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Four-way modeling of 4.2 K time-resolved excitation emission fluorescence data for the quantitation of polycyclic aromatic hydrocarbons in soil samples

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ABSTRACT

A screening method for the soil analysis of 15 Environmental Protection Agency-polycyclic aromatic hydrocarbons (EPA-PAHs) is reported. The new method is based on the collection of 4.2 K fluorescence time-resolved excitation–emission cubes (TREECs) via laser-excited time-resolved Shpol'skii spectroscopy. 4.2 K fluorescence TREECs result from the superposition of fluorescence time-resolved excitation emission matrices recorded at different time windows from the laser excitation pulse. Potential interference from unknown sample concomitants is handled by processing the four-way 4.2 K fluorescence TREEC data arrays with either parallel factor analysis (PARAFAC) or unfolded partial least-squares/residual-trilinearization (U-PLS/RTL). The sensitivity of the two approaches makes possible to determine PAHs at the ng g^{-1} to pg g^{-1} concentration level with no need for sample pre-concentration. Its selectivity eliminates sample clean-up steps and chromatographic separation. These features reduce PAH loss, analysis time and cost. The method is environmentally friendly as the complete screening of the 15 EPA-PAHs takes only 250 μL of organic solvent per sample.

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1. Introduction

Considerable efforts have been made to develop analytical techniques capable to determine trace concentration levels of polycyclic aromatic hydrocarbons (PAHs) in environmental samples [1–10]. One of the main reasons for this motivation is the carcinogenic and toxicological nature of some PAHs. Under this prospective, particular attention has been paid to the 16 PAHs included in the U.S. Environmental Protection Agency (EPA) priority pollutant list. Their monitoring in air, water and soil samples is recommended to prevent human exposure to PAH contamination.

Environmental monitoring of EPA-PAHs follows the general pattern of sample clean up, pre-concentration and chromatographic analysis. Sample preparation simplifies matrix composition and pre-concentrates PAHs to achieve detectable concentrations by chromatographic techniques. High-performance liquid chromatography (HPLC) with absorption and/or fluorescence detection and gas chromatography–mass spectrometry (GC–MS) are the basis of current EPA methodology. When HPLC is applied to unfamiliar samples, a supporting technique such as gas-chromatography–mass spectrometry (GC–MS) is often used for confirmation purposes [11–14].

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The time consuming procedures of traditional methodology makes the development of screening techniques particularly attractive for the routine monitoring of numerous samples. Screening techniques capable to provide a “yes or no” answer to PAH contamination prevent unnecessary scrutiny of un-contaminated samples via conventional methods, reduce analysis cost and expedite turnaround time for decision making and remediation purposes. A recent trend for the direct determination—i.e. no chromatographic separation—of targeted species in matrixes of unknown composition refers to processing multidimensional spectroscopic data with second-order multivariate calibration methods [15–24]. The determination of phenanthrene and benzo[k]fluoranthene in urban run-off water samples [25], benzo[a]pyrene and dibenzo[a,h]anthracene in underground, tap and mineral water samples [26], and the analysis of chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]anthracene, benzo[a]pyrene and dibenzo[a,h]anthracene in river water and sludge samples [27] has been demonstrated on the basis of room-temperature fluorescence excitation-emission matrices (EEMs) combined to either parallel factor analysis (PARAFAC) or unfolded partial least-squares/residual-bilinearization (U-PLS/RBL).

This article deals with the direct determination of 15 EPA-PAHs in soil samples. Soil is one of the most important reservoirs for PAHs, which are deposited in the gaseous state or associated to air-borne particles, even at sites far from the petroleum industry

[28–31]. Once present in the soil, PAHs become a long-term source of environmental health risk due to their rather low water solubility, intrinsic chemical stability and high resistance to biodegradation. Our approach—which we have named laser-excited time-resolved Shpol'skii spectroscopy (LETRSS)—takes advantage of the full dimensionality of fluorescence spectroscopy combining spectral and lifetime information in multidimensional formats known as wavelength time matrices (WTM) [32] and time-resolved excitation-emission matrixes (TREEMs) [33].

A WTM consists of a series of emission spectra recorded under one excitation wavelength and different time delays after the laser excitation pulse. A TREEM is an excitation–emission matrix recorded at a certain time window during the total fluorescence decay of the sample. The term time window refers to the variation of both the delay and the gate times after the short duration of the excitation pulse. Recording WTMs during the fluorescence or phosphorescence decay of the sample provides an additional parameter for PAH identification (lifetime). Unambiguous PAH determination is made possible on the basis of spectral and lifetime information. Fluorescence or phosphorescence lifetimes also report on spectral peak purity, i.e. an essential condition for the accurate quantitative determination of PAHs without previous chromatographic separation [34,35]. The choice of an appropriate time window for TREEM collection enhances the fluorescence of targeted compounds over the fluorescence interference of sample concomitants [36].

LETRSS measurements are made at liquid helium temperature (4.2 K) with the aid of a cryogenic fiber optic probe (FOP); frozen samples are prepared in a matter of seconds [32–36]. The main reason for reducing the sample temperature is to promote the spectral narrowing that one needs to determine numerous EPA-PAHs without previous chromatographic separation. We take advantage of the FOP with a straightforward procedure for the LETRSS analysis of soil samples [37]. Upon sample sonication with microliters of *n*-octane in the vessel of the FOP, PAHs partition into the organic solvent for WTM collection. Since sample handling is limited to weighing milligrams of soil into the vial of the FOP, contamination risks and/or PAH loss is kept to a minimum. The small vial of the FOP (750 μ L) and the small volume of extracting solvent facilitate the simultaneous extraction of numerous samples. The entire experimental procedure—including the 30 min of sonication time—takes less than 40 min per sample. The method is environmentally friendly as the complete screening of the 15 EPA-PAHs takes only 250 μ L of organic solvent per sample.

Accurate determination of EPA-PAHs via 4.2 K WTM analysis forces the analyst to check for potential interference via lifetime analysis. This is particularly true for the analysis of samples with unknown composition. A single exponential decay with a lifetime equivalent to the lifetime of the pure standard provides a strong argument to claim accurate PAH determination [34,35,37]. The approach we present here is fundamentally different as we base PAH determination on 4.2 K fluorescence time-resolved excitation–emission cubes (TREECs). 4.2 K fluorescence TREECs result from the superposition of fluorescence TREEMs recorded at different time windows from the laser excitation pulse. Potential interference from unknown sample concomitants is handled by processing four-way 4.2 K fluorescence TREEC data arrays with PARAFAC and U-PLS/RTL.

To the extent of our literature search, this is the first report on the analysis of soil samples via 4.2 K fluorescence TREEC coupled to second-order multivariate calibration methods. The same is true for the hyphenation of PARAFAC or U-PLS/RBL to 4.2 K fluorescence TREEC data. The only article that exists on processing high-resolution data with second-order calibration methods deals with the combination of PARAFAC to 4.2 K excitation modulated phosphorescence WTMs (EMWTMs). EMWTMs were generated

with the superposition of five 4.2 K phosphorescence WTMs recorded at five excitation wavelengths. Each WTM was recorded using the same delay (50 μ s) and gate (1100 ms) times. The EMWTMs/PARAFAC approach was successfully applied to the analysis of 2,3,7,8-tetrachloro-dibenzo-para-dioxin in solid-phase water extracts [38].

The four-way 4.2 K fluorescence TREEC data arrays used here for the determination of the fifteen EPA-PAHs were recorded during the nanosecond time domain of the fluorescence decay. TREECs were generated from 4.2 K fluorescence TREEMs recorded using an excitation range common to the 15 EPA-PAHs. The sensitivity of TREEC/four-way modeling made possible to determine PAHs at the ng g^{-1} to pg g^{-1} concentration level with no need for sample pre-concentration. Its selectivity is demonstrated with analytical recoveries statistically equivalent to those obtained with classic methodology.

2. Experimental section

2.1. Chemicals

All solvents were HPLC grade and purchased from Fisher. Unless otherwise noted, Nanopure water—from a Barnstead Nanopure Infinity water system—was used throughout. All chemicals—including those used for PAH extraction—were analytical reagent grade and used without further purification. A soil sample of known composition—Natural Matrix Reference Material CRM 104-100; PAH contaminated soil/sediment from Southern Branch of the Elizabeth River, Chesapeake Bay area—was acquired from Resource Technology Corporation, Laramie, WY. A Supelco PAH mixture (EPA 610) in methanol:methyl chloride (1:1 v/v) from Supelco was used as the reference standard for HPLC analysis. PAH standards for LETRSS analysis were purchased from Aldrich at their highest available purity. Rhodamine 6G was acquired from Exciton and used with the tunable dye laser according to specifications.

Note: Use extreme caution when handling PAHs known to be extremely toxic.

2.2. Analysis of soil samples via classic methodology

Saponification and sonication procedures for PAH extraction from soil samples followed previously reported methodology [39]. The same is true for the HPLC analysis of soil extracts [37]. The complete description of the saponification and sonication procedures, the instrumentation for HPLC analysis and the experimental conditions for the chromatographic separation of EPA-PAHs is provided in the supplemental information section of this article.

2.3. PAH screening via 4.2 K LETRSS

Complete description of the instrumentation for LETRSS analysis is provided in the supplemental information section of this article. The same is true for the cryogenic FOP. A known amount (0.05 g) of soil sample was mixed with 250 μ L of *n*-octane in the vessel of the FOP. The mixture was submitted to 30 min of sonication at room temperature in a Branson sonication bath (Model 3210). After 5 min of settling time, the sample extract was analyzed via LETRSS. 4.2 K measurements were done by coupling the sample vial of the FOP to the copper tubing of the fiber assembly. The tip of the fiber assembly was kept \sim 0.5 cm above the surface of the liquid layer. Sample freezing was accomplished by lowering the copper tubing into the liquid helium, which was held in a Dewar flask with 60 L storage capacity. The liquid

helium would typically last 3 weeks with daily use, averaging 15–20 samples per day. Complete sample freezing took less than 90 s per sample. Replacing the frozen sample involved removing the sample vial from the cryogen container and melting the frozen sample with a heat gun. Because no physical contact between the tip of the fiber-optic bundle and the sample ever occurred during measurements, probe cleanup between measurements was not necessary. The entire freeze, thaw, and sample replacement cycle took no longer than 5 min. PAH concentrations were determined with the multiple standard additions method.

2.4. Collection of TREEC data arrays

The four-way 4.2 K fluorescence TREEC data arrays used for EPA-PAH determination result from the superposition of four TREECs recorded from soil extracts containing different concentrations of individual PAHs. PAHs concentrations were adjusted via the multiple standard addition procedure. The first TREEC reflected the original PAH composition of the soil sample; i.e. no standard addition. The other TREEC—one per standard addition—were obtained after adding 5, 10 and 15 μL of a standard EPA-PAH mixture to the 250 μL volume of soil extract in the sample vial of the FOP. All standard additions were made at room temperature.

The 4.2 K fluorescence TREEMs used to generate the four TREECs were recorded using an excitation range (280–295 nm) common to the 15 EPA-PAHs. The tunable dye laser was stepped at 0.5 nm increments, generating a total of twenty two (22) excitation wavelengths. Fluorescence was recorded within the 300–500 nm wavelength range using the following delay (D) times: 10, 30, 60, 90, 120 and 150 ns. The gate width for the first TREEM ($D=10$ ns) was 20 ns. The gate widths for the remaining TREEMs were 30 ns. These parameters provided the following time windows ($D-D+G$) for TREEM collection: TREEM₁=10–30 ns, TREEM₂=30–60 ns, TREEM₃=60–90 ns, TREEM₄=90–120 ns, TREEM₅=120–150 ns, and TREEM₆=150–180 ns.

2.5. Chemometric algorithms and software

All calculations were done using MATLAB 7.6 [40–44], with the aid of the MVC3 graphical toolbox and a user friendly MATLAB graphical interface available on internet [45].

3. Results and discussion

3.1. Analysis of soil samples with classic methodology

Several methods exist to extract PAHs from soil samples [39,46–49]. Our choice for methanolic saponification was based on its comparatively high PAH extraction efficiency [39]. Saponification under methanolic conditions breaks down polymeric structures of organic matter—which is frequently present in soil samples and chemically associated to PAHs—and increases the accessibility of the solvent for PAH extraction. The sonication method was selected because of its relatively short extraction time and easy implementation [39,46]. The solvent we used for sonication—hexane:acetone:toluene (10:5:1 v/v/v)—was reported to yield higher extraction efficiencies than other sonication solvents [39,46]. It should be noticed, however, that even after careful optimization of extraction parameters—such as solvent composition and extraction time—PAH recoveries with sonication tend to be lower than with other extraction techniques.

HPLC analysis of soil extracts was done with previously reported methodology [37]. Fig. S-1 depicts a typical chromatogram of the soil sample. Peak assignments were solely based on the retention times of pure standards. Acenaphthylene showed no

fluorescence under the conditions of the separation. Table S-1 summarizes the retention times and the limits of detection (LOD) of the HPLC method along with the PAH recoveries obtained via saponification-HPLC and sonication-HPLC analysis. The HPLC-LOD were calculated according to the formula $\text{LOD}=3S_B/m$; where S_B is the standard deviation of the average blank signal estimated from one-fifth of the peak-to-peak noise ($N_{p-p}/5$) and m is the slope of the calibration curve [50]. The N_{p-p} was measured at the base peak of each PAH elution over a sufficiently wide region of the chromatogram. Calibration curves were built with synthetic mixtures of pure standards using a minimum of five linear concentrations per PAH. The slopes of the calibration curves were calculated from the linear dynamic ranges (data not shown) using the least squares method for statistical fitting [51].

All PAH concentrations determined in the soil extracts were at the parts-per-million ($\mu\text{g}/\text{mL}$) level. Average saponification values varied from 0.55 $\mu\text{g}/\text{mL}$ (fluorene) to 18.5 $\mu\text{g}/\text{mL}$ (fluoranthene). Sonication concentrations varied from 0.27 $\mu\text{g}/\text{mL}$ (fluorene) to 5.46 $\mu\text{g}/\text{mL}$ (pyrene). All LODs were at the parts-per-billion level (ng mL^{-1}) and, therefore, well below the concentrations of EPA-PAHs in soil extracts. As expected, the PAH recoveries with methanolic saponification were considerably higher than those obtained with sonication. The relative standard deviations (RSD) of the average recoveries obtained with saponification varied from 2.4% (fluoranthene) to 8.9% (fluorene). The RSD values obtained with the sonication method varied from 1.5% (chrysene) to 12.5% (naphthalene). These facts can be attributed to several factors, including uncontrolled PAH loss during extraction via saponification and sonication [39,46].

3.2. 4.2 K LETRSS analysis of EPA-PAHs

For the specific case of EPA-PAHs, the solvent matching criterion leads to one of the following five n -alkanes: n -pentane (naphthalene, acenaphthene, and acenaphthylene), n -hexane (phenanthrene and pyrene), n -heptane (fluorene, fluoranthene, benzo[*g,h,i*]perylene, benzo[*b*]fluoranthene and anthracene), n -octane (benzo[*a*]pyrene, dibenz[*a,h*]anthracene, chrysene and benz[*a*]anthracene), and n -nonane (indeno[1,2,3-*cd*]pyrene and benzo[*k*]fluoranthene) [52]. Using the best matching solvent for each PAH would provide the best spectral resolution possible. Considering the use of five organic solvents per sample practically unattractive for screening purposes, we carried out the analysis of the 15 EPA-PAHs with n -octane. Our preference was based on preliminary studies showing better sonication extractions with n -octane than with the other four solvents.

Acenaphthylene was the only compound with no fluorescence emission in frozen octane. Its lack of fluorescence persisted after sample de-oxygenation or analyte freezing in n -pentane, n -hexane, n -heptane and n -nonane. Table S-2 summarizes the 4.2 K fluorescence figures of merit of the remaining PAHs in n -octane. The excitation and emission ranges include all the peaks analytically meaningful for each PAH, i.e. with a signal-to-noise ratio equal to or higher than 3 ($S/N \geq 3$). All PAHs showed well-behaved single exponential fluorescence decays in n -octane. The reported lifetimes (τ) were measured at the maximum emission (λ_{em}) wavelength of each PAH. Sample excitation was at 283.2 nm, i.e. an excitation wavelength common to the 15 EPA-PAHs that provides their determination at trace concentration levels (ng/mL). The LODs were calculated with the formula $\text{LOD}=3 \times S_B/m$; where S_B is the standard deviation of the blank ($N=16$) and m is the slope of the calibration curve [53]. Calibration curves were built with pure standards using a minimum of five linear concentrations per PAH. The slopes of the calibration curves were calculated from the linear dynamic ranges (data not shown) using the least squares method for statistical fitting.

Fig. S-2 shows the normalized fittings of the fluorescence decays of the 15 PAHs. According to their time decay profiles, EPA-PAHs belong to one of the following three groups: short- ($\tau \leq 11.9$ ns), medium- ($40.7 \text{ ns} \leq \tau \leq 55.2$ ns) and long-lived ($\tau \geq 123.8$ ns) PAHs. Within the context of time-resolved measurements with fixed time delays, discrimination of short- and medium-lived PAHs should be possible with time delays of 30 and 170 ns, respectively. These delay values are based on the residual and often negligible fluorescence observed after delay times $\geq 3 \times \tau$ [54]. Because time-discrimination of long-lived PAHs is not possible, their potential interference to the determination of both short-lived and medium-lived PAHs is a matter of concern. The same is true for the determination of short-lived PAHs and the potential interference from medium-lived PAHs.

3.3. TREEC analysis

One possibility to circumvent this limitation is based on the spectral narrowing EPA-PAHs experience under Shpol'skii conditions at 4.2 K. Because WTMs provide the analyst with numerous peaks, there is always the possibility to find a set of excitation and fluorescence wavelengths free from matrix interference. In the analysis of samples with unknown composition, the analyst should always check for potential interference via lifetime analysis. A single exponential decay with a lifetime equivalent to the lifetime of the standard constitutes a strong argument for the accurate determination of the targeted PAH [37].

The approach we present here is fundamentally different as we base PAH determination on the appropriate selection of time windows for TREEM collection. By shortening the gate of the ICCD, one can reduce the spectral contribution of medium- and long-lived PAHs and still collect most of the fluorescence emitted by short-lived PAHs. By placing the time window at an intermediate time interval within the total fluorescence decay of the sample, it is possible to enhance the spectral features of medium-lived PAHs, time-discriminate the fluorescence of short-lived PAHs and reduce the contribution of long-lived PAHs.

Fig. 1 depicts TREEM₁ ($D-D+G=10\text{--}30$ ns), TREEM₃ ($D-D+G=60\text{--}90$ ns) and TREEM₆ ($D-D+G=150\text{--}180$ ns) recorded from the soil extract with no standard addition. Visual comparison of the spectral profiles of EPA-PAHs within the emission range of the

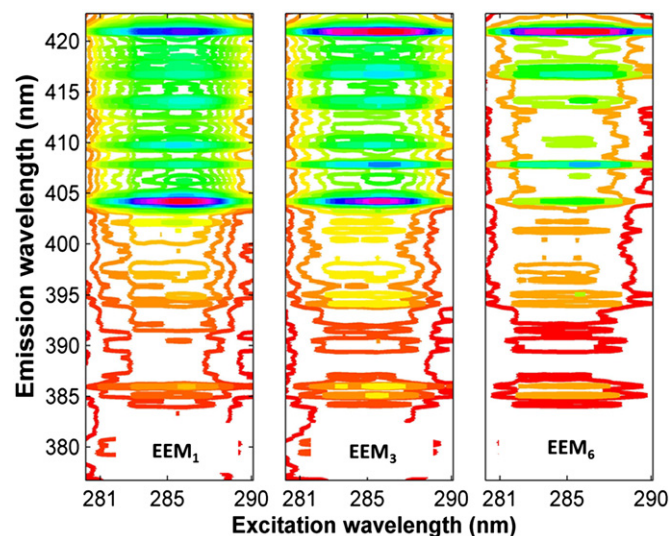


Fig. 1. 4.2 K TREEM recorded from soil extract with the following time windows: 10–30 ns (EEM₁), 60–90 ns (EEM₃) and 150–180 ns (EEM₆). Excitation wavelength was stepped at 0.5 nm increments. Each emission spectrum corresponds to the accumulation of 100 laser pulses.

TREEM (375–425 nm) leads to the following PAHs as the main contributors of the total fluorescence of the extract: benzo[*a*]anthracene, anthracene, dibenzo[*a,h*]anthracene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, benzo[*k*]fluoranthene, and fluoranthene. The relative contribution of each PAH to the total fluorescence of the sample varies with the time window of the TREEM. The fluorescence of benzo[*a*]pyrene—which appears in TREEM₁ and TREEM₃ at ~ 401.2 nm—practically vanishes in TREEM₆. Benzo[*a*]pyrene is a medium-lived PAH with $\tau = 42.8 \pm 1.6$ ns. The same is not true for the prominent fluorescence at 422.5 nm. The main reason for its presence in TREEM₆ is that belongs to benzo[*g,h,i*]perylene, a long-lived PAH ($\tau = 123.8 \pm 2.5$ ns). The superposition of TREEM_{1–6} leads to a TREEC with 540,672 data points. The superposition of TREEM_{1–6} corresponds to the TREEC of the soil extract.

3.4. PARAFAC and U-PLS/RTL modeling

The theory of PARAFAC and U-PLS/RTL is well documented [40–43]. Briefly, the decomposition of the four-way TREEC data array with PARAFAC allows the analyst to extract emission, excitation and time profiles of EPA-PAH along with their relative concentrations. The score of each PAH is then used to predict its concentration in the unknown sample. The principle of operation of U-PLS/RTL is fundamentally different as the original cube data is transformed into uni-dimensional arrays (vectors) by concatenating (unfolding) the original three-dimensional information. Concentration information is first used with no data from the unknown sample. A usual U-PLS model is calibrated with the included data and the vector of calibration concentrations. If there are no unsuspected interferences in the test sample, the concentration of each PAH can then be estimated using the same parameters used in the calibration step. The sample scores will be un-suited for PAH prediction when un-calibrated components occur in the test sample. In this case, the residuals of the U-PLS prediction step are abnormally large in comparison to the typical instrumental noise assessed by replicate measurements. The residual tri-linearization procedure—which is based on a Tucker3 decomposition—makes possible to model interference effects and accurately predict the concentration of each PAH in the unknown sample.

EPA-PAH concentrations in the soil extracts were obtained via the multiple standard additions procedure. This calibration method accounts for possible matrix interference due to inner filter and synergistic effects. Table 1 summarizes the concentrations of PAH standards used in the synthetic mixtures for the first, second and third standard additions. PARAFAC and U-PLS/RTL modeling were carried out generating a total of four TREECs, i.e. one TREEC for the soil sample—zero standard addition—and one TREEC per standard addition in Table 1.

The emission and excitation wavelength ranges, the time windows, the number of PARAFAC factors (responsive components) and the number of latent variables for U-PLS/RTL modeling (U-PLS/RTL factors) are listed in Table 2. The number of U-PLS/RTL factors was estimated via the cross validation method [55]. Each PLS model was built by subtracting the TREEC of the sample (zero standard addition) to those recorded after the first, second and third standard additions. The number of PARAFAC factors was estimated following the internal parameter procedure—also known as the core consistency procedure—originally introduced by Bro [56]. Fig. 2 shows the loading matrices for emission (A), excitation (B) and time decay (C) modes for benzo[*a*]pyrene predicted with the settings in Table 3. The labeling of components 1, 2 and 3 follows the order assigned by the model in the four-way array data of the sample. The order reflects their relative contribution to the overall variance. Benzo[*a*]pyrene (1) is the main fluorescence contributor in the four-way array data.

The fluorescence contribution of interferences **2** and **3** is practically negligible. The remarkable similarity among the predicted spectral profiles and the experimental spectra of benzo[a]pyrene—denoted as short dash dotted gray lines—is confirmed by the values of correlation coefficients; i.e. $r^2=0.889$ (Fig. 2A) and $r^2=0.846$ (Fig. 2B). The same is true for the predicted decay profile of benzo[a]pyrene (Fig. 2C). The predicted value of the fluorescence lifetime (34.2 ns) obtained with **D** is close to the experimental fluorescence lifetime of benzo[a]pyrene (38.6 ± 0.3 ns, $N=3$). The fact that the decay profile follows a well behaved, single exponential decay confirms the negligible contributions of **2** and **3** to the total fluorescence of the sample. Similar results were obtained for the remaining EPA-PAHs.

3.5. Comparison of TREEC–Chemometric modeling to sonication–HPLC data

Table 3 summarizes the PAH recoveries obtained via TREEC/Chemometric modeling and HPLC. In all the cases, PAH extraction was carried out via the sonication screening method. The statistical comparisons of PAH recoveries at different concentration levels was carried out with the Bonferroni's adjustment test [57]. The alpha value— $\alpha'=1-(1-\alpha)^{1/k}$ —was calculated for an overall

Table 1
Concentrations ^a used for multiple standard additions and chemometric modeling.

PAH ^b	First ^c	Second ^c	Third ^c
Napthalene	1.0	3.0	2.0
Acenaphthene	2.0	1.0	3.0
Fluorene	3.0	2.0	1.0
Phenanthrene	2.0	6.0	4.0
Anthracene	1.0	2.0	3.0
Fluoranthene	3.0	6.0	9.0
Pyrene	9.0	3.0	6.0
Benzo[a]anthracene	6.0	2.0	4.0
Chrysene	4.0	6.0	2.0
Benzo[b]fluoranthene	4.0	6.0	2.0
Benzo[k]fluoranthene	2.0	3.0	1.0
Benzo[a]pyrene	2.0	1.0	3.0
Indeno[1,2,3-cd]pyrene	3.0	1.0	2.0
Dibenzo[a,h]anthracene	3.0	2.0	1.0
Benzo[g,h,i]perylene	1.0	2.0	3.0

^a All concentrations are in $\mu\text{g}/\text{mL}$.

^b PAH standards were prepared in n-octane.

^c 5, 10 and 15 μL of standard solution were added to 250 μL of soil extract.

Table 2
Parameters for PARAFAC and U-PLS/RTL analysis.

PAH	Excitation ^a (nm)	Emission ^a (nm)	EEM ^b #	Time windows ^b (ns)	PARAFAC factors	U-PLS/RTL factors
Napthalene	280.5–285.5	319.9–320.7	4, 5	90/120, 120/150	2	1
Acenaphthene	283.0–289.5	319.3–319.1	1–3	10/30, 30/60, 60/90	3	2
Fluorene	281.5–287.5	303.6–305.1	1, 2	10/30, 30/60	2	1
Phenanthrene	286.0–290.0	350.4–351.7	1, 2	10/30, 30/60	2	1
Anthracene	282.5–295.0	383.6–384.4	1–5	10/30, 30/60, 60/90, 90/120, 120/150	3	2
Fluoranthene	280.0–290.0	402.7–403.8	1–5	10/30, 30/60, 60/90, 90/120, 120/150	3	2
Pyrene	280.5–290.0	370.1–371.2	4, 5	90/120, 120/150	3	2
Benzo[a]anthracene	281.0–288.5	381.6–382.4	1–3	10/30, 30/60, 60/90	3	2
Chrysene	280.0–286.5	362.1–362.8	1–3	10/30, 30/60, 60/90	3	2
Benzo[b]fluoranthene	284.5–290.0	398.6–399.5	1–4	10/30, 30/60, 60/90, 90/120	3	2
Benzo[k]fluoranthene	280.0–289.5	401.5–402.4	1, 2	10/30, 30/60	3	2
Benzo[a]pyrene	283.0–287.5	400.5–401.5	1, 2	10/30, 30/60	3	2
Indeno[1,2,3-cd]pyrene	283.5–290.0	401.5–402.4	1–3	10/30, 30/60, 60/90	2	1
Dibenzo[a,h]anthracene	281.0–295.0	390.9–392.1	1–3	10/30, 30/60, 60/90	3	2
Benzo[g,h,i]perylene	283.0–288.5	404.6–405.3	1–5	10/30, 30/60, 60/90, 90/120, 120/150	3	2

^a Wavelength ranges are fractions of recorded EEMs. The complete wavelength ranges of the recorded EEMs were 280–295 nm for excitation and 300–500 nm for emission.

^b EEM # refers to the TREEM recorded during the following delay (D)/delay + gate (D+G) time windows: 1 = 10/30 ns, 2 = 30/60 ns, 3 = 60/90 ns, 4 = 90/120 ns, 5 = 120/150 ns, 6 = 150/180 ns.

significance level of 0.05 and 15 PAH comparisons ($k=15$). Because the calculated value ($\alpha'=3.5 \times 10^{-3}$) was considerably smaller than the critical t value ($t_{(\alpha',3-1)}=13.9$), it was concluded that the experimental recoveries of the 15 EPA-PAHs obtained with the three methods—i.e. TREEC/PARAFAC, TREEC/U-PLS-RTL and HPLC—were statistically equivalent.

Examination of HPLC data in Table 3 and Table S-1 provides a direct comparison between the screening extraction method and classic sonication methodology. The main difference is the inability of the screening method to detect the presence of anthracene and indeno[1,2,3-cd]pyrene. The observed difference can be attributed to the smaller mass of soil used with the screening method. The extraction of 0.05 g of soil—as opposed to 20 g of sample—probably leads to extract concentrations lower than the LODs of HPLC. The same is not true for the two TREEC/Chemometric modeling methods. The LODs of 4.2 K LETRSS analysis (see Table S-2) make possible to determine the presence of both anthracene and indeno[1,2,3-cd]pyrene in the extracts of 0.05 g of soil.

The presence of constant and proportional biases in the two TREEC methods was tested by comparing their results to HPLC data in Table 3. The statistical comparisons were made with the aid of the bivariate least-squares (BLS) regression method and the elliptic joint confidence region (EJCR) test [58,59]. The plots of the TREEC/PARAFAC and TREEC/U-PLS/RTL data versus the sonication–HPLC data provided the following slope/intercept results: $0.944 \pm 0.20/5.4 \pm 7.7$ (TREEC/PARAFAC) and $0.941 \pm 0.08/2.9 \pm 3.2$ (TREEC/U-PLS/RTL). The better precision of U-PLS/RTL is in good agreement with its superior predictive ability, which probably results from its latent variable properties [56]. The EJCRs of the slopes and the intercepts are shown in Fig. 3. The elliptical domains obtained with TREEC/PARAFAC and with TREEC/U-PLS/RTL include the theoretically predicted value of the slope (1) and the intercept (0). This fact rules out the possible presence of constant and proportional biases in the two approaches.

4. Conclusion

We have demonstrated for the first time the possibility to monitor 15 EPA-PAHs in soil samples processing high-resolution fluorescence data with third-order multivariate calibration methods. Its experimental relies on a previously reported procedure with various desirable features for routine screening of numerous

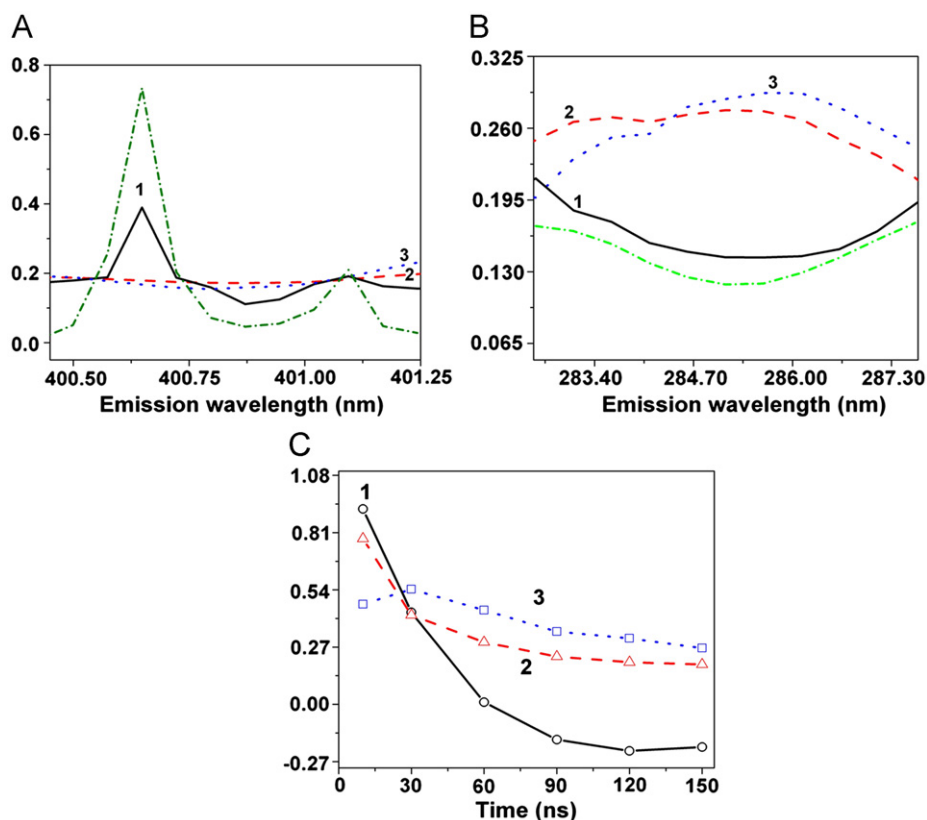


Fig. 2. PARAFAC modeling profiles obtained from soil extract. (A) Emission spectral profile, (B) excitation spectral profile and (C) fluorescence decay profile of benzo[a]pyrene. Numbers 1, 2 and 3 indicate the factor order. The short dash dotted (— • —) green line in (A) corresponds to the normalized emission spectrum of benzo[a]pyrene. (For interpretation of references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3

Comparison of PAH recoveries obtained via TREEC/PARAFAC, TREEC/U-PLS/RTL and HPLC analyses^a.

PAH	HPLC (%)	TREEC/PARAFAC (%)	TREEC/U-PLS/RTL (%)	<i>t</i> -student ^b		
				<i>t</i> ₁	<i>t</i> ₂	<i>t</i> ₃
Napthalene	44.8 ± 4.0	42.2 ± 3.8	45.6 ± 3.9	0.17	0.13	0.22
Acenaphthene	40.9 ± 2.3	39.6 ± 1.7	39.2 ± 1.6	0.52	0.70	0.14
Fluorene	43.0 ± 0.3	44.3 ± 0.8	44.1 ± 0.8	2.73	2.19	0.29
Phenanthrene	40.5 ± 1.4	42.9 ± 1.3	41.4 ± 1.4	1.31	0.52	0.82
Anthracene	–	45.7 ± 2.8	45.1 ± 2.8	–	–	0.08
Fluoranthene	24.3 ± 0.8	27.0 ± 1.0	26.5 ± 0.9	3.29	3.03	0.06
Pyrene	30.3 ± 2.3	31.1 ± 1.8	31.3 ± 1.9	0.18	0.29	0.06
Benzo[<i>a</i>]anthracene	31.8 ± 1.3	27.9 ± 1.8	30.9 ± 1.8	1.58	0.36	0.92
Chrysene	58.5 ± 5.6	55.4 ± 4.3	57.8 ± 4.1	0.12	0.02	0.13
Benzo[<i>b</i>]fluoranthene	55.4 ± 5.0	51.9 ± 3.5	53.8 ± 3.4	0.18	0.10	0.16
Benzo[<i>k</i>]fluoranthene	39.3 ± 1.1	43.6 ± 1.4	41.3 ± 1.4	2.71	1.26	1.17
Benzo[<i>a</i>]pyrene	49.9 ± 2.1	40.4 ± 2.1	48.9 ± 1.9	2.15	0.25	2.11
Indeno[1,2,3- <i>cd</i>]pyrene	–	46.5 ± 3.6	47.1 ± 3.4	–	–	0.05
Dibenzo[<i>a,h</i>]anthracene	37.5 ± 3.2	46.7 ± 3.1	43.1 ± 3.4	0.95	0.88	0.34
Benzo[<i>g,h,i</i>]perylene	38.6 ± 0.6	40.5 ± 1.0	38.4 ± 0.6	2.75	0.55	3.09

^a HPLC, TREEC/PARAFAC and TREEC/U-PLS/RTL analyses were performed in soil extracts obtained via the sonication screening procedure. Each reported value is the average of three independent extractions of soil samples.

^b *t*-student = experimental values calculated for $N=3$ and $p < 0.0035$ ($\alpha' = 1 - (1 - \alpha)^{1/k}$, Bonferroni's adjustment test); *t*₁: comparison between HPLC and TREEC/PARAFAC; *t*₂: comparison between HPLC and TREEC/U-PLS/RTL; *t*₁ corresponds to comparison between HPLC and TREEC/PARAFAC; *t*₃: comparison between both chemometric models. Reported values should be compared to *t* critical = 13.9.

soil samples [37]. PAH determination is based on the collection of 4.2 K fluorescence TREEMs recorded at six time windows away from the laser excitation pulse. Potential interference from unknown sample concomitants was successfully handled by processing four-way 4.2 K fluorescence TREEC data arrays with PARAFAC and U-PLS/RTL. The analytical recoveries of the 15 EPA-PAHs were in good agreement with those obtained via classic

sonication-HPLC methodology. When handling intrinsically complex samples of unknown composition processing 4.2 K WTM data with univariate calibration methods forces the analyst to check for potential interference via lifetime analysis. Depending on the complexity of the sample, situations might arise where finding an appropriate set of excitation and fluorescence wavelengths is not possible. The TREEC/PARAFAC and TREEC/U-PLS/RTL

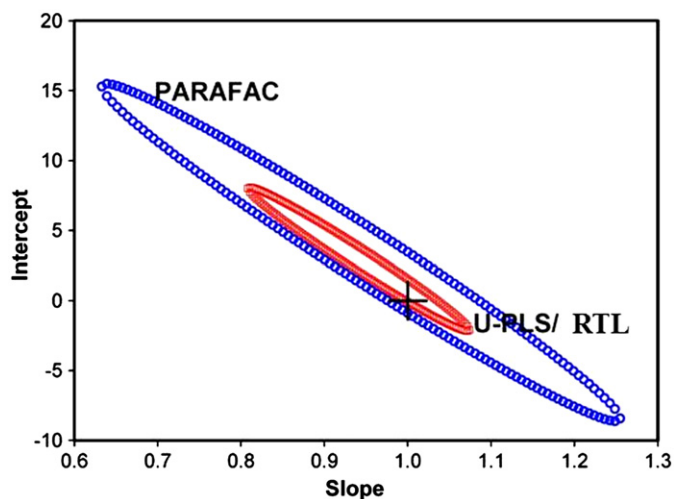


Fig. 3. Elliptic joint confidence region obtained with the bivariate least-squares regression method from the plot of the TREEM/PARAFAC and TREC/U-PLS data in Table 3.

approaches presented here provide a general solution to the ubiquitous problem of spectral interference.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.talanta.2012.09.035>.

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