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Normal-phase liquid chromatography retention behavior of polycyclic aromatic hydrocarbon and their methyl-substituted derivatives on an aminopropyl stationary phase

Walter B. Wilson^{a,*}, Hugh V. Hayes^b, Lane C. Sander^a, Andres D. Campiglia^b, and Stephen A. Wise^a

^aChemical Sciences Division, Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899.

^bDepartment of Chemistry, University of Central Florida, Orlando, Florida 32816.

Abstract

Retention indices for 124 polycyclic aromatic hydrocarbons (PAHs) and 62 methyl-substituted (Me-) PAHs were determined using normal-phase liquid chromatography (NPLC) on a aminopropyl (NH₂) stationary phase. PAH retention behavior on the NH₂ phase is correlated to the total number of aromatic carbons in the PAH structure. Within an isomer group, non-planar isomers generally elute earlier than planar isomers. MePAHs generally elute slightly later but in the same region as the parent PAHs. Correlations between PAH retention behavior on the NH₂ phase and PAH thickness (*T*) values were investigated to determine the influence of non-planarity for isomeric PAHs with four to seven aromatic rings. Correlation coefficients ranged from $r = 0.19$ (five-ring *peri*-condensed molecular mass (MM) 252 Da) to $r = -0.99$ (five-ring *cata*-condensed MM 278 Da). In the case of the smaller PAHs (MM 252 Da), most of the PAHs had a planar structure and provided a low correlation. In the case of larger PAHs (MM 278 Da), nonplanarity had a significant influence on the retention behavior and good correlation between retention and *T* was obtained for the MM 278 Da, MM 302 Da, MM 328 Da, and MM 378 Da isomer sets.

Keywords

normal-phase liquid chromatography; retention index; polycyclic aromatic hydrocarbons; non-planarity

*Corresponding author. walter.wilson@nist.gov, Phone number: 301-975-3438.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of environmental pollutants that have been identified as potential carcinogens and studies have shown that exposure to PAHs can lead to an increased risk of cancer [1–3]. These compounds often exist in complex mixtures of condensed multi-ring benzenoid compounds that originate from natural and anthropogenic sources. Methods used to quantify PAHs in complex matrix samples typically employ a single chromatographic technique or a combination of techniques such as normal-phase liquid chromatography (NPLC), reversed-phase (RP)-LC, and/or gas chromatography (GC). RPLC separations on a polymeric octadecylsilane (C_{18}) stationary phase [4] and GC [5] on a 50% phenyl methylpolysiloxane (MPS) phase or 50% liquid crystalline dimethylpolysiloxane stationary phase has been shown to provide excellent separations of isomeric PAHs. NPLC is often used as a low resolution fractionation technique to isolate PAHs for further characterization by RPLC and/or GC [6–19].

Silica and alumina were the classical adsorbents used for decades with nonpolar solvents in open column chromatography for the isolation of aliphatic and aromatic hydrocarbons (including PAHs). However, because of high absorptivity and the influence of moisture content, the use of these adsorbents for routine NPLC fractionation of PAHs was unreliable. In the late 1970s with the emergence of chemically-bonded LC stationary phases, the use of polar functionalized stationary phases in a NP mode became a viable alternative. Wise et al. [10] introduced the use of an aminopropyl (NH_2) stationary phase for the separation and isolation of PAHs from complex mixtures. Wise et al. [10] investigated the retention behavior of 42 alkyl-substituted benzenes and two-ring to five-ring PAHs on the NH_2 phase and compared the retention characteristics with classical silica [20] and alumina [21] data from the literature. They concluded that with the NH_2 phase.... “when a nonpolar mobile phase such as *n*-hexane is used, a hydrocarbon class separation similar to that obtained on silica and alumina is achieved, i.e., saturated hydrocarbons elute before olefinic and aromatic hydrocarbons, and the elution volumes for the aromatic hydrocarbons increase with the number of condensed rings.”. In contrast to classical adsorbents where the addition of alkyl groups to the aromatic ring generally increased retention, they observed that the presence of an alkyl group had only a slight effect on retention, thereby providing a more distinct class (aromatic ring) fractionation with more reproducible retention than silica or alumina [10]. Wise et al. [10] attributed the retention behavior on the NH_2 phase primarily to “the interaction between the aromatic π electrons of the PAHs and the polar amino group of the stationary phase.”. Following this initial study, Wise et al. [22] published additional NPLC retention data, as retention indices, for 31 parent PAHs with two to six aromatic rings and 38 alkyl-substituted PAHs commenting that retention was “... based on the number of condensed aromatic rings and on some steric considerations.”. A later study focusing on six-ring PAHs of molecular mass (MM) 302 Da reported the retention indices for 19 of these six-ring isomers [6].

Since these initial studies reporting retention indices [10, 22], Wise and coworkers have used the NH_2 stationary phase extensively for fractionation of complex PAH matrices [6–8, 11–14] without expanding the investigation of the retention behavior to a greater number of PAHs. The original studies [10, 14, 22] were incorrect in looking at the separation as based

on increasing number of condensed aromatic rings. In subsequent studies [6–8, 11–13] using the NH₂ phase for PAH fractionation, this concept changed to the retention being based on the number of aromatic carbons rather than aromatic rings. May and Wise [11] first stated that the retention of PAHs on the NH₂ phase was based on number of aromatic carbons, and they reported retention indices for seven deuterated PAHs, which were used as internal standards in PAH fractions isolated on the NH₂ phase. In those studies, however, there was no formal evaluation or discussion of the retention based on number of aromatic carbons. In the current study, we revisit the NPLC retention of PAHs on the NH₂ phase for a significantly greater number (i.e., 186) of PAHs and alkyl-PAHs and investigate the relationship of retention and number of aromatic carbons and the influence of non-planarity on the retention behavior. A similar study of NPLC retention behavior for polycyclic aromatic sulfur heterocycles (PASHs) and their alkylated derivatives is published elsewhere [23]. In a companion study [24] the NPLC retention behavior of PAHs and MePAHs reported here is used as the basis to develop a NPLC fractionation procedure combined with GC/MS for characterizing the complex mixture of PAHs in a coal tar sample.

Materials and methods

Materials

Chemicals—Reference standards were obtained from several commercial sources including Bureau of Community Reference (Brussels, Belgium), Chiron AS (Trondheim, Norway), W. Schmidt (Ahrensburg, Federal Republic of Germany), Pfaltz and Bauer, Inc. (Waterbury, CT), Fluka Chemie AG. (Buchs, Switzerland), and the National Cancer Institute of Chemical Carcinogen Repository (Bethesda, MD). Additional reference standards were obtained from J. Jacob and G. Grimmer (Biochemical Institute for Environmental Carcinogens, Ahrensburg, Federal Republic of Germany), J. Fetzer (Chevron Research Co., Richmond CA), and A. K. Sharma (Penn State University, College of Medicine, Department of Pharmacology, Hershey, Pennsylvania, USA). The names and abbreviations for these standards are listed in Tables 1 – 5. HPLC grade *n*-hexane, dichloromethane (DCM), and toluene were purchased from Fisher Scientific (Pittsburgh, PA, USA).

Instrumentation and chromatographic columns—NPLC-UV was performed using a liquid chromatograph (1200 series, Agilent Technologies, 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 an NH₂ analytical column purchased from Waters (Milford, MA) with the following characteristics: 25.0 cm length, 4.6 mm inner diameter, and 5 μm average particle diameters.

Methods

Thickness Calculations—The procedure for calculating the thickness (*T*) dimension has been described in detail previously [25, 26]. Typically, non-substituted PAHs and MePAHs with $T \approx 3.90 \text{ \AA}$ and $T \approx 4.20 \text{ \AA}$, respectively, are considered to be planar molecules. As the *T* value increases, the degree of non-planarity increases for the PAH structure.

NPLC retention index data—NPLC retention index values ($\log I$) were calculated according to Eq. (1) with the following index markers: (2) Naph, (3) Phe, (4) BaA, (5) BbC, (6) DBbkC, (7) BbN12kC [26].

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

R is the corrected retention volume, x represents the solute, and n and $n + 1$ represent the lower and higher eluting PAH standards. All retention index data was obtained at room temperature using the NH_2 analytical column with a mobile phase of 98 % n -hexane and 2 % DCM with a flow rate of 1.0 mL/min except for the seven-ring MM 352 Da, seven-ring MM 378 Da isomer group, and the eight-ring PAHs (80 % n -hexane and 20 % DCM). Previous studies have investigated the retention behavior of PAHs on NH_2 stationary phase using $\log I$ values as their basis for retention indices using index markers 2 – 5 [26, 6]. DBahP was used as the six-ring index marker because of a lack of reference standard at that time for DBbkC. DBbkC and BbN12kC represent the six-ring and seven-ring, respectively, of the series of *cata*-condensed PAHs used as retention standards (Fig. S1). The $\log I$ values are based on three chromatographic runs obtained from reference standards. The standard deviation of $\log I$ values was equal to or less than ± 0.02 . Baseline resolution of two components could be achieved with a difference of ~ 0.08 $\log I$ units on the NH_2 analytical column.

Statistical t-test calculations—The method for calculating statistical t -test for linear correlations between L/B and T has been described in detail previously using Eq. (2) [27, 28]:

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

where r is the correlation coefficient, df is the degrees of freedom ($n - 2$), and n is the number of data points. Based on the df , the correlation coefficient shows a significant linear trend if the calculated t_{cal} value is greater than the critical t -test value (t_{crit}). These statistical calculations will be used in the results and discussion section to correlate the NPLC retention behavior of isomeric PAHs and their structural non-planarity (T).

Results and Discussion

The NPLC retention indices ($\log I$), molecular mass (MM), number of aromatic carbons, and thickness (T) values for the two-ring and three-ring, four-ring, five-ring, six-ring, and seven-ring and eight-ring PAHs are summarized in Tables 1 – 5, respectively. The retention indices in Tables 1 – 5 represent the most extensive compilation of NPLC retention data for PAHs on the NH_2 phase to be published to date. The $\log I$ values reported here and the values from the original papers by Wise et al. [6, 10, 11, 22] discussed previously are generally in agreement relative to elution orders. However, because of the improvements in LC packing

materials (10 μm irregular particles vs. 5 μm spherical particles), the analytical column used to generate the $\log I$ data in this study is much more efficient. Thus, PAHs with differences in retention indices of 0.07 – 0.10 $\log I$ are baseline resolved in the current study whereas in the original studies a difference of 0.10 to 0.12 $\log I$ units was generally required to separate two PAHs [22].

Since the original studies [10, 14, 22], researchers have reported retention behavior for a very limited number of PAHs (especially isomers) with the following polar functionalized stationary phases: NH_2 [15, 29], triamine [30, 31], silica [30], alumina [30], 2,4-dinitroanilinoctylsilica [31], 2,3,6-trinitroanilinoctylsilica [31], aminocyano [15, 32], dihydroxypropyl propyl ether [33], nitrophenyl [32], poly(4-vinylpyridine)-grafted silica [34], 3-(2,4-Dinitroanilino)propyl [30, 31, 35], and hypercrosslinked polystyrene [35]. Despite these additional polar stationary phases, the NH_2 phase has been the predominant phase used for NPLC to isolate isomeric PAHs based on the number of aromatic carbon atoms [6, 7, 9]. Herein, we investigate this correlation by plotting the $\log I$ averages vs. the total number of aromatic carbon atoms in Fig. 1a. The error bars represent the standard deviation for the $\log I$ averages, which demonstrate that the retention behavior varies for PAHs with the same number of aromatic carbon atoms. This correlation is better illustrated in Fig. 1b, which plots the $\log I$ values vs. the total number of aromatic carbon atoms for each of the 124 unsubstituted parent PAHs. The MePAHs provided similar results as the unsubstituted parent PAHs, and the corresponding plots are provided in Figs. S2a and S2b. Differences among the retention of isomeric PAHs or MePAHs on the NH_2 phase may be due to differences in molecular shape and/or non-planarity, which will be discussed in the following sections.

In this study, 85 of the 124 parent PAHs included in this study have planar molecular structures, as indicated by the T parameter ($T = 3.88 \text{ \AA}$ to $4.24 \text{ \AA}$). The remaining PAHs have various degrees of non-planarity within the following ranges for T : $T = 4.25 \text{ \AA}$ to $5.00 \text{ \AA}$ (27 PAHs), $T = 5.01 \text{ \AA}$ to $5.50 \text{ \AA}$ (12 PAHs), and $T > 5.50 \text{ \AA}$ (10 PAHs). As shown in Fig. 1b, three of the five PAHs with the highest degree of non-planarity ($T > 5.50 \text{ \AA}$) are less retentive than the PAHs with the same number of aromatic carbon atoms and $T < 5.50 \text{ \AA}$. The four isomeric groups of PAHs with 22, 24, 26, and 30 aromatic carbon atoms are plotted in Fig. 1c using the T parameter as a third dimension (size of the circle) to illustrate the influence of non-planarity on the retention behavior of PAHs. One of the isomer groups included in Fig. 1c is the six-ring PAHs with MM 302 Da (24 aromatic carbons), which primarily consist of two structural subsets: pyrene backbone (10 isomers) and fluoranthene backbone (12 isomers). The pyrene backbone for the MM 302 Da isomers consists of four benzene rings and the fluoranthene backbone consists of three benzene rings and one cyclopenta-1,3-diene ring. Based on the MM 302 Da isomer structure schemes shown in Fig. S3a and S3b, the average $\log I$ values for the PAHs are plotted vs. the total number of aromatic carbon atoms in Fig. 1d. In each case, the fluoranthene backbone PAHs were slightly more retentive than the pyrene backbone PAHs.

Two-ring and three-ring PAHs

The retention indices ($\log I$) and T values for the two-ring and three-ring PAHs and MePAHs are summarized in Table 1. Ant and Phe are the two parent PAHs in this isomer group (Fig. S4). Although both isomers have the same T value, they are baseline resolved on the NH_2 phase with Ant eluting first. Ant is the linear structure and Phe has the presence of a *bay*-region as shown in Fig S4, which is discussed in more detail in the next section. Similar studies have shown the importance of a *bay*-region for the retention behavior of PASH in RPLC on a polymeric C_{18} stationary phase [27]. In the case of MePAHs, the two of the four possible MeFlu isomers (MM 180 Da), three possible MeAnt isomers (MM 192 Da), and four of the five possible MePhe isomers (MM 192 Da) were evaluated on the NH_2 phase (Fig S5, Fig S6, and Fig S7, respectively). The only isomer with MM of 192 Da with a non-planar structure is 4-MePhe with $T = 4.69 \text{ \AA}$, and it elutes slightly before the three other MePhe isomers, which all elute after Phe.

Four-ring PAHs

The retention indices ($\log I$) and T values for the four-ring PAHs and MePAHs are summarized in Table 2. NPLC chromatograms obtained from individual reference standards of the four-ring PAHs are shown in Fig 2. The elution order among the four-ring PAH groups follows the increasing MM and number of aromatic carbon atoms of each isomer groups, i.e., MM 202 Da, MM 216 Da, and MM 228 Da. The three MM 202 Da isomers are nearly baseline resolved, while the three MM 216 Da isomers co-elute. NPLC chromatograms obtained from individual reference standards of the MeFluor and MePyr isomers are shown in Fig. S8 and Fig. S9, respectively. MeFluor and MePyr isomers have similar retention behaviors within the group due to their planar structures ($T \approx 4.21 \text{ \AA}$). Comparisons with their parent PAH, i.e., Fluor and Pyr, reveal some differences in their retention behavior. Pyr elutes at $\log I$ of 3.44 and the three MePyr elute with $\log I$ values ranging from 3.38 to 3.43 while Fluor has a $\log I$ value of 3.51 and the four MeFluor elute later with $\log I$ values ranging from 3.57 to 3.65.

In the case of the *cata*-condensed MM 228 Da isomers, BaA and Chr co-elute but are resolved from the remaining three isomers, which are baseline resolved. PAHs with similar T values would be expected to have similar retention behaviors on the NH_2 stationary phase. BaA, Chr, and Nap are the three planar PAHs with MM 228 Da. Similar to the retention behaviors of Ant and Phe, the linear isomer Nap elutes first followed by BaA and Chr that have *bay*-regions. BcPhe and TriPhe are non-planar with T values of 4.99 and 4.37 $\text{\AA}$, respectively. BcPhe elutes earlier than the planar PAHs due to its non-planarity. TriPhe elutes last among the five MM 228 Da isomers despite the moderately non-planar structure but it does have three *bay*-regions in the structure. A plot of retention on the NH_2 stationary phase vs. T for the five MM 228 Da isomers is shown in Fig S10 with a correlation coefficient of $r = -0.70$, which provides a t_{cal} value of 1.71 ($t_{\text{crit}} = 3.18$, $\alpha = 0.05$, $n = 5$) indicating that there is not a significant linear trend between PAH retention and T values [36].

In the case of the 26 methyl-substituted four-ring *cata*-condensed PAHs (MM 242 Da), a plot of retention on the NH_2 stationary phase vs. T is shown in Fig. 3a with a correlation

coefficient of $r = -0.79$, which provides a t_{cal} value of 6.21 ($t_{\text{crit}} = 2.07$, $\alpha = 0.05$, $n = 26$) indicating that there is a significant linear trend between the 26 *cata*-condensed MePAH retention and T values [36]. Based on the $\log I$ values reported in Table 2, non-planarity does play a significant role in the retention behavior of MePAHs on the NH_2 stationary phase. Two examples of the importance of non-planarity are illustrated with BcPhe and Chr, their molecular structures are shown in Fig. 3b. The NPLC chromatograms obtained from individual standards of BcPhe and six MeBcPhe isomers are shown in Fig 3c. 1-MeBcPhe elutes prior to BcPhe on the NH_2 stationary phase. This early elution behavior can be attributed to the higher degree of non-planarity as indicated by the larger T value ($T = 5.94 \text{ \AA}$). Similar results were obtained for the MeBaA isomers, where 1-Me ($T = 5.04 \text{ \AA}$) and 12-MeBaA ($T = 5.16 \text{ \AA}$) eluted prior to BaA. Of the *cata*-condensed four-ring MePAH isomers, the six MeChr isomers provided the best correlation for their retention on the NH_2 stationary phase vs. T providing a correlation coefficient of $r = -0.99$ and a t_{cal} value of 14.14 indicating that there is a significant linear trend ($t_{\text{crit}} = 2.78$, $\alpha = 0.05$, $n = 6$) [36]. The NPLC chromatograms obtained from individual MeChr standards are shown in Fig. 3d. The earlier elution for 4-Me and 5-MeChr in comparison to the other four MeChr isomers can be explained by the larger T values of $4.97 \text{ \AA}$ and $5.01 \text{ \AA}$, respectively. NPLC chromatograms obtained from individual reference standards of the MeBaA and MeTriPhe isomers are shown in Fig. S11 and Fig. S12, respectively.

Five-ring PAHs

The retention indices ($\log I$) and T values for the five-ring PAHs and MePAHs are summarized in Table 3. NPLC chromatograms obtained from individual reference standards of the 12 five-ring MM 252 Da PAHs and 11 five-ring MM 278 Da PAHs are shown in Fig. 4. In the case of the MM 252 Da isomers, the T values for the MM 252 Da isomers are $\sim 3.90 \text{ \AA}$ indicating planarity except for BiAcep ($4.73 \text{ \AA}$). Because of the planarity of this PAH isomer group, their retention on the NH_2 stationary phase and T values did not provide a significant linear correlation ($r = 0.09$, data not shown).

In the case of the five-ring MM 278 Da PAH isomers (*cata*-condensed), non-planarity was expected to play an important role in their retention behaviors based on their T values and previous discussions including the four-ring *cata*-condensed PAHs. BbC, Pic, DBajA, Pent, BaNap, DBahA, and DBacA are planar with T values of $\approx 3.89 \text{ \AA}$. DBcgP, DBbgP, BcC, and BgC are non-planar with T values ranging from $5.23 \text{ \AA}$ to $6.24 \text{ \AA}$. The chromatograms in Fig. 4 clearly indicate the effect of non-planarity on the retention behavior. The seven planar isomers elute within 24 min – 27 min, while the non-planar isomers ($T \approx 5.30 \text{ \AA}$) elute earlier at 18.5 min – 20 min and the highly non-planar isomer DBcgP ($T \approx 6.24 \text{ \AA}$), elutes at ~ 15.5 min. The correlation of retention on the NH_2 phase and T value for the 11 MM 278 Da isomers is shown in Fig. 5a with a correlation coefficient of $r = -0.99$. The t_{cal} value is 23.45 ($t_{\text{crit}} = 2.16$, $\alpha = 0.05$, $n = 11$) indicating that there is a significant linear trend between the retention of *cata*-condensed five-ring PAH isomers and T values [36].

A number of five-ring MePAH isomer sets were investigated including two *peri*-condensed isomer sets (12 MeBaP and 3 MePer) and one *cata*-condensed MePAH isomer set (5 MePic). NPLC chromatograms obtained from individual reference standards for these isomers are

shown in Fig. S13, Fig. S14, and Fig. S15, respectively. In the case of the *peri*-condensed isomer sets, the MePAH isomers with the methyl group located in the *bay*-region of the structure provided the highest degree of non-planarity with $T = 4.89 \text{ \AA}$ (1-MePer, 10-MeBaP, and 11-MeBaP). Similar behaviors were observed for 1-Me, 6-Me, and 13-MePic with T values of $5.39 \text{ \AA}$, $5.56 \text{ \AA}$, and $5.42 \text{ \AA}$, respectively. In all cases, the MePAHs eluted later than the parent PAH and the non-planar MePAH eluted earlier than the other MePAH isomers (exception is 1-MePer).

Six-ring PAHs

The retention indices ($\log I$) and T values for the six-ring PAHs are summarized in Table 4. The six-ring PAHs consist of the following isomer groups: (1) MM 276 Da; (2) MM 302 Da; and (3) MM 328 Da. NPLC chromatograms obtained from individual reference standards of the MM 276 Da PAH isomers are shown in Fig. S16. A large number of non-planar PAH isomers are included in the MM 302 Da and MM 328 Da groups with seven isomers ($T = 4.84 \text{ \AA}$) and six isomers ($T = 4.85 \text{ \AA}$), respectively. In addition, the MM 328 Da PAH isomers represent the group with the largest differences in T values among all isomer sets investigated with T ranging from $3.88 \text{ \AA}$ (DBaNap) to $7.41 \text{ \AA}$ (Phe34cPhe).

NPLC chromatograms obtained on the NH_2 phase with individual reference standards of the 25 six-ring MM 302 Da PAHs are shown in Fig. 6. Wise et al. [6] reported $\log I$ values for 19 isomers of MM 302 Da using different retention marker PAHs for the six-ring and seven-ring markers. The elution order of the 19 isomers in the original report and this study are similar. Of particular interest is the fact that 6 of the 8 non-planar isomers elute distinctively before ($< 29 \text{ min}$) the remaining 18 investigated isomers ($> 30 \text{ min}$) investigated. The other two nonplanar isomers, BbPer and DBNap, showed the highest affinity for the NH_2 , which could be influenced by the presence of *bay*-regions in their molecular structures (1 and 2, respectively). In addition to the strongly non-planar isomers ($T = 4.84 \text{ \AA}$), there is a group of five MM 302 Da isomers that are moderately non-planar with T values ranging from $4.19 \text{ \AA}$ (DBaP and N21aP) to $4.64 \text{ \AA}$ (DBbF). With the exception of DBbF, the moderately non-planar PAHs elute with the planar MM 302 Da isomers ($T \approx 3.90 \text{ \AA}$) between $30 - 37 \text{ min}$. A plot of retention on the NH_2 stationary phase vs. T for the MM 302 Da isomers is shown in Fig. 5b. A correlation coefficient of $r = -0.71$ and a t_{cal} value of 4.80 ($t_{\text{crit}} = 2.08$, $\alpha = 0.05$, $n = 25$) indicates the existence of a linear trend. Similar to the MM 278 Da isomers, the general trend for elution on the NH_2 phase for the MM 302 Da isomers is influenced by structure non-planarity.

NPLC chromatograms obtained on the NH_2 phase with individual reference standards of the 17 six-ring MM 328 Da *cata*-condensed PAHs are shown in Fig. 7. For this isomer set, 5 of the 6 non-planar isomers elute ($< 35 \text{ min}$) distinctively before the remaining 12 isomers ($> 40 \text{ min}$). The extreme non-planarity of Phe34cPhe, DBgpC, and DBcpC resulted in similar $\log I$ values as the MM 276 Da and the non-planar MM 302 Da isomers. In the case of DBgpC and Phe34cPhe, their non-planarity has been used extensively to characterize C_{18} stationary phases in RPLC [37–39]. BhPen with a $T = 4.85 \text{ \AA}$ elutes $\sim 15 \text{ min}$ later than N12aNap with a $T = 5.00 \text{ \AA}$. Based on previous discussions, the three *bay*-regions in the molecular structure for BhPen may influence the later retention than N12aNap with no *bay*-

regions. Similar observations were made for the MM 302 Da isomers of BbPer ($T = 4.84 \text{ \AA}$) and DBNap ($T = 4.89 \text{ \AA}$), which elute later than N21bF ($T = 4.92 \text{ \AA}$). A plot of retention on the NH_2 stationary phase vs. T for the MM 328 Da isomers is shown in Fig. 5c. The plot provided a correlation coefficient of $r = -0.91$ and a t_{cal} value of 8.60 ($t_{\text{crit}} = 2.13$, $\alpha = 0.05$, $n = 17$) indicating a clear linear trend.

Seven-ring and eight-ring PAHs

The retention indices ($\log I$) and T values for the seven- and eight-ring PAHs are summarized in Table 5. These isomer groups consisted of Cor (MM 300 Da), eight MM 326 Da isomers, Trux (MM 342 Da), three MM 350 Da isomers, three MM 352 Da isomers, two MM 376 Da isomers, and 20 MM 378 Da isomers. NPLC chromatograms obtained on the NH_2 phase with individual reference standards of these PAHs are shown in Fig. S17 – Fig. S20. Cor was the only MM 300 Da isomer included in this study. A total of eight MM 326 Da isomers were available for this study and they can be divided into two sub-groups based on the perylene and fluoranthene structural backbone. The five perylene-based PAHs elute before the three fluoranthene-based PAHs. Additional MM 326 Da reference standards are required to fully explore this trend in more detail. In the case of the perylene-based MM 326 Da isomers, DBcdImPer elutes at ~28 min while the remaining four isomers elute between 37 – 42 min. The three fluoranthene-based isomers are baseline resolved over a 10 min time interval. No correlation of retention vs T was observed.

The molecular structures of the seven-ring *cata*-condensed PAH isomers with MM 378 Da are shown in Fig. S21. This isomer group represents the largest number of non-planar isomers in this study with 10 isomers, which have T values ranging from $4.28 \text{ \AA}$ to $5.95 \text{ \AA}$. A plot of retention on the NH_2 stationary phase vs. T for the MM 378 Da isomers is shown in Fig. 5d. The plot provided a correlation coefficient of $r = -0.74$ and a t_{cal} value of 6.71 ($t_{\text{crit}} = 2.09$, $\alpha = 0.05$, $n = 20$) indicating that there is a significant linear trend. Similar to the MM 278 Da, MM 302 Da, MM 328 Da isomers, the general trend for elution on the NH_2 phase for the MM 378 Da isomers is influenced by non-planarity. The 10 non-planar isomers can be split into three subsets based on the same T values used in Fig. 1: (1) Phe12bC ($4.28 \text{ \AA}$), TriNap ($4.51 \text{ \AA}$), DBacPen ($4.57 \text{ \AA}$), TriBacjNap ($4.77 \text{ \AA}$), and Phe910bC ($4.98 \text{ \AA}$); (2) BaN12jNap ($5.28 \text{ \AA}$), Phe 43bC ($5.33 \text{ \AA}$), Phe34bTriPhe ($5.47 \text{ \AA}$), and BcN21pC ($5.49 \text{ \AA}$); and 3) Phe910bTriPhe ($5.95 \text{ \AA}$).

DBacPen was the only PAH from subset 1 to elute earlier than the planar isomers. It is interesting to note that DBacPen has the fewest number of *bay*-regions (three) and has a linear structural backbone (Nap) with no additional benzene ring attachments. As discussed for the three and four-ring *cata*-condensed PAH isomers, these two structural features result in these PAHs being less retentive on the NH_2 stationary phase. Of the four isomers in subset 2, BcN21pC and Phe34bTriPhe are less retentive than Phe43bC and BaN12jNap, which could be a result of the slight difference in T values ($\sim 0.14 \text{ \AA}$). Phe910bTriPhe (subset 3) was expected to be less retentive than the PAHs in subset 2 based on the results for the non-planar five- and six-ring *cata*-condensed PAHs. However, Phe910bTriPhe eluted after Phe34bTriPhe and BcN21pC. Phe910bTriPhe and Phe34bTriPhe have similar molecular structure backbone except for the placement of one benzene ring, which results in

Phe910bTriPhe having six *bay*-regions and Phe34bTriPhe having three *bay*-regions and one *ff*-region.

Conclusions

The results presented in this paper represent the most extensive investigation of NPLC retention behavior of PAHs and MePAHs on a polar stationary phase (i.e., the NH₂ phase). The NPLC retention of PAHs on the NH₂ phase is primarily based on the number of aromatic carbon atoms in the structure. In the case where there are differences in retention behavior, two main structure features are shown to influence the retention of PAHs on the NH₂ phase. First, the non-planar isomers are less retentive than the planar isomers. Correlations between the non-planarity (*T*) of the MM 278 Da, MM 302 Da, MM 328 Da, and MM 378 Da isomers and their retention on the NH₂ phase were reported with correlation coefficients ranging from $r = -0.71$ (MM 302 Da isomers) to $r = -0.99$ (MM 278 Da isomers). Second, the presence of a *bay*-region in the structure of isomeric PAHs resulted in the PAHs being more retentive on the NH₂ phase. The retention data reported in this study will provide the basis for developing NPLC fractionation strategies to characterize complex PAH mixtures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.

Plots of the number of aromatic carbon atoms vs. the average log I value for the PAHs (a) and the log I value for each PAH (b). The uncertainty listed with each value is the standard deviation for the average log I value for each set of aromatic carbon atoms. Plot of the number of aromatic carbon atoms vs. the average log I and T value for the six-ring and seven-ring PAHs (c). Plot of the number of aromatic carbon vs. the average log I value for the dibenzopyrene and dibenzofluoranthene backbone isomer subsets (d).

Fig 2.
NPLC separation of the four-ring PAH isomers with MM 202 Da, 216 Da, and 228 Da on the NH_2 stationary phase

Fig 3.

(a) Plot of retention on the NH_2 stationary phase versus T values for the 26 methyl-substituted four-ring cata-condensed PAHs. (b) Molecular structures of BcPhe and Chr and their methyl-substitution position numbering. (c) NPLC separation of BcPhe and six MeBcPhe isomers on the NH_2 stationary phase. (d) NPLC separation of Chr and six MeChr isomers on the NH_2 stationary phase

Fig 4.
NPLC separation of the 12 MM 252 Da PAH isomers and the 11 MM 278 Da isomers on the NH_2 stationary phase

Fig 5.

Plots of retention on the NH_2 stationary phase versus T values for the a) 11 MM 278 Da PAH isomers, b) 25 MM 302 Da PAH isomers, c) 17 MM 328 Da PAH isomers, and d) 20 MM 378 Da PAH isomers

Fig 6.
NPLC separation of the 25 MM 302 Da PAH isomers on the NH₂ stationary phase

Fig 7.
NPLC separation of the 17 MM 328 Da PAH isomers on the NH_2 stationary phase

Table 1

NPLC retention indices for the two-ring and three-ring PAHs.

PAHs	Abbreviations	MM (Da)	No. of Aromatic Carbon	Thickness (Å)	Log I NH ₂
Naphthalene	Naph	128	10	3.88	2.00
Acenaphthylene	Acey	152	12	3.88	2.68
Acenaphthene	Ace	154	10	4.22	2.27
Fluorene	Flu	166	12	4.24	2.72
1-Methylfluorene	1-MeFlu	180	12	5.13	2.82
2-Methylfluorene	2-MeFlu	180	12	5.02	2.74
Anthracene	Ant	178	14	3.88	2.93
1-Methylantracene	1-MeAnt	192	14	4.21	2.92
2-Methylantracene	2-MeAnt	192	14	4.20	2.96
9-Methylantracene	9-MeAnt	192	14	4.14	3.03
Phenanthrene	Phe	178	14	3.89	3.00
1-Methylphenanthrene	1-MePhe	192	14	4.22	3.05
2-Methylphenanthrene	2-MePhe	192	14	4.20	3.06
4-Methylphenanthrene	4-MePhe	192	14	4.69	3.02
9-Methylphenanthrene	9-MePhe	192	14	4.22	3.03

Table 2

NPLC retention indices for the four-ring PAHs.

PAHs	Abbreviations	MM (Da)	No. of Aromatic Carbon	Thickness (Å)	Log I NH ₂
Accephenanthrene	Acephe	202	16	3.89	3.59
Fluoranthene	Fluor	202	16	3.88	3.51
1-Methylfluoranthene	1-MeFluor	216	16	4.22	3.63
3-Methylfluoranthene	3-MeFluor	216	16	4.20	3.65
7-Methylfluoranthene	7-MeFluor	216	16	4.22	3.57
8-Methylfluoranthene	8-MeFluor	216	16	4.20	3.59
Pyrene	Pyr	202	16	3.89	3.44
1-Methylpyrene	1-MePyr	216	16	4.20	3.43
2-Methylpyrene	2-MePyr	216	16	4.20	3.38
4-Methylpyrene	4-MePyr	216	16	4.21	3.42
7H-Benzo[c]fluorene	7H-BcF	216	16	4.24	3.72
11H-Benzo[a]fluorene	11H-BaF	216	16	4.24	3.68
11H-Benzo[b]fluorene	11H-BbF	216	16	4.24	3.73
Benzo[a]anthracene	BaA	228	18	3.89	4.00
1-Methylbenzo[a]anthracene	1-MeBaA	242	18	5.04	3.98
2-Methylbenzo[a]anthracene	2-MeBaA	242	18	4.21	4.06
3-Methylbenzo[a]anthracene	3-MeBaA	242	18	4.21	4.08
4-Methylbenzo[a]anthracene	4-MeBaA	242	18	4.22	4.08
5-Methylbenzo[a]anthracene	5-MeBaA	242	18	4.22	4.05
6-Methylbenzo[a]anthracene	6-MeBaA	242	18	4.21	4.15
7-Methylbenzo[a]anthracene	7-MeBaA	242	18	4.57	4.17
8-Methylbenzo[a]anthracene	8-MeBaA	242	18	4.21	4.02
9-Methylbenzo[a]anthracene	9-MeBaA	242	18	4.20	4.06
10-Methylbenzo[a]anthracene	10-MeBaA	242	18	4.20	4.12
11-Methylbenzo[a]anthracene	11-MeBaA	242	18	4.22	3.96
12-Methylbenzo[a]anthracene	12-MeBaA	242	18	5.16	3.97
Benzo[c]phenanthrene	BcPhe	228	18	4.99	3.66

PAHs	Abbreviations	MM (Da)	No. of Aromatic Carbon	Thickness (Å)	Log I NH ₂
1-Methylbenzo[c]phenanthrene	1-MeBcPhe	242	18	5.94	3.60
2-Methylbenzo[c]phenanthrene	2-MeBcPhe	242	18	5.18	3.77
3-Methylbenzo[c]phenanthrene	3-MeBcPhe	242	18	5.00	3.76
4-Methylbenzo[c]phenanthrene	4-MeBcPhe	242	18	5.39	3.74
5-Methylbenzo[c]phenanthrene	5-MeBcPhe	242	18	5.28	3.73
6-Methylbenzo[c]phenanthrene	6-MeBcPhe	242	18	5.05	3.69
Chrysene	Chr	228	18	3.92	4.00
1-Methylchrysene	1-MeChr	242	18	4.22	4.11
2-Methylchrysene	2-MeChr	242	18	4.20	4.12
3-Methylchrysene	3-MeChr	242	18	4.20	4.12
4-Methylchrysene	4-MeChr	242	18	5.01	4.05
5-Methylchrysene	5-MeChr	242	18	4.97	4.04
6-Methylchrysene	6-MeChr	242	18	4.22	4.11
Triphenylene	TriPhe	228	18	4.37	4.10
1-Methyltriphenylene	1-MeTriPhe	242	18	5.19	4.08
2-Methyltriphenylene	2-MeTriPhe	242	18	4.18	4.15
Naphthacene	Nap	228	18	3.89	3.92

Table 3

NPLC retention indices for the five-ring PAHs.

PAHs	Abbreviations	MM (Da)	No. of Aromatic Carbon	Thickness (Å)	Log I NH ₂
Benzo[ghi]fluoranthene	BghiF	226	18	3.88	3.70
Cyclopenta[cd]pyrene	CPcdP	226	18	3.89	3.95
Benzo[e]aceanthrylene	BeAce	252	20	3.89	4.58
Benzo[j]aceanthrylene	BjAce	252	20	3.89	4.64
Benzo[k]acephenanthrylene	BkAcep	252	20	3.89	4.54
Benzo[k]acephenanthrylene	BkAcep	252	20	3.90	4.51
Benzo[l]acephenanthrylene	BlAcep	252	20	4.73	4.50
Benzo[a]fluoranthene	BaF	252	20	3.89	4.44
Benzo[b]fluoranthene	BbF	252	20	3.88	4.43
Benzo[j]fluoranthene	BjF	252	20	3.94	4.52
Benzo[k]fluoranthene	BkF	252	20	3.89	4.41
Benzo[e]pyrene	BeP	252	20	3.89	4.40
Benzo[a]pyrene	BaP	252	20	3.89	4.30
1-Methylbenzo[a]pyrene	1-MeBaP	266	20	4.20	4.42
2-Methylbenzo[a]pyrene	2-MeBaP	266	20	4.21	4.37
3-Methylbenzo[a]pyrene	3-MeBaP	266	20	4.22	4.45
4-Methylbenzo[a]pyrene	4-MeBaP	266	20	4.21	4.38
5-Methylbenzo[a]pyrene	5-MeBaP	266	20	4.22	4.39
6-Methylbenzo[a]pyrene	6-MeBaP	266	20	4.36	4.52
7-Methylbenzo[a]pyrene	7-MeBaP	266	20	4.22	4.40
8-Methylbenzo[a]pyrene	8-MeBaP	266	20	4.20	4.42
9-Methylbenzo[a]pyrene	9-MeBaP	266	20	4.20	4.41
10-Methylbenzo[a]pyrene	10-MeBaP	266	20	4.98	4.36
11-Methylbenzo[a]pyrene	11-MeBaP	266	20	4.89	4.36
12-Methylbenzo[a]pyrene	12-MeBaP	266	20	4.21	4.42
Perylene	Per	252	20	3.91	4.48
1-Methylperylene	1-MePer	266	20	5.27	4.50

PAHs	Abbreviations	MM (Da)	No. of Aromatic Carbon	Thickness (Å)	Log I NH ₂
2-Methylperylene	2-MePer	266	20	4.46	4.49
3-Methylperylene	3-MePer	266	20	4.44	4.59
Benzo[<i>a</i>]naphthacene	BaNap	278	22	3.89	4.97
Benzo[<i>b</i>]chrysene	BbC	278	22	3.89	5.00
Benzo[<i>c</i>]chrysene	BcC	278	22	5.39	4.56
Benzo[<i>g</i>]chrysene	BgC	278	22	5.32	4.56
Dibenz[<i>a,c</i>]anthracene	DBacA	278	22	3.89	5.04
Dibenz[<i>a,h</i>]anthracene	DBahA	278	22	3.89	5.03
Dibenz[<i>a,j</i>]anthracene	DBajA	278	22	3.89	4.96
Dibenzo[<i>b,g</i>]phenanthrene	DBbgP	278	22	5.23	4.50
Dibenzo[<i>c,g</i>]phenanthrene	DBcgP	278	22	6.24	4.16
Pentaphene	Pen	278	22	3.89	4.96
Picene	Pic	278	22	3.90	5.00
1-Methylpicene	1-MePic	292	22	5.39	5.03
2-Methylpicene	2-MePic	292	22	4.20	5.12
3-Methylpicene	3-MePic	292	22	4.20	5.12
6-Methylpicene	6-MePic	292	22	5.56	5.10
13-Methylpicene	13-MePic	292	22	5.42	5.06

Table 4

NPLC retention indices for the six-ring PAHs.

PAHs	Abbreviations	MM (Da)	No. of Aromatic Carbon	Thickness (Å)	Log I NH ₂
Anthanthrene	Anth	276	22	3.88	4.66
Benzol[ghi]perylene	BghiPer	276	22	3.89	4.75
Indeno[1,2,3- <i>cd</i>]pyrene	I123cdP	276	22	3.88	4.89
Benzo[<i>a</i>]perylene	BaPer	302	24	5.47	4.97
Benzo[<i>b</i>]perylene	BbPer	302	24	4.84	5.49
Dibenzo[<i>a,e</i>]pyrene	DBaeP	302	24	4.19	5.43
Dibenzo[<i>a,h</i>]pyrene	DBahP	302	24	3.90	5.35
Dibenzo[<i>a,i</i>]pyrene	DBaiP	302	24	3.89	5.31
Dibenzo[<i>a,l</i>]pyrene	DBalP	302	24	5.17	4.88
Dibenzo[<i>e,l</i>]pyrene	DBelP	302	24	3.89	5.45
Dibenzo[<i>a,e</i>]fluoranthene	DBaeF	302	24	3.90	5.39
Dibenzo[<i>a,f</i>]fluoranthene	DBafF	302	24	3.97	5.40
Dibenzo[<i>a,k</i>]fluoranthene	DBakF	302	24	3.89	5.35
Dibenzo[<i>b,e</i>]fluoranthene	DBbeF	302	24	4.64	5.14
Dibenzo[<i>b,k</i>]fluoranthene	DBbkF	302	24	3.90	5.37
Dibenzo[<i>j,l</i>]fluoranthene	DBjlF	302	24	4.41	5.48
Dibenzo[<i>de,mm</i>]naphthacene	DBNap	302	24	4.89	5.53
Naphtho[1,2- <i>a</i>]pyrene	N12aP	302	24	5.13	4.91
Naphtho[1,2- <i>e</i>]pyrene	N12eP	302	24	5.15	4.92
Naphtho[2,1- <i>a</i>]pyrene	N21aP	302	24	4.19	5.32
Naphtho[2,3- <i>a</i>]pyrene	N23aP	302	24	3.89	5.27
Naphtho[2,3- <i>e</i>]pyrene	N23eP	302	24	4.41	5.35
Naphtho[1,2- <i>b</i>]fluoranthene	N12bF	302	24	3.90	5.44
Naphtho[1,2- <i>k</i>]fluoranthene	N12kF	302	24	3.91	5.51
Naphtho[2,1- <i>b</i>]fluoranthene	N21bF	302	24	4.92	5.15
Naphtho[2,3- <i>b</i>]fluoranthene	N23bF	302	24	3.89	5.39
Naphtho[2,3- <i>j</i>]fluoranthene	N23jF	302	24	3.93	5.52

PAHs	Abbreviations	MM (Da)	No. of Aromatic Carbon	Thickness (Å)	Log I NH ₂
Naphtho[2,3- <i>k</i>]fluoranthene	N23kF	302	24	3.90	5.39
Benzo[<i>b</i>]picene	BbPic	328	26	3.89	6.03
Benzo[<i>c</i>]picene	BcPic	328	26	3.90	5.78
Benzo[<i>c</i>]pentaphene	BcPen	328	26	3.90	6.05
Benzo[<i>h</i>]pentaphene	BhPen	328	26	4.85	6.05
Dibenzo[<i>a,h</i>]naphthacene	DBajNap	328	26	3.95	6.03
Dibenzo[<i>a,i</i>]naphthacene	DBalNap	328	26	3.88	6.06
Dibenzo[<i>c,p</i>]chrysene	DBcpC	328	26	5.62	4.97
Dibenzo[<i>g,p</i>]chrysene	DBgpC	328	26	6.15	4.84
Dibenzo[<i>b,k</i>]chrysene	DBbkC	328	26	3.89	6.00
Dibenzo[<i>a,c</i>]naphthacene	DBacNap	328	26	3.89	6.04
Naphtho[1,2- <i>b</i>]triphenylene	N12bTriPhe	328	26	4.14	6.04
Naphtho[1,2- <i>a</i>]naphthacene	N12aNap	328	26	5.00	5.45
Naphtho[2,1- <i>a</i>]naphthacene	N21aNap	328	26	3.89	6.02
Naphtho[1,2- <i>b</i>]chrysene	N12bC	328	26	3.89	6.08
Naphtho[2,3- <i>g</i>]chrysene	N23gC	328	26	5.43	5.39
Hexaphene	Hexa	328	26	3.89	5.97
Phenanthro[3,4- <i>c</i>]phenanthrene	Phe34cPhe	328	26	7.41	4.89

Table 5

NPLC retention indices for the seven- and eight-ring PAHs.

PAHs	Abbreviations	MM (Da)	No. of Aromatic Carbon	Thickness (Å)	Log I NH ₂
<i>Seven-Ring PAHs</i>					
Coronene	Cor	300	24	3.89	5.03
Acenaphtho[1,2- <i>g</i>]fluoranthene	A12fF	326	26	4.63	5.90
Acenaphtho[1,2- <i>k</i>]fluoranthene	A12kF	326	26	3.89	6.11
Dibenzo[<i>b,g,h</i>]perylene	DBbghiPer	326	26	3.90	5.68
Dibenzo[<i>e,g,h</i>]perylene	DBeghiPer	326	26	3.89	5.74
Dibenzo[<i>c,d,m</i>]perylene	DBcdlmPer	326	26	3.90	5.28
Diindeno[1,2,3- <i>de</i> ,1',2',3'- <i>k</i>]anthracene	Rubicine	326	26	3.89	5.82
Naphtho[1,2,3,4- <i>gh</i>]perylene	N1234-ghiPer	326	26	4.07	5.71
Naphtho[8,1,2- <i>bcd</i>]perylene	N812bcdPer	326	26	4.74	5.77
Benzo[<i>a</i>]naphtho[8,1,2- <i>cde</i>]naphthacene	BaN812cdeNap	352	28	4.37	6.50
Benzo[<i>vw</i>]hexaphene	BvwxHexa	352	28	3.89	6.35
Dibenzo[<i>h,rs</i>]pentaphene	DBhrsPenta	352	28	4.31	6.57
Benzo[<i>a</i>]naphtho[1,2- <i>j</i>]naphthacene	BaN12jNap	378	30	5.28	6.46
Benzo[<i>a</i>]naphtho[1,2- <i>i</i>]naphthacene	BaN12iNap	378	30	4.05	6.97
Benzo[<i>a</i>]naphtho[2,1- <i>j</i>]naphthacene	BaN21jNap	378	30	3.95	7.00
Benzo[<i>b</i>]naphtho[1,2- <i>k</i>]chrysene	BbN12kC	378	30	3.89	7.00
Benzo[<i>b</i>]naphtho[2,1- <i>k</i>]chrysene	BbN21kC	378	30	3.89	6.91
Benzo[<i>c</i>]naphtho[2,1- <i>p</i>]chrysene	BcN21pC	378	30	5.49	5.46
Dibenzo[<i>a,c</i>]pentacene	DBacPen	378	30	4.57	6.25
Dibenzo[<i>a,f</i>]pentacene	DBafPen	378	30	3.89	6.93
Heptaphene	Hepta	378	30	3.90	6.87
Dibenzo[<i>b,n</i>]picene	DBbnPic	378	30	3.90	6.95
Dibenzo[<i>c,m</i>]pentaphene	DBcmPenta	378	30	3.90	7.10
Naphtho[2,3- <i>c</i>]pentaphene	N23cPenta	378	30	4.01	6.96
Phenanthro[1,2- <i>b</i>]chrysene	Phe12bC	378	30	4.28	7.08
Phenanthro[2,1- <i>b</i>]chrysene	Phe21bC	378	30	3.89	6.97

PAHs	Abbreviations	MM (Da)	No. of Aromatic Carbon	Thickness (Å)	Log I NH ₂
Phenanthro[4,3- <i>b</i>]chrysene	Phe43bC	378	30	5.33	6.54
Phenanthro[9,10- <i>b</i>]chrysene	Phe910bC	378	30	4.98	7.04
Phenanthro[3,4- <i>b</i>]triphenylene	Phe34bTriPhe	378	30	5.47	5.28
Phenanthro[9,10- <i>b</i>]triphenylene	Phe910bTriPhe	378	30	5.95	6.04
Tribenzo[<i>a,c,j</i>]naphthacene	TriBacjNap	378	30	4.77	7.07
Trinaphthylene	TriNaph	378	30	4.51	7.01
<i>Eight-Ring PAHs</i>					
Truxene	Trux	342	24	4.26	5.99
Benzo[<i>a</i>]coronene	BaCor	350	28	3.89	6.17
Benzo[<i>pqr</i>]naphtho[8,1,2- <i>bcd</i>]perylene	BpqrN812bcdPer	350	28	4.26	6.13
Phenanthro[5,4,3,2- <i>abcd</i>]perylene	Phe5432abcdePer	350	28	3.89	6.16
Pyranthrene	Pyrant	376	30	3.94	6.39
Tribenzo[<i>a,cd,l,m</i>]perylene	TriBacdImPer	376	30	5.58	6.21