Electronic Supplementary Material (ESI) for Analytical Methods.

Supplemental Information

On the co-elution of Benzo[a]pyrene and Dibenzo[a,l]pyrene in chromatographic fractions and their unambiguous determination in tobacco extracts via Laser-Excited Time Resolved Shpol'skii Spectroscopy

Ahmed Comas, Anthony Santana, and Andres D. Campiglia*

Department of Chemistry, University of Central Florida, 4000 Central Florida Blvd., Physical Science Room 255, Orlando, FL, 3281-2366, United States

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* Corresponding author (andres.campiglia@ucf.edu

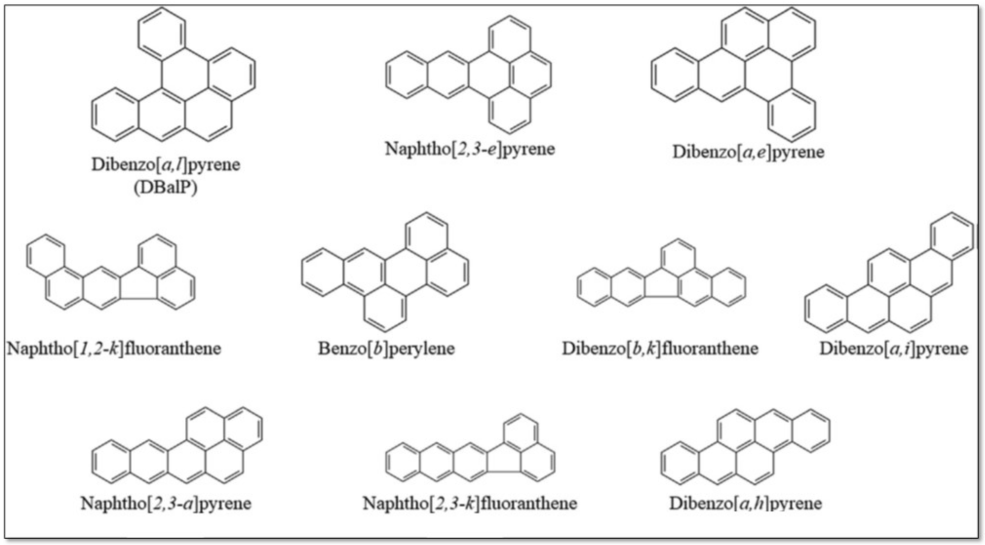


Fig. S1 Molecular structures of PAH isomers with MM 302 Da

Table S1: Modifications Made to EPA Method 610 for the Separation of EPA-PAHs

Parameter	EPA Method 610	Modified EPA Method 610
Column Type	Reverse Phase HC-ODS Sil-X	Reverse Phase Eclipse PAH Column
Particle Size	5-micron	5-micron
Column Dimensions	250 mm x 2.6 mm	250 mm x 4.6 mm
Column Temperature	Ambient	Ambient
Mobile Phase	Acetonitrile/Water	Acetonitrile/Water
Flow Rate	1.5 mL/min	2.0 mL/min[1]
Injection Volume	20 microliters	20 microliters
Elution	Gradient	Gradient

[1] Volume flow rate = [cross sectional area of chromatographic column] x [linear velocity] = [πr^2] x [2mm/sec] = [$\pi(2.3\text{mm})^2$] x [2mm/sec] = 33.238 mm^3/sec; Flow rate in mm^3/min = [33.328 mm^3/sec] x 60 sec/min = 1,994.28 mm^3/min; Flow rate in mL/min = 1,994.28 mm^3/min x 1 mL / 1000mm^3 ≈ 2 mL/min.

Table S2. Analyte recoveries of sample procedure prior to HPLC analysis

PAH	A_0 (µV*s) [a]	A_1 (µV*s) [b]	Recovery (%) [c]	t-test [d]
BaP	55937 ± 417	57755 ± 2179	103 ± 4	1.29
DBalP	147383 ± 1607	150878 ± 996	102 ± 1.0	3.46

[a] A_0 = Average chromatographic areas of PAH standards directly injected into the HPLC system. A_0 is based on three sample injections of the same analyte concentration. Injection volume = 20 µL.

[b] A_1 = Average chromatographic areas of PAH standards submitted to the entire sample preparation procedure prior to HPLC analysis. A_1 is based on three sample injections of the same analyte concentration. Injection volume = 20 µL.

[c] Recoveries were calculated with the formula $100*(A_1/A_0)$.

[d] t-test comparing experimental recoveries to 100%; t critical ($\alpha = 0.05$; $N = 3$) = 4.3.

Table S3. Analyte recoveries for LETRSS analysis of HPLC fractions

PAH	Recovery (%) [a]	t-test [b]
BaP	89 ± 2.8	6.35
DBalP	103 ± 3.5	1.48

[a] Recoveries were calculated with the formula $100*(M_1/M_0)$; where M_1 is the mass of PAH in the HPLC fraction obtained via MSA and M_0 is the theoretical mass of PAH in the HPLC fraction assuming no analyte loss; i.e.,100% recovery. M_1 values were based on five data points in the calibration graphs. Each data point was the average of three signal measurements. All the signals plotted in the graph were subtracted from blank signals recorded from n-octane. At the maximum emission wavelength of the PAH.

[b] t-test comparing experimental recoveries to 100%; t critical ($a = 0.05$; $N = 3$) = 4.3.

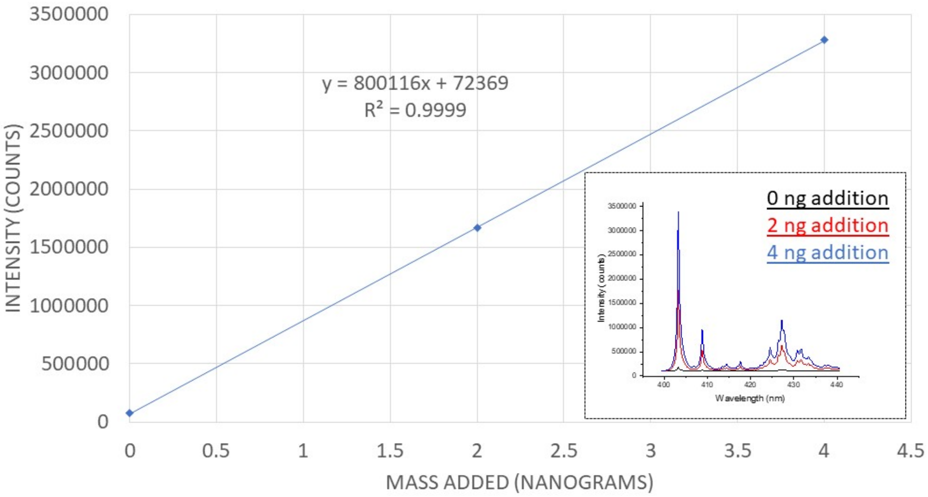


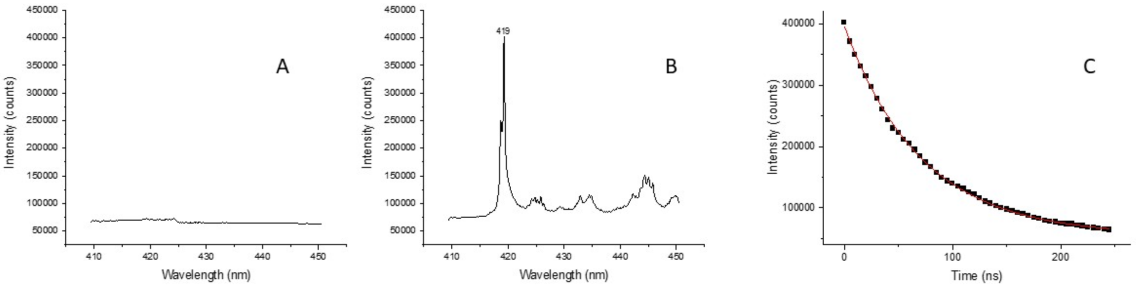
Fig. S2. Calibration plot for the MSA determination of BaP in an HPLC fraction of a tobacco extract. The mass of BaP found by the MSA procedure was 0.09 ng ± 0.01 ng. The standard deviation was calculated according to the equation:

$$s_c = \frac{s_{y/x}}{b} * \left[\frac{1}{N} + \frac{\bar{y}}{b^2 \sum_i (x_i - \bar{x})^2}\right]^{\frac{1}{2}}$$

Where S_c is the standard deviation of the concentration, $S_{y/x}$ is the standard deviation of the regression, m is the slope from the MSA linear fit, $\bar{y}$ and $\bar{x}$ are the averages of the y and x values, respectively, from the MSA graph, and x_i is the i^{th} x value. Note: The mass of BaP = 0.09 ng ± 0.01 ng is equivalent to 68.94 ng ± 10.01 ng of BaP per gram of tobacco sample. This value was calculated by taking into consideration the recovery of the method (89 %) and all the dilutions made in the entire HPLC-LETRSS procedure described in section 2.7. Step-by-step calculations are as follows:

Step 1: 5 mL aliquot from 50 mL tobacco extract was used for HPLC analysis.
Mass of BaP in 5 mL aliquot = Total mass of BaP in tobacco sample x [5 mL / 50 mL]
Step 2: 5 mL aliquot was evaporated to dryness and re-constituted in 1 mL of acetonitrile.
Step 3: 20 mL of the 1 mL solution prepared in step 2 was injected in the HPLC system.
Mass of BaP injected in the HPLC system = 20 mL / 1000 mL x {Total mass of BaP in tobacco sample x [5 mL / 50 mL]}
Step 4: HPLC fraction is evaporated to dryness and reconstituted in 1 mL of octane.
Step 5: 90 mL aliquot of the solution in step 4 was used for the MSA procedure:
90 mL / 1000 mL x 20 mL / 1000 mL x {Total mass of BaP in tobacco sample x [5 mL / 50 mL]}. Therefore: 0.09 ng ± 0.01 ng = 90 mL / 1000 mL x 20 mL / 1000 mL x {Total mass of BaP in tobacco sample x [5 mL / 50 mL]}. Total mass of BaP in tobacco sample = 500 ng ± 55.6 ng.

Taking into consideration the recovery of the HPLC-LETRSS procedure: Total mass of BaP in tobacco sample = 500 ng ± 55.6 ng / 0.89 = 562 ng ± 62.5 ng. Since the total mass of tobacco was 8.094 g, the mass of BaP per gram of tobacco was: 562 ng ± 62.5 ng / 8.094 g = 69.43 ± 7.72 ng.



HPLC fraction	τ_{HPLC}	τ_{std}	T_{exp}	T_{crit}	Statistically Equivalent
No Spike	-	74.70 ± 3.21	-	-	-
Spiked with DBalP	71.3 ± 2.15	74.70 ± 3.21	1.52	2.776	Y

Fig. S3. LETRSS analysis of an HPLC fraction of a tobacco extract. (A) Fluorescence spectrum of the un-spiked HPLC fraction; (B) Fluorescence spectrum of the HPLC fraction spiked with DBalP; and (C) Fluorescence decay of the HPLC fraction spiked with DBalP. Fluorescence spectra and fluorescence decays were recorded under optimum instrumental parameters for the determination of DBalP. T_{crit} = t critical ($\alpha = 0.05$; N = 3) and T_{exp} = t experimental ($\alpha = 0.05$; N = 3).