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Determination of polycyclic aromatic hydrocarbons with molecular mass 302 in Standard Reference Material 1597a by reversed-phase liquid chromatography and stop-flow fluorescence detection

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Abstract

The identification of isomeric polycyclic aromatic hydrocarbons (PAHs) in complex samples via reversed-phase liquid chromatography (RPLC) with fluorescence detection (FL) is normally based on matching the chromatographic retention times of suspected peaks of interest with reference standards. Since no spectral information is obtained during the chromatographic run, the accurate identification of co-eluting PAHs with similar chromatographic behaviors requires confirmation with additional chromatographic methods. This is particularly true for the analysis of PAH isomers with the relative molecular mass (MM, g/mol) 302. The work presented here explores the information content of room-temperature fluorescence spectra for the analysis of PAHs with MM 302 in the Standard Reference Material (SRM) 1597a. Fluorescence spectra were recorded under stop-flow conditions with the aid of a commercial HPLC system. Of the 21 MM 302 PAHs known to be present in the SRM 1597a, 20 were tentatively identified based on retention times and the presence of 18 was confirmed based on excitation and emission spectral profiles.

1. Introduction

Polycyclic aromatic compounds (PACs) are considered environmental pollutants originating from a vast assortment of anthropogenic and natural sources. A subclass of PACs, polycyclic aromatic hydrocarbons (PAHs), has been at the forefront of studied environmental pollutants for many decades due to their highly carcinogenic and mutagenic potential.^{1–3} Among the large group of PAHs, the molecular mass (MM, g/mol) 302 PAH isomers (Fig. S1) is of extreme importance due to the presence of dibenzo[*a,l*]pyrene (DBaP), which is roughly

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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 the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Conflict of interest

There are no conflicts of interest to declare.

100 times more toxic than the most potent PAH (benzo[*a*]pyrene) included in the Environmental Protection Agency Priority Pollutant List.^{4,5} The MM 302 PAH isomers have been identified in combustion-related samples such as coal tar,^{6–13} crude oil,^{14,15} and diesel particulate.¹⁶

Gas chromatography/mass spectrometry (GC/MS)^{6, 9–11, 13, 16–18} and reverse-phase liquid chromatography with ultraviolet absorption (RPLC/UV)^{17–20} or fluorescence detection (RPLC/FL)^{7,8,12,14} are popular techniques for the analysis of PAHs in combustion-related samples. Similar chromatographic behaviors make the analysis of MM 302 PAHs a particularly challenging task.^{6,9,11} Since MM 302 isomers present virtually identical mass fragmentation patterns, unambiguous identification via GC/MS requires their complete separation in the chromatographic column. Complementary separation schemes have been developed for this purpose based on stationary phases with different selectivity. These include a 5 % phenyl-substituted methylpolysiloxane (phenyl-MPS) phase,⁶ a 50 % phenyl phase,^{6,9,11} and a 50 % liquid crystalline dimethylpolysiloxane (LC-DMPS) phase.⁶

The unambiguous determination of MM 302 isomers in RPLC fractions has been accomplished via Shpol'skii spectroscopy.^{7,21} The research presented here investigates the specificity of room-temperature fluorescence (RTF) spectra for the purpose at hand. Although fluorescence detection is widely used in RPLC for retention time assignments and quantitative purposes, qualitative analyses based on RTF spectra have been limited to a small number of non-isomeric PAH standards with no application to complex samples.^{22,23} The new RPLC/FL method presented here is applied for the analysis of 23 MM 302 PAH isomers in the Standard Reference Material (SRM) 1597a, a complex mixture of PACs from coal tar.

Prior to these studies, the National Institute of Standards and Technology (NIST) has identified 21 MM 302 PAH isomers in SRM 1597a.^{6,9,10,13,17} Mass fraction values were assigned for 17 of these PAH isomers based on five different analytical approaches.¹⁰ Three of the analytical approaches included the use of a normal-phase-LC (NPLC) fractionation procedure of the coal tar sample on a semi-preparative aminopropyl (NH₂) column prior to GC/MS or RPLC/FL analysis. More recently, Wilson et al. improved the NPLC fractionation procedure for the identification of PAHs⁹ and polycyclic aromatic sulfur heterocycles (PASHs)²⁴ in SRM 1597a and other combustion-related samples via GC/MS.

The same NPLC fractionation approach^{9,24} is applied here prior to the analysis of the coal tar sample via RPLC/FL. RTF spectra (excitation and emission) are recorded with the aid of a commercial HPLC instrument under stop-flow conditions. The spectral profiles recorded at the maximum retention times of the chromatogram provide valuable information to confirm the presence of MM 302 PAHs in SRM 1597a. The aims of this study are to demonstrate a new analytical methodology for this class of isomeric PAHs in complex samples and to show the importance for the analyst to not rely solely on RPLC retention time data for PAH identification.

2. Experimental Section

2.1. Materials and reagents

SRM 1597a (Complex Mixture of PAHs from Coal Tar) was obtained from the Office of SRMs at NIST (Gaithersburg, MD, USA). PAH reference standards were purchased from three different sources: Bureau of Community Reference (BCR, Brussels, Belgium), Chiron (Trondheim, Norway), and A. K. Sharma (College of Medicine and Department of Pharmacology at Penn State University, Hershey, Pennsylvania). HPLC grade water, acetonitrile, dichloromethane (DCM), and n-hexane were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Normal-phase liquid chromatography fractionation

The NPLC fractionation instrument, column, separation conditions, and fractionation procedure are reported in an earlier publication⁹ using a NH₂ semi-prep column from Waters (Milford, MA) with the following characteristics: 25.0 cm length, 10 mm internal diameter, and 5 µm average particle diameter. A total of 14 fractions were collected based on published NPLC retention data for these PAHs.²⁴

2.3. Reversed-phase liquid chromatography and stop-flow room-temperature fluorescence spectra

RPLC analysis was 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, Thermo Scientific). Separations were carried out on a polymeric (Zorbax Eclipse PAH) C₁₈ column purchased from Agilent (Avondale, PA) with the following characteristics: 25 cm length, 4.6 mm diameter, and 5 µm average particle diameter. Separations were achieved using a 100 % acetonitrile mobile phase and a flow rate of 1.5 mL/min. RTF spectra were recorded using a stop-flow function on the instrument at the apex of each chromatographic peak. The analytical standard flow cell had a cell volume of 8 µL. The excitation source was a xenon flash lamp with broadband illumination from 200 nm to 880 nm. The excitation and emission monochromators have the same spectral bandwidth (20 nm), accuracy (± 2 nm), and repeatability (± 0.2 nm). The fluorescence detector can measure up to four channels with independent parameters simultaneously. A single channel detection with a max data collection rate of 100 Hz was selected for the collection of RTF spectra. A programmable filter wheel consisting of five wavelengths was utilized throughout the analysis. Detection was made with a photomultiplier tube with spectral response from 200 nm to 800 nm.

2.4. Gas chromatography/mass spectrometry

Mass spectra of the MM 302 PAHs were obtained using a GC/MS method published previously using a 50 % phenyl stationary phase (SLB-PAHms) obtained from Supelco (Bellefonte, PA) with the following characteristics: 60.0 m length, 0.25 mm internal diameter, and 0.25 µm film thickness.⁹

3. Results and Discussion

3.1. Room-Temperature Fluorescence Spectra

FL spectroscopy is a highly selective and sensitive detection method for LC and has been widely applied in the determination of PAHs in environmental^{19,26–30} and combustion-related samples.^{7,8,10,12,13,17,31} Most applications are based on the acquisition of retention times using a single or a multiple set of excitation and emission wavelengths throughout the entire chromatogram. Since no spectral information is obtained at each chromatographic peak, peak assignment is solely based on retention times. To obtain unambiguous identification of fluorophores of interest and confirmation of peak purities for calibration purposes, analysts are required to perform further analyses of RPLC fractions via GC/MS.^{7,8,17} In the case of MM 302 PAHs, however, confirmation in RPLC fractions requires multiple GC/MS measurements using stationary phases with different selectivities.^{6,10} This is due to the virtually identical mass fragmentation patterns of MM 302 PAH isomers (see Fig. S2 for mass spectra of BaPer, DBalP, N12eP, and N23kF) which prevent confirmation on the bases of a single GC separation.

Previous publications on the FL properties of MM 302 PAHs have shown distinctive spectral profiles. In previous studies, Campiglia and co-workers developed methodology for the analysis of DBalP, DBaeP, DBaiP, DBelP, DBahP and N23aP in environmental extracts on the bases of spectral and lifetime data.^{7,20,21,32–34} Fluorescence spectra and lifetimes were recorded from *n*-octane solutions at liquid nitrogen (77 K) and liquid helium (4.2 K) temperatures. The spectral resolution achieved under Shpol'skii conditions provided vibrational fingerprints for the determination of the six isomers with or without previous RPLC separation. Single exponential time decays obtained at the maximum fluorescence wavelengths of the isomers confirmed the lack of spectral interference from concomitants in the sample. The comparison of fluorescence lifetimes to those recorded from pure standards provided an additional parameter for the unambiguous identification of each isomer in the studied samples.

In the present study, we investigate the qualitative potential of stop-flow RTF spectra for the analysis of 23 MM 302 isomers in NPLC fractions from a complex coal tar sample (SRM 1597a). Excitation and emission spectra were recorded in the stop-flow mode at the maximum fluorescence intensities of chromatographic peaks with specific retention times. The chromatographic retention times, excitation wavelengths, and emission wavelengths for the studied PAHs are summarized in Table 1. Individual reference standards were measured using a 100 % acetonitrile mobile phase, 1.5 mL/min flow rate, and 25 °C column temperature. These separation conditions were selected based on published RPLC/FL methods¹² and the obtain fluorescence spectra for PAHs may change slightly using a different mobile phase such as methanol. The total time of spectral collection at each chromatographic peak was approximately 10 s. The fluorescence spectra recorded from individual reference standards of BaPer, DBalP, N12eP, and N23kF are shown in Fig. 1. Unlike the mass spectra in Fig. S2, the excitation and emission spectra in Fig. 1 provide distinctive profiles for each one of the four isomers.

3.2. Reverse-phase liquid chromatography separation of MM 302 PAHs

In previous studies, Wise et al.¹² separated 10 isomers with MM 302 using a polymeric C₁₈ stationary phase, 100 % acetonitrile mobile phase, a flow rate of 1.5 mL/min, a column temperature of 29 °C, a single UV wavelength detector, and a multi-wavelength FL detector. DBaP, DBaH, DBaI, dibenzo[*b,k*]fluoranthene (DBbkF), N23aP, naphtho[2,3-*e*]pyrene (N23eP), naphtho[1,2-*k*]fluoranthene (N12kF), naphtho[2,3-*b*]fluoranthene (N23bF), and N23kF were the only isomers studied due to the limited number of authentic reference standards. In the present study, there were a total of 23 MM 302 PAHs analyzed using the same chromatographic conditions except for the column temperature. The column temperature was held constant at room temperature (25 °C) for all separations presented here.

PAH detection was carried out in the UV detection mode at 254 nm, which is commonly used in the literature for the determination of PAHs via RPLC/UV.^{17,19,20} The RPLC/UV chromatogram obtained for the 23 MM 302 PAH isomers is shown in Fig. S3. Naphtho[1,2-*b*]fluoranthene (N12bF), DBaP, N23kF, and DBaH were the only isomers to be baseline separated. The remaining 19 isomers had either significant chromatographic interference from other isomers or the sensitivity was too low to be detected at 254 nm. The following isomer sets co-eluted in the RPLC separation and will be discussed below in detail: (1) DBaP, BaPe, and N12eP; (2) dibenzo[*j,l*]fluoranthene (DBjlF) and dibenzo[*b,e*]fluoranthene (DBbeF); (3) dibenzo[*a,k*]fluoranthene (DBakF) and dibenzo[*a,e*]fluoranthene (DBaeF); (4) N23eP and naphtho[2,1-*b*]fluoranthene (N21bF); and (5) naphtho[2,3-*j*]fluoranthene (N23jF) and N12kF.

The RPLC/FL chromatograms obtained for the 23 MM 302 PAH isomers are shown in Fig. 2. PAH detection was carried out using the following excitation/emission wavelengths: (1) 281/423 nm, (2) 286/435 nm, (3) 248/402 nm, and (4) 290/475 nm. All excitation and emission wavelengths selected correspond to a compromise among the maximum excitation and emission wavelengths listed in Table 1. In several cases, the chromatographic peaks for individual PAHs are present at multiple wavelengths, but the chromatogram that provided the highest fluorescence intensity is labeled.

The RPLC/FL chromatogram shown in Fig. 3a was obtained from a standard mixture with DBaP, N12eP, and BaPe (isomer set 1) at the excitation and emission wavelengths of 281 nm and 423 nm, respectively. Under these conditions, BaPe could not be detected in the chromatogram but DBaP and N12eP could be detected. The RPLC/FL chromatograms shown in Fig. 3b – 3d were obtained using optimal wavelengths for DBaP, N12eP, and BaPe, respectively. Under these conditions, DBaP was identified in Fig. 3b but with significant contributions from N12eP. N12eP was identified in Fig. 3c with minimal contribution from DBaP. BaPe was identified in Fig. 3d with no contribution from DBaP or N12eP. Similar results were obtained for isomer set 2; i.e. the RPLC/FL chromatogram at the maximum emission wavelengths of DBbeF (406 nm) and DBjlF (507 nm) with no interference from each other. Based on previous work by Wilson et al. [9], the wavelengths selected for the RPLC/FL chromatograms in Fig. 2 did not include specific fluorescence wavelengths for DBjlF and DBbeF because both isomers elute in different NPLC fractions

of SRM 1597a. In the case of isomer set 3, 4, and 5, the co-eluting isomers have similar excitation and emission wavelengths that prevent their selective determination.

3.3. Analysis of Standard Reference Material 1597a

SRM 1597a is a natural complex mixture of PAHs in coal tar that has been extensively characterized for the identification and quantification of 21 MM 302 Da PAHs. Mass fraction values for 17 of the 21 isomers were assigned based on the combination of RPLC/FL and GC/MS.¹⁰ Recently, Wilson et al.⁹ demonstrated the use of a detailed NPLC fractionation procedure to simplify the complex sample matrix prior to GC/MS measurements. The NPLC fractionation procedure was developed based on recently published retention data.²⁵ The NPLC fractionation procedure included the collection of 14 fractions over 90 min. The MM 302 PAH isomers were identified in three NPLC fractions with the following distribution: (fraction 10) N12eP, DBaIP, N12aP, and BaPer; (fraction 11) DBbeF, N23aP, DBaIP, and DBaHP; and (fraction 12) N12bF, N12kF, N23jF, N23bF, DBbkF, DBaKF, DBjIF, N23kF, N23eP, DBaeP, N21aP, DBelP, and BbPer. The identification of the MM 302 Da PAHs was based on NPLC and GC retention times of pure reference standards. In the present study, the feasibility of using RPLC/FL and RTF spectra for the identification of 21 MM 302 Da PAH isomers was investigated using the same NPLC fractionation described previously.⁹

3.3.1. Fraction 10—The RPLC/FL chromatogram obtained with an excitation wavelength of 281 nm and emission wavelength of 423 nm for fraction 10 is shown in Fig. 4. The excitation and emission spectra of DBaIP, N12eP and N12aP inserted in Fig. 4 were recorded from fraction 10 at the retention times listed in Table 1. Comparison to the standard spectra in Fig. 1 provides evidence of the presence of the three isomers in the chromatographic fraction. In the case of DBaIP, approximately 1 nm and 4 nm shifts were observed in its maximum excitation and emission wavelengths, respectively. The emission spectra recorded from the chromatographic peak show two additional spectral peaks at 372 nm and 401 nm. These additional peaks could be attributed to the presence of other FL components in fraction 10 such as PAHs⁹ and/or PASHs.²⁴ Similar observations were made for N12eP and N12aP. As discussed earlier, the attempt to determine BaPer at its maximum excitation (462 nm) and emission (524 nm) wavelengths provided no indication of its presence in the chromatographic fraction (data not shown).

3.3.2. Fraction 11—The RPLC/FL chromatograms obtained at the four selected wavelengths for fraction 11 are shown in Fig. 5. The excitation and emission spectra recorded from reference standards of DBbeF, DBaIP, N23aP, and DBaHP (solid line) and from fraction 11 (dotted line) are shown in Fig. 6a – 6d, respectively. The excitation and emission spectra of N23aP in fraction 11 provided an almost identical match to the reference spectra indicating no presence of interfering components. In the case of DBbeF and DBaIP, the excitation and emission spectra from fraction 11 provided very similar profiles to their respective reference spectra. However, slight differences can be observed between 275 – 300 nm in the excitation spectra of DBbeF and at 230 nm in the excitation spectra of DBaIP. In the case of DBaHP, its identification is made possible on the bases of excitation spectra; its emission spectrum shows the contribution from other fluorophores of unknown identity.

3.3.3. Fraction 12—The RPLC/FL chromatograms obtained at the four selected wavelengths for fraction 12 is shown in Fig. 7. The excitation and emission spectra obtained from reference standards of N12bF, DBaeP, BbPer, DBbkF, and N23bF (solid line) and from fraction 12 (dotted line) are shown in Fig. 8a – 8d, respectively. The excitation and emission spectra collected for N12bF, DBbkF, DBaeP, BbPer, N23jF, and N23bF in fraction 12 matched their respective reference spectra indicating the absence of interfering components. The excitation and emission spectra collected for N21aP, DBjlF, DBelP, DBakF, N23eP, N12kF, and N23kF are shown in Fig. S4 – S10. N21aP, DBjlF, DBelP, and N23eP were identified based on their retention times and excitation spectra. Their emission spectra showed clear interference from other fluorophores in the chromatographic peak. DBakF was identified based on its retention time and excitation/emission spectra with peaks at 483 nm and 512 nm; the peak at 532 nm shows significant interference. The excitation spectra recorded from the chromatographic peak clearly indicates the presence of interference. In the case of N12kF and N23kF, the excitation and emission spectra of the chromatographic peak do not match their respective reference standard spectra. The excitation and emission spectra recorded at the maximum wavelengths of N12kF clearly match the spectra of N23jF. This is a strong indication of the absence of N12kF in the chromatographic peak.

4. Conclusions

Due to the similar chromatographic behaviors and virtually identical mass fragmentation patterns, the unambiguous determination of PAH isomers with MM 302 in SRMs calls for the combination of NPLC, RPLC and GC/MS. Isomer identification is based on NPLC, RPLC, and GC retention times and predominant mass ion peaks.^{9,10} The method presented here adds a new layer of qualitative information to the chromatographic analysis of MM 302 PAHs. We have shown that recording excitation and emission spectra for suspected RPLC chromatographic peaks for MM 302 PAHs in NPLC fractions allowed for their positive identification in SRM 1597a. Of the 21 MM 302 PAHs previously identified in SRM 1597a,^{9,10} only N12kF and N23kF could not be confirmed on the bases of excitation and/or emission spectra. BaPer could not be identified either chromatographically or spectroscopically.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Canha N, I Lopes ED Vicente AM Vicente BAM Bandowe S.M. Almeida et al. Environ. Sci. Pollut. Res 2016, 23(11), 10799–807.
2. de Souza MR, da Silva FR, de Souza CT, Niekraszewicz L, Dias JF, Premoli S et al. Chemosphere. 2015, 139, 512–7. [PubMed: 26295689]

3. Olson GM, Meyer BM, Portier RJ. Chemosphere. 2016, 145, 322–8. [PubMed: 26692508]
4. Cavalieri EL, Higginbotham S, RamaKrishna NVS, Devanesan PD, Todorovic R, Rogan EG et al. Carcinogenesis. 1991, 12(10), 1939–44. [PubMed: 1934274]
5. Devanesan P, Ariese F, Jankowiak R, Small GJ, Rogan EG, Cavalieri EL. Chem Res Toxicol. 1999, 12(9), 789–95. [PubMed: 10490499]
6. Schubert P, Schantz MM, Sander LC, Wise SA. Anal Chem. 2003, 75(2), 234–46. [PubMed: 12553757]
7. Moore AFT, Goicoechea HC, Barbosa F, Campiglia AD. Anal Chem. 2015, 87(10), 5232–9. [PubMed: 25902285]
8. Wilson WB, Alfarhani B, Moore AFT, Bisson C, Wise SA, Campiglia AD. Talanta. 2016, 148, 444–53. [PubMed: 26653471]
9. Wilson WB, Hayes HV, Sander LC, Campiglia AD, Wise SA. Anal Bioanal Chem. 2017, 409(21), 5171–83. [PubMed: 28664340]
10. Wise SA, Poster DL, Leigh SD, Rimmer CA, Mossner S, Schubert P et al. Anal Bioanal Chem. 2010, 398(2), 717–28. [PubMed: 20644914]
11. Ona-Ruales JO, Sharma AK, Wise SA. Anal Bioanal Chem. 2015, 407(30), 9165–76. [PubMed: 26449848]
12. Wise SA, Deissler A, Sander LC. Polycyclic Aromatic Compounds. 1993, 3(3), 169–84.
13. Wise SA, Benner BA, Byrd GD, Chesler SN, Rebbert RE, Schantz MM. Anal Chem. 1988, 60(9), 887–94.
14. Kozin I, Gooijer C, Velthorst N, Harmsen J, Wieggers R. Intern J Environ Anal Chem. 1995, 61(4), 285–97.
15. Kozin IS, Gooijer C, Velthorst NH. Anal Chem. 1995, 67(9), 1623–6.
16. Schantz MM, McGaw E, Wise SA. Anal Chem. 2012, 84(19), 8222–31. [PubMed: 22963542]
17. Wise SA, Benner BA, Liu HC, Byrd GD, Colmsjo A. Anal Chem. 1988, 60(7), 630–7. [PubMed: 3382016]
18. Durant JL, Lafleur AL, Plummer EF, Taghizadeh K, Busby WF, Thilly WG. Environ. Sci. Technol. 1998, 32(13), 1894–906.
19. Wise SA, Benner BA, Chesler SN, Hilpert LR, Vogt CR, May WE. Anal Chem. 1986, 58(14), 3067–77.
20. Yu SJ, Campiglia AD. Appl Spectrosc. 2004, 58(12), 1385–93. [PubMed: 15606949]
21. Wilson WB, Campiglia AD. J Chromatogr A. 2011, 1218(39), 6922–9. [PubMed: 21872256]
22. Smalley MB, Shaver JM, McGown LB. Anal Chem. 1993, 65(23), 3466–72.
23. Dvorak MA, Oswald GA, Van Benthem MH, Gillispie GD. Anal. Chem 1997, 69(17), 3458–64. [PubMed: 21639268]
24. Wilson WB, Hayes HV, Campiglia AD, Wise SA. Under review with Anal. Bioanal. Chem 2018.
25. Wilson WB, Hayes HV, Sander LC, Campiglia AD, Wise SA. Anal Bioanal Chem. 2017, 409(22), 5291–305. [PubMed: 28717893]
26. Wise SA, Poster DL, Kucklick JR, Keller JM, VanderPol SS, Sander LC et al. Anal Bioanal Chem. 2006, 386(4), 1153–90. [PubMed: 17047949]
27. Howerton SB, Goodpaster JV, McGuffin VL. Anal Chim Acta. 2002, 459(1), 61–73.
28. Kicinski H, Adamek S, Kettrup A. Chromatographia. 1989, 28(3), 203–8.
29. Capelo J, Galesio M, Felisberto G, Vaz C, Pessoa JC. Talanta. 2005, 66(5), 1272–80. [PubMed: 18970118]
30. Manoli E, Samara C. Chromatographia. 1996, 43(3–4), 135–42.
31. Kim Oanh NT, Reutergårdh LB, Dung NT. Environ Sci Technol. 1999, 33(16), 2703–9.
32. Moore AFT, Barbosa F Jr, Campiglia AD. Appl Spectrosc. 2014, 68, 14–25. [PubMed: 24405949]
33. Wilson WB, Campiglia AD. Analyst. 2011, 136, 3366–3374. [PubMed: 21725549]
34. Yu S, Campiglia AD. Anal Chem. 2005, 77, 1440–1447. [PubMed: 15732929]

Fig. 1.
Excitation and emission spectra collected from individual reference standards for BaPer, DBalP, N12eP, and N23kF.

Fig. 2.

Fluorescence chromatograms obtained for the 23 MM 302 PAH isomers at multiple wavelength conditions.

Fig. 3. Fluorescence chromatograms obtained for DBaIP, N12eP, and BaPer at multiple wavelength conditions.

Fig. 4.

Fluorescence chromatograms for fraction 10 of SRM 1597a at 281/423 nm and the excitation and emission spectra of DBaIP, N12eP, and N12aP.

Fig. 5. Fluorescence chromatograms for fraction 11 of SRM 1597a at multiple wavelengths. The asterisk label represents the PAHs that were identified with interfering components.

Fig. 6. Excitation and emission spectra collected from fraction 11 of SRM 1597a (dash line) and reference standards (solid line): a) DBbF, b) DBaiP, c) N23aP, and d) DBahP.

Fig. 7. Fluorescence chromatograms for fraction 12 of SRM 1597a at multiple wavelengths. The asterisk label represents the PAHs that were identified with interfering components.

Fig. 8. Excitation and emission spectra collected from fraction 12 of SRM 1597a (dash line) and reference standards (solid line): a) N12bF b) DBaeP, c) N12kF, d) BbPer, e) DBbkF, and f) N23bF.

Table 1

Stop-flow excitation and emission wavelengths for the 23 MM 302 PAH isomers in acetonitrile

PAHs	Abbreviations	Retention Time ^a (min)	Excitation Wavelengths (nm)	Emission Wavelengths (nm)
Benzo[<i>a</i>]perylene	BaPer	7.76 ± 0.12	238, <u>274</u> , 462	<u>524</u> , 555
Dibenzo[<i>a,h</i>]pyrene	DBaIP	8.41 ± 0.17	245, 268, <u>297</u>	428, 447
Naphtho[1,2- <i>c</i>]pyrene	N12eP	8.53 ± 0.29	249, <u>296</u> , 357	<u>402</u> , 416
Dibenzo[<i>b,e</i>]fluoranthene	DBbeF	10.59 ± 0.15	<u>251</u> , 277, 300	<u>406</u> , 429
Dibenzo[<i>j,k</i>]fluoranthene	DBjIF	10.81 ± 0.17	239, <u>245</u>	444, 471, <u>507</u>
Naphtho[1,2- <i>b</i>]fluoranthene	N12bF	11.88 ± 0.03	253, <u>279</u> , 383	<u>439</u>
Dibenzo[<i>a,k</i>]fluoranthene	DBakF	12.75 ± 0.07	249, <u>269</u>	<u>483</u> , 512
Dibenzo[<i>a,e</i>]fluoranthene	DBaeF	12.82 ± 0.09	<u>249</u> , 415	490, <u>508</u>
Naphtho[2,1- <i>b</i>]fluoranthene	N21bF	13.39 ± 0.08	238, 246, <u>295</u>	<u>446</u>
Naphtho[2,3- <i>c</i>]pyrene	N23eP	13.57 ± 0.07	<u>247</u> , 296, <u>315</u>	<u>413</u> , 433, 463
Naphtho[1,2- <i>a</i>]pyrene	N12aP	14.05 ± 0.09	270, <u>298</u> , 383	<u>440</u> , <u>455</u>
Dibenzo[<i>a,e</i>]pyrene	DBaeP	14.83 ± 0.15	277, <u>294</u> , 359	<u>402</u> , 420
Naphtho[2,3- <i>j</i>]fluoranthene	N23jF	15.14 ± 0.04	<u>251</u> , 261, 290	<u>536</u>
Naphtho[1,2- <i>k</i>]fluoranthene	N12kF	15.52 ± 0.13	<u>247</u> , <u>289</u> , 317	435, 443
Benzo[<i>a</i>]perylene	BbPer	16.34 ± 0.14	<u>232</u> , 253	<u>443</u> , 470
Dibenzo[<i>e,h</i>]pyrene	DBeIP	17.01 ± 0.12	268, <u>277</u> , 359	<u>389</u> , 503, 524
Dibenzo[<i>b,k</i>]fluoranthene	DBbkF	21.75 ± 0.24	<u>248</u> , 302, 386	413, 439
Naphtho[2,3- <i>b</i>]fluoranthene	N23bF	22.80 ± 0.23	251, <u>281</u> , 314	<u>427</u> , 450
Naphtho[2,1- <i>a</i>]pyrene	N21aP	29.90 ± 0.03	<u>247</u> , 260, 382	<u>420</u> , 443
Dibenzo[<i>a,l</i>]pyrene	DBaIP	42.45 ± 0.51	239, <u>293</u> , <u>387</u>	440, 465, 499
Naphtho[2,3- <i>a</i>]pyrene	N23aP	48.10 ± 0.33	<u>247</u> , <u>291</u> , 418	<u>467</u> , <u>484</u>
Naphtho[2,3- <i>k</i>]fluoranthene	N23kF	55.49 ± 0.36	<u>264</u> , 289, 331	<u>458</u>
Dibenzo[<i>a,h</i>]pyrene	DBahP	58.30 ± 0.89	249, <u>303</u>	<u>455</u> , 481

^aThe uncertainty listed with each value is the standard deviation for the average retention time for each individual PAH (n = 3).

^bMaximum excitation and emission wavelengths are indicated by underline.