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RESEARCH PAPER

Qualitative characterization of SRM 1597a coal tar for polycyclic aromatic hydrocarbons and methyl-substituted derivatives via normal-phase liquid chromatography and gas chromatography/mass spectrometry

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Abstract A normal-phase liquid chromatography (NPLC) fractionation procedure was developed for the characterization of a complex mixture of polycyclic aromatic hydrocarbons (PAHs) from a coal tar sample (Standard Reference Material (SRM) 1597a). Using a semi-preparative aminopropyl (NH_2) LC column, the coal tar sample was separated using NPLC based on the number of aromatic carbons; a total of 14 NPLC fractions were collected. SRM 1597a was analyzed before and after NPLC fractionation by using gas chromatography/mass spectrometry (GC/MS) with a 50% phenyl stationary phase. The NPLC-GC/MS method presented in this study allowed for the identification of 72 PAHs and 56 MePAHs. These identifications were based on the NPLC retention times for authentic reference standards, GC retention times for authentic reference standards, and the predominant molecular ion peak in the mass spectrum. Most noteworthy was the determination of dibenzo[*a,l*]pyrene, which could not be measured directly by GC/MS because of low concentration and co-elution with dibenzo[*j,l*]fluoranthene. The NPLC-GC/MS procedure also allowed for the tentative identification of 74 PAHs and 117 MePAHs based on the molecular ion peak only. This study represents the most comprehensive qualitative characterization of SRM 1597a to date.

Keywords Polycyclic aromatic hydrocarbons · Normal-phase liquid chromatography fractionation · Gas chromatography/mass spectrometry · Coal tar · Standard reference material

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are an important class of environmental pollutants originating from a wide variety of natural and anthropogenic sources. Studies have shown that exposure to PAHs can lead to an increased risk of cancer and as such, PAHs are classified as potential carcinogens [1–3]. PAHs have been detected in environmental and combustion-related samples such as coal tar [4–10], diesel particulate matter [11], crude oil [12], air particulate matter [13], mussels [14], sediments [14], and cigarette smoke particulate [15]. The National Institute of Standards and Technology (NIST) has developed a variety of natural matrix Standard Reference Materials (SRMs) for use in validating new and current analytical methodologies for the determination of PAHs [14]. The sample under investigation in this report (SRM 1597a) is a complex mixture of PAHs from coal tar for which mass fractions of individual PAHs of selected isomer groups have been measured and included in the Certificate of Analysis (COA) [8]. The distribution of the molecular mass (MM) isomer groups for PAHs reported in the

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COA is as follows: MM 178 Da (2), MM 202 Da (2), MM 226 Da (2), MM 228 Da (4), MM 252 Da (7), MM 276 Da (3), MM 278 Da (7), MM 300 Da (1), and MM 302 Da (17); and the distribution for methyl-substituted (Me) PAHs is as follows: MM 192 Da (6), MM 216 Da (4), and MM 242 Da (1). In addition, quantitative values for PAHs with MM 326 Da [4] and MM 328 Da [5] in the SRM are reported in the literature. In addition to the parent PAHs, a limited number of Me-PAHs are reported in the COA for the following isomer groups: MM 192 Da (6), MM 216 Da (4), and MM 242 Da (1).

Methods used to quantify PAHs in complex natural-matrix SRMs at NIST have typically employed a combination of the following: extraction, fractionation by normal-phase liquid chromatography (NPLC) or cleanup by solid-phase extraction, gas chromatography/mass spectrometry (GC/MS) and/or reversed-phase (RP)-LC coupled to a fluorescence detector (FLD). NPLC is now widely employed to separate complex PAH mixtures into fractions using polar stationary phases: aminopropyl (NH_2) [4, 5, 7, 16, 17], nitrophenyl (NO_2) [18], dihydroxypropyl propyl ether (Diol) [19], aminocyano (NH_2CN) [20], and silica [21] to best isloate individual compounds for selective identification and quantification. GC/MS and RPLC-FLD methods have been used as complementary methods of analysis to determine PAHs in natural matrix SRMs for over 40 years at NIST [14]. The GC/MS approach typically requires analysis of extracts with various stationary phases with different selectivity for PAHs to characterize the samples. Stationary phases that exhibit different selectivity toward PAHs include a 5% phenyl-substituted methylpolysiloxane (phenyl-MPS) phase, 50% phenyl-MPS phase, and/or 50% liquid crystalline dimethylpolysiloxane (LC-DMPS) phase [4–8, 11, 13, 16]. In contrast, the RPLC-FLD approach usually involves the separation of PAHs with a polymeric octadecylsilane (C_{18}) stationary phase, which has been shown to provide excellent separation of isomeric PAHs and MePAHs based on shape of the PAH isomer [22–25]. The molecular shape of PAH isomers has been described previously using the length-to-breadth ratio (*L/B*) and thickness (*T*) values. In general, retention of PAH isomers was observed to increase with increasing *L/B* values; however, non-planar PAHs eluted earlier than expected based on *L/B* values [22].

In the current study, a NPLC fractionation procedure coupled with UV detection was developed based on retention data reported elsewhere for over 180 PAHs and MePAHs [26] and applied to a coal tar sample (SRM 1597a). The NPLC fractionation of SRM 1597a allowed for the identification of 72 PAHs and 56 MePAHs through comparison of GC retention data with pure reference standards. In addition, a total of 74 PAHs and 117 MePAHs were tentatively identified in SRM 1597a based on the predominant molecular ion peak in the GC mass spectra. A similar study for polycyclic aromatic sulfur heterocycles (PASHs) and their alkylated derivatives in SRM 1597a will be published elsewhere.

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 of all PAHs and MePAHs included in this study are listed Table S1 (see Electronic Supplementary Material, ESM). SRM 1597a (Complex Mixture of PAHs from Coal Tar) were obtained from the Office of Standard Reference Materials at the NIST (Gaithersburg, MD, USA). HPLC grade *n*-hexane and dichloromethane (DCM) were purchased from Fisher Scientific (Pittsburgh, PA, USA).

Instrumentation and chromatographic columns

NPLC-UV fractionation was performed using a Varian 9012 Solvent Delivery System (Agilent, Santa Clara, CA) coupled to Jasco UV-1570 Intelligent UV-vis detector (Easton, MD) with a NH_2 semi-prep column from Waters (Milford, MA) with the following characteristics: 25.0 cm length, 10 mm internal diameter, and 5 μm average particle diameters. GC/MS analysis was performed on a gas chromatograph (HP 6890 series GC, Agilent Technologies, Avondale, PA) coupled to a quadrupole mass spectrometer with electron impact (EI) ionization (HP 5973 MSD, Agilent). The GC was equipped with an on-column injector and an autosampler. Separations were carried out on an 50% phenyl stationary phase (SLB-PAHms column) obtained from Supelco (Bellefonte, PA) with the following characteristics: 60.0 cm length, 0.25 mm internal diameter, 0.25 μm film thickness, and a maximum programmable temperature of 360 °C.

Methods

NPLC fractionation

SRM 1597a was concentrated by N_2 evaporation to near dryness (≈100 μL) and reconstituted with the *n*-hexane/DCM mixture (98/2 *vol/vol*) to the volume of 1.5 mL. This allowed

for multiple sample injections at the maximum workable level for the fractionation system, i.e. below the overloading limit of the NH_2 column. The fractionation procedure consisted of injecting 0.25 mL of the sample solution into the NH_2 column using a mobile phase of 98% *n*-hexane, 2% DCM, and a flow rate of 4.0 mL/min. Fourteen fractions were collected using an in-house collection system over a 90 min time interval. The collected fractions were concentrated by N_2 evaporation to individual volumes of ≈ 0.25 mL.

GC/MS measurements

PAHs were determined in a non-fractionated SRM 1597a sample and in the NPLC fractions using GC/MS with on-column injection and the mass spectrometer programmed in selected-ion monitoring (SIM) mode for the following molecular ions of each individual parent PAH isomer groups: *m*/*z* 202, 228, 252, 278, 300, 302, 326, and 328. The SIM mode was used for the following molecular ions of the MePAHs: *m*/*z* 180, 192, 216, 242, 266, and 292. The GC oven was temperature programmed as follows for the two-ring to five-ring PAHs: isothermal at 60 °C for 2 min then 5 °C/min to 300 °C, and isothermal at 300 °C for 50 min. In the case of six- and seven-ring PAHs, the GC oven temperature was programed to be isothermal at 100 °C for 1 min, with 45 °C/min to 200 °C, 2 °C/min to 310 °C for 130 min, 45 °C/min to 325 °C, and isothermal at 325 °C for 60 min.

Results and discussion

SRM 1597a is a natural complex mixture of PAHs derived from coal tar for which a selected number of PAHs have been assigned a mass fraction value. SRM 1597a has certified mass fraction values for 34 PAHs, reference mass fraction values for 36 PAHs, and reference mass fraction values for 10 PASHs [8]. The mass fraction values for these PAHs were assigned based on combining results from several methods including GC/MS on a 5% or 50% phenyl stationary phase, GC/MS on a 50% liquid crystalline-dimethylpolysiloxane stationary phase (50% LC-DMPS), and/or RPLC-FLD [8]. PASH mass fraction values were based on measurements performed with GC/MS and GC coupled to an atomic emission detector (AED) using the same stationary phases used for the PAHs [8, 27]. For detailed characterization of specific PAH isomer groups, the coal tar sample has been fractionated by NPLC using a semi-preparative NH_2 column to isolate isomeric fractions of PAHs in previous studies [4, 5, 16]. A similar approach, based on extensive NPLC PAH retention data [26], was implemented in the present study prior to PAH determination by GC/MS using a recently developed 50% phenyl phase GC column (SLB-PAHms).

The NPLC chromatogram for the fractionation of SRM 1597a is shown in Fig. 1. A total of 14 fractions were collected over a 90 min time interval. Based on GC/MS analysis of the fractions, the distribution and identification of the parent three-ring, four-ring, five-ring, six-ring, and seven-ring PAHs in fractions 3–14 are summarized in Table 1. The distribution and identification of the three-ring, four-ring, and five-ring MePAHs in fractions 3–11 are summarized in Table 2. Fractions 1 and 2 contained one and two aromatic-ring compounds, which were not of interest in this investigation. Individual identifications in fractions 3–14 are summarized in Table S1 (see ESM). The identification of the individual PAHs was based on three criteria: (1) the NPLC retention times of authentic reference standards, (2) GC retention times of authentic reference standards, and (3) the predominant molecular ion peak in the mass spectrum. Using these criteria, a total of 72 PAHs and 56 MePAHs were identified in fractions 3–14. The tentatively identified number of PAHs (74) and MePAHs (117) listed in Tables 1 and 2 (within parentheses) is based solely on criterion 3. The following sections will discuss in detail the identification of both the parent PAHs and MePAHs.

Three-ring PAHs

The GC/MS chromatograms obtained for the three-ring MM 166 Da PAHs and MM 180 Da MePAH isomers in SRM 1597a and F3 are shown in Fig. S1 (see ESM). Fluorene (Flu) is one of the five possible three-ring MM 166 Da PAHs and is the only isomer reported in the COA for SRM 1597a. The remaining four isomers are 3*H*–benz[*e*]indene, 1*H*–benz[*e*]indene, 1*H*–benz[*f*]indene, and phenalene [29]. No reference standards were available for their identification

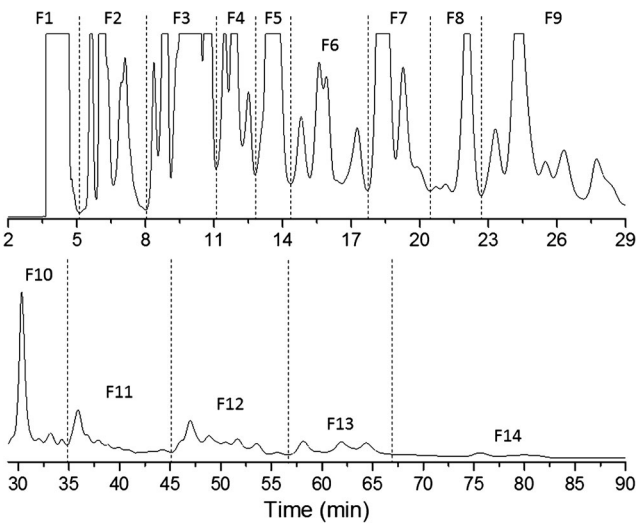

Fig. 1 NPLC fractionation chromatogram of SRM 1597a with UV-vis detection at 254 nm

Table 1 Distribution of the identified[a] and tentatively[b] identified non-substituted three-ring, four-ring, five-ring, six-ring, and seven-ring PAHs in NPLC fractions

Fraction	Three-Ring PAHs		Four-Ring PAHs		Five-Ring PAHs		Six-Ring PAHs		Seven-Ring PAHs	
	MM 166	MM 178	MM 202	MM 228	MM 252	MM 278	MM 302	MM 328	MM 300	MM 326
F3	1	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F4	n.d.	n.d.	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F5	n.d.	n.d.	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F6	n.d.	n.d.	n.d.	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F7	n.d.	n.d.	n.d.	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
F8	n.d.	n.d.	n.d.	n.d.	1	n.d.	n.d.	n.d.	n.d.	n.d.
F9	n.d.	n.d.	n.d.	n.d.	8 (2)	3	n.d.	n.d.	n.d.	n.d.
F10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4 (1)	(14)	1 (2)	n.d.
F11	n.d.	n.d.	n.d.	n.d.	n.d.	7	4 (3)	(3)	(4)	(2)
F12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13 (5)	3 (10)	n.d.	(10)
F13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1 (4)	n.d.	5 (12)
F14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11	n.d.	(2)

[a] Identified based on retention times of reference standards

[b] Tentatively identified based on the predominant molecular ion peak in the mass spectrum. These are represented in the table by the values located in the parentheses

[c] n.d. = Molecular ion not detected in this fraction

in the coal tar sample, but there were four peaks tentatively identified as MM 166 Da PAH isomers (Fig. S1, peaks 1–4). There are a total of 36 possible MM 180 Da MePAH isomers for the five MM 166 Da PAH isomers [28]. 1-Me and 2-MeFlu, the only methyl-substituted isomers identified for Flu, and six additional methyl isomers were tentatively identified in the coal tar sample (peaks 1–6).

The GC/MS chromatograms obtained for the three-ring MM 178 Da PAHs and MM 192 Da MePAH isomers in SRM 1597a and F3 are shown in Fig. S2 (see ESM). Anthracene (Ant) and phenanthrene (Phe) are reported in the COA of the coal tar sample with relatively high mass fraction values and have been successfully analyzed in the sample without the need of NPLC fractionation previously [8]. 2-Me, 3-Me, and 9-MePhe isomers are identified with no interference in the GC/MS chromatograms. 1-Me and 4-MePhe co-elute in the GC/MS chromatograms. Similar observations were obtained by Poster et al. [29] for measuring the five

Table 2 Distribution of the identified[a] and tentatively[b] identified methyl-substituted three-ring, four-ring and five-ring MePAHs in NPLC fractions

Fractions	Three-Ring PAHs		Four-Ring PAHs		Five-Ring PAHs	
	MM 180	MM 192	MM 216	MM 242	MM 266[d]	MM 292
F3	2 (6)	8	n.d.	n.d.	n.d.	n.d.
F4	n.d.	n.d.	2	n.d.	n.d.	n.d.
F5	n.d.	n.d.	5	n.d.	n.d.	n.d.
F6	n.d.	n.d.	n.d.	7	(5)	n.d.
F7	n.d.	n.d.	n.d.	13	(3)	(5)
F8	n.d.	n.d.	n.d.	2	(3)	(7)
F9	n.d.	n.d.	n.d.	n.d.	13 (16)	(15)
F10	n.d.	n.d.	n.d.	n.d.	(15)	(6)
F11	n.d.	n.d.	n.d.	n.d.	n.d.	4 (36)

[a] Identified based on the retention times obtained with reference standards

[b] Tentatively detected based on the predominant molecular ion peak in the mass spectrum. These are represented in the table by the values located in the parentheses

[c] n.d. = Molecular ion not detected in this fraction

[d] The tentatively identified MM 266 Da MePAH isomers may correspond to the five-ring *cata*-condensed MM 266 parent PAH isomers

MePhe isomers in diesel particulate-related SRM samples. Previous work by Wise et al. [8] has demonstrated the ability to identify 1-Me and 4-MePhe in the coal tar sample using different GC stationary phases. In this study, the three possible MeAnt isomers are identified in the coal tar sample, but 2-MeAnt is the only isomers previously reported in the COA [8]. The signal in the GC/MS chromatogram for 1-Me and 2-MeAnt are about 2 orders of magnitude higher than 9-MeAnt.

Four-ring PAHs

The GC/MS chromatograms obtained for the *peri*-condensed MM 202 Da PAH isomers in SRM 1597a, F4, and F5 are shown in Figs. S3a, S3b, and S3c, respectively (see ESM). The GC/MS chromatograms obtained for the *peri*-condensed MM 216 Da MePAH isomers in SRM 1597a, F4, F5, and F6 are shown in Fig. S4a, S4b, S4c, and S4d, respectively (see ESM). In the case of MM 202 Da PAHs, three of the four possible isomers were identified and baseline resolved in the GC/MS chromatogram of the unfractionated coal tar sample. In the case of the MM 216 Da MePAH isomers, the three MePyrene (MePyr) isomers were well resolved but there was co-elution between three of the four MeFluoranthene (MeFluor) isomers. In the unfractionated sample, the three predominate peaks (48–49 min) in the GC/MS chromatograms are from the four-ring *cata*-condensed MM 216 Da PAH isomers: 7*H*–benzo[*c*]fluorene, 11*H*–benzo[*a*]fluorene, and 11*H*–benzo[*b*]fluorene. These PAH isomers elute in F6 based on the GC/MS chromatogram shown in Fig. S4. The three MePyr isomers are isolated in F4 and F5. The four MeFluor isomers are isolated in F5 and F6. One additional MM 216 Da MePAH isomer (peak 1), presumably a MeFluor isomer, was tentatively identified in the coal tar sample (F5).

The GC/MS chromatograms obtained for the five *cata*-condensed MM 228 Da isomers in SRM 1597a, F6, and F7 are shown in Fig. S5a, S5b, and S5c, respectively (see ESM). Benzo[*c*]phenanthrene (BcPhe), benz[*a*]anthracene (BaA), triphenylene (TriPhe), and chrysene (Chr) are detected in the sample and are reported in the COA. Naphthacene (Nap) is not reported in the COA but was identified in the unfractionated sample (ESM Fig. S5a) and F6 (ESM Fig. S5b). Based on the retention behavior reported by Wilson et al. [26], BcPhe and Nap elute in the NPLC fraction before BaA, TriPhe and Chr. The GC/MS chromatograms obtained for the *cata*-condensed MM 242 Da MePAH isomers in SRM 1597a, F6, F7, and F8 are shown in Fig. 2a, b, c, and d, respectively. Based on these results, 22 of the possible 29 isomers were identified in F6, F7, and F8 (ESM Table S1).

Six of the seven methyl-substituted isomers identified in F6 have a high degree of non-planarity with *T* values of 5.00 Å (3-MeBcPhe) to 5.39 Å (4-MeBcPhe). 11-MeBaA is the one planar isomer to elute in F6, an expected behavior that confirms previously reported retention index (*log I*) data [26]. The largest number of MM 242 Da isomers were identified in F7 (13 total), which included the non-planar 4-Me and 5-MeChr isomers (T = 5.01 Å and 5.28 Å, respectively). For no apparent reason, these two isomers are retained on the NH_2 phase slightly longer than the non-planar isomers in F6 [26]. The only three isomers for which no reference standards were available are 1-Me, 2-Me, and 12-MeNap. Three unidentified peaks (peaks 1–3) in the GC/MS chromatogram of F7 were tentatively identified to have a MM of 242 Da, however; these three isomers would be expected to elute in F6 with Nap because of their planar molecular structure.

The benefit of using NPLC fractionation is demonstrated with all three fractions (F6, F7, and F8). 2-Me and 11-MeBaA co-elute in the unfractionated sample; on the contrary, after the NPLC fractionation, 11-MeBaA is identified in F6 and 2-MeBaA is identified in F7. In the case of 4-Me, 5-Me, and 6-MeBcPhe, the contribution of an unidentified compound that is present in the unfractionated sample is removed from the sample in F6. 7-MeBaA and 2-MeTriPhe are separated in the NPLC fractionation procedure from other interfering isomers. There are still cases were isomers co-elute after fractionation, and in these cases, analysis of the fractions could be beneficial using multiple GC stationary phases with different selectivities.

Five-ring PAHs

The GC/MS chromatograms obtained for the five-ring *peri*-condensed MM 252 Da PAH isomers in SRM 1597a, F7, and F8 are shown in Fig. 3a, b, and c, respectively. Benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*j*]fluoranthene (BjF), benzo[*a*]fluoranthene (BaF), benzo[*e*]pyrene (BeP), benzo[*a*]pyrene (BaP), and perylene (Per) are identified in the GC/MS chromatogram of the unfractionated sample and reported in the COA. After fractionation, BaP was identified in F8 and the other six PAHs were identified in F9. The five remaining PAHs that are not reported in the COA include benz[*k*]acephenanthrylene (BkAcep), benz[*l*]acephenanthrylene (BlAcep), benz[*j*]-acephenanthrylene (BjAcep), benz[*j*]aceanthrylene (BjAce), and benz[*e*]aceanthrylene (BeAce). Based on the GC retention of reference standards, the following isomers co-elute with each other in the GC/MS chromatogram: (1) BaF and BkAcep; (2) BlAcep and BjAcep; and (3) BjAce and BeAce. Nalin et al. [30] has recently shown the ability to separate and identify BaF and BkAcep in SRM 1597a using a 50% LC-DMPS stationary phase. BlAcep and BjAcep co-elute on the 50% phenyl phase but in the study by Nalin et al. [30] both isomers are separated using a 50% LC-DMPS phase. By combining the current results with the results of Nalin et al. [30], we can identify the presence of BlAcep in SRM 1597a. BjAce and BeAce co-elute on both stationary

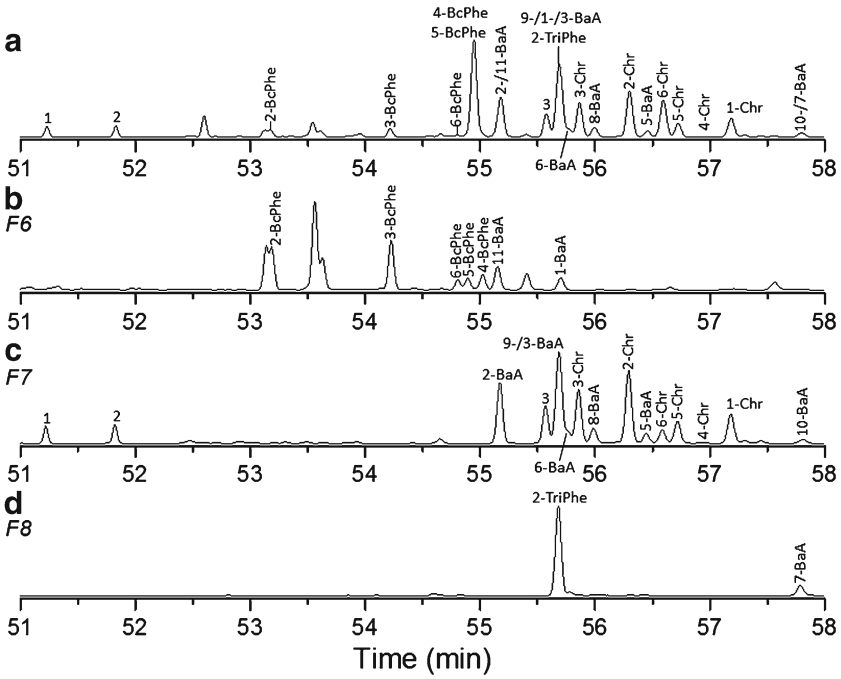

Fig. 2 GC/MS chromatograms in SIM mode for the *m/z* 242 ion for the following samples: (**a**) SRM 1597a, (**b**) F6, (**c**) F7, and (**d**) F8

phases and because of their relatively low response in the GC/MS chromatograms, identification was not possible in the coal tar sample.

For the 12 MM 252 Da isomers in this study, there are a total of 130 possible MM 266 Da MePAH isomers but only 15 reference standards were available (12 MeBaP isomers and 3 MePer isomers). The GC/MS chromatograms obtained for the five-ring *peri*-condensed MM 266 Da MePAH isomers in SRM 1597a, F6, F7, F8, F9, and F10 are shown in Fig. 4a, b, c, d, e, and f, respectively. 1-Me, 2-Me, 3-Me, 4-Me, 5-Me, 6-Me, 8-Me, 9-Me, and 12-MeBaP were identified in F9 along with 2-Me and 3-MePer. 10-Me and 11-MeBaP were not detected in the coal tar sample but would have eluted in F8 due to their non-planarity (T = 4.98 Å and 4.89 Å, respectively) [26]. Based on reference standards, 7-MeBaP and 1-MePer would elute in F9 of the NPLC chromatogram. Both of these isomers co-elute in the GC/MS chromatogram (Fig. 4e) not allowing for the confirmation that both or one of the two isomers are identified in coal tar sample. In addition to the 11 MePAHs identified based on reference standards, there were a total of 42 MM 266 Da MePAH isomers tentatively identified in the coal tar sample in the following fractions (peaks 1–42): (F6) 5, (F7) 3, (F8) 3, (F9) 16, and (F10) 15. It is important to note that some of the tentatively identified peaks in these fractions could be from the five-ring MM 266 Da *cata*-condensed PAH isomer group (i.e., 7*H*–Dibenzo[*c,g*]fluorene) [28], which is a benzene ring extension to the four-ring MM 216 Da *cata*-condensed PAH isomers identified in Fig. S4 (see ESM). The MM 266 Da PAH and MePAH isomer groups have the same number of aromatic carbon atoms (i.e., 20) and would be expected to elute in the same NPLC fractions [26].

The GC/MS chromatograms obtained for the five-ring *cata*-condensed MM 278 Da PAH isomers in SRM 1597a, F9, and F10 are shown in Fig. 3d, e, and f, respectively. Dibenzo[*b,g*]phenanthrene (DBbgPhe), benzo[*g*]chrysene (BgC), benzo[*c*]chrysene (BcC), dibenzo[*a,j*]anthracene (DBajA), dibenzo[*a,c*]anthracene (DBacA), dibenzo[*a,h*]-anthracene (DBahA), pentaphene (Pen), benzo[*b*]chrysene (BbC), benzo[*a*]naphthacene (BaNap), and picene (Pic) are identified in the unfractionated sample with co-elution between DBahA and Pen. Nalin et al. [30] have identified DBahA and Pen in SRM 1597a by separating these two isomers using a 50% LC-DMPS phase. The theoretical number of isomers for the MM 278 Da PAHs is 12 [28, 31], and these isomers differ significantly in terms of non-planarity. DBbgPhe, BgC, and BcC have T values of 5.23 Å, 5.32 Å, and 5.40 Å, respectively. These three MM 278 Da isomers elute in F9 and the planar isomers ($T \approx 3.89$ Å) eluted in the F11. No MM 278 Da PAH isomers were detected in F10. Dibenzo[*c,g*]phenanthrene (DBcgPhe) and pentacene (penta) were not identified in the coal tar sample but they would have eluted in F9 and F11, respectively, based on NPLC retention data [26].

The GC/MS chromatograms obtained for the five-ring *cata*-condensed MM 292 Da MePAH isomers in SRM 1597a, F7, F8, F9, F10, and F11 are shown in Fig. 5a, b, c, d, e, and f, respectively. There are a total of 130 possible isomers for the methyl-substituted five-ring *cata*-condensed PAHs [28]. In the current study, reference standards were only

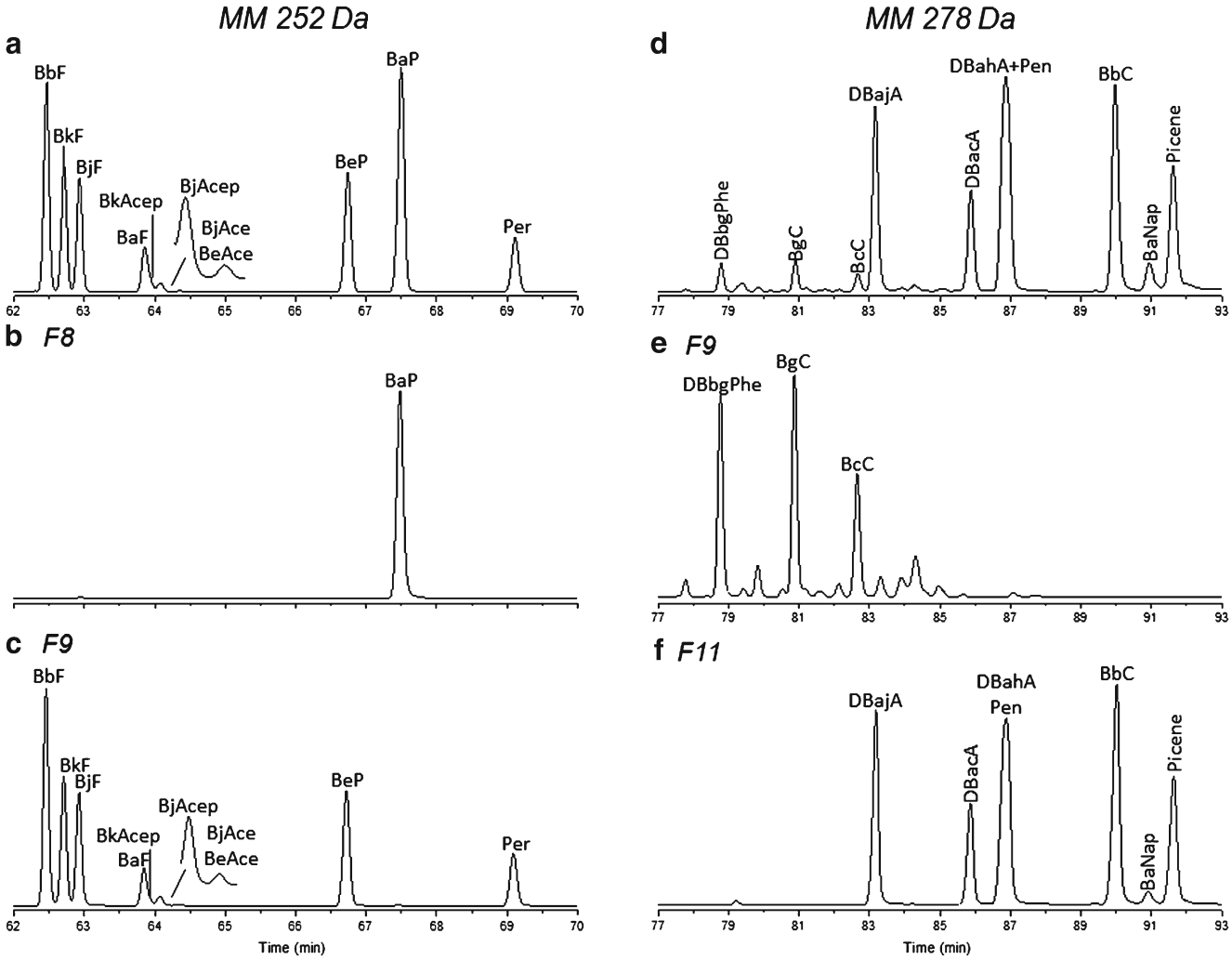

Fig. 3 GC/MS chromatograms in SIM mode for the <i > m/z</*i* > 252 ion for the following samples: (**a**) SRM 1597a, (**b**) F8 and (**c**) F9. GC/MS analysis with SIM for the *m/z* 278 ion for the following samples: (**d**) SRM 1597a, (**e**) F9 and (**f**) F11

available for 1-Me, 2-Me, 3-Me, 6-Me, and 13-MePic isomers. Despite the planarity differences for 1-Me, 2-Me, 3-Me, and 6-MePic (T = 4.20 Å – 5.56 Å), all four isomers were identified in F11. There are a total of 69 possible MM 292 Da MePAH isomers tentatively identified in the coal tar sample in the following fractions: (F7) 5, (F8) 7, (F9) 15, (F10) 6, and (F11) 36 (peaks 1–69). Even though standards were not available for positive identification, the power of the NPLC fractionation to provide potential identification and eventual quantification is evident.

Six-ring PAHs

The MM 302 Da isomers are the largest group of PAH isomers previously characterized in SRM 1597a [6, 8, 16, 32]. NIST has quantified a total of 17 MM 302 Da isomers and identified an additional 6 isomers. The mass fraction concentrations assigned to the 17 MM 302 Da isomers reported in the COA are based on the combination of results from five separate analytical approaches as described elsewhere [6–8, 16, 32]. In addition, several recent publications have supplied additional qualitative and quantitative information on these isomers in SRM 1597a [5, 9, 10]. Wilson et al. [9] demonstrated the use of 4.2 K laser-excited time-resolved Shpol'skii spectroscopy to determine the dibenzo[*a,l*]pyrene (DBalP), dibenzo[*a,e*]pyrene (DBaeP), dibenzo[*a,i*]pyrene (DBaiP), dibenzo[*a,h*]pyrene (DBahP), and naphtho[2,3-*a*]pyrene (N23aP) isomers in RPLC fractions of SRM 1597a. Moore et al. [10] quantified the same five isomers using the combination of 4.2 K excitation-emission matrices and parallel factor analysis without a chromatographic separation. Qualitative measurements have been performed by Oña-Ruales et al. [10] to demonstrate the advantage of using NPLC to separate the MM 302 Da isomers based on planarity differences prior to GC/MS analysis. Recently, Wilson et al. [26] reported NPLC retention data that explains the observations previously reported by Oña-Ruales et al. [10]. In the

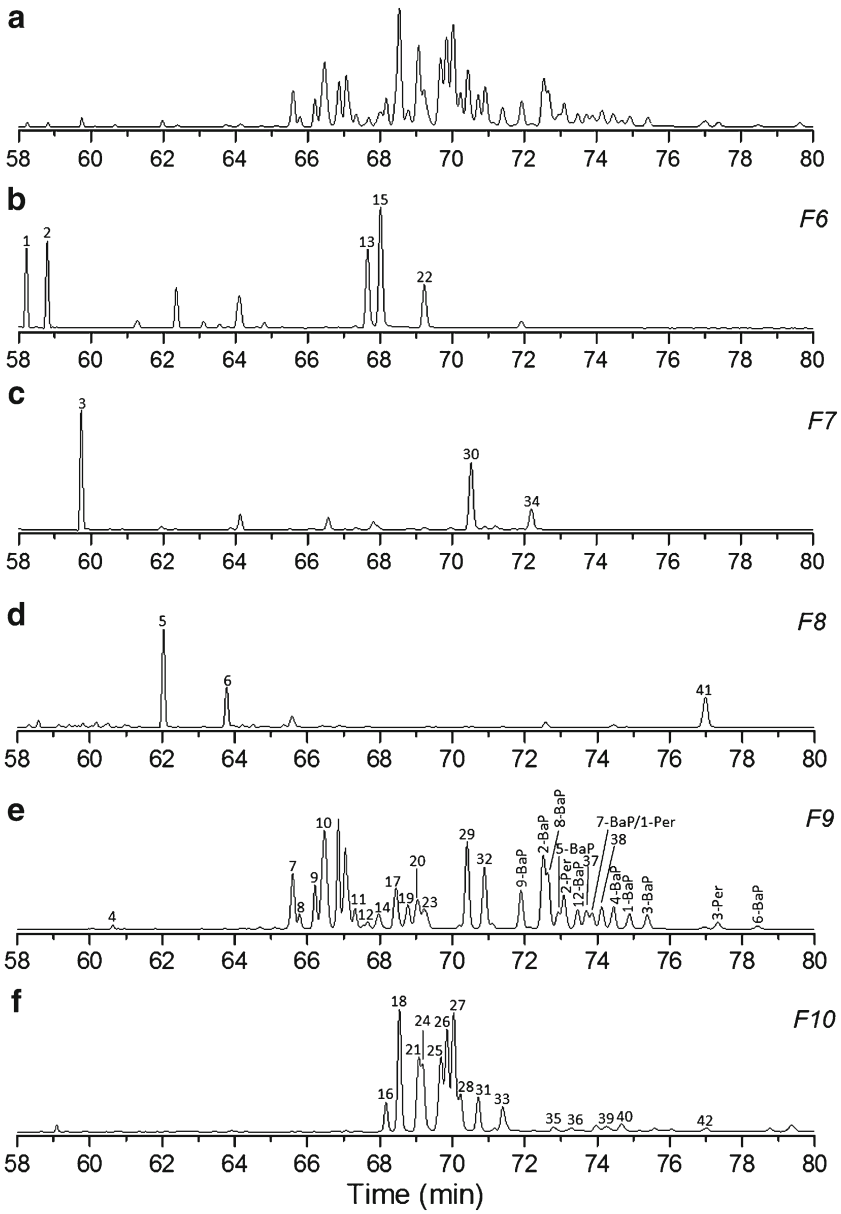

Fig. 4 GC/MS chromatograms in SIM mode for the *m/z* 266 ion for (**a**) SRM 1597a, (**b**) F6, (**c**) F7, (**d**) F8, (**e**) F9, and (**f**) F10

present study, we refine and expand the use of NPLC to improve on the separation and characterization of MM 302 Da isomers in SRM 1597a.

The GC/MS chromatogram obtained for the MM 302 Da isomers in SRM 1597a (unfractionated) is shown in Fig. 6a using the SLB-PAHms column (50% phenyl), which is similar to the results published previously by Schubert et al. [6] using a DB-17 ms (50% phenyl) GC column with identical dimensions (60 m × 0.25 mm i.d. × 0.25 μm film thickness). A number of the isomers remain unresolved despite the long column lengths and extended analysis time. Among the NPLC fractions isolated, the MM 302 Da isomers were identified in F10, F11, and F12 as shown in Fig. 6b, c, and d, respectively. Based on criteria 1 and 2 discussed previously, the number of MM 302 Da PAH isomers identified in F10, F11, and F12 were 4, 4, and 13, respectively. Based on criterion 3, eight additional MM 302 Da PAH isomers (peaks 1–8 in Fig. 6) were tentatively identified in F10, F11, and F12.

The GC analyses of NPLC fractions reported in this study were obtained using the same chromatographic conditions previously published by Schubert et al. [6]. The elution profiles on both GC columns were expected to be similar since both stationary phases are 50% phenyl phases; however, there were some differences in selectivity. One of the differences involved the elution order of naphtho[1,2-*a*]pyrene (N12aP) and naphtho[2,3-*k*]fluoranthene (N23kF). Schubert et al. [6] identified N23kF eluting before N12aP on a DB-17 ms column. N12aP elutes before N23kF in the present study on the SLB-PAHms column. The second difference involves the retention behavior of naphtho[1,2-*e*]pyrene (N12eP). In the

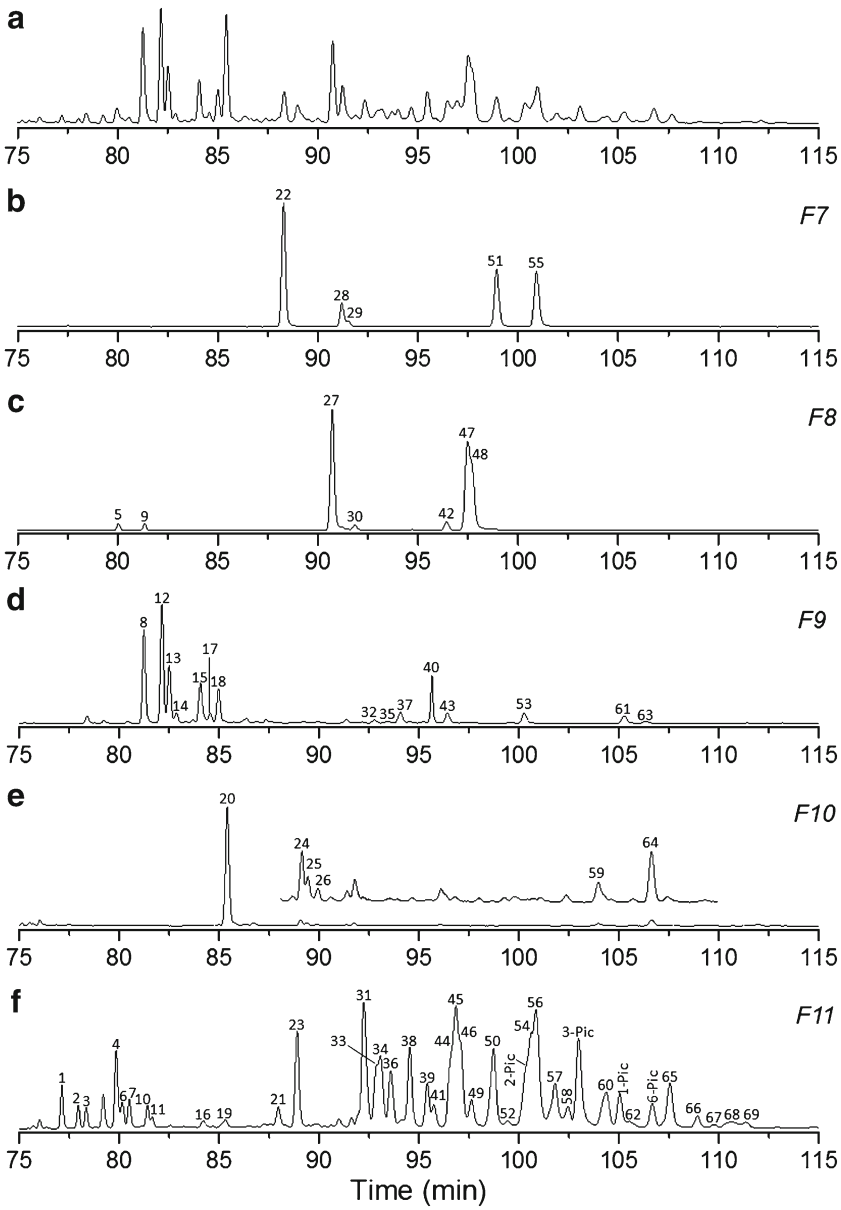

Fig. 5 GC/MS chromatograms in SIM mode for the *m/z* 292 ion for (**a**) SRM 1597a, (**b**) F7, (**c**) F8, (**d**) F9, (**e**) F10, and (**f**) F11

current study N12eP co-elutes with dibenzo[*a,k*]fluoranthene (DBakF); where as in the Schubert et al. [6] study, N12eP co-eluted with dibenzo[*j,l*]fluoranthene (DBjlF). The retention behavior in the current study was confirmed based on comparison of retention times of authentic reference standards.

The third separation difference involves the elution of DBalP, which is the most carcinogenic PAH reported [33, 34], and its accurate determination in combustion-related samples is of considerable importance but difficult to achieve. Schubert et al. [6] reported the separation of DBalP from DBjlF; however, in the present study both MM 302 Da isomers co-elute in the GC/MS chromatogram obtained for the SRM 1597a without NPLC fractionation (Fig. 6a). Due to the differences in structural non-planarity of DBalP ($T = 5.17$ Å) and DBjlF ($T = 4.40$ Å), DBalP is separated from DBjlF in the NPLC fractionation step. As clearly shown in Fig. 6b, DBalP can be determined easily in the GC/MS chromatogram of F10.

The theoretical number of isomers for the MM 328 Da PAHs is 37, and the isomers differ significantly in terms of non-planarity [28, 31]. Reference standards were available for a total of 17 isomers of MM 328 Da PAHs. Direct qualitative or quantitative analysis of the MM 328 Da isomers via GC/MS (no NPLC fractionation) is extremely difficult due to low concentration and co-eluting peaks as shown in Fig. 7a. The MM 328 Da isomers were expected in F11 and later based on the retention times obtained with reference standards on the NH_2 column: (F11) phenanthro[3,4-*c*]phenanthrene (Phe34cPhe) and dibenzo[*g,p*]chrysene (DBgpC); (F12) dibenzo[*c,p*]chrysene (DBcpC), naphtho[2,3-*g*]chrysene (N23gC), and naphtho[1,2-*a*]naphthacene (N12aNap); (F13)

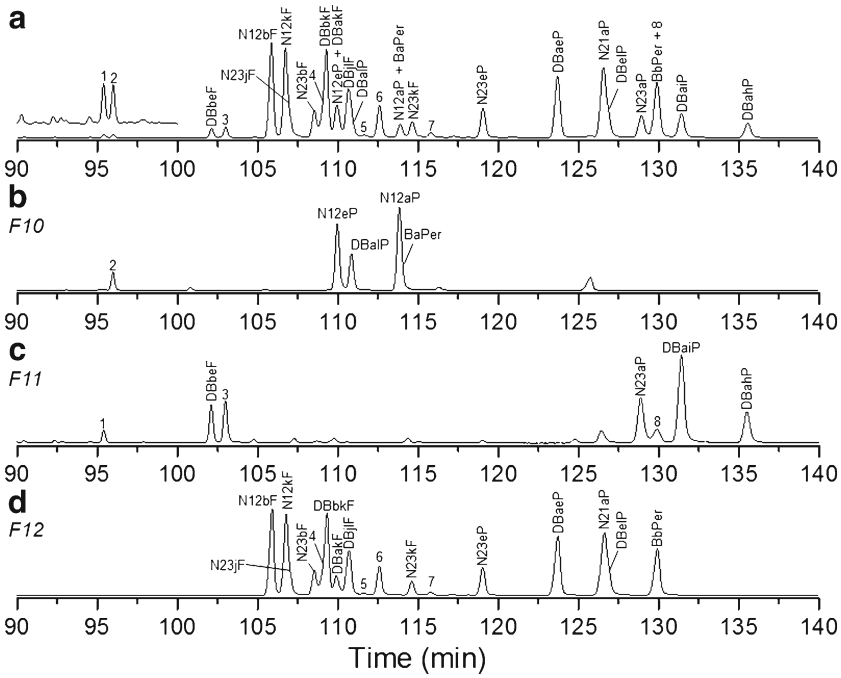

Fig. 6 GC/MS chromatograms in SIM mode for the *m/z* 302 ion for the following samples: (**a**) SRM 1597a, (**b**) F10, (**c**) F11, and (**d**) F12

benzo[*c*]picene (BcPic); and (F14) naphtho[1,2-*b*-]triphenylene (N12bTriPhe), benzo[*h*]pentaphene (BhPen), benzo[*c*]pentaphene (BcPen), dibenzo[*a,c*]naphthacene (DBacNap), dibenzo[*a,j*]naphthacene (DBajNap), dibenzo[*a,l*]naphthacene (DBalNap), naphtho[1,2-*b*]chrysene (N12bC), dibenzo[*b,k*]chrysene (DBbkC), hexaphene (Hexap), benzo[*b*]picene (BbPic), and naphtho[2,1-*a*-]naphthacene (N21aNap) [26]. DBgpC and Phe34cPhe were expected to elute in F11 with T = 6.15 Å and T = 7.41 Å, respectively [26]. DBcpC, N23gC, and N12aNap elute together in F12 because of their similar T values of 5.62 Å, 5.43 Å, and 5.00 Å, respectively. The non-planar BhPen (T = 4.85 Å) elutes in F14 with the 10 planar isomers. For the planar isomers ($T \approx 3.90$ Å), 10 of 11 isomers eluted within the same fraction (F14). BcPic was the one planar isomer that eluted in F13. With the exception of BhPen, all the non-planar isomers eluted in the fractions prior to the planar isomers. The retention behavior of BcPic and BhPen was expected based on their NPLC retention behavior [26].

The GC/MS chromatograms obtained for F11 and F12 are shown in Fig. 7b and c, respectively. Phe34cPhe and DBgpC were not detected in F11 while DBcpC, N23gC, and N12aNap were identified in F12. In the case of DBcpC and N23gC, they were baseline resolved in both GC/MS chromatograms without (Fig. 7a) and with (Fig. 7c) NPLC fractionations. N12aNap was better resolved in the GC/MS chromatogram of F12 because of the elimination of other PAHs during the NPLC fractionation. In addition to the 6 non-planar isomers available in this study, there are 15 unidentified non-planar isomers shown in Fig. S6 (see ESM) with T values ranging from 5.23 Å (N23aTe) to 6.57 Å (DBcgC). In the case of F11, there were two unidentified MM 328 Da isomers identified (peaks labeled 1 and 10). Based on the T values of the 15 non-planar isomers for which no reference standards were available, these two peaks could possibly be the extremely non-planar isomers, i.e., DBclC (T = 5.97 Å), N21cC (T = 6.00 Å), BsPic (T = 6.00 Å), N12gC (T = 6.11 Å), N12aTe (T = 6.24 Å) and/or DBcgC (T = 6.57 Å). In the case of F12, there were nine additional MM 328 Da isomers tentatively identified (peaks labeled 2, 3, 7, 9, 10, and 12–15 in Fig. 7). Those nine tentatively identified MM 328 Da isomers could be the nine remaining non-planar isomers for which no reference standards were available, which have T values ranging from 5.23 Å (N23aTe) to 5.44 Å (N23cC and BfPic).

The GC/MS chromatograms obtained for F13 and F14 are shown in Fig. 7d and e, respectively. BcPic was the only MM 328 Da isomer detected in F13 with an available reference standard. The largest number of MM 328 Da isomers (11) were found in F14. In comparison to the GC/MS chromatogram shown in Fig. 7a, the chromatogram of F14 (Fig. 7e) was improved significantly by the removal of a substantial portion of the MM 326 Da isomers through the NPLC fractionation step (F13, Table 1). In addition, four MM 328 Da isomers were tentatively identified in F13 and one in F14, which are most likely the five planar isomers shown in Fig. S7 (see ESM) with $T \approx 3.90$ Å.

The NPLC conditions used in the present study allowed for BcPic and N21aNap to be determined in separate fractions. Oña-Ruales et al. [5] previously reported the semi-quantitative determination of BcPic and N21aNap together using a similar NPLC fractionation procedure that had both isomers elute in the same NPLC fraction. Quantitative analysis of some MM

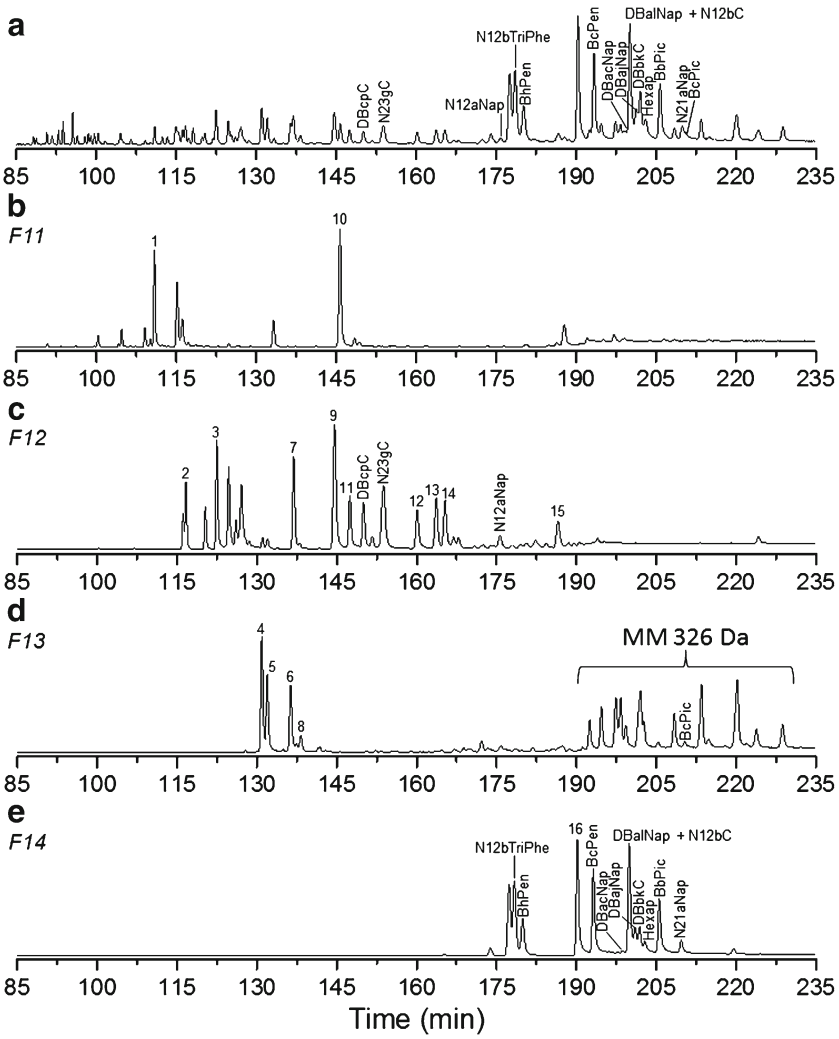

Fig. 7 GC/MS chromatograms in SIM mode for the *m/z* 328 ion for the following samples: (**a**) SRM 1597a, (**b**) F11, (**c**) F12, (**d**) F13, and (**b**) F14

328 Da isomers is still limited despite the benefits of the NPLC fractionation conditions in the present study. DBalNap, N12bC, DBajNap, DBbkC, and Hexap were collected in the same fraction (F14) and are only partially resolved in the GC/MS chromatogram (Fig. 7e) obtained with the 50% phenyl phase.

Seven-ring PAHs

Coronene (Cor) is typically the only PAH of MM 300 Da quantitatively measured in combustion-related [5–8] and environmental [6] samples due to the lack of authentic reference standards for additional isomers. Because of their mass fragmentation similarities with the MM 302 Da PAHs, quantitation via direct analysis with GC/MS is often difficult depending on the stationary phase [6]. The GC/MS chromatogram obtained for the MM 300 Da isomers in SRM 1597a is shown in Fig. S8a (see ESM), which easily demonstrates this issue. Cor was the only MM 300 Da isomer identified based on all three criteria discussed previously. Small contributions are observed in the chromatogram from the MM 302 Da isomers because of the presence of a *m/z* 300 fragmentation peak in their mass spectra. The presence of the MM 302 Da isomers in the chromatogram would prevent Cor from being baseline resolved from other peaks and possibly leading to inaccurate quantitative measurement. The GC/MS chromatograms shown in ESM Fig. S8b (F10) and ESM Fig. S8c (F11) illustrate the benefits of the NPLC fractionation step prior to GC/MS measurements. The contribution of the MM 302 Da isomers shown in ESM Fig. S8a is significantly reduced. Six additional MM 300 Da isomers were tentatively identified in F10 and F11 of the coal tar sample (peaks 1–6).

The GC/MS chromatograms obtained for the seven-ring MM 326 Da PAH isomers in SRM 1597a, F11, F12, F13, and F14 are shown in Fig. S9a, S9b, S9c, S9d, and S9e, respectively (see ESM). Dibenzo[*b,ghi*]perylene (DBbghiPer), dibenzo[*b,qpr*]perylene (DBbqprPer), naphtho[1,2,3,4-*ghi*]-perylene (N1234ghiPer), naphtho[8,1,2-*bcd*]perylene (N812bcdPer), and dibenzo[*cd,lm*]perylene (DBcdlmPer) are identified in the unfractionated sample (ESM Fig. S9)a and in

F13 (ESM Fig. S9d). Reference standards were available for acenaphtho[1,2-*j*]fluoranthene (A12jF), acenaphtho[1,2-*k*-]fluoranthene (A12kF), and diindeno[1,2,3-de,1′,2′,3′-*kl*]-anthracene (Rubicene) but they were not identified in the sample. Due to the NPLC fractionation, there was a total of 24 additional MM 326 Da isomers tentatively identified in the coal tar sample (peaks 1–24). From examining the GC/MS chromatograms of F12, F13 and F14, it is clearly shown that the NPLC fractionation separated two interfering MM 326 Da isomers into different fractions from the five identified isomers. In the case of N1234ghiPer, the interfering MM 326 Da PAH isomer (peak 24) elutes earlier in F12. In the case of DBbqprPer, the interfering MM 326 Da PAH isomer (peak 23) elutes in F14. Similar observations were reported previously by Oña-Ruales et al. [4] using different fractionation conditions and a slightly different GC/MS stationary phase.

Conclusions

A NPLC fractionation procedure using a NH_2 column was developed and used in combination with GC/MS for the identification of three-ring to seven-ring PAHs in a coal tar sample (SRM 1597a). Direct comparison between GC/MS analysis with and without NPLC fractionation clearly illustrates the benefit of the NPLC fractionation procedure. A total of 72 PAHs and 56 MePAHs were identified based on comparison with authentic standards, and an additional 74 PAHs and 117 MePAHs were tentatively identified. The results presented in this paper represent the most extensive qualitative characterizations of PAHs and MePAHs in SRM 1597a and will serve as a guide for future measurements of PAHs in environmental and petroleum SRM samples.

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Compliance with ethical standards

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

References

1. Canha N, Lopes I, Vicente ED, Vicente AM, Bandowe BAM, Almeida SM, et al. Mutagenicity assessment of aerosols in emissions from domestic combustion processes. Environ Sci Pollut Res. 2016;23(11):10799–807. doi:10.1007/s11356-016-6292-2.
2. Olson GM, Meyer BM, Portier RJ. Assessment of the toxic potential of polycyclic aromatic hydrocarbons (PAHs) affecting gulf menhaden (*Brevoortia patronus*) harvested from waters impacted by the bp deepwater horizon spill. Chemosphere. 2016;145:322–8. doi:10.1016/j.chemosphere.2015.11.087.
3. de Souza MR, da Silva FR, de Souza CT, Niekraszewicz L, Dias JF, Premoli S. Evaluation of the genotoxic potential of soil contaminated with mineral coal tailings on snail *Helix aspersa*. Chemosphere. 2015;139:512–7. doi:10.1016/j.chemosphere.2015.07.071.
4. Ona-Ruales JO, Ruiz-Morales Y, Wise SA. Identification and quantification of seven fused aromatic rings C26H14 peri-condensed benzenoid polycyclic aromatic hydrocarbons in a complex mixture of polycyclic aromatic hydrocarbons from coal tar. J Chromatogr A. 2016;1442:83–93. doi:10.1016/j.chroma.2016.02.082.
5. Ona-Ruales JO, Sharma AK, Wise SA. Identification and quantification of six-ring C26H16 cata-condensed polycyclic aromatic hydrocarbons in a complex mixture of polycyclic aromatic hydrocarbons from coal tar. Anal Bioanal Chem. 2015;407(30):9165–76. doi:10.1007/s00216-015-9084-5.
6. 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(2):234–46. doi:10.1021/ac0259111.
7. Wise SA, Benner BA, Byrd GD, Chesler SN, Rebbert RE, Schantz MM. Determination of polycyclic aromatic-hydrocarbons in a coal-tar standard reference material. Anal Chem. 1988;60(9):887–94. doi:10.1021/ac00160a012.
8. Wise SA, Poster DL, Leigh SD, Rimmer CA, Mossner S, Schubert P, et al. Polycyclic aromatic hydrocarbons (PAHs) in a coal tar standard reference material-SRM 1597a updated. Anal Bioanal Chem. 2010;398(2):717–28. doi:10.1007/s00216-010-4008-x.
9. Wilson WB, Alfarhani B, Moore AFT, Bisson C, Wise SA, Campiglia AD. Determination of high-molecular weight polycyclic aromatic hydrocarbons in high performance liquid chromatography fractions of coal tar standard reference material 1597a via solid-phase nanoextraction and laser-excited time-resolved shpol'skii spectroscopy. Talanta. 2016;148:444–53. doi:10.1016/j.talanta.2015.11.018.
10. Moore AFT, Goicoechea HC, Barbosa F, Campiglia AD. Parallel factor analysis of 4.2 K excitation–emission matrices for the direct determination of dibenzopyrene isomers in coal-tar samples with a cryogenic fiber-optic probe coupled to a commercial spectrofluorimeter. Anal Chem. 2015;87(10):5232–9. doi:10.1021/acs.analchem.5b00147.
11. Schantz MM, McGaw E, Wise SA. Pressurized liquid extraction of diesel and air particulate standard reference materials: effect of extraction temperature and pressure. Anal Chem. 2012;84(19):8222–31. doi:10.1021/ac301443v.
12. Zhao Y, Hong B, Fan YQ, Wen M, Han X. Accurate analysis of polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs homologs in crude oil for improving the gas chromatography/mass spectrometry performance. Ecotoxicol Environ Saf. 2014;100:242–50. doi:10.1016/j.ecoenv.2013.10.018.
13. Schantz MM, Cleveland D, Heckert NA, Kucklick JR, Leigh SD, Long SE, et al. Development of two fine particulate matter standard reference materials (< 4 mu m and < 10 mu m) for the determination of organic and inorganic constituents. Anal Bioanal Chem. 2016;408(16):4257–66. doi:10.1007/s00216-016-9519-7.
14. Wise SA, Poster DL, Kucklick JR, Keller JM, VanderPol SS, Sander LC, et al. Standard reference materials (SRMs) for determination of organic contaminants in environmental samples. Anal Bioanal Chem. 2006;386(4):1153–90. doi:10.1007/s00216-006-0719-4.
15. Zhang XT, Hou HW, Chen H, Liu Y, Wang A, Hu QY. Quantification of 16 polycyclic aromatic hydrocarbons in cigarette smoke condensate using stable isotope dilution liquid chromatography with atmospheric-pressure photoionization tandem mass

spectrometry. J Sep Sci. 2015;38(22):3862–9. doi:10.1002/jssc.201500623.
16. Wise SA, Benner BA, Liu HC, Byrd GD, Colmsjo A. Separation and identification of polycyclic aromatic hydrocarbon isomers of molecular-weight 302 in complex-mixtures. Anal Chem. 1988;60(7):630–7. doi:10.1021/ac00158a006.
17. Regueiro J, Matamoros V, Thibaut R, Porte C, Bayona JM. Use of effect-directed analysis for the identification of organic toxicants in surface flow constructed wetland sediments. Chemosphere. 2013;91(8):1165–75. doi:10.1016/j.chemosphere.2013.01.023.
18. Olsson P, Sadiktsis I, Holmback J, Westerholm R. Class separation of lipids and polycyclic aromatic hydrocarbons in normal phase high performance liquid chromatography - a prospect for analysis of aromatics in edible vegetable oils and biodiesel exhaust particulates. J Chromatogr A. 2014;1360:39–46. doi:10.1016/j.chroma.2014.07.064.
19. Fang ML, Getzinger GJ, Cooper EM, Clark BW, Garner LVT, Di Giulio RT. Effect-directed analysis of elizabeth river porewater: developmental toxicity in zebrafish (*Danio rerio*). Environ Toxicol Chem. 2014;33(12):2767–74. doi:10.1002/etc.2738.
20. Marvin CH, McCarry BE, Lundrigan JA, Roberts K, Bryant DW. Bioassay-directed fractionation of pah of molecular mass 302 in coal tar-contaminated sediment. Sci Total Environ. 1999;231(2–3):135–44. doi:10.1016/s0048-9697(99)00096-0.
21. Gilgenast E, Boczkaj G, Przyjazny A, Kaminski M. Sample preparation procedure for the determination of polycyclic aromatic hydrocarbons in petroleum vacuum residue and bitumen. Anal Bioanal Chem. 2011;401(3):1059–69. doi:10.1007/s00216-011-5134-9.
22. Sander LC, Pursch M, Wise SA. Shape selectivity for constrained solutes in reversed-phase liquid chromatography. Anal Chem. 1999;71(21):4821–30. doi:10.1021/ac9908187.
23. Sander LC, Wise SA. Influence of stationary-phase chemistry on shape-recognition in liquid-chromatography. Anal Chem. 1995;67(18):3284–92. doi:10.1021/ac00114a027.
24. Sander LC, Wise SA. Shape selectivity in reversed-phase liquid-chromatography for the separation of planar and nonplanar solutes. J Chromatogr A. 1993;656(1–2):335–51. doi:10.1016/0021-9673(93)80808-l.
25. Wise SA, Sander LC, Lapouyade R, Garrigues P. Anomalous behavior of selected methyl-substituted polycyclic aromatic-hydrocarbons in reversed-phase liquid-chromatography. J Chromatogr. 1990;514(2):111–22. doi:10.1016/s0021-9673(01)89383-3.
26. Wilson WB, Hayes HV, Sander LC, Campiglia AD, Wise SA. Normal-phase liquid chromatography retention behavior of polycyclic aromatic hydrocarbon and their methyl-substituted derivatives on an aminopropyl stationary phase. Anal Bioanal Chem. 2017; in preparation.
27. Mössner SG, Wise SA. Determination of polycyclic aromatic sulfur heterocycles in fossil fuel-related samples. Anal Chem. 1999;71(1):58–69. doi:10.1021/ac980664f.
28. Sander LC, Wise SA. Polycyclic Aromatic Hydrocarbon Structure Index, NIST Special Publication 922 (http://www.nist.gov/mml/csd/special-publication-922-polycyclic-aromatic-hydrocarbon-structure-index), U.S. Government Printing Office, Washington, DC, 1997. Access 17.01.03.
29. 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(2):113–39. doi:10.1080/10406630308059.
30. Nalin F, Sander LC, Wilson WB, Wise SA. Gas chromatographic retention behavior of polycyclci aromatic hydrocarbons and alkyl-substituted pahs on two stationary phases of different selectivity. Anal Bioanal Chem 2017, in preparation.
31. Dias JR. Handbook of polycyclic hydrocarbons. New York: Elseveir; 1987.
32. 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(3):169–84. doi:10.1080/10406639308047869.
33. Cavalieri EL, Higginbotham S, RamaKrishna NVS, Devanesan PD, Todorovic R, Rogan EG. Comparative dose—response tumorigenicity studies of dibenzo[a,l]pyrene versus 7, 12-dimethylbenz[a]anthracene, benzo[a and two dibenzo[a,l]pyrene dihydrodiols in mouse skin and rat mammary gland. Carcinogenesis. 1991;12(10):1939–44. doi:10.1093/carcin/12.10.1939.
34. Devanesan P, Ariese F, Jankowiak R, Small GJ, Rogan EG, Cavalieri EL. Preparation, isolation, and characterization of dibenzo[a,l]pyrene diol epoxide−deoxyribonucleoside monophosphate adducts by hplc and fluorescence line-narrowing spectroscopy. Chem Res in Toxicol. 1999;12(9):789–95. doi:10.1021/tx980202x.