Anal Bioanal Chem (2015) 407:9165–9176
DOI 10.1007/s00216-015-9084-5

RESEARCH PAPER

Identification and quantification of six-ring $C_{26}H_{16}$ *cata*-condensed polycyclic aromatic hydrocarbons in a complex mixture of polycyclic aromatic hydrocarbons from coal tar

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Received: 11 August 2015 / Revised: 21 September 2015 / Accepted: 25 September 2015 / Published online: 8 October 2015

Abstract We applied a combination of normal-phase liquid chromatography (NPLC) with ultraviolet–visible spectroscopy and gas chromatography with mass spectrometry (GC/MS) for the fractionation, identification, and quantification of six ring $C_{26}H_{16}$ *cata*-condensed polycyclic aromatic hydrocarbons, PAHs, in the Standard Reference Material 1597a, Complex Mixture of PAHs from Coal Tar. For the characterization analysis, we calculated the GC retention indices of 17 $C_{26}H_{16}$ PAH authentic reference standards using the Rxi-PAH and DB-5 GC columns. Then, we used NPLC with ultraviolet–visible spectroscopy to isolate the fractions containing the $C_{26}H_{16}$ PAHs, and subsequently, we used GC/MS to establish the identity and quantity of the $C_{26}H_{16}$ PAHs using authentic reference standards. Following this procedure, 12 $C_{26}H_{16}$ *cata*-condensed PAHs benzo[*c*]pentaphene, dibenzo[*f,k*]tetraphene, benzo[*h*]pentaphene, dibenzo[*a,l*]tetracene, dibenzo[*c,k*]tetraphene, naphtho[2,3-*c*]tetraphene, dibenzo[*a,c*]tetracene, benzo[*b*]picene, dibenzo[*a,j*]tetracene, naphtho[2,1-*a*]tetracene, dibenzo[*c,p*]chrysene, and dibenzo[*a,f*]tetraphene were identified and quantified for the first time, and benzo[*c*]picene was quantified for the first time in an environmental combustion sample.

Keywords Coal tar · Polycyclic aromatic hydrocarbons (PAHs) · Standard reference material (SRM) · Normal-phase liquid chromatography with ultraviolet–visible spectroscopy (NPLC/UV–vis) · Gas chromatography with mass spectrometry (GC/MS)

Introduction

Polycyclic aromatic hydrocarbons (PAHs) of the $C_{26}H_{16}$ group include 37 benzenoid *cata*-condensed isomers with molecular mass 328 Da listed in Fig. 1 [1]. Reports about the separation and unquestionable identification of the PAHs in this group are scarce. Bemgard et al. [2] reported the gas chromatographic (GC) analysis of the $C_{26}H_{16}$ *cata*-condensed PAHs focusing on the evaluation of the performance of four different chromatographic columns for the separation of fourteen isomers and the respective identification of these isomers in a coal tar sample, i.e., Standard Reference Material (SRM) 1597. In that report, the calculation of the chromatographic retention indices allowed the identification of benzo[*c*]picene and the tentative identifications of benzo[*c*]pentaphene, dibenzo[*f,k*]tetraphene, dibenzo[*c,k*]tetraphene, naphtho[2,3-*c*]tetraphene, and benzo[*b*]picene. The retention indices calculations were performed using two series of PAHs as index markers. The first series composed of phenanthrene, chrysene, picene, benzo[*c*]picene, and dinaphtho[2,1-*a*:2,1-*h*]anthracene, and the second series composed of coronene and benzo[*a*]coronene. In a more comprehensive analysis, Bergvall et al. [3], reported the determination of the PAHs from 252 to 302 Da and the tentative identification of PAHs from 316 to 376 Da in urban dust (SRM 1649a) and diesel

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Electronic supplementary material The online version of this article (doi:10.1007/s00216-015-9084-5) contains supplementary material, which is available to authorized users.

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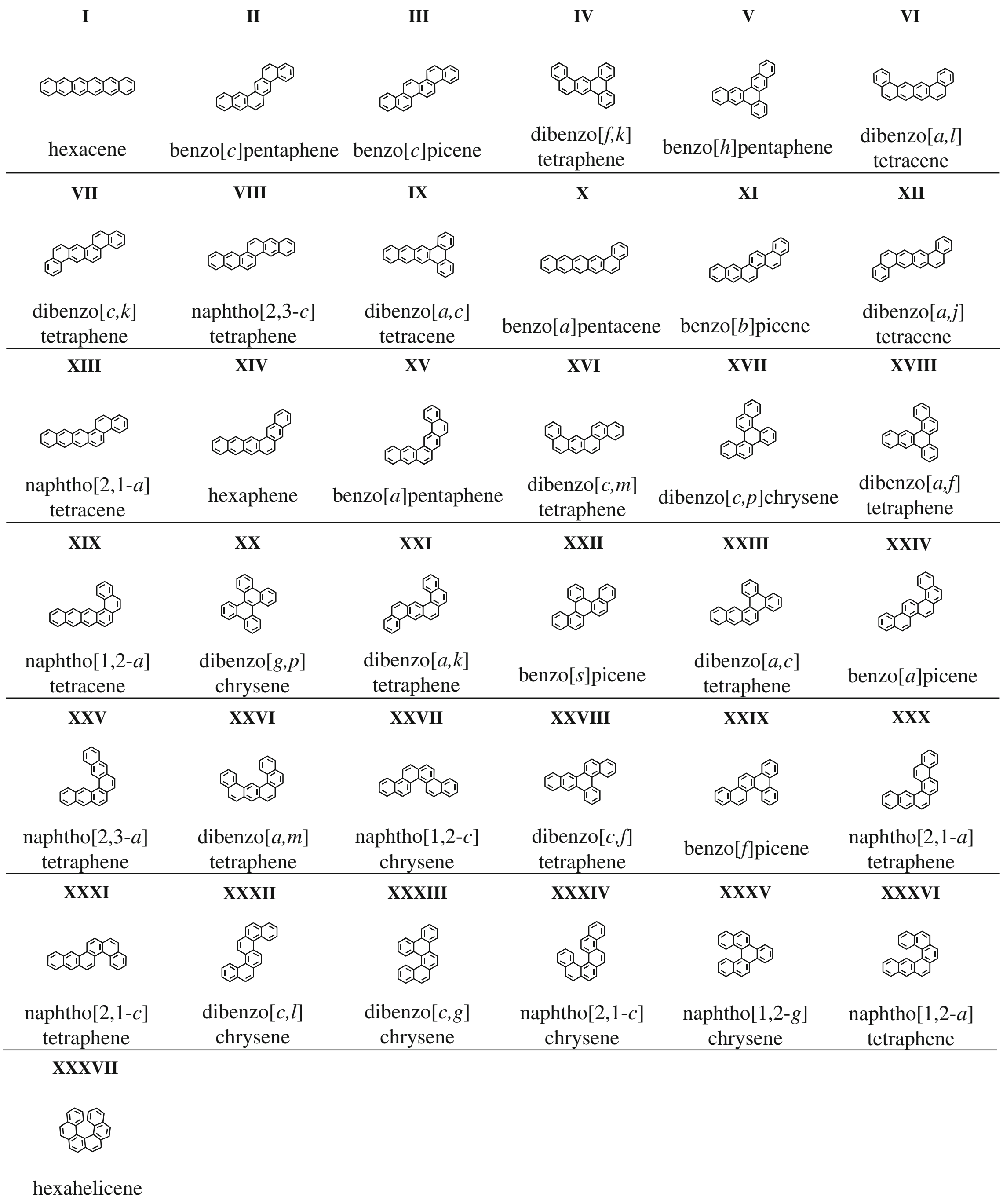


Fig. 1 The 37 $C_{26}H_{16}$ *cata*-condensed benzenoid PAHs ordered with respect to the increase in steric hindrance irregularities. **I** with no steric hindrance, **II** to **XVI** with one or more bay regions, **XVII** to **XXXII** with one or more cove regions, **XXXIII** to **XXXVI** with one fjord region, and **XXXVII** with one helical region

particulate matter (SRM 1650 and SRM 2975). An accelerated extraction method followed by qualitative and quantitative analysis using gas chromatography with mass spectrometry (GC/MS) was applied for the characterization of the PAHs. In the studies of Bemgard et al. [2] and Bergvall et al. [3], normal-phase liquid chromatography (NPLC) was used for the isolation of the fraction of interest and GC/MS was used for the identification of the PAHs. Although the two studies provided interesting results regarding the tentative presence of some $C_{26}H_{16}$ *cata*-condensed PAHs in a variety of samples, the lack of reference standards and the broad NPLC fractions from the samples precluded unquestionable identification of a significant number of $C_{26}H_{16}$ *cata*-condensed PAHs.

With the availability of authentic standards, one strategy for the identification of the $C_{26}H_{16}$ *cata*-condensed PAHs is use of a combination of NPLC with ultraviolet–visible spectroscopy (NPLC/UV–vis), and GC/MS. Initially, NPLC/UV–vis using a polar stationary phase, e.g., aminopropyl ($NH_2CH_2CH_2CH_2$-) with a nonpolar mobile phase such as hexane-dichloromethane mixtures, allows separation of the PAH isomer groups based on the number of aromatic carbons [4–8]. GC/MS then provides the separation and molecular mass determination for identification of the isomers through comparison of the elution times of the unknown PAHs with elution times of authentic standards of $C_{26}H_{16}$ *cata*-condensed PAHs. This method is analogous to the approach used in the characterization of PAHs with molecular mass up to 302 Da in two SRM air particulate samples [8]. In that report, NPLC/UV–vis was used for the separation of eight fractions and the combination of GC/MS and reversed-phase liquid chromatography with UV–vis was used for the separation, identification, and quantification of the PAHs. Using this approach, 55 unsubstituted PAHs and polycyclic aromatic sulfur heterocycles (PASHs) were identified and 75 unsubstituted PAHs and PASHs were characterized based on molecular mass only [8].

The sample under investigation in this report is a complex mixture of PAHs from coal tar for which mass fractions of selected PAHs have been assigned and which is disseminated as SRM 1597a [9–11] (an updated version of the above mentioned SRM 1597). For SRM 1597a, certified values for 34 PAHs, reference values for 36 PAHs, and reference values for 10 PASHs are reported in the Certificate of Analysis [9]. In spite of the comprehensive characterization of SRM 1597a, there are still numerous PAHs that have not been identified or quantified in this material. Thus, the interest in the application of new experimental methods for the qualitative and quantitative elucidation of additional PAHs in this mixture remains active.

The steric hindrance or nonplanar geometry of PAHs is linked to the mutagenic/carcinogenic potential of PAHs [12]. Among the 37 $C_{26}H_{16}$ *cata*-condensed PAHs in Fig. 1, 16 include a cove region (XVII, XVIII, XIX, XX, XXI, XXII, XXIII, XXIV, XXV, XXVI, XXVII, XXVIII, XXIX, XXX, XXXI, XXXII), 4 include a fjord region (XXXIII, XXXIV, XXXV, XXXVI), and 1 includes a helical region (XXXVII). However, only 14 out of these 21 nonplanar structures have procedures of synthesis reported in the literature (i.e., XVIII, XX, and XXXVII [13]; XVII [14]; XIX in unreported results from W Schmidt; XXI and XXV [15]; XXII [16]; XXIII and XXXIII [17]; XXIV, XXVI, and XXXIV [18]; and XXXV [19]), and only 1 of these 21 nonplanar structures, XVII, has the carcinogenic potential evaluated and demonstrated [14, 20]. Consequently, it is evident that only the identification of $C_{26}H_{16}$ *cata*-condensed PAHs in actual samples will encourage the syntheses of the other 7 remaining nonplanar PAHs (XXVII, XXVIII, XXIX, XXX, XXXI, XXXII, XXXVI) and the evaluation of the biological behavior of the 20 untested nonplanar structures. For this study, reference standards of the following nineteen $C_{26}H_{16}$ *cata*-condensed PAHs were available: I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XVII, XVIII, XIX, XX, and XXXVII. In parallel theoretical research efforts, the characterization of the aromatic sextets [21] and the prediction of the locations of maximum absorbance in the UV–vis spectra [22] of *cata*-condensed PAHs, i.e., $C_{26}H_{16}$, were analyzed so as to have a comprehensive view of the behavior of these PAHs.

In this study we identified and quantified the presence of 13 $C_{26}H_{16}$ *cata*-condensed isomers in a coal tar extract (SRM 1597a): benzo[*c*]pentaphene, II; benzo[*c*]picene, III; dibenzo[*f,k*]tetraphene, IV; benzo[*h*]pentaphene, V; dibenzo[*a,l*]tetracene, VI; dibenzo[*c,k*]tetraphene, VII; naphtho[2,3-*c*]tetraphene, VIII; dibenzo[*a,c*]tetracene, IX; benzo[*b*]picene, XI; dibenzo[*a,j*]tetracene, XII; naphtho[2,1-*a*]tetracene, XIII; dibenzo[*c,p*]chrysene, XVII; and dibenzo[*a, f*]tetraphene, XVIII. As far as we know, this is the first time that III has been quantified and the first time that II, IV, V, VI, VII, VIII, IX, XI, XII, XIII, XVII, and XVIII have been identified and quantified in an environmental combustion sample.

Materials and methods

Materials

Chemicals

SRM 1597a was obtained from the Office of Reference Materials, National Institute of Standards and Technology, Gaithersburg, Maryland. The $C_{26}H_{16}$ *cata*-condensed PAH standards were obtained from the following sources: I from W Schmidt, Institut für PAH-Forschung, Greifenberg, Germany and TJ Chow, Department of Chemistry, National Taiwan Normal University, Taipei, Taiwan; II, III, IV, V, VII, VIII, IX, X, XI, XIII, XIV, XVIII, XIX, XX, and XXXVII from W Schmidt, Institut fur PAH-Forschung, Greifenberg, Germany; and VI, XII, and XVII from AK Sharma, Penn State University, College of Medicine, Department of Pharmacology, Hershey, Pennsylvania. The purity of these compounds was not assessed. The $C_{24}H_{14}$ *peri*-condensed PAH standards used in this study were from the same sources as reported by Schubert et al. [23]. Hexanes (99.9 %) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Dichloromethane (99.8 %) was purchased from JT Baker (Center Valley, PA, USA).

Instrumentation and chromatographic columns

NPLC/UV–vis was performed using a Varian 9012 Solvent Delivery System (Agilent, Santa Clara, CA) coupled to Jasco UV-1570 Intelligent UV/Vis Detector (Jasco, Easton, MD) using a Waters Spherisorb 5 μm NH_2 10×250 mm semi-prep LC column (Waters, Milford, MA). GC/MS analyses were performed on an Agilent 6890 N Network Gas Chromatograph coupled to Agilent 5973 Inert Mass Selective Detector (Agilent, Santa Clara, CA) using a Restek Rxi-PAH GC Column (Restek, Bellefonte, PA), 60 m length, 0.25 mm id, 0.10 μm film thickness, maximum programmable temperature 360 °C, and minimum bleed at 350 °C, and using an Agilent DB-5 GC Column (Agilent, Santa Clara, CA), 5 % phenylmethylpolysiloxane stationary phase, 60 m length, 0.25 mm id, 0.25 μm film thickness, maximum programmable temperature 350 °C, and minimum bleed at 325 °C.

Methods

Qualitative analysis: fractionation

For the NPLC fractionation, 1 mL of the original sample of SRM 1597a was concentrated by evaporation to 0.5 mL and solvent-exchanged from toluene (the original solvent in SRM 1597a) to the solvent mixture hexane/dichloromethane (90/10 *vol/vol*). Next, the sample solution (0.5 mL) was added to 1 mL of the hexane/dichloromethane mixture so as to have the concentration of the sample at the maximum workable level for the fractions of interest, i.e., just below the overloading limit of the LC column during the NPLC/UV–vis analysis. The NPLC fractionation consisted of injection of five aliquots of 0.25 mL of the sample solution on the amino semi-prep column using a mobile phase of hexane/dichloromethane (80/20 *vol/vol*) at a flow rate of 2 mL/min. The following six fractions were collected: **F0** (from 14.7 to 15.4 min), **F1** (from 15.4 to 17.0 min), **F2** (from 17.0 to 19.2 min), **F3** (from 19.2 to 20.7 min), **F4** (from 20.7 to 22.7 min), and **F5** (from 22.7 to 25.7 min). Finally, the collected volumes were concentrated by evaporation to individual volumes of 0.5 mL.

Qualitative analysis: identification

The concentrated fractions were analyzed to determine the presence of $C_{26}H_{16}$ *cata*-condensed PAH isomers using GC/MS with on-column injection and the mass spectrometer programmed in selected-ion monitoring (SIM) mode for the *m/z* 328 ion. The GC was temperature programmed as follows: isothermal at 140 °C for 2 min then 20 °C/min to 325 °C, and isothermal at 325 °C for 90 min. The identification of the $C_{26}H_{16}$ *cata*-condensed PAHs was accomplished by matching the retention times of the reference standards with the retention times of the unknown compounds in the GC/MS analysis of the fractions.

Quantitative analysis

The quantification of the identified $C_{26}H_{16}$ *cata*-condensed PAHs in SRM 1597a was performed using the mass fractions reported in the Certificate of Analysis for SRM 1597a [9] for coronene, dibenzo[*a,l*]pyrene, and dibenzo[*a,h*]pyrene as reference internal standards. The detector response was assumed to be equivalent for all of the molecular mass 328 Da isomers and equivalent to the response for the reference internal standards. These results should be considered as semi-quantitative using this approach.

Results and discussion

Selection of GC column for the separation of the $C_{26}H_{16}$ *cata*-condensed PAHs

The Rxi-PAH and DB-5 GC columns were evaluated for this analytical study due to the high thermal limit of their stationary phases (i.e., 360 and 350 °C) which is necessary for the elution of high-molecular-mass PAHs. The Rxi-PAH column is a proprietary phase with retention behavior for PAHs similar to a 50 % phenylmethylpolysiloxane phase, and the DB-5 is a 5 % phenylmethylpolysiloxane phase. The GC/MS chromatograms for the separation of 17 $C_{26}H_{16}$ *cata*-condensed PAHs using the Rxi-PAH and the DB-5 columns are shown in Fig. 2a and b illustrating a similar behavior for the two columns and demonstrating the resolving power of the phenylmethylpolysiloxane stationary phases for the high-molecular-mass PAH isomers investigated in this study. The $C_{26}H_{16}$ *cata*-condensed PAHs hexacene (**I**) and benzo[*a*]pentacene (**X**) were available; however, they are not shown in Fig. 2a and b due to their low solubility in dichloromethane solution and high photo-reactivity.

Calculation of retention indices

The retention indices for the $C_{26}H_{16}$ *cata*-condensed PAH standards were calculated using the original work of Lee et al. [24] based on the series naphthalene (two rings), phenanthrene (three rings), chrysene (four rings), and picene (five rings). Since the retention time of all of the $C_{26}H_{16}$ *cata*-condensed PAH standards available exceeds the retention time of picene in the series of Lee, an extended series using benzo[*c*]picene (six rings) was used. The equation for the calculation of the retention index is the following [24]:

$$I = 100\,\frac{T_{R(substance)} - T_{R(C_z)}}{T_{R(C_{z+1})} - T_{R(C_z)}} + 100\,z$$

Where, I = retention index, $T_{R(substance)}$ = retention time of the substance under investigation, $T_R\ (C_z)$ and $T_R\ (C_{z+1})$ =

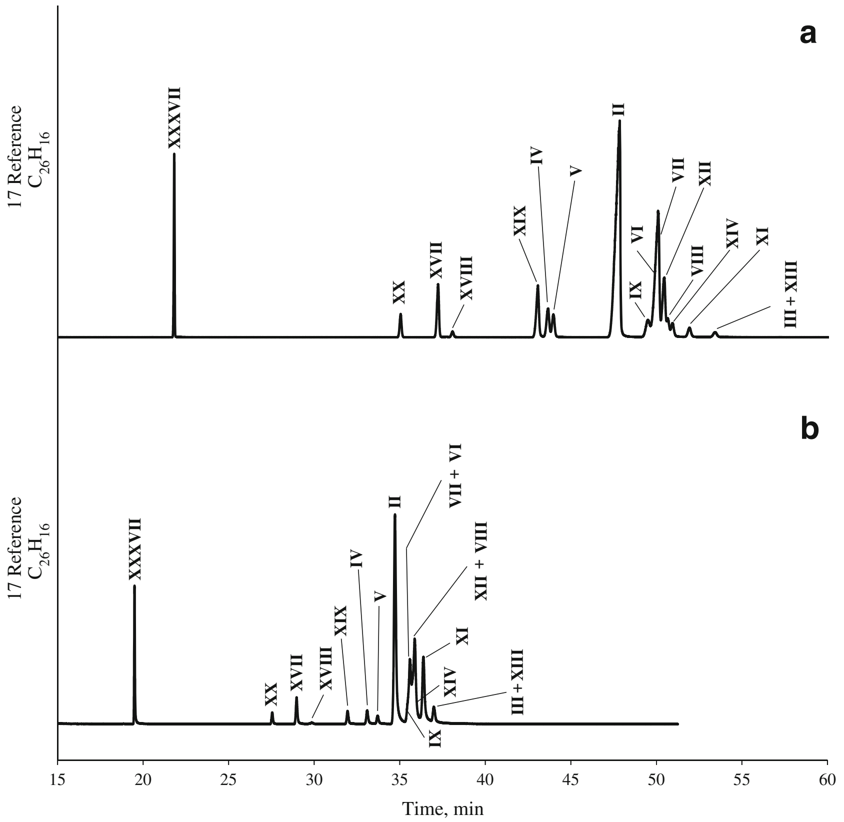


Fig. 2 GC/MS analyses of the 17 $C_{26}H_{16}$ *cata*-condensed PAH standards available with SIM for the *m/z* 328 ion in the following columns: **a** Rxi-PAH, **b** DB-5

retention times of the PAH standards, e.g., picene and benzo[*c*]picene, bracketing the substance under investigation, and z = number of carbon atoms in the PAH standard eluting immediately before the substance under investigation. The calculated retention indices for the 17 $C_{26}H_{16}$ PAHs standards available (i.e., II, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XVII, XVIII, XIX, XX, and XXXVII) using the Rxi-PAH and DB-5 GC columns are presented in Table 1. The study of Bemgard et al. [2] compared four GC columns, i.e., Rtx-200 with a trifluoropropylmethylpolysiloxane phase, CP-Sil 8 with a 5 % diphenyl, 95 % dimethyl phase, XTI-5 with a 5 % phenyl phase, and a silabiphenyl phase (silarylene/siloxane copolymer) with 25 % biphenyl. At the time of the Bemgard et al. [2] study, these four columns represented the typical nonpolar 5 % phenylmethylpolysiloxane-like phases used for PAH separations. The Rxi-PAH and the DB-5 columns provide elution of the $C_{26}H_{16}$ PAH over 20 and 10 min (excluding XXXVII), respectively, whereas they elute in about a 5 min range on the columns in the Bemgard study. All of the index standards elute between picene (retention index 500) and benzo[*c*]picene (retention index 600). Therefore, only two retention index standards (i.e., picene and benzo[*c*]picene) were necessary for calculation of the retention indices for the Rxi-PAH and DB-5 columns in comparison to three index standards (i.e., picene, benzo[*c*]picene,

Table 1 Average retention indices and standard deviations for the seventeen $C_{26}H_{16}$ *cata*-condensed PAHs with standards available

$C_{26}H_{16}$ PAH	Average retention index		Standard deviation[a]	
	Rxi-PAH	DB-5	Rxi-PAH	DB-5
II	581.64	587.39	0.03	0.30
III	600.00	600.00		
IV	569.69	578.14	0.02	0.46
V	570.67	581.49	0.02	0.46
VI	589.34	592.14	0.16	0.36
VII	589.63	592.14	0.15	0.36
VIII	591.69	594.30	0.12	0.25
IX	588.10	591.36	0.03	0.36
XI	595.04	596.71	0.04	0.18
XII	590.31	594.30	0.09	0.25
XIII	600.00	600.00		
XIV	592.26	595.17	0.11	0.20
XVII	550.00	555.52	0.01	0.32
XVIII	552.65	560.31	0.03	0.37
XIX	567.81	571.89	0.03	0.41
XX	543.34	547.72	0.01	0.31
XXXVII	502.77	503.64	0.01	0.02

[a] Based on 5 determinations

and dinaphtho[2,1-*a*:2,1-*h*)anthracene) used by Bemgard et al. [2] to calculate retention indices for the Rtx-200, CP-Sil 8, and XTI-5 columns. These are the first reported retention indices for the $C_{26}H_{16}$ PAH on a 50 % phenyl-like stationary phase.

Qualitative analysis

Although there is a similar chromatographic behavior between the Rxi-PAH and the DB-5 columns, the $C_{26}H_{16}$ PAHs elute over a longer time span for the Rxi-PAH column (20 min) than for the DB-5 (10 min), and the separation of the PAHs pairs VI and VII and VIII and XII is accomplished only with the Rxi-PAH column (see Fig. 2a and b); therefore, only the Rxi-PAH column was used for the analyses (qualitative and quantitative). As shown in Fig. 3, when no NPLC fractionation is involved, the GC/MS chromatogram with SIM for the *m/z* 328 ion indicates the presence of a very limited number of $C_{26}H_{16}$ *cata*-condensed PAHs with molecular mass 328 Da. Only two $C_{26}H_{16}$ *cata*-condensed PAHs (II and XI) were identified based on comparison of retention times with the 17 $C_{26}H_{16}$ *cata*-condensed PAH reference standards illustrated in Fig. 2a, and two additional isomers were characterized based only on molecular mass. The remaining peaks in the chromatogram belong to either PAHs with lower molecular mass, e.g., 326 Da, which generate a small 328 ion or to co-eluting PAHs (e.g., alkyl-PAHs of lower molecular mass) that elute in the same time range as the $C_{26}H_{16}$ *cata*-condensed PAHs. Consequently, the identification and subsequent quantification of $C_{26}H_{16}$ *cata*-condensed PAHs based on direct GC/MS analysis of SRM 1597a is not viable.

Due to the limitations of direct GC/MS analysis, an approach involving an NPLC fractionation of the coal tar mixture was investigated. The NPLC/UV–vis chromatogram from the fractionation of SRM 1597a is illustrated in Fig. 4. Six fractions were collected (**F0**, **F1**, **F2**, **F3**, **F4**, and **F5**) as described in the Materials and methods section. Prior to the qualitative and quantitative characterization of the unknown $C_{26}H_{16}$ *cata*-condensed PAHs in SRM 1597a, the elution behavior of the $C_{26}H_{16}$ *cata*-condensed PAH reference standards was studied using NPLC/UV–vis so as to locate the particular fraction in which each of these standards elute. The results indicated that XVII, XX, and XXXVII elute in **F1**; XVIII and XIX elute in **F2**; and II, III, IV, V, VI, VII, VIII, IX, XI, XII, XIII, and XIV elute in **F4**. There was no indication of the presence of $C_{26}H_{16}$ *cata*-condensed PAHs in **F0**, **F3**, and **F5** (i.e., negligible *m/z* 328 response or no elution of available standards); therefore, **F0**, **F3**, and **F5** were disregarded as retention time intervals of interest for the identification of $C_{26}H_{16}$ *cata*-condensed PAHs. The distribution of the detected ions in **F0**, **F1**, **F2**, **F3**, **F4**, and **F5** using scan mode (from 278 to 374 *m/z*) is summarized in Table 2.

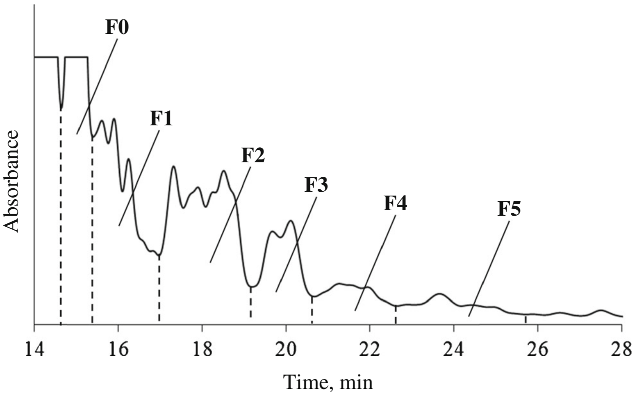


Fig 4 NPLC/UV–Vis chromatogram of the fractionation of the concentrated SRM 1597a in six consecutive fractions, **F0** (from 14.7 to 15.4 min), **F1** (from 15.4 to 17.0 min), **F2** (from 17.0 to 19.2 min), **F3** (from 19.2 to 20.7 min), **F4** (from 20.7 to 22.7 min), and **F5** (from 22.7 to 25.7 min)

Among these fractions, Fraction 1 (**F1**), Fraction 2 (**F2**), and Fraction 3 (**F3**) were also analyzed as retention time intervals of interest for the identification of $C_{24}H_{14}$ (**F1** and **F2**) and $C_{26}H_{14}$ (**F3**) *peri*-condensed PAHs, i.e., molecular mass 302 and 326 Da. GC/MS analyses of **F1** with SIM for the *m/z* 302 ion is illustrated in Fig. 5a with peaks identified based on

Fig. 3 GC/MS chromatogram of the unfractionated SRM 1597a with SIM for the *m/z* 328 ion

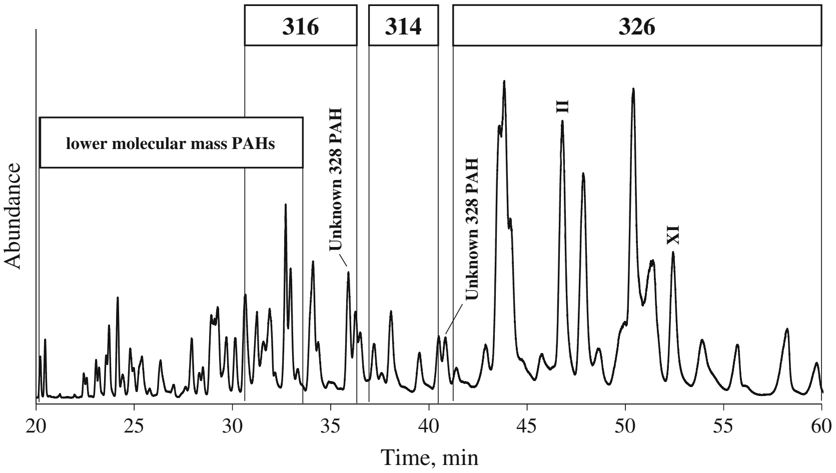


Table 2 Distribution of the detected ions in **F0**, **F1**, **F2**, **F3**, **F4**, and **F5** using scan mode (from 278 to 374 *m/z*)

		Fraction					
		F0	F1	F2	F3	F4	F5
ion m/z	278	●●	●●	●	○	○	○
	300	○	●	●	●	●	●
	302	●	●	●●	●	○	○
	326	○	●	●	●●	●	○
	328	○	●	●	●	●●	○
	350	●	○	○	●	●	●
	352	○	○	●	●	●	●●
	374	○	○	○	○	○	○

Black circle denotes presence of ions. White circle denotes absence of ions. Two black circles denotes the most abundant ion

retention time of authentic standards as dibenzo[*b,e*]fluoranthene, naphtho[1,2-*e*]pyrene, dibenzo[*a,l*]pyrene, naphtho[1,2-*a*]pyrene, and benzo[*a*]perylene (See Electronic Supplementary Material (ESM) Fig. S3 for structures and names of molecular mass 302 Da PAH isomers). GC/MS analysis of **F2** with SIM for the *m/z* 302 ion is shown in Fig. 5b with the following peaks identified based on retention time of authentic standards: naphtho[1,2-*b*]fluoranthene, naphtho[1,2-*k*]fluoranthene, naphtho[2,3-*j*]fluoranthene, naphtho[2,3-*b*]fluoranthene, dibenzo[*b,k*]fluoranthene, dibenzo[*a,k*]fluoranthene, dibenzo[*j,l*]fluoranthene, naphtho[2,3-*k*]fluoranthene, naphtho[2,3-*e*]pyrene, dibenzo[*a,e*]pyrene, naphtho[2,1-*a*]pyrene, dibenzo[*e,l*]pyrene, naphtho[2,3-*a*]pyrene, benzo[*b*]perylene, dibenzo[*a,i*]pyrene, and dibenzo[*a,h*]pyrene. Although the focus of this study was not the molecular mass 302 Da PAHs, the NPLC fractionation provides some new information on the characterization of the coal tar SRM relative to these isomers. As illustrated in Fig. 5a and b, the 302 molecular mass isomers are distributed in both **F1** and **F2**. Even though the NPLC fractionation of PAHs is based on the number of aromatic carbons in the PAHs, isomers with nonplanar structures typically elute earlier than the planar isomers [4, 7]. Based on the NPLC retention data published previously for molecular mass 302 Da PAH isomers [7] and the characterization of the location of the various 328 molecular mass in the selected fractions mentioned above, dibenzo[*a,l*]pyrene, benzo[*a*]perylene, and dibenzo[*b,e*]fluoranthene have retention indices of 4.60, 5.12, and 5.36, respectively, and therefore they would elute earlier than the other molecular mass 302 Da isomers with NPLC retention indices ranging from 5.90 to 6.42. NPLC retention indices were not published for naphtho[1,2-*e*]pyrene and naphtho[1,2-*a*]pyrene since standards for these isomers were not available in the earlier study [7]. However, the five 302 Da molecular mass isomers identified in **F1** are nonplanar based on the thickness values published by Sander and Wise [1] and later in Schubert et al. [23], with thickness values of 4.64 Å, 5.13 Å, 5.15 Å, 5.17 Å, and 5.47 Å for dibenzo[*b,e*]fluoranthene, naphtho[1,2-*a*]pyrene, naphtho[1,2-*e*]pyrene,

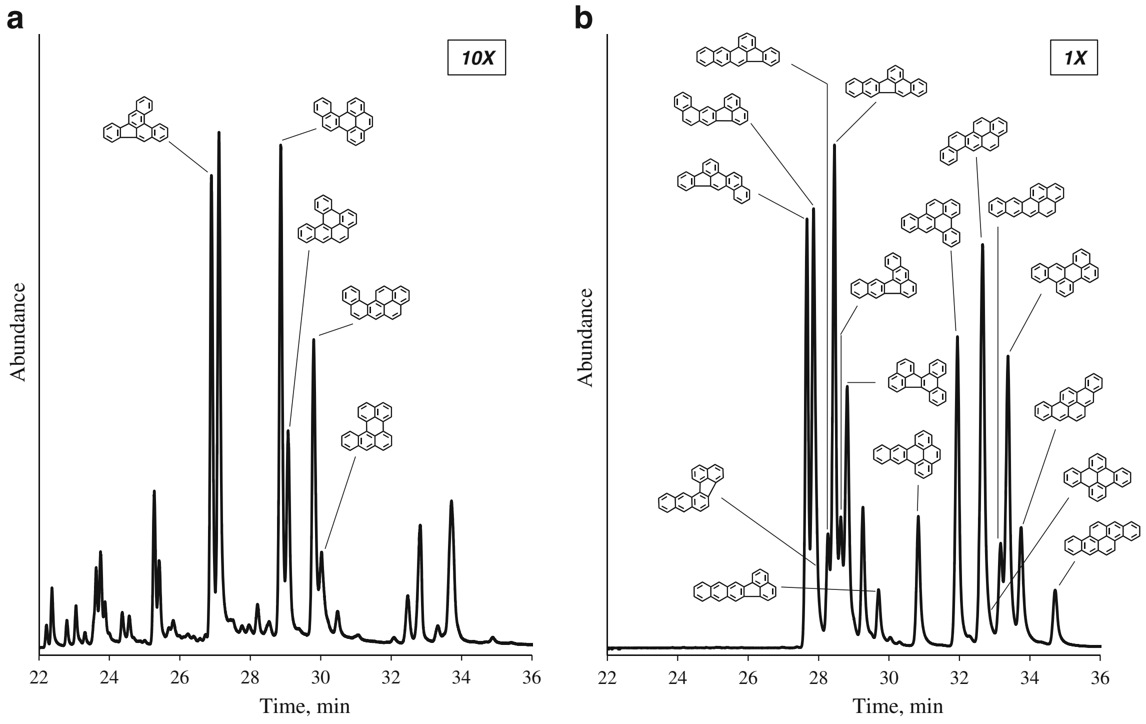


Fig. 5 GC/MS analyses with SIM for the *m/z* 302 ion of the following samples: **a** Fraction 1 (**F1**), **b** Fraction 2 (**F2**). The names of the structures are given in Figure S3 of the supporting information

dibenzo[*a,l*]pyrene, and benzo[*a*]perylene, respectively, compared to thickness values of typically 3.9 Å for most of the isomers in **F2**. The results of the qualitative and quantitative analysis of the $C_{26}H_{14}$ *peri*-condensed PAHs in **F3** will be published elsewhere [25].

The GC/MS analyses of **F1** with SIM for the *m/z* 300 ion showing the reference internal standard of coronene and **F1** with SIM for the *m/z* 302 ion are shown in Fig. 6b and c, respectively. Also, the GC/MS analysis of **F1** with SIM for the *m/z* 328 ion is shown in Fig. 6d and it is compared with the GC/MS analysis of the 17 $C_{26}H_{16}$ *cata*-condensed benzenoid PAH reference standards illustrated in Fig. 6a. Furthermore, the GC/MS analyses of **F2** with SIM for the *m/z* 302 ion, **F2** with SIM for the *m/z* 328 ion, and **F4** with SIM for the *m/z* 328 ion are shown in Fig. 7b, c, and d and are compared with the GC/MS analysis of the 17 $C_{26}H_{16}$ *cata*-condensed benzenoid PAH reference standards illustrated in Fig. 7a.

Fraction 1, **F1**

As shown in Fig. 6d, the GC/MS chromatogram with SIM for the *m/z* 328 ion of **F1** is composed of 10 unknown peaks *A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*, *I*, and *J*. Of the 10 unknown peaks observed in **F1**, peak *F* is identified by matching its observed retention time with the retention time of XVII. The peak *J* belongs to a $C_{26}H_{14}$ *peri*-condensed PAHs with molecular mass 326 Da, which generates a small 328 ion, and the unknown peaks *A*, *B*, *C*, *D*, *E*, *G*, *H* and *I* could not be identified based on retention times compared with standards. A retention time match between *G* and *H*, with XVIII is disregarded because XVIII elutes in NPLC in the retention time interval of fraction **F2** and not in the retention time interval of fraction **F1**. Most of the $C_{26}H_{16}$ isomers for which authentic standards were not available (i.e., XXII through XXXVI) are nonplanar structures with thickness values of between 5.23 Å and 6.20 Å, [1] these nonplanar isomers would be expected to elute in **F1** or **F2**, if they are present in the coal tar. Therefore, it is highly likely

Fig. 6 GC/MS analyses of the following samples: **a** The 17 $C_{26}H_{16}$ *cata*-condensed PAH standards available with SIM for the *m/z* 328 ion, **b** Fraction 1 (**F1**) with SIM for the *m/z* 300 ion, **c** Fraction 1 (**F1**) with SIM for the *m/z* 302 ion, **d** Fraction 1 (**F1**) with SIM for the *m/z* 328 ion. The names of the structures are given in Figure S3 of the supporting information

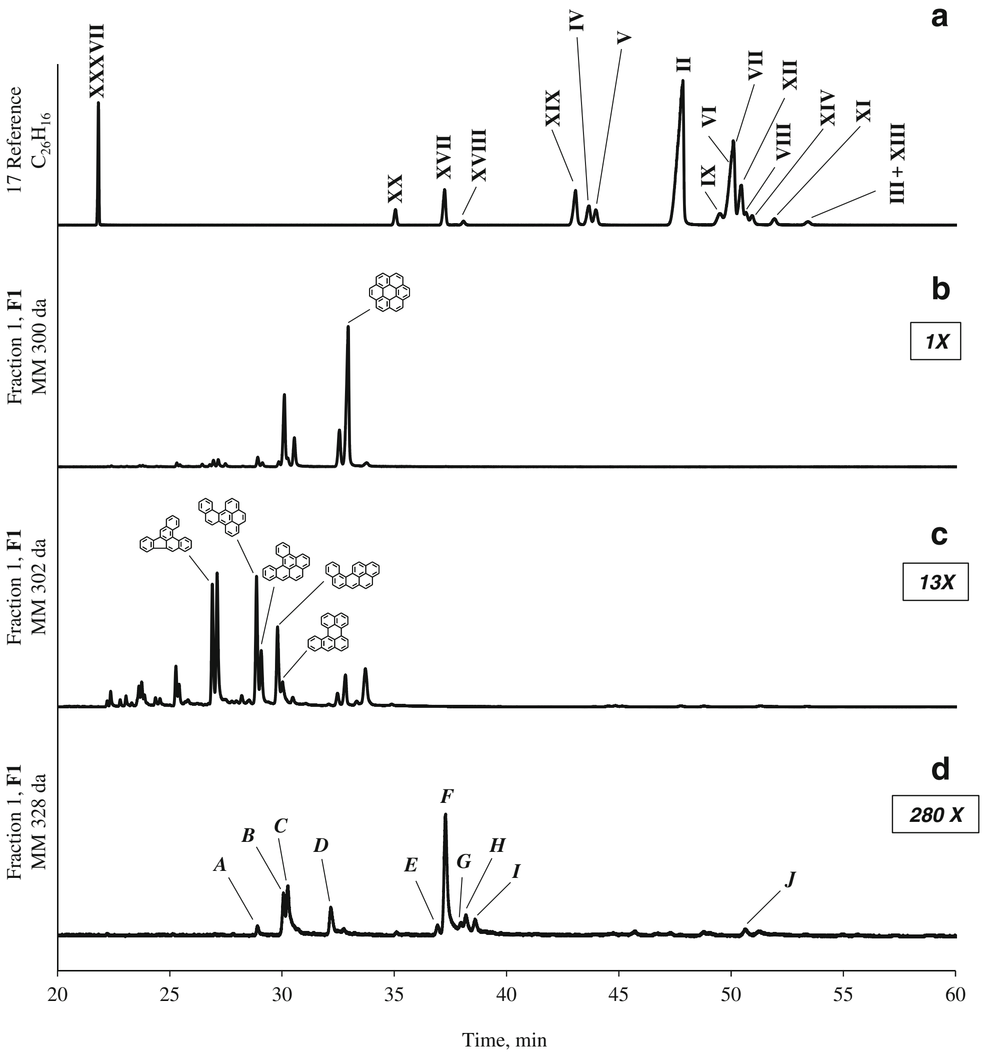
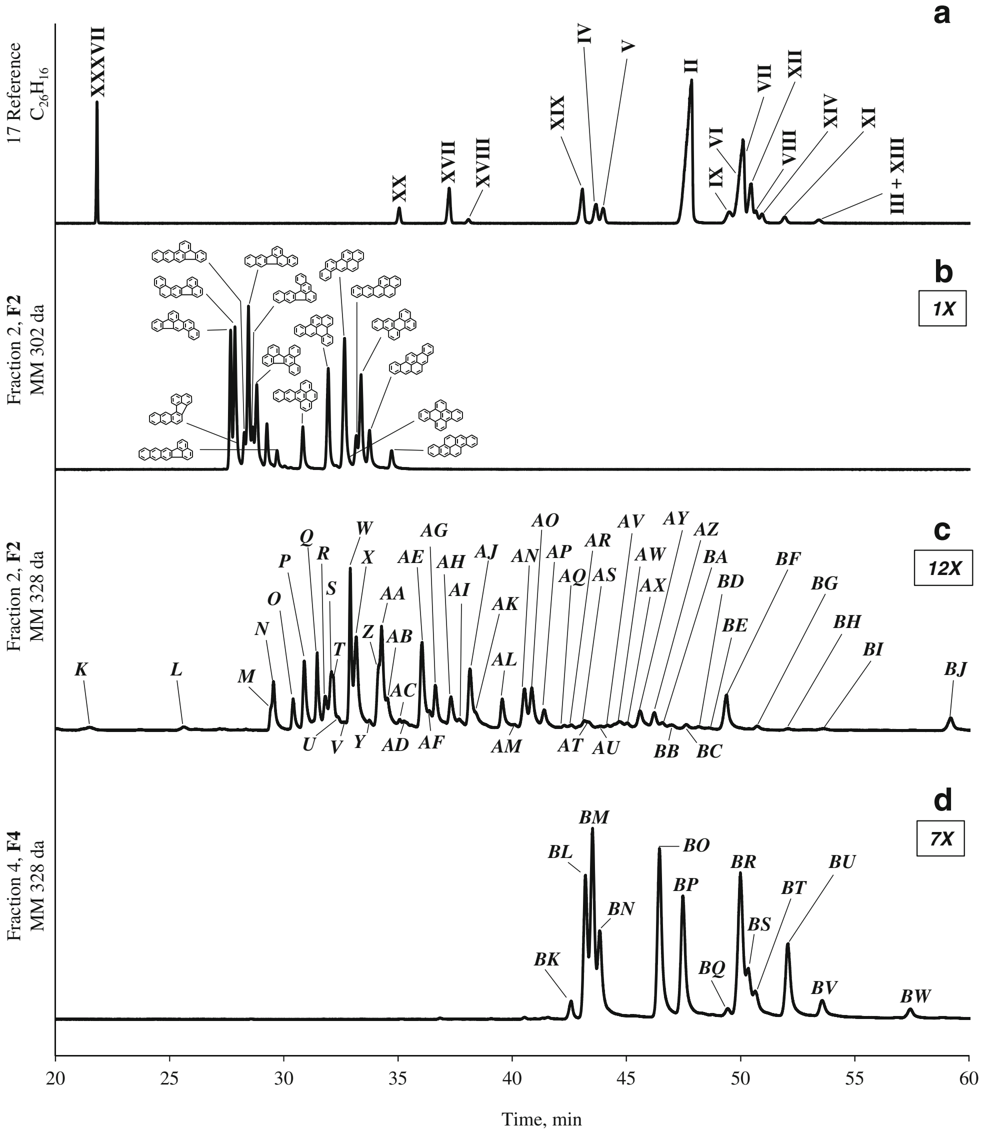


Fig. 7 GC/MS analyses of the following samples: **a** The 17 $C_{26}H_{16}$ *cata*-condensed PAH standards available, **b** Fraction 2 (**F2**) with SIM for the *m/z* 302 ion, **c** Fraction 2 (**F2**) with SIM for the *m/z* 328 ion, **d** Fraction 4 (**F4**) with SIM for the *m/z* 328 ion. The names of the structures are given in Figure S3 of the supporting information



that peaks *A-I* (except *F*) in **F1** could be some of these nonplanar isomers.

Fraction 2, **F2**

As illustrated in Fig. 7c, the GC/MS chromatogram with SIM for the *m/z* 328 ion of **F2** is composed of 52 unknown peaks *K-Z*, *AA-AZ*, and *BA-BJ*. Of the 52 unknown peaks observed in **F2**, peak *AJ* is identified by matching its retention time with the retention time of XVIII. In addition, there is no retention time match between XIX, which elutes in **F2** as it was stated, and any of the 51 remaining peaks. The peaks *AP*, *AU*, *AW*, *AX*, *AY*, *AZ*, *BA*, *BB*, *BC*, *BD*, *BE*, *BF*, *BG*, and *BJ* belong to $C_{26}H_{14}$ *peri*-condensed PAHs with molecular mass 326 Da that generate a small 328 ion. The other unknown peaks *K*, *L*, *M*, *N*, *O*, *P*, *Q*, *R*, *S*, *T*, *U*, *V*, *W*, *X*, *Y*, *Z*, *AA*, *AB*, *AC*, *AD*, *AE*, *AF*, *AG*, *AH*, *AI*, *AK*, *AL*, *AM*, *AN*, *AO*, *AQ*, *AR*, *AS*, *AT*, *AV*, *BH*, and *BI*, were not identified because their retention times do not match with any of the retention times of the other $C_{26}H_{16}$ *cata*-condensed PAH reference standards. The retention time matches between *K* with XXXVII, *AC* with XX, *AH* with XVII, and *AT* with IV are disregarded because XXXVII, XX, and XVII elute in NPLC in the retention time interval of fraction **F1**, and IV elutes in the retention time interval of fraction **F4**. As mentioned above, some of these unknown peaks could be the nonplanar isomers for which no authentic standards were available.

Fraction 4, **F4**

The GC/MS chromatogram from the analysis of **F4**, which contains most of the $C_{26}H_{16}$ PAH, is shown in Fig. 7d. Of the 13 unknown peaks: *BK*, *BL*, *BM*, *BN*, *BO*, *BP*, *BQ*, *BR*, *BS*, *BT*, *BU*, *BV*, and *BW* observed in **F4**, 9 peaks are identified based on retention times according to the following assignments: *BM*=IV, *BN*=V, *BP*=II, *BQ*=IX, *BR*=VI in co-elution with VII, *BS*=XII, *BT*=VIII, *BU*=XI, and *BV*=III in co-elution with XIII. In addition, this result is verified by the above mentioned NPLC elution analysis of the $C_{26}H_{16}$ standards that indicates IV, V, II, IX, VI, VII, XII, VIII, XI, III, and XIII elute in **F4**. The peak *BW* belongs to a $C_{26}H_{14}$ *peri*-condensed PAH with molecular mass 326 Da that generates a small 328 ion. The unknown peaks *BK*, *BL*, and *BO* are not identified because their retention times do not match any of the retention times of the other $C_{26}H_{16}$ *cata*-condensed PAH reference standards. However, based on planarity, [1] two of the three unknown isomers would likely be XV, thickness 3.9 Å, and XVI, thickness 3.92 Å. Fraction 4, **F4**, contains the largest number of $C_{26}H_{16}$ *cata*-condensed PAH isomers identified among the six fractions analyzed.

Quantitative analysis

The preferred quantitative approach using an internal standard added to SRM 1597a prior to the analytical procedure was not feasible because of the lack of suitable internal standard PAHs (e.g., isotopically labeled PAHs). Therefore, the following alternative approaches, which are semi-quantitative, were used for the determination of $C_{26}H_{16}$ *cata*-condensed PAHs in **F1** and **F4**. Coronene and dibenzo[*a,l*]pyrene, which were previously quantified in SRM 1597a [9–11], and which elute in fraction **F1** were used as reference internal standards for quantification. For the quantification of $C_{26}H_{16}$ isomers in **F2** and **F4**, known quantities of **F1** containing coronene and dibenzo[*a,l*]pyrene were added to **F2** and **F4** to facilitate the quantification. The details of the semi-quantitative approach are presented in the ESM (Figs. S1 and S2). A second direct analysis GC/MS approach was compared using the dibenzo[*a,h*]pyrene (mass fraction previously determined) as an internal reference standard for the determination of XVIII in **F2**. The results for the calculated mass fractions (mg/kg) are summarized in Table 3 for 13 $C_{26}H_{16}$ *cata*-condensed PAHs. The mass fractions for the 12 PAH isomers of molecular mass 328 Da determined using dibenzo[*a,l*]pyrene as the internal standard are a factor of 2.2 times higher than when using coronene as the internal reference standard. Using the direct GC/MS approach (no NPLC fractionation) (see Fig. 3) with benzo[*ghi*]perylene (mass fraction 50.5±0.6 mg/kg reported in the Certificate of Analysis for SRM 1597a [9]) as an internal reference standard to quantify **II** provided a mass fraction of 0.76 mg/kg compared to the value of 0.75 mg/kg±0.13 mg/kg obtained using coronene as the internal reference value following NPLC fractionation. This result indicates that the lower mass fraction obtained using coronene as the internal standard may be more reliable than those using dibenzo[*a,l*]pyrene. Determination of dibenzo[*a,l*]pyrene in **F1** using coronene as the internal standard provides a mass fraction of 0.53±0.14 mg/kg (*n*=5) compared with the 1.12±0.17 mg/kg reported in the Certificate of Analysis [9], which was determined by GC/MS analysis on a 50 % phenylmethylpolysiloxane stationary phase with SIM for the *m/z* 302 ion as described by Schubert et al. [23]. As described above, the NPLC fractionation places dibenzo[*a,l*]pyrene in **F1** and avoids the problems associated with quantifying a small

Table 3 Calculated mass fractions (mg/kg) of the thirteen identified $C_{26}H_{16}$ *cata*-condensed PAHs **II**, **III** + **XIII**, **IV**, **V**, **VI** + **VII**, **VIII**, **IX**, **XI**, **XII**, **XVII**, and **XVIII** using coronene, dibenzo[*a,l*]pyrene, and dibenzo[*a,h*]pyrene as reference internal standards

		Reference internal standard		
PAH[a]	n	Coronene 8.7±1.8 mg/kg [8] Mass fraction mg/kg	Dibenzo[*a,l*]pyrene 1.12±0.17 mg/kg [8] Mass fraction mg/kg	Dibenzo[*a,h*]pyrene 2.57±0.30 mg/kg [8] Mass fraction mg/kg
II	5	0.75±0.13	1.6±0.3	
III + XIII	5	0.12±0.02	0.28±0.08	
IV	5	0.93±0.15	2.0±0.4	
V	5	0.67±0.16	1.5±0.3	
VI+VII	5	0.83±0.18	1.8±0.4	
VIII	5	0.21±0.05	0.45±0.05	
IX	5	0.042±0.009	0.09±0.02	
XI	5	0.47±0.08	1.0±0.2	
XII	5	0.26±0.05	0.58±0.08	
XVII (F1)	3	0.10±0.02	0.28±0.06	
XVIII (F2)	3			0.25±0.08

n = number of independent replicates. Measure uncertainties mean standard deviations from the number of replicates stated in the table

[a] All PAHs identified in **F4** except as noted

chromatographic peak eluting closely to other larger isomers.

As shown in Table 3, the mass fractions for the 328 Da molecular mass isomers range from 0.042 mg/kg (IX) to 0.93 mg/kg (IV). The most abundant 328 molecular mass isomers are more than 10 times lower than the mass fraction of the most abundant molecular mass 302 Da PAHs, 100 times lower than benzo[*a*]pyrene, and 1000 times lower than naphthalene. Intrinsic in these results is the tendency of the combustion reaction to favor the formation of slightly or non-distorted molecules, e.g., IV (molecular thickness=4.138 Å [1]), instead of highly distorted molecules, e.g., XVII (molecular thickness=5.615 Å [1]).

Conclusions

The accurate identification and quantification of $C_{26}H_{16}$ *cata*-condensed PAHs requires isolation and enrichment of the isomer fraction using NPLC prior to GC/MS analysis. This is the first report of the identification and quantification of 13 $C_{26}H_{16}$ *cata*-condensed PAHs isomers in an environmental combustion sample (coal tar) including benzo[*c*]pentaphene (II), dibenzo[*f,k*]tetraphene (IV), benzo[*h*]pentapheme (V), dibenzo[*a,l*]tetracene (VI), dibenzo[*c,k*]tetraphene (VII), naphtho[2,3-*c*]tetraphene (VIII), dibenzo[*a,c*]tetracene (IX), benzo[*b*]picene (XI), dibenzo[*a,j*]tetracene (XII), naphtho[2,1-*a*]tetracene (XIII), dibenzo[*c,p*]chrysene (XVII), dibenzo[*a,f*]tetraphene (XVIII), and benzo[*c*]picene (III).

The experimental procedure shown here is adaptable for the qualitative and quantitative analysis of large PAHs, e.g., PAHs with molecular masses exceeding 300 Da. As it is shown in Table 2, GC/MS signals with up to 352 Da were detected in the NPLC separation range under analysis (14.7 min to 25.7 min). In practice, the NPLC separation range can be extended for the identification and quantification of larger compounds depending on the focus of the analysis. However, the high volatility and the low concentration of large PAHs, the general low ratio of the number of reference standards available to the number of theoretical isomers for large PAH families, and the weak stabilities of GC stationary phases above 350 °C would preclude the prolongation of the fractionation to longer NPLC elution times. In that scenario, the isomer families viable for NPLC/UV–vis with GC/MS would include the $C_{26}H_{14}$, $C_{28}H_{14}$, and $C_{28}H_{16}$. The $C_{26}H_{14}$, $C_{28}H_{14}$, and $C_{28}H_{16}$ families show a noticeable percentage of reference standards available (56, 63, and 39 %, respectively) [1] that would provide insightful information about the composition of large PAHs in complex environmental samples.

Acknowledgments The authors want to acknowledge Jacolin A. Murray from NIST for technical discussions on GC analyses of PAHs.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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