituted polycyclic aromatic sulfur heterocycles in reversed-phase liquid chromatography. *J. Chromatogr. A.* in preparation.
39. Sander, LC.; Wise, SA. Washington, DC: 1997. PAH Structure Index, NIST Special Publication 922. <http://ois.nist.gov/cfdocs/nistpubs/sp/922/>
40. Miller, MJ.; Miler, JC. Statistics and Chemometrics for Analytical Chemistry. 6th. New York: Pretice-Hall, Inc.; 2000.

Fig. 1.
Molecular structures of the three-ring and four-ring PASHs included in the present study.

Fig. 2.
LC separations of the four three-ring PASH isomers on the monomeric and polymeric C₁₈ phases.

Fig. 3.
LC separations of the 13 four-ring PASH isomers on the monomeric and polymeric C₁₈ phases.

Fig. 4.

Plot of retention ($\log I$) on the polymeric C_{18} phase versus L/B value for the 13 four-ring PASHs with MM 234 Da. Data point assignment: (1) P34T, (2) P32T, (3) P23T, (4) P910T, (5) BbN12T, (6) P43T, (7) A21T, (8) P21T, (9) A12T, (10) P12T, (11) BbN23T, (12) BbN21T, and (13) A23T (See Fig. 1 for structures). PASHs with the sulfur atom located in the bay-region are identified with an asterisk (*) in the graph.

Fig. 5.
Molecular structures of the five-ring peri-condensed PASHs isomers with MM 258 Da included in the present study.

Fig. 6.
Molecular structures of the five-ring cata-condensed PASHs isomers with MM 284 Da included in the present study.

Fig. 7.

LC separations of the 10 five-ring peri-condensed PASH isomers with MM 258 Da on the monomeric and polymeric C₁₈ phases. The graph insert shows the plot of retention ($\log I$) on the polymeric C₁₈ phase versus L/B value for the ten five-ring PASHs isomers with MM 258 Da. Data point assignment for the plot for the insert: (1) B12P43T, (2) TriP45T, (3) Py45T, (4) B12P34T, (5) Py21T, (6) B45P19T, (7) B45P91T, (8) Py12T, (9) C45T, and (10) TeP112T.

Fig. 8.

LC separations of the 20 five-ring PASH isomers with MM 284 Da on the monomeric and polymeric C₁₈ phases.

Fig. 9.

Plot of retention ($\log I$) on the polymeric C_{18} phase versus L/B value for the (A) 20 five-ring PASHs isomers with MM 284 Da. (B) Plot of retention ($\log I$) on the polymeric C_{18} phase versus L/B value for the six non-planar five-ring PASHs isomers with MM 284 Da. (C) Plot of retention ($\log I$) on the polymeric C_{18} phase versus T value for the six nonplanar five-ring PASH isomers with MM 284 Da. Data point assignment for the plot in B and C: (1) BbP43T, (2) DiN[21:12]T, (3) TriP21T, (4) TriP23T, (5) B34P12T, (6) TriP12T, (7) A21BT, (8) BbP32T, (9) DiN[21:23]T, (10) BbP910T, (11) BbP23T, (12) A23BT, (13) BbP34T, (14)

DiN[23:23]T, (15) DiN[12:12]T, (16) A12BT, (17) DiN[12:21]T, (18) BbP12T, (19) BbP21T, and (20) DiN[12:23]T.

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Fig. 10.
Molecular structures of the six-ring PASHs with MM 282 Da and MM 334 Da included in the present study.

Fig. 11.
LC separations of the 12 six-ring PASH isomers with MM 334 Da on the monomeric and polymeric C₁₈ phases.

Fig. 12.

(A) Plot of retention ($\log I$) on the polymeric C_{18} phase versus L/B value for the 12 six-ring PASHs isomers with MM 334 Da. (B) Plot of retention ($\log I$) on the polymeric C_{18} phase versus L/B value for the nine nonplanar six-ring PASHs isomers with MM 334 Da. (C) Plot of retention ($\log I$) on the polymeric C_{18} phase versus T value for the nine nonplanar six-ring PASH isomers with MM 334 Da. Data point assignment: (1) BbB56P43T, (2) N[21:34]P12T, (3) BbTriP12T, (4) BbC21T, (5) BbC43T, (6) N21P910T, (7) BbB56P23T, (8) BbB56P12T, (9) BbC23T, (10) BbB56P21T, (11) BbTriP21T, and (12) BbC12T.

Fig. 13.

Molecular structures of the seven-ring PASHs with MM 306 Da, MM 332 Da and MM 384 Da included in the present study.

Fig. 14. LC separations of seven-ring PASHs with MM 306 Da and MM 332 Da on the monomeric (A) and polymeric (B) C₁₈ phases, and LC separations of seven-ring PASHs with MM 384 Da on the monomeric (C) and polymeric (D) C₁₈ phases.

Table 1

LC conditions for separating isomeric 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 PASHs					
<i>MM 184 Da</i>	8.0 ^b	85	15	1.0	254
Four-ring PASHs					
<i>MM 208 and 234 Da</i>	30.0 ^b	85	15	1.0	254
Five-ring PASHs					
<i>MM 258 Da</i>	20.0 ^b	85	15	1.0	254
<i>MM 284 Da</i>	0.0	85	15	1.5	254
	30.0	100	0		
	60	100	0		
Six-ring PASHs					
<i>MM 282 Da</i>	30.0 ^b	100	0	1.5	294
<i>MM 334 Da</i>	0.0	85	15	1.5	254
	30.0	100	0	1.5	
	31.0	100	0	2.0	
	60	100	0	2.0	
Seven-ring PASHs					
<i>MM 306 and 332 Da</i>	65.0 ^b	100	0	2.0	313
<i>MM 384 Da</i>	70.0 ^b	100	0	2.0	304

^aVolume fraction.

^bIsocratic conditions for the total run time.

Table 2

LC retention indices and molecular shape parameters for three and four-ring PASHs isomers.

PASHs	Molecular Dimensions			Log I		
	L (Å)	W (Å)	T (Å)	L/B	Monomeric C ₁₈	Polymeric C ₁₈
Three-ring PASHs						
MM 184 Da						
DBT	11.62	8.02	4.06	1.45	3.28	2.84
N12T	11.28	8.07	4.16	1.40	2.84	2.90
N21T	11.28	8.07	4.06	1.40	2.78	2.74
N23T	11.67	7.52	4.07	1.55	2.91	2.89
Four-ring PASHs						
MM 208 Da						
P19T	11.08	9.34	4.06	1.19	3.47	3.33
MM 234 Da						
A12T	13.39	8.70	4.06	1.54	3.92	3.97
A21T	13.51	8.56	4.05	1.58	3.76	3.78
A23T	14.12	7.49	4.05	1.88	3.90	4.45
B6N12T	12.72	9.31	4.39	1.37	4.00	3.75
B6N21T	13.66	8.13	4.06	1.68	4.13	4.22
B6N23T	13.90	8.17	4.06	1.70	4.05	4.05
P12T	13.49	8.11	4.15	1.66	3.90	3.99
P21T	13.35	8.12	4.05	1.65	3.70	3.82
P23T	13.43	8.75	4.05	1.54	3.70	3.67
P32T	13.74	8.75	4.06	1.57	3.66	3.64
P34T	12.27	9.23	4.06	1.32	3.73	3.50
P43T	12.27	9.23	4.06	1.33	3.86	3.75
P9,10T	11.83	10.50	4.06	1.13	3.88	3.71

Table 3

LC retention indices and molecular shape parameters for the five-ring PASH isomers.

PASHs	Molecular Dimensions				Log I	
	L (Å)	W (Å)	T (Å)	L/B	Monomeric C_{18}	Polymeric C_{18}
<i>MM 258 Da</i>						
B12P34T	11.93	10.44	4.05	1.14	4.63	4.44
B12P43T	11.92	10.46	4.05	1.14	5.02	4.09
B45P19T	13.28	9.35	4.05	1.42	4.47	4.55
B45P91T	13.31	9.37	4.10	1.42	4.47	4.55
C45T	13.81	9.28	4.05	1.49	4.76	4.74
Py12T	13.51	9.34	4.06	1.45	4.59	4.66
Py21T	13.28	9.34	4.06	1.42	4.43	4.52
Py45T	11.43	10.58	4.05	1.08	4.54	4.42
TeP112T	13.64	9.32	4.05	1.46	4.97	4.96
TriP45T	11.83	10.62	4.06	1.11	4.60	4.39
<i>MM 284 Da</i>						
A12BT	15.85	8.81	4.06	1.80	5.37	5.08
A21BT	14.45	10.06	4.06	1.44	4.99	4.49
A23BT	14.64	9.30	4.07	1.95	5.06	4.72
B34P12T	13.52	10.57	4.84	1.28	4.89	4.25
B8P12T	15.02	9.38	4.36	1.60	5.22	5.16
B8P21T	15.92	8.17	4.07	1.95	5.36	5.43
B8P23T	15.98	8.76	4.06	1.83	4.51	4.70
B8P32T	14.99	9.46	4.06	1.59	4.91	4.52
B8P34T	14.47	9.68	4.10	1.50	5.32	4.79
B8P43T	12.40	10.30	5.35	1.20	4.91	4.06
B8P910T	13.72	10.64	4.33	1.29	5.30	4.65
DiN[12:12]T	15.11	9.34	4.06	1.62	5.40	4.91
DiN[12:21]T	15.58	8.58	4.06	1.82	5.66	5.12
DiN[12:23]T	16.14	8.23	4.06	1.96	5.47	5.43

PASHs	Molecular Dimensions				<i>Log I</i>	
	<i>L_z</i> (Å)	<i>W</i> (Å)	<i>T</i> (Å)	<i>L/B</i>	Monomeric C ₁₈	Polymeric C ₁₈
DiN[21:12]T	12.60	10.23	5.22	1.23	4.97	4.15
DiN[21:23]T	14.53	9.746	4.20	1.49	4.99	4.53
DiN[23:23]T	16.23	8.94	4.06	1.82	5.19	4.81
TriP12T	13.73	10.69	4.09	1.29	4.76	4.39
TriP21T	13.28	10.56	4.79	1.26	4.64	4.16
TriP23T	13.70	11.28	4.07	1.22	4.51	4.21

Table 4
LC retention indices and molecular shape parameters for the six and seven-ring PASH isomers.

PASHs	Molecular Dimensions				Log I	
	L (Å)	W (Å)	T (Å)	L/B	Monomeric C ₁₈	Polymeric C ₁₈
Six-ring PASHs						
MM 282 Da						
B1011C45T	13.49	9.78	4.08	1.38	5.83	5.31
B45TriP112T	11.86	10.54	4.06	1.13	5.57	4.78
Per112T	11.71	10.60	5.06	1.11	5.05	4.03
MM 334 Da						
BbB56P12T	16.26	9.50	5.40	1.71	6.05	4.96
BbB56P21T	16.49	9.84	4.96	1.68	6.33	5.38
BbB56P23T	16.04	10.20	5.06	1.57	5.82	4.86
BbB56P43T	11.79	11.73	6.70	1.04	5.14	4.15
BbC12T	16.91	9.77	4.13	1.73	6.16	5.58
BbC21T	18.10	8.16	4.06	2.22	5.70	4.61
BbC23T	18.29	8.81	4.06	2.08	5.00	5.18
BbC43T	14.69	10.74	5.52	1.37	5.89	4.67
BbTriP12T	14.24	10.51	5.83	1.36	5.78	4.48
BbTriP21T	15.93	10.64	4.56	1.50	6.27	5.46
N21P910T	14.46	10.56	5.47	1.37	6.19	4.70
N[21:34]P12T	13.54	11.93	5.76	1.14	5.55	4.37
Seven-ring PASHs						
MM 306 Da						
B67Per112T	12.03	11.84	4.06	1.03	6.41	5.22
MM 332 Da						
Diacen[12:12]T	15.29	9.82	4.06	1.56	6.25	5.83
N[218:345]Py12T	13.53	11.84	4.07	1.14	5.43	4.22
MM 384 Da						
BbB56C12T	16.79	11.02	5.90	1.52	6.70	5.13

PASHs	Molecular Dimensions				Log I	
	L_z (Å)	W (Å)	T (Å)	L/B	Monomeric C ₁₈	Polymeric C ₁₈
BbB56C21T	18.03	10.55	5.25	1.71	7.03	6.12
BbB56C43T	15.94	10.83	6.53	1.47	6.43	5.29
DiP[910:910]T	15.30	11.07	5.66	1.38	7.37	5.36

Table 5Correlation 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
Three-ring PASHs <i>MM 184 Da</i>	4	$y = 0.47x + 2.17$	0.45	2	2.78	0.71 No
Four-ring PASHs <i>MM 234 Da</i>	13	$y = 0.97x + 2.38$	0.75	11	2.23	3.76 Yes
Five-ring PASHs <i>MM 258 Da</i>	10	$y = 1.02x + 3.19$	0.75	8	2.31	3.21 Yes
Five-ring PASHs <i>MM 284 Da</i>	20	$y = 1.44x + 2.47$	0.89	18	2.10	8.31 Yes
Six-ring PASHs <i>MM 334 Da</i>	12	$y = 0.66x + 3.83$	0.51	10	2.23	1.88 No
Seven-ring PASHs <i>MM 384 Da</i>	4	$y = 2.54x + 1.61$	0.80	2	4.30	1.89 No