Development of a reversed-phase liquid chromatography and fluorescence method with multichannel selective wavelength detection for the determination of benzo[a]pyrene and six of its isomers

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The baseline separation of benzo[a]pyrene in complex samples via reversed-phase liquid chromatography (RPLC) is particularly challenging due to the potential for interferences from other molecular mass (MM) 252 (g mol⁻¹) polycyclic aromatic hydrocarbons (PAHs). The work presented here explores the use of different types of RPLC stationary phases and different fluorescence (FL) detection conditions for application to method development. Six different stationary phases were investigated with SRM 869b and SRM 1647f for potential use in the development of PAH methods: polymeric C₁₈, monomeric C₁₈, narrow pore C₃₀, wide pore C₃₀, phenylhexyl, and pentfluorophenyl phases. Although the best chromatographic separation was obtained with the polymeric C₁₈ stationary phase, seven MM 252 PAH isomers were not fully resolved and selective detection was utilized to eliminate interferences. Stop-flow fluorescence (excitation and emission) spectra were recorded for the seven PAHs; these spectra differed significantly among the isomers. Based on these spectra, the appropriate excitation/emission wavelengths were determined to be 406/440 nm, 243/509 nm, and 290/411 nm. These conditions were used in the new RPLC/FL method and the seven MM 252 PAH isomers were baseline resolved in their respective chromatograms. The new RPLC/FL method was used to identify the MM 252 PAHs in a diesel particulate extract (SRM 1975).

Keywords: Reversed-Phase Liquid Chromatography; Fluorescence Detection; Polycyclic Aromatic Hydrocarbons; Standard Reference Materials; Stationary Phases
Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent a diverse class of environmentally important chemicals produced from a wide variety of natural and anthropogenic sources [1-3]. Because of their carcinogenic nature, the U.S. Environmental Protection Agency (EPA) includes 16 PAHs as priority pollutants and recommends their routine environmental monitoring (Figure S1) [4]: naphthalene (Nap), acenaphthene (Ace), acenaphthylene (Acen), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fluor), pyrene (Pyr), chrysene (Chry), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), benzo[g,h,i]perylene (BghiPer), dibenz[a,h]anthracene (DBahA), and indeno[1,2,3-cd]pyrene (I123cdP). Among the 16 EPA-PAHs, BaP is the most carcinogenic and often used as a marker for PAH measurements. BaP has been identified in a wide range of environmental and combustion-related samples such as sediment [5-7], air particulate [8], coal tar [9, 10], crude oil [11, 12], and diesel particulate [13-15]. The presence of molecular mass (MM) 252 g mol\(^{-1}\) isomers represents a significant challenge for the determination of BaP in these natural matrix samples. These isomers include benzo[a]fluoranthene (BaF), BbF, benzo[j]fluoranthene (BjF), BkF, benzo[e]pyrene (BeP), and perylene (Per).

Reversed-phase liquid chromatography (RPLC) with chemically bonded octadecylsilane (C\(_{18}\)) stationary phases has been used since the early 1970's for the separation of PAHs [16]. Over the years, column manufacturers have developed a variety of C\(_{18}\) stationary phases that exhibit different selectivity toward isomeric PAHs and these selectivity differences for isomeric PAHs have been evaluated for numerous C\(_{18}\) RPLC columns [17, 18]. Significant differences in selectivity among C\(_{18}\) stationary phases occur based on differences in the synthetic approach, classified as monomeric or polymeric syntheses. Polymeric C\(_{18}\) phases typically exhibit enhanced
selectivity for PAHs based on the molecular shape of PAHs and provide significantly better separations for isomeric PAHs than do monomeric C$_{18}$ phases [19-22]. The molecular shape of PAHs can be described by the length-to-breadth ratio ($L/B$), which is defined by a box drawn around the PAH structure, oriented to provide the maximum $L/B$ value. Typically, PAH retention increases as a function of $L/B$ for polymeric C$_{18}$ phases unless the PAH has a non-planar structure.

In the current study, PAH separations on six RPLC stationary phases were investigated: (1) polymeric C$_{18}$ phase, (2) monomeric C$_{18}$ phase, (3) narrow pore C$_{30}$ phase, (4) wide pore C$_{30}$ phase, (5) phenylhexyl (PH) phase, and (6) pentafluorophenyl (PFP) phase. Selectivity for these stationary phases was characterized using three PAH-containing solution reference materials. SRM 869b is a column test mixture that can be used to predict the RPLC separation selectivity for PAHs. SRM 1647f is a calibration solution for the 16 EPA-PAHs. SRM 2260a is a calibration solution containing 35 PAHs with a higher number of isomeric PAHs. By using these materials to characterize column selectivity, a new reversed-phase liquid chromatography (RPLC) method with fluorescence (FL) detection was developed for the determination of BaP and six of its MM 252 PAH isomers. The research presented here investigates the potential use of FL detection to simultaneously determine these seven MM 252 PAHs in complex samples using multichannel wavelength conditions.

Experimental

Reagents and Materials

SRM 869b (Column Selectivity Test Mixture for Liquid Chromatography), SRM 1647f (Priority Pollutant Polycyclic Aromatic Hydrocarbons in Acetonitrile), SRM 2260a (Aromatic Hydrocarbons in Toluene), and SRM 1975 (Diesel Particulate Extract) were obtained from the
Office of Standard Reference Materials at the National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA). HPLC grade toluene, n-hexane, water, and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA, USA).

**Reversed-Phase Liquid Chromatography**

RPLC separations were performed using an Ultimate 3000 Dionex HPLC system (Thermo Scientific, Sunnyvale, California) equipped with the following components: a pump, an UV absorption detector, a FL detector, and an online degasser. The instrument was computer controlled using commercial software (Chromeleon version 6.8, Thermo Scientific). Separations were carried out on the six stationary phases listed in Table 1. The separations of SRM 869b were with a mobile phase of 85/15 (v/v) acetonitrile/water, a flow rate of 1.5 mL min⁻¹, and absorbance detection at 254 nm. The separations of SRM 1647f were with the following mobile phase gradient: 50/50 (volume fraction) acetonitrile/water for 3 min, linear gradient to 100 % acetonitrile over 15 min, and isocratic conditions for 20 min. The mobile phase flow rate and absorbance detection wavelength were 1.5 mL min⁻¹ and 254 nm, respectively. The separations of SRM 2260a and SRM 1975 were the same as SRM 1647f except the linear gradient was adjusted from 15 min to 20 min. The optimal separation and fluorescence detection conditions for each SRM 2260a and SRM 1975 sample are discussed in the following sections.

**Stop-Flow Fluorescence Spectra Collection**

Fluorescence spectra were collected using a stop-flow function on the Dionex HPLC instrument at the apex of each chromatographic peak with an 8 µL flow cell. Chromatographic peaks were excited with a xenon flash lamp with broadband illumination from 200 nm to 880 nm.
The excitation and emission monochromators have a 20 nm spectral bandwidth, 2 nm accuracy, and 0.2 nm repeatability. The fluorescence detector has the potential to measure up to four channels simultaneously with independent parameters with a photomultiplier tube covering the range of the xenon flash lamp. A single channel fluorescence detector is required for the collection of stop-flow fluorescence spectra with a maximum data collection rate of 100 Hz. A programmable filter wheel consisting of five wavelengths was utilized throughout the analysis.

Results and Discussion

Comparison of Different Stationary Phases

C₁₈ stationary phases have been shown to provide excellent separation capabilities for isomeric PAHs [19-21], alkylated PAHs [18], and their heterocyclic analogs such as polycyclic aromatic sulfur heterocycles [23, 24]. Two approaches used in preparation of alkyl stationary phases are often described as monomeric and polymeric syntheses, and the resulting stationary phases exhibit significant selectivity differences toward PAHs [20, 25]. Polymeric C₁₈ phases are prepared using di- or trifunctional silanes (with the addition of water); monofunctional silanes are used to prepare monomeric C₁₈ phases. Other types of RP stationary phases are known to exhibit selectivity differences towards PAH isomers. In this study, two commercially prepared C₁₈ phases (polymeric and monomeric), two C₃₀ phases (narrow and wide pore), PH phase, and a PFP phase were selected to investigate the RPLC selectivity for separating mixtures of PAHs (Table 1).

SRM 869b is a column selectivity test mixture that contains three PAHs (Figure S2). The elution order of these solutes provides a sensitive indicator of monomeric-like or polymeric-like selectivity and the selectivity factor α_{TBN/BaP} can be used to characterize overall selectivity for PAHs related to molecular shape (i.e. shape selectivity) [18, 26]. Polymeric C₁₈ phases usually
exhibit $\alpha_{\text{TBN/BaP}} \leq 1$, intermediate polymeric C$_{18}$ phases are represented by $\alpha_{\text{TBN/BaP}}$ values between 1.0 to 1.7, and monomeric C$_{18}$ phases typically produce values for $\alpha_{\text{TBN/BaP}} \geq 1.7$. The RPLC separations of SRM 869b using these six stationary phases are shown in Figure 1 with a mobile phase of 85/15 (v/v) acetonitrile/water, a flow rate of 1.5 mL min$^{-1}$, and absorbance detection at 254 nm. The polymeric and monomeric C$_{18}$ stationary phases provided $\alpha_{\text{TBN/BaP}}$ values of 0.49 and 1.97, respectively, which are consistent with the criteria listed above. The narrow pore C$_{30}$ and wide pore C$_{30}$ stationary phases used in this study provided $\alpha_{\text{TBN/BaP}}$ values of 0.51 and 0.48, respectively, indicating that differences in pore size and surface area have little influence on the $\alpha_{\text{TBN/BaP}}$ values for columns prepared by the same surface modification chemistry. The PH and PFP phases were the only non-alkyl-chain stationary phases selected here and provided $\alpha_{\text{TBN/BaP}} = 2.05$ and 0.83, respectively.

Additional selectivity comparisons were conducted using a calibration solution (SRM 1647f) that contained the 16 priority pollutant PAHs designated by the EPA. The mixture was separated with each of the six stationary phases using the following mobile phase gradient: 50/50 (volume fraction) acetonitrile/water for 3 min, linear gradient to 100 % acetonitrile over 15 min, and isocratic conditions for 20 min. The mobile phase flow rate and absorbance detection wavelength were 1.5 mL min$^{-1}$ and 254 nm, respectively. Separations of SRM 1647f are shown in Figure 2 for the polymeric C$_{18}$ phase, the narrow pore C$_{30}$ phase, and the PFP phase. Separations of SRM 1647f are shown in Figure S3 for the monomeric C$_{18}$ phase, wide pore C$_{18}$ phase, and PH phase. The 16 EPA-PAHs were baseline resolved with the polymeric C$_{18}$ phase, which are similar to previous results [27]. With the monomeric C$_{18}$ phase, significant co-elution occurred for two pairs of PAHs: (1) Ace and Flu and (2) Chr and BaA. In general, better separations of PAH isomers were achieved with the polymeric C$_{18}$ phases compared with the monomeric C$_{18}$ phases.
C<sub>30</sub> stationary phases have seen increased usage over the past decade for the separation of carotenoids [28, 29], lipids [30], fatty acids [31, 32], triacylglycerol regioisomers [32, 33], and PAHs [26, 34]. For separations of PAHs, Sander and Wise [26] observed a significant change in selectivity with changes in stationary phase alkyl chain length. Using SRM 869b, BaP eluted first on a C<sub>12</sub> phase, second on a C<sub>22</sub>, and third for the monomeric C<sub>30</sub> phase. In the case of polymeric phases, the change is less dramatic but a similar trend was observed. Selectivity coefficients (α<sub>TBN/BaP</sub>) decreased with increasing chain lengths for both monomeric and polymeric phases. Similar results were observed in the current study and values for α<sub>TBN/BaP</sub> were identical for the C<sub>30</sub> polymeric-like phases (α<sub>TBN/BaP</sub> ≈ 0.50). Bonding chemistry has less influence on PAH selectivity for long alkyl chain length phases than for C<sub>18</sub> phases [26].

Recently, Zhang et al. [34] characterized two C<sub>30</sub> stationary phases (Sil-CBM-C<sub>30</sub> and Develosil C<sub>30</sub>) using SRM 869b and SRM 1647e (SRM 1647e is a prior lot of SRM 1647f). Develosil C<sub>30</sub> is a commercially available column and Sil-CBM-C<sub>30</sub> was synthesized to include an embedded polar carbamate group linker between the silica and C<sub>30</sub> chain. The introduction of the carbamate group was characterized by α<sub>TBN/BaP</sub> = 0.29, compared with α<sub>TBN/BaP</sub> = 0.60 for the Develosil C<sub>30</sub> column. The polar embedded C<sub>30</sub> column provided a better separation for the 16 EPA-PAHs. In the present study, conventionally synthesized narrow and wide pore C<sub>30</sub> columns were selected to provide a better representation for the majority of C<sub>30</sub> columns commercially available (no polar linker). Both C<sub>30</sub> phases provided the same selectivity towards the 16 EPA-PAHs as observed with the polymeric C<sub>18</sub> phases; however, the separation obtained with the polymeric C<sub>18</sub> was better than that obtained with either C<sub>30</sub> phases. PFP phases were not originally designed for PAH separations, but these phases exhibit unique selectivity towards aromatic compounds due to π-electron interactions [35]. Poorer
resolution of the 16 EPA-PAHs was obtained with the PFP phase column in comparison to polymeric C18 and C30, and the retention behavior of several PAHs (e.g., Flu, Ace, BkF, BbF, DBahA, and 1123cdP) was altered compared with the alkyl phase columns. The PH phase provided the same separation of the 16 EPA-PAHs as the monomeric C18 phase, which is consistent with similar selectivity factors for the two phase columns. Based on the results discussed in this section, the polymeric C18 phase was selected for further studies.

**RPLC/FL Analysis of Isomeric PAHs with MM 252**

Much of the interest in the analysis of PAHs is placed on compounds present in environmental samples e.g., BaP, BbF, and BkF. Other studies have identified additional MM 252 PAH isomers in environmental [8] and combustion-related samples [10]. Most often, methods for PAH identification and quantitation are based on gas chromatography/mass spectrometry (GC/MS), with different columns that provide a measure of selectivity choices. An alternate independent method was needed for comparison with GC/MS methods, and the current research details this effort to develop a RPLC/FL method for measuring more complex mixtures of the MM 252 PAH isomers. SRM 2260a is a calibration solution of aromatic hydrocarbons in toluene containing a mixture of 35 PAHs, and it is intended for the calibration of chromatographic instrumentation used for the determination of PAHs. This solution contains seven MM 252 PAH isomers as shown in Figure S4 and the SRM was utilized in the development of the new RPLC/FL method.

Wise and Sander [22] discussed the retention behavior for MM 252 PAH isomers using a polymeric C18 stationary phase with the following retention order: BaF, BjF, BeP, BbF, Per, BkF, and BaP. The current RPLC/UV separation of SRM 2260a shown in Figure 3 exhibited the same elution order for these isomers (see Table 2). Because partial co-elution occurs between (1) BjF
and BeP; and (2) BbF and Per, the separation conditions were modified to lengthen the gradient from 15 to 20 min. Better separation of these isomers or selective detection methods are needed for accurate quantitation.

Selective detection was studied as a way of eliminating interferences from unresolved constituents. FL detectors are commonly used in methods for the determination of PAHs to provide a highly selective and sensitive detection method [36-40]. Previous studies have demonstrated how FL spectral profiles are significantly different for isomeric PAHs [36]. A similar study was performed here to provide multiple FL wavelength options for detection of the MM 252 PAHs in the chromatographic separation. Excitation and emission (FL) spectra were recorded at the apex of the chromatographic peaks for each PAH using a stop-flow function on the data system. The chromatographic retention times, excitation wavelengths, and emission wavelengths of the seven MM 252 PAHs present in SRM 2260a are summarized in Table 2. The FL spectra recorded from the individual reference standards for the seven PAHs are shown in Figure 4 and Figure S5.

In the case of the first co-eluting isomer pair in Figure 3, BjF has maximum excitation/emission wavelengths of 243/509 nm and BeP has maximum excitation/emission wavelengths of 286/393 nm. Based on their spectral profiles, neither isomer fluoresces at the excitation wavelength of the other, and by using the two wavelength maximum conditions, the PAHs can be determined without interference. A similar approach can be utilized for the second co-eluting isomer pair (BbF and Per). These PAHs have similar maximum fluorescence wavelengths of 250/439 nm and 249/440 nm, respectively, and other differences in their fluorescence profiles must be exploited. BbF has a secondary excitation peak at 295 nm that is not present in Per excitation spectrum. Per has a secondary excitation peak at 406 nm that is not present in BbF excitation spectrum. These excitation differences allow BbF and Per to be determined with excitation/emission wavelengths...
of 295/439 nm and 406/440 nm, respectively. BaP and BkF have similar excitation and emission wavelengths compared to BeP, and BaF has similar excitation/emission wavelengths compared to BjF. The RPLC/FL chromatograms obtained for the seven MM 252 PAH isomers in SRM 2260a are shown in Figure 5. PAHs were detected using a multi-channel FL detector with the following excitation/emission wavelengths: (1) 406/440 nm, (2) 243/509 nm, and (3) 290/411 nm. The excitation and emission wavelengths selected for channels 1 and 2 are based on the previous discussions to determine BjF and Per without interference from co-eluting PAHs. The excitation and emission wavelengths selected for channel 3 represent the best choice to reduce measurement bias for concurrent determination of BeP, BbF, BkF, and BaP (FL spectral profiles are listed in Table 2).

The feasibility of the new RPLC/FL method for the determination of the seven MM 252 PAH isomers in a complex sample matrix was investigated using a diesel particulate extract (SRM 1975). The diesel extract sample was prepared from a 24 h extraction with dichloromethane of an industrial forklift diesel particulate matter reference material also available at NIST (SRM 2975). SRM 1975 only has certified mass fraction values for BbF, BkF, and BeP; however, BjF, BaF, and BaP are listed in the certificate of analysis for the source material. The RPLC chromatograms obtained with UV and FL detection for SRM 1975 are shown in Figure 6. Under the selective fluorescence wavelengths, the chromatographic resolution was significantly improved compared to the UV separation providing the direct determination of BaF, BjF, BeP, BbF, and BkF. Per and BaP were not determined via the new RPLC/FL method or the certification methods of SRM 1975. In the case of BaP, its concentration in the source material (SRM 2975) is significantly lower than those of the other MM 252 PAH isomers. To determine BaP in SRM 1975, the sample was first cleaned-up using a recently published normal-phase (NP) LC fractionation procedure.
prior to analysis via RPLC/FL [10]. The RPLC/FL chromatogram shown in Figure S6 obtained after NPLC fractionation clearly allowed for the identification of BaP.

Conclusions

Knowledge of selectivity differences among RPLC column stationary phases is requisite to informed column selection during method development, particularly for isomeric PAHs. In the present study, six commercial columns were evaluated with a column test mixture (SRM 869b) and a mixture of EPA priority pollutant PAHs (SRM 1647f). Similar to polymeric C18 phase, narrow and wide pore C30 phases were classified as polymeric-like and the column stationary phases provided a similar separation for the 16 priority pollutant PAHs. The PFP phase was also classified as polymeric-like but the stationary phase column exhibited different selectivity toward PAHs, and was unable to resolve multiple constituents in SRM 1647f. The PH stationary phase was classified as monomeric-like and provided a similar separation for the 16 EPA-PAHs as the monomeric C18 phase. Using the polymeric C18 phase, a new RPLC/FL method was developed for the separation and selective detection of BaP and six of its isomers in a 35 PAH calibration solution (SRM 2260a). A multichannel fluorescence detector was utilized to determine identify chromatographically unresolved PAHs under selective excitation and emission wavelengths, thereby eliminating interferences. The research presented here provides a new RPLC/FL method for the targeted analysis of PAHs in environmental or combustion-related samples.

Disclaimer
Certain commercial equipment or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

**Compliance with Ethical Standards**

**Conflict of interest**

There are no conflicts of interest to declare.

**Ethical Approval**

This article does not contain any studies with human participants or animals performed by any of the authors.
References


Figure 1. RPLC/UV chromatograms of SRM 869b on the six stationary phases investigated.
Figure 2. RPLC/UV chromatograms of SRM 1647f on the polymeric C_{18} phase, narrow pore C_{30} phase, and PFP phases.
Figure 3. RPLC/UV chromatogram of the seven MM 252 PAH isomers in SRM 2260a on the polymeric C18 phase.
**Figure 4.** Excitation and emission spectra collected from individual reference standards for the two pairs of co-eluting isomers: (1) BjF and BeP and (2) BbF and Per.
Figure 5. RPLC/FL chromatograms obtained for the seven MM 252 PAH isomers in SRM 2260a using multiple wavelength conditions and on the polymeric C18 phase.
Figure 6. RPLC chromatograms obtained for the diesel particulate extract (SRM 1975) with UV and FL detection on the polymeric C\textsubscript{18} phase. The asterisk in the chromatogram represents were BaP was identified in the chromatograms in Figure S6 after NPLC fractionation.
Table 1 Analytical RPLC columns investigated in the present study

<table>
<thead>
<tr>
<th>Column</th>
<th>Stationary Phase</th>
<th>Particle Size (µm)</th>
<th>Pore Size (Å)</th>
<th>Surface Area (m² g⁻¹)</th>
<th>Carbon Load (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zorbax Eclipse PAH</td>
<td>Polymeric C₁₈</td>
<td>5</td>
<td>95</td>
<td>160</td>
<td>14</td>
</tr>
<tr>
<td>Agilent 5</td>
<td>Monomeric C₁₈</td>
<td>5</td>
<td>170</td>
<td>290</td>
<td>17</td>
</tr>
<tr>
<td>ProntoSil 120</td>
<td>Polymeric C₃₀</td>
<td>3</td>
<td>120</td>
<td>300</td>
<td>25</td>
</tr>
<tr>
<td>ProntoSil 300</td>
<td>Polymeric C₃₀</td>
<td>3</td>
<td>300</td>
<td>100</td>
<td>13</td>
</tr>
<tr>
<td>Zorbax Eclipse Plus</td>
<td>PH</td>
<td>5</td>
<td>95</td>
<td>160</td>
<td>9</td>
</tr>
<tr>
<td>Poroshell 120 PFP</td>
<td>PFP</td>
<td>4</td>
<td>120</td>
<td>130</td>
<td>5.1</td>
</tr>
</tbody>
</table>

*a Data included here was provided by the manufacturers.
**Table 2** Stop-flow excitation and emission wavelengths for the seven MM 252 PAH isomers in SRM 2260a

<table>
<thead>
<tr>
<th>PAHs</th>
<th>Retention Time (min)</th>
<th>Excitation Wavelengths (nm)</th>
<th>Emission Wavelengths (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaF</td>
<td>23.24</td>
<td>262, 366, 425, 458</td>
<td>498, 514</td>
</tr>
<tr>
<td>BjF</td>
<td>23.63</td>
<td>243, 304, 385, 461</td>
<td>509</td>
</tr>
<tr>
<td>BeP</td>
<td>23.79</td>
<td>286, 322</td>
<td>393</td>
</tr>
<tr>
<td>BbF</td>
<td>24.24</td>
<td>250, 295, 350</td>
<td>439</td>
</tr>
<tr>
<td>Per</td>
<td>24.42</td>
<td>210, 249, 406</td>
<td>440, 468, 502</td>
</tr>
<tr>
<td>BkF</td>
<td>25.51</td>
<td>253, 299, 367</td>
<td>413, 433</td>
</tr>
<tr>
<td>BaP</td>
<td>26.57</td>
<td>263, 290, 366</td>
<td>411, 431</td>
</tr>
</tbody>
</table>

*a* Maximum excitation and emission wavelengths are indicated by underline.
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Figure S1. Molecular structures of the PAHs in SRM 1647f designated as priority pollutants by the EPA.
Figure S2. Molecular structures of the PAHs in SRM 869b.
**Figure S3.** RPLC/UV chromatograms of SRM 1647f on the monomeric C$_{18}$ phase, wide pore C$_{30}$ phase, and PH phases.
Figure S4. Molecular structures of the MM 252 PAH isomers in SRM 2260a.
Figure S5. Excitation and emission spectra collected from individual reference standards for BkF, BaP, and BaF.
Figure S6. RPLC/FL chromatogram obtained for the diesel particulate extract (SRM 1975) after NPLC fractionation.