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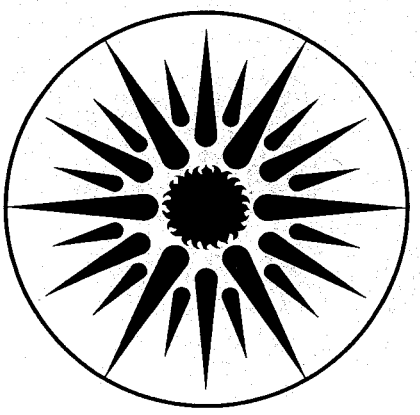

ENERGY & ENVIRONMENT DIVISION

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SELECTIVE FLUORESCENCE DETECTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN ENVIRONMENTAL TOBACCO SMOKE AND OTHER AIRBORNE PARTICLES

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ABSTRACT

An analytical method is described for the simultaneous quantitation of polycyclic aromatic hydrocarbons [PAHs] and alkyl-derivatives [alkyl-PAHs] in "real world" samples using microbore reversed-phase high performance liquid chromatography in conjunction with two programmable fluorescence detectors. Sensitivity and selectivity were enhanced by analyzing PAHs under their optimum fluorescence wavelengths. The accuracy of the analytical method was evaluated by determination of PAHs in 5 mg of standard reference material SRM 1649. The method was also successfully employed to analyze major parent PAHs and some alkyl-PAHs from environmental tobacco smoke [ETS] with a sample size of 2 mg using class-selective fluorescence wavelengths. Some alkyl-PAHs were tentatively identified even in the absence of standard compounds. Coeluting pairs were identified and analyzed by careful selection of excitation and emission wavelengths for each compound. Identities of the signals were confirmed by comparing both the retention behavior and the peak-height ratios at two or more different excitation and emission wavelength combinations.

INTRODUCTION

Reversed-phase high performance liquid chromatography [RPLC] on chemically bonded octadecyl stationary phases is by far the most popular liquid chromatographic method for the separation of polycyclic aromatic hydrocarbons [PAHs]. The main attraction of RPLC is its unique selectivity for the separation of PAH isomers that are often difficult to separate by other chromatographic methods. In addition, the compatibility of RPLC with gradient elution techniques and the rapid equilibration of these columns to changes in mobile phase composition make RPLC a convenient separation technique. Another major advantage of high performance liquid chromatography [HPLC] is the availability of sensitive and

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selective dual-monochromator fluorescence detectors. The specificity of the fluorescence detectors is due to the availability of two wavelengths, excitation and emission, for detection. Therefore, the combination of RPLC with fluorescence detection is a reliable method for the quantitative determination of PAHs in environmental extracts.

However, difficulties can be encountered with complex matrices such as environmental tobacco smoke [ETS]. A serious problem associated with the identification and quantitation of particular PAHs based only on RPLC data is the coelution of alkyl-PAHs with fewer aromatic rings [1]. This problem was addressed by Wise *et. al.*, and two methods were developed based on pre-fractionation or selective detection [1-6]. The pre-fractionation method exploits the differences in retention of PAHs in normal-phase high performance liquid chromatography [NPLC] and RPLC. The retention of PAHs on the polar chemically-bonded stationary phases, used with a nonpolar mobile phase [normal-phase], increases with the number of condensed aromatic rings [or number of aromatic carbon atoms]. However, the presence of an alkyl group on the PAH has virtually no effect on the retention [7-8]. In contrast, a nonpolar chemically-bonded stationary phase with a polar mobile phase [reversed-phase] separates alkyl-substituted PAHs [9-15]. NPLC was used to fractionate the total PAHs/Alkyl-PAHs mixture into several fractions, each containing the same number of aromatic carbons. Then each fraction was quantitated by RPLC-UV/fluorescence. Loss of analytical precision, volatility loses, contamination during intermediate collection and reconcentration steps, and the time required for multi-step separation are the major drawbacks of such methods.

In the alternative selective detection method, the PAH mixture was analyzed without NPLC pre-fractionation. Fluorescence wavelengths were programmed to enhance the specificity and the selectivity of individual PAHs in the mixture and minimize interferences from coeluting species. Several fluorescence wavelength programs were developed by other research groups to quantitate PAHs [16-18]. However, none of these programs were reported to quantitate alkyl-PAHs simultaneously with parent compounds. Although this is not usually a problem for PAH samples collected in outdoor air in U.S. cities [5,19], indoor air is frequently impacted by ETS which contains alkyl-PAHs at levels similar to those of the parent compounds. A few of the reported methods were tested only on the synthetic mixtures, but real world samples are considerably more complex.

In this paper we describe a highly reliable, precise method to overcome the coelution problems, using a dual-detector programmable fluorescence system. In early development of this method [20], extracts of indoor airborne particulate matter were separated by means of a reversed-phase microbore column, and the compounds were detected with only a single programmable fluorescence detector. Each sample was analyzed twice using the same solvent program but two different wavelength programs for

detection. For complex environmental samples such as ETS, the quantitation obtained from this method was imprecise due to background interferences and coelution problems.

In the method reported here, a second programmable fluorescence detector was connected in series so that analysis with two wavelength programs could be accomplished with a single injection. Peak broadening and loss of resolution due to the extra dead volume of the second detector assembly were minimal, and the performances were evaluated. Background interferences from the polar compounds in ETS were nearly eliminated by cleaning the sample using a silica SEP-PAK column [21]. Some of the initially unresolved peaks were separated by modifying the solvent program to have a less steep gradient. Constant retention and resolution of the analytes were maintained by isothermal separation of PAHs at elevated temperatures. Hence, both the accuracy and precision of the analytical results were improved substantially.

Multiple detection methods provide improved specificity, selectivity, qualitative identification, quantitation, and decreased overall analysis time [22-27]. In these methods, the ratio of the peak heights or peak areas of fluorescence or ultraviolet [UV] signals as well as mass spectra were used to confirm the identity and purity of the chromatographic signal. The only multi-detection fluorescence method reported in literature [27] used the peak-height ratios at two sets of fixed wavelengths to identify PAHs.

Since fluorescence spectroscopy is nondestructive, coupling of two programmable fluorescence detectors allows simultaneous detection of PAHs under two sets of excitation and emission wavelength combinations. Therefore, by careful selection of excitation and emission wavelengths, a high degree of specificity and selectivity can be obtained. This selection permits the determination of PAHs in a multi-component system even when complete resolution of the PAHs by HPLC is not achieved; hence, coeluting pairs can be quantitated simultaneously. Coeluting pairs can be easily identified from the broadening of the chromatographic signal as well as by comparing the variation of the intensity of the chromatographic signal with fluorescence wavelength change. The components in the coeluting mixture can be identified from the fluorescence excitation and emission spectra scanned from the peak upslope, apex and the downslope.

This method can also be used to help identify some alkyl-PAHs, even in the absence of their respective standards. The qualitative identification is three-fold. First, the identity of the unknown compound is hypothesized from the retention time of the signal. In RPLC, methyl-PAHs are eluted after the parent PAHs because of the greater hydrocarbonaceous contact area of the methyl-PAHs. In general, these methyl-PAHs are resolved on polymeric octadecyl-bonded phases and eluted in order of increasing rod-like shape which is characterized by the length-to-breadth ratio of the isomer [9]. This characteristic

can be used to pick out unidentified signals as methyl derivatives of previously eluted parent PAHs. Secondly, these preliminary identifications are further supported by comparing the enhancement or suppression of the fluorescence signal with that of the parent compound under different fluorescence wavelength combinations. Finally, peak-height ratios of the signal are matched with those of the available standards. If necessary, further confirmation can be obtained by scanning the excitation and emission spectra of the signal peak.

EXPERIMENTAL SECTION

The reversed-phase HPLC separations were done on a model 1090M liquid chromatography system [Hewlett-Packard, Mountain View, CA] equipped with a DR5 binary solvent system and a temperature-controlled column compartment. An external thermostat-controlled water bath was also employed to control the temperature of the column compartment at 28.0 ± 0.2°C. ChemStation software equipped with foreground-background capability was used to control the instrument, record chromatograms and spectra and quantitate PAHs concentrations. A Rheodyne 8125 sample injector with a 5 microliter sample loop was used. The HPLC analysis was carried out on a Vydac 201TP5215 reversed-phase C18 analytical column [2.1 mm x 15 cm, 5 micron particles] from The Separations Group, Hesperia CA. The analytical column was protected by a guard column cartridge packed with 10 micron Vydac 201TP C18 particles.

The HPLC detection system consisted of two dual-monochromator programmable fluorescence detectors with xenon lamps in series Hewlett Packard HP1046A. The first fluorescence detector was directly coupled to the ChemStation equipped with software to scan the excitation and emission spectra of the analytes in both the "on fly" and "stopped-flow" modes. The second fluorescence detector was coupled to the ChemStation with an analog-to-digital converter (HP Interface 35900). Excitation and emission wavelengths and other parameters were set using the detector's stand-alone controls. The two detectors were purchased three years apart.

PAH standard compounds were obtained from the following suppliers and were used without purification: anthracene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, coronene, 9,10-dimethylanthracene, 3,6-dimethylphenanthrene, fluoranthene, 1-methylfluoranthene, phenanthrene, perylene, pyrene and triphenylene from Aldrich Chemical Company, Milwaukee, WI; 7,12-dimethylbenz[a]anthracene from Eastman-Kodak, Rochester NY; 1,2-benzofluorene from Accu Standards, New Haven, CT; benz[a]anthracene, dibenz[a,c]anthracene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene from Pfaltz and Bauer, Stamford, CT; cyclopenta[cd]pyrene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, 6,12-dimethylchrysene, 3-

methylbenz[a]anthracene, 2-methylbenzo[b]fluoranthene, 10-methylbenzo[b]fluoranthene, 8-methylbenzo[a]pyrene, 11-methylbenzo[a]pyrene, 8-methylfluoranthene and 4-methylpyrene; from Midwest Research Institute, Kansas City, MO; 1-methylanthracene from Alfa-Johnson Matthey Company, Ward Hill, MA; benzo[j]fluoranthene, 1-methylbenz[a]anthracene and all six methylchrysene isomers from the Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium; SRM 1649 urban dust, and SRM 1647a standard mixture, from the US Department of Commerce, National Institute of Standards and Technology, Washington DC, and deuterated fluoranthene and benzo[e]pyrene from MSD Isotopes, Quebec, Canada. HPLC-grade acetonitrile and tetrahydrofuran were obtained from Burdick and Jackson Lab. Inc., Muskegan, MI. Locally-deionized water, acetonitrile and tetrahydrofuran were shown to be free of fluorescence impurities.

Stock solutions of the standards were prepared by dissolving weighed amounts [using a Model 25 automatic electrobalance, Cahn Instruments, Cerritos, CA] of the PAHs in acetonitrile. Standard solution A contained naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene-D_{10}, fluoranthene, pyrene, 3,6-dimethylphenanthrene, triphenylene, 1,2-benzofluorene, benz[a]anthracene, chrysene, 7,12-dimethylbenz[a]anthracene, benzo[e]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, 10-methylbenzo[b]fluoranthene, dibenzo[a,l]pyrene, benzo[ghi]perylene, indeno[1,2,3-cd]pyrene, dibenzo[a,e]pyrene, 3,6-dimethylbenzo[a]pyrene, coronene, dibenzo[a,i]pyrene and dibenzo[a,h]pyrene, in the concentration range of 2-50 ng/ml in acetonitrile. Standard solution B contained 1-methylanthracene, 2-methylphenanthrene, 8-methylfluoranthene, 4-methylpyrene, chrysene, 5-methylchrysene, 1-methylbenz[a]anthracene, benzo[j]fluoranthene, perylene, 1-methylchrysene, dibenz[a,c]anthracene, 2-methylbenzo[b]fluoranthene, 11-methylbenzo[a]pyrene, benzo[ghi]perylene and 8-methylbenzo[a]pyrene in the concentration range of 20-200 ng/ml. Working standard mixtures were prepared at ng/ml concentration by diluting in acetonitrile. Both stock and standard solutions were stored in the dark at -15°C when not in use. Standard solutions were brought to room temperature and sonicated briefly before use.

The reproducibility of retention times was highly dependent on the thermal stability of the analytical columns. Initial separations at sub-ambient temperatures [15-20°C] demonstrated a decrease of retention times throughout the day. Slightly elevated temperatures [30°C] were maintained using circulation from an external heated water bath, and the retention times were then reproducible.

Secondary organic modifiers (1-propanol and tetrahydrofuran) were tested to improve the retention of PAHs (prior to elevation of column temperature), but the retention times were not reproducible. However, the presence of tetrahydrofuran in the mobile phase led to sharper and more intense peaks for high molecular weight PAHs. Moreover, the presence of tetrahydrofuran in the solvent

mixture resulted in faster column re-equilibration. Therefore tetrahydrofuran was introduced into the mobile phase mixtures.

The mobile phase components were vacuum-degassed during the preparation of the solvent mixtures and were helium-degassed before use. This also minimized the loss of fluorescence efficiency by removing the dissolved oxygen, a known fluorescence quencher, from the mobile phase. The mobile phase consisted of two solvent mixtures. Solvent A consisted of a 95:5 [v:v] mixture of acetonitrile and tetrahydrofuran, and solvent B was a 38:2:60 [v:v:v] mixture of acetonitrile, tetrahydrofuran and water. Initial flow through the column was 0.45 ml/min and the flow rate was linearly increased up to 0.75 ml/min between 22 and 34 min. The flow rate was 0.75 ml/min between 34 and 42 min. Then the flow rate was stepped down at 42.05 min to the initial flow rate. The gradient elution program started with 10% of A, and the solvent strength was increased linearly between 1 and 4 min to 13.5% A, 4 and 8 min to 17.5% A, 8 and 12 min to 23.5% A, 12 and 16 min to 28% A, 16 and 24 min to 40% A, 24 and 30 min to 55% A, 30 and 39.5 min to 100% A. The mobile phase was isocratic between 39.50 and 42.1 min., and the gradual decrease of the solvent strength between 42.1 and 46 min brought the mobile phase to initial conditions. The HPLC pump-controlled solvent program was stopped at 47 min, and then the column was equilibrated for 15 minutes at the initial conditions before the next injection.

In order to maximize selectivity and sensitivity for the analysis, the best excitation and emission wavelengths were determined under stopped-flow conditions using individual pure compounds. As the fluorescence peak appeared, the LC pumps were stopped in order to trap the compound of interest in the fluorescence cell of the first detector. For each compound, the excitation spectrum was rapidly scanned at zero order emission [collecting all light above > 305 nm using only a cut-off filter]. Then, the emission spectrum was scanned at 254 nm excitation wavelength. Excitation and emission maxima were selected, and the emission spectrum was rescanned at the best excitation wavelength. Similarly, the excitation spectrum was also rescanned under the best emission wavelength.

The intensity of the fluorescence signals from standard compounds decreased throughout the day. The benzo[a]pyrene signal dropped by 28% for the first detector and 10% for the second detector over 10 injections. Therefore, the 254 nm excitation and zero order emission condition was selected as the reference fluorescence condition under which all the compounds produced a relatively intense signal. The standard solutions were analyzed under this reference fluorescence condition at the beginning of the day and then after every four injections. The information from the reference injection was used to calculate drift-corrected peak heights for the samples. Peak-height ratios were calculated from the normalized peak heights.

Extracts of urban dust (National Institute of Standards and Technology Standard Reference Material, SRM 1649) and environmental tobacco smoke (ETS) were prepared in cyclohexane by 15-30 min sonication of the particles (5 mg and 2 mg respectively) and cleaned using silica SEP-PAK columns [21]. An aliquot of the cleaned extract in cyclohexane was diluted in an aliquot of tetrahydrofuran and three aliquots of acetonitrile before the HPLC analysis. Alternatively, the cleaned extract was solvent-exchanged to acetonitrile by means of a second SEP-PAK column. ETS extracts were analyzed under twelve different excitation and emission wavelength combinations. The retention times of the signals and the fluorescence peak-height ratios were compared with those of the standards as the primary identification of the peaks. Purities of the peaks were further assessed by comparing the peak-height ratios as well as the changes of peak intensities with changes of fluorescence wavelengths. Once the signals were identified, two fluorescence programs were developed for the two detectors to quantitate the PAHs/alkyl-PAHs of interest. Peak heights rather than peak areas were measured as the analytical parameter because they were more reproducible, and their use minimized problems with peak resolution in the samples caused by the software-defined integration limits. Fluoranthene-D_{10} was used as an internal standard to correct for any PAH losses during sample preparation. (Deuterated benzo(e)pyrene, used as an internal standard in earlier studies [20], was found to coelute with other species of interest.)

Safety Considerations: Many of the PAH/Alkyl PAH are carcinogenic. Personal protection such as gloves should be worn, and a glove box should be used when weighing the standards. Avoid exposure to acetonitrite and tetrahydrofuran by performing as many operations as possible in a laboratory hood. Store tetrahydrofuran in the dark to avoid peroxide formation.

RESULTS AND DISCUSSION

DUAL FLUORESCENCE DETECTORS

The performance of the dual detectors was evaluated by analyzing standard mixture A at 254 nm excitation and zero order [> 305 nm] emission using both the detectors. Peak widths were expected to be broadened at the second detector due to extra connecting tubing and the second detector cell volume. Therefore, signal broadening, measured as peak band width at half height, w, and resolution, R, were selected as analytical parameters for the evaluation. Band widths at half heights were measured for non-baseline resolved signals. Resolution of the latter signal of the pair was calculated using the formula [28]:

$$R = 1.18 [t_2 - t_1] / [w_1 + w_2]$$

where t_1 and t_2 are the retention times of the first and second signals of the pair, respectively.

R and W are compared in Table I. The decrease in resolution between the two detectors was less than 13% for all non-baseline-resolved signals. This implies that diffusion of PAHs was small after the first detector. Also, the broadening of signals was less than 10% which minimized errors in analytical quantitation.

DETERMINATION OF THE OPTIMUM FLUORESCENCE WAVELENGTH COMBINATIONS

The wavelength maxima of the excitation and emission spectra of individual PAHs are listed in Table II with their relative retentions calculated with respect to naphthalene. As retention theory predicts, planar PAHs eluted in order of increasing number of aromatic carbon atoms in the PAH molecule. Rod-like PAHs/alkyl-PAHs eluted later than the more spherical PAHs/alkyl-PAHs. Also the alkyl-PAHs eluted later than the parent compounds. Maximum excitation and emission wavelengths also showed several trends. Both the excitation and emission maximum wavelengths increased with the number of aromatic carbon atoms in the molecule. Also the addition of alkyl groups to the parent molecule did not change the excitation and emission wavelengths significantly. This similarity is clearly illustrated in Fig. 1 which shows the excitation and emission spectra of chrysenes: all the possible methylchrysenes, chrysene and 6,12-dimethylchrysene. Fig. 1 indicates that all the alkyl isomers and chrysene can be quantitated as a group using the same excitation and emission wavelength combinations. Since all the methyl derivatives of PAHs were not commercially available we evaluated the feasibility of using the fluorescence similarity to tentatively identify the unknown signals with a minimum number of available standards, as discussed below.

EVALUATION OF THE PEAK-HEIGHT RATIOS

Both standard solutions A and B were analyzed under twelve different fluorescence wavelength combinations for peak-height ratio determinations. These fluorescence wavelength combinations were selected such that, under each fluorescence wavelength combination, detection of a class of targeted compounds [parent PAH with methyl derivatives] was enhanced. For example, in particulate ETS semi-volatile PAHs were the targated compounds, and special attention was paid to 3–5 membered ring PAHs and their methyl derivatives.

Peak-height ratios for benzo[b]fluoranthene and two of its methyl derivatives at twelve different fluorescence wavelength combinations are compared in Table III as an example. From these studies two

important conclusions can be drawn. First, under these wavelength combinations the peak-height ratios of the parent and alkyl-PAHs are generally very similar. Some variations of the peak-height ratios could be expected for parent and alkyl-PAHs eluted at significantly different solvent polarities because the excited molecules in different solvent mixtures experienced different solvent relaxation processes before the occurrence of fluorescence. In any case, similar peak-height ratios for parent and alkyl-PAHs for all the analyzed compounds suggested that the alkyl-PAHs can be tentatively identified even without the alkyl standards. Second, the peak-height ratios of PAHs/alkyl-PAHs were dependent on which detector was used for their measurement, because the response for a given signal was not same from the two detectors. The differences of peak height ratios indicate a significant difference between the optics and/or electronics of the two detectors. Therefore, a reference PAH must analyzed in both the standard and extracts using the same detector under identical chromatographic and fluorescence wavelength combinations.

Figure 2 compares chromatograms of standard mixture A at 12 different fluorescence wavelength combinations. These fluorescence chromatograms illustrate the specificity of fluorescence detection. Even though 28 PAHs/alkyl-PAHs were present in the standard mixture A, some of the compounds could be totally "turned off" while the others were "turned on." For example, chromatogram 2a has a strong signal for phenanthrene (5), and fluoranthene signals are totally "turned off", whereas chromatogram 2f has strong signals for fluoranthenes (7,8), and the phenanthrene signal is "turned off." Therefore, each member of a possible coelution pair of an alkyl-phenanthrene and fluoranthene could be quantified unambiguously. Also chromatogram 3f illustrates that 244 nm excitation and 480 nm emission is very specific for fluoranthenes. Therefore, alkyl-fluoranthenes could be quantitated using these wavelengths without any interferences from pyrene, triphenylene, chrysene or benz[a]anthracene and their alkyl-derivatives. Similarly 263 nm excitation and 371 nm emission in 2h exhibits the ability to "turn off" benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene signals. Therefore alkyl-chrysenes which elute in that region can be quantitated without those interferences.

Silica-cleaned [21] ETS extracts were also analyzed under a similar set of fluorescence wavelength combinations, and the chromatograms are shown in Fig. 3. The presence of some major PAHs in the ETS extract can be easily recognized from their retention times. But the purity of the signals was unknown. Therefore, peak-height ratios of the signals under some fluorescence wavelength combinations were compared with those of the standards and tabulated in Table IV. Matching of the peak-height ratios indicates that the signal was pure at those two sets of fluorescence wavelengths. However, matching ratios could be mathematically possible from two signals, both of which had interferences. Therefore peak-height ratios were compared under several fluorescence wavelength combinations to identify the purity of the signals. A large deviation (>15%) from the standards clearly indicates the presence of a fluorescence impurity at one of the fluorescence wavelength combinations.

This was well illustrated in the comparison of B[b]F peak-height ratios. Peak-height ratios associated with the 245 nm excitation and 391 nm emission (combination b in Table IV) deviated from the standard values. Careful comparison of those ratios reveals that closely eluting 3-methylchrysene and 2-methylchrysene signals interfered with both the B[b]F and 10-mB[b]F signals, respectively. Peak-height ratios for B[b]F associated with other wavelength combinations deviated less from the standard ratios because at those wavelengths chrysene signals were also suppressed. However, peak-height ratios associated with 244 nm excitation 480 nm emission wavelengths (combination f) also deviated, even though chrysene was "turned off" at these wavelengths. This suggests that another unidentified compound is interfering at this wavelength; therefore, this set of wavelengths would not be appropriate for quantitation.

As discussed earlier, coelution of a tentatively identified alkyl-phenanthrene with fluoranthene is illustrated in Fig. 3. Two fluorescence signals labelled as x-mPH and y-mPH in chromatogram a, b, h and j are not due to fluoranthene-D_{10} and fluoranthene. The peak-height ratios of x-mPH and y-mPH are compared in Table 4. This comparison suggests that those two signals are due to alkyl-phenanthrenes. In addition to this example, a signal corresponding to an alkyl-pyrene [x-mPY] was also tentatively identified.

Two sets of dual-detector fluorescence programs were constructed to analyze ETS extracts (Table V). These fluorescence programs were based on the information acquired from peak-height ratio comparisons with standards (Table IV), peak shapes (Fig. 3), known coeluting pairs or closely eluting pairs (Table II) and the fluorescence behavior of the parent PAHs (Fig. 2). In addition, attempts were made to analyze all the possible PAH signals using a minimum number of sample injections and a minimum number of wavelength changes for a single analysis. For ETS extracts, two injections are required even with a dual-detector system, because of the complexity of the mixture.

A single injection is sufficient to analyze all the PAHs of interest from a less complicated matrix such as SRM 1649, Figure 4. Here, slightly different excitation and emission wavelengths were selected (compared to the previous study [20]) to further minimize possible interferences. The dual-detector fluorescence program constructed to quantitate PAHs from SRM 1649 is given in Table VI. The PAH concentrations are in generally good agreement with the published values for SRM 1649, Table VII. We now have preliminary evidence that our high value for phenanthrene in SRM 1649 was due to an artifact from the cleanup column.

In general, the following considerations were included in selecting fluorescence programs for maximum sensitivity and selectivity: Optimum fluorescence wavelength combinations were selected for

each compound, and attempts were made to detect each compound at its best fluorescence wavelengths. But the choice of excitation and emission wavelengths is highly dependent on other PAHs present in the sample as well as the sample matrix. Common fluorescence wavelength combinations were selected for pairs such as benzo[b]fluoranthene and benzo[k]fluoranthene which could be highlighted under the same wavelength combination. For two closely-eluting or coeluting compounds, fluorescence wavelength combinations were set at each detector such that one compound was totally "turned-off" while the other was "turned-on" and vice-versa for the other detector. If such wavelength combinations are not possible both compounds were highlighted under two different common fluorescence wavelength combinations and the quantitation could be done by solving two simultaneous equations. For ETS each family of alkyl-PAHs and parent PAH was highlighted under one fluorescence wavelength combination. Stepwise wavelength changes were made at retention times corresponding to minima in fluorescence signals between peaks.

The limits of detection, LOD, *i.e.* the lowest concentration of PAHs that can be reliably detected, were evaluated for selected PAHs in the standard mixtures, ETS and SRM 1649 samples (Table VIII). A signal to noise ratio of three was used as the criterion for the LOD. The LOD of PAHs in ETS and SRM 1649 were expressed in micrograms per gram of the particles, assuming a 500 μL total extract volume. PAH and alkyl PAH concentrations in ETS are given in reference 21.

ADVANTAGES OF THE METHOD

The combination of the resolving power of microbore reversed-phase columns with specific and sensitive fluorescence detection yields several advantages in the determination of PAHs. Since the optimum wavelengths were used for quantitation, the detection limit has been improved to the sub-pg level, which is roughly one thousand times more sensitive than UV absorbance detection. The semi-micro extraction and clean-up protocols developed with this method [21] required smaller initial sample size for a complete PAHs analysis. For example, SRM 1649 required only 5 mg [21] of the sample matrix instead of 1 g [1]. Therefore, sampling times for airborne particulate matter and other environmental matrices can be shortened significantly or lower air flow rates can be used. Two mg of total ETS particles (collected from less than 5% of the air in a roomsized environmental chamber) from 3 cigarettes within a 2 hour sampling period was more than sufficient for a complete analysis of PAHs [21]. The analysis time and the hazardous solvent waste volume generated for a complete analysis were also dramatically reduced. The reduction in solvent waste is due to the smaller sample size and the use of a microbore column. Since pre-fractionation and multi-separation methods were replaced by a simple SEP-PAK clean-up procedure, uncertainties accompanying those extra steps such as the loss of volatile compounds and the contamination of PAHs from solvent artifacts were reduced. The most significant advantage of this

method is the ability to quantitate chromatographically inseparable or difficult to separate compounds using selective detection. A single separation with two detectors improved the precision of the detection method compared to earlier work [20]. In addition, the problems of coelution were minimized, and the concentrations of both components of a pair could be quantitated from a single analysis. Thereby the overall analytical precision of the method was improved. The use of class-selective fluorescence wavelength combinations also provides a method to tentatively identify the alkyl-PAH derivatives and estimate their concentrations even in the absence of the respective standard compounds.

Although this method can be successfully applied to more complex matrices, construction of the dual-fluorescence program requires a large amount of preliminary work, including the qualitative identification and purity analysis of the signals. Prospective users must be especially careful in adapting the fluorescence programs presented here to detectors from other manufacturers. Different excitation lamp types will produce different excitation and emission maxima for the same PAH.

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FIGURE CAPTIONS

Figure 1. Excitation and emission spectra of chrysene and its alkyl-derivatives. Identification: 0= chrysene, 1= 1-methylchrysene, 2= 2-methylchrysene, 3= 3-methylchrysene, 4= 4-methylchrysene, 5= 5-methylchrysene, 6= 6-methylchrysene, 7= 6,12-dimethylchrysene. Absorbance and fluorescence are given in arbitrary units.

Figure 2. Standard mixture A at different fluorescence wavelength combinations. Peak identification: 1= naphthalene, 2= acenaphthene, 3= acenaphthylene, 4= fluorene, 5= phenanthrene, 6= anthracene, 7= fluoranthene-D_{10}, 8= fluoranthene, 9= pyrene, 10= 3,6-dimethylphenanthrene, 11= triphenylene, 12= 1,2-benzofluorene, 13= benz[a]anthracene, 14= chrysene, 15= 7,12-dimethylbenz[a]anthracene, 16= benzo[e]pyrene, 17= benzo[b]fluoranthene, 18= benzo[k]fluoranthene, 19= benzo[a]pyrene, 20= 10-methylbenzo[b]fluoranthene, 21= dibeno[al]pyrene, 22= benzo[ghi]perylene, 23= indeno[1,2,3-cd]pyrene, 24= dibenzo[ae]pyrene, 25= 3,6-dimethylbeno[a]pyrene, 26= coronene, 27= dibenzo[ai]pyrene, 28= dibenzo[ah]pyrene.

Figure 3. ETS extract at different fluorescence wavelength combinations. Peak identification: PH= phenanthrene, F= fluoranthene, BaA= benz[a]anthracene, CH= chrysene, BbF= benzo[b]fluoranthene, BkF= benzo[k]fluoranthene, BaP= benzo[a]pyrene, BghiP= benzo[ghi]perylene, IND= indeno[1,2,3-cd]pyrene], 10-mBbF= 10 methylbenzo[b]fluoranthene, x-mPH and y-mPH= suspected methylphenanthrenes, x-mPY= suspected methylpyrene.

Figure 4. Analysis of PAHs in SRM 1649. Peak identification: PH= phenanthrene, F-D10= fluoranthene-D_{10} [internal standard], F= fluoranthene, BaA= benz[a]anthracene, CH= chrysene, BeP= benzo[e]pyrene, BbF= benzo[b]fluoranthene, BkF= benzo[k]fluoranthene, BaP= benzo[a]pyrene, BghiP= benzo[ghi]perylene, IND= indeno[1,2,3-cd]pyrene.

TABLES

Table I. Comparison of resolution for two fluorescence detectors in series

	First Detector			Second Detector		
PAH	t [a]	R [b]	w [c]	t [a]	R [b]	w [c]
fluoranthene-D_{10}	11.69		0.29	11.83		0.32
fluoranthene	12.30	1.22	0.30	12.42	1.11	0.32
3,6-dimethyphenanthrene	15.59		0.31	15.71		0.32
triphenylene	16.12	1.03	0.31	16.25	0.99	0.32
benz[a]anthracene	19.31		0.34	19.43		0.36
chrysene	20.04	1.24	0.36	20.17	1.17	0.39
benzo[k]fluoranthene	26.91		0.31	27.03		0.32
benzo[a]pyrene	27.75	1.60	0.31	27.85	1.51	0.32
dibenzo[al]pyrene	30.79		0.32	30.89		0.36
benzo[ghi]perylene	31.18	0.74	0.31	31.27	0.64	0.35
dibenzo[ae]pyrene	33.96		0.24	34.04		0.25
3,6-dimethylbenzo[a]pyrene	34.61	1.68	0.22	34.68	1.62	0.22

[a] t = retention time in min.

[b] R = resolution = $1.18\ (t_2-t_1)(w_1+w_2)$
where w_1 and w_2 are peak widths at half height and t_1, t_2 are retention times of the first and second compounds, respectively.

[c] w = peak widths at half height in min.

Table II. Excitation and emission maxima in nm for PAHs and alkyl-PAHs

Relative retention [a]	PAH/Alkyl PAH	Excitation Maxima [b]				Emission Maxima
1.00	naphthalene	**220**				330
1.63	acenaphthene	**225**	291			330
1.63	acenaphthylene	**225**	289	340		330
1.79	fluorene	**262**	294			315
2.14	phenanthrene	**245**				359
2.53	anthracene	**245**				391
2.60	1-methylfluorene	**263**	295			310
2.88	fluoranthene-D_{10}	**230**	282	354		445
3.03	fluoranthene	**231**	281	357		447
3.23	1-methylanthracene	**246**				397
3.29	pyrene	**234**	266	317	330	382
3.31	2-methylphenanthrene	**245**				367
3.76	3,6-dimethylphenanthrene	**246**	293			365
4.00	triphenylene	**249**				362
4.30	8-methylfluoranthene	**230**	240	290		441
4.34	1,2-benzofluorene	228	245	**255**	298	359
4.41	2,3-benzofluorene	231	**257**	308		348
4.47	4-methylpyrene	**234**	271	335		383
4.84	benz[a]anthracene	228	245	254	**266**	399
5.07	cyclopenta[cd]pyrene	230	241	**300**	359	417
5.09	chrysene	227	247	**263**		371
5.57	5-methylchrysene	**264**				381
5.58	6-methylchrysene	**263**				373
5.65	benzo[e]pyrene-D_{12}	231	242	**285**		390
5.66	4-methylchrysene	**264**				379
5.68	1-methylbenz[a]anthracene	**280**				399
5.93	7,12-dimethylbenz[a]anthracene	230	**292**	362		414
5.93	benzo[e]pyrene	229	240	**285**	328	390
5.93	benzo[j]fluoranthene	**234**	292	367	390	437
6.14	perylene	226	**245**			443
6.15	6,12-dimethylchrysene	226	**264**			378
6.24	benzo[b]fluoranthene	229	**245**	294	354	434
6.24	3-methylchrysene	**263**				375
6.54	1-methylchrysene	**263**				373
6.61	3-methylbenz[a]anthracene	225	240	**281**	400	401
6.69	benzo[k]fluoranthene	**232**	241	304	324	423
6.69	dibenzo[a,c]anthracene	230	243	**289**		405
6.86	2-methylbenzo[b]fluoranthene	229	**245**	296		430
6.88	benzo[a]pyrene	227	254	**292**	367	414
6.89	2-methylchrysene	**264**				375
7.30	10-methylbenzo[b]fluoranthene	229	**245**	296	365	425
7.36	11-methylbenzo[a]pyrene	227	255	**292**	385	419
7.47	dibenzo[a,l]pyrene	233	241	264	**298**	429
7.63	benzo[ghi]perylene	235	245	266	**292**	415
7.90	indeno[1,2,3-cd]pyrene	233	**244**	300	360	480
8.16	8-methylbenzo[a]pyrene	226	265	**292**	380	415
8.22	dibenzo[a,e]pyrene	231	241	**292**	327	405
8.39	3,6-dimethylbenzo[a]pyrene	226	255	**295**	385	419
8.78	coronene	**297**	338			435
9.32	dibenzo[a,i]pyrene	231	243	**296**	330	417
9.63	dibenzo[a,h]pyrene	230	246	262	**304**	457

[a] Relative retention with respect to naphthalene signal

[b] The most intense wavelength is given in bold face.

Table III. Peak height ratios for benzo[b]fluoranthene and two methylbenzo[b]fluoranthenes at various excitation and emission wavelengths

Ratio [a]	c/e	c/d	c/b	c/f	c/i	c/j	c/k	c/l	e/d	e/b	e/f	e/j	e/k	e/l
BbF [b,c]	0.55	0.67	2.16	0.75	6.24	2.53	1.29	0.51	1.20	3.90	1.36	4.57	2.33	0.92
2mBbF [b]	0.70	0.71	3.43	1.46	5.75	2.52	1.23	0.63	1.01	4.92	2.10	3.61	1.77	0.90
10mBbF [b]	0.55	0.58	1.51	0.78	-	2.00	1.18	0.51	1.06	2.74	1.43	3.65	2.15	0.94
BbF [d]	0.30	0.33	1.50	0.49	3.37	1.86	1.45	0.30	1.12	5.03	1.65	6.25	4.88	1.02
2mBbF	0.38	0.41	1.60	0.58	3.02	1.93	1.49	0.37	1.07	4.22	1.54	5.08	3.92	0.96
10mBbF	0.28	0.30	1.08	0.52	-	1.46	1.36	0.30	1.04	3.82	1.84	5.15	4.80	1.05

Ratio [a]	d/b	d/f	k/l	d/j	d/k	d/l	b/f	b/j	b/k	b/l	f/j	f/k	f/l	j/k	j/l
BbF [c]	3.24	1.13	0.40	3.80	1.93	0.76	0.35	1.17	0.60	0.24	3.36	1.71	0.68	0.51	0.20
2mBbF	4.86	2.08	0.51	3.57	1.75	0.89	0.43	0.73	0.36	0.18	1.72	0.84	0.43	0.49	0.25
10mBbF	2.59	1.35	0.44	3.45	2.03	0.89	0.52	1.33	0.78	0.34	2.56	1.51	0.66	0.59	0.26
BbF [d]	4.49	1.47	0.21	5.58	4.36	0.91	0.33	1.24	0.97	0.20	3.79	2.96	0.62	0.78	0.16
2mBbF	3.95	1.44	0.25	4.76	3.68	0.90	0.36	1.20	0.93	0.23	3.31	2.55	0.63	0.77	0.19
10mBbF	3.67	1.76	0.22	4.94	4.60	1.01	0.48	1.35	1.26	0.28	2.81	2.62	0.57	0.93	0.20

[a] Excitation/emission codes in nm:

a - 245/359	e - 245/434	i - 234/382
b - 245/391	f - 244/480	j - 266/399
c - 292/414	g - 254/ > 305	k - 288/405
d - 232/423	h - 263/371	l - 245/443

[b] BbF = benzo[b]fluoranthene, mBbF = methylbenzo[b]fluoranthene

[c] Detector # 1

[d] Detector # 2

Table IV. Peak height ratios for PAHs/alkyl-PAHs in a standard mixture and in ETS [a]

phenanthrene

RATIO [b,c]	a/b	a/h	a/j	b/h	b/j	h/j
STD	1.99	3.81	14.2	1.92	7.17	3.73
PH	1.84	3.43	11.8	1.87	6.43	3.44
x-mPH	1.59	2.59	8.31	1.63	5.23	3.21
y-mPH	1.95	2.57	10.3	1.32	5.26	4.00

fluoranthene

RATIO	d/e	d/l	d/f	e/l	e/f	l/f
STD	1.22	1.30	1.47	0.94	1.20	1.13
F [ETS]	1.17	1.06	1.50	0.91	1.28	1.41

pyrene

RATIO	d/e	d/b	d/k	d/i	d/j	e/b	e/k	e/i	e/j	b/k	b/i	b/j	k/i	k/j	i/j
STD	2.20	0.51	9.39	0.36	1.36	0.23	4.26	0.16	0.62	18.6	0.71	2.69	0.04	0.15	3.80
PY	2.39	0.52	6.15	0.34	1.23	0.22	2.57	0.14	0.51	11.7	0.65	2.34	0.06	0.20	3.59
x-mPY	1.78	0.47	8.33	0.44	1.71	0.27	4.67	0.24	0.96	17.6	0.92	3.60	0.05	0.21	3.92

benz[a]anthracene

RATIO	d/b	d/c	d/k	d/i	d/j	b/c	b/k	b/i	b/j	c/k	c/i	c/j	k/i	k/j	i/j
STD	0.98	0.81	0.53	1.29	0.99	0.82	0.54	1.31	1.01	0.66	1.61	1.23	2.44	1.87	0.77
BaA	0.73	0.88	0.54	0.92	1.02	1.20	0.74	1.25	1.39	0.62	1.05	1.16	1.69	1.87	1.11

chrysene

RATIO	a/b	a/h	a/i	a/j	b/h	b/i	b/j	h/j	i/j
STD	0.69	0.31	0.82	0.44	0.44	1.18	0.63	1.43	0.53
CH	0.95	0.40	0.77	0.64	0.42	0.82	0.68	1.61	0.83

benzo[b]fluoranthene

RATIO	d/e	d/f	d/c	d/l	e/f	e/b	e/l	f/b	f/l	b/l
STD	0.89	1.47	4.49	0.91	1.65	5.03	1.02	3.05	0.29	0.20
BbF	0.88	1.58	0.56	0.79	1.79	0.64	0.90	0.36	0.50	1.27
x-mBbF	0.95	2.53	0.84	1.17	2.65	0.88	1.23	0.33	0.46	6.02

benzo[k]fluoranthene

RATIO	d/e	d/f	d/c	d/k	d/l	e/f	e/c	e/k	e/l	f/c	f/k	f/l	c/k	c/l	k/l
STD	0.96	3.94	2.32	3.74	1.15	4.11	2.41	3.90	1.20	0.59	0.95	0.29	1.62	0.50	0.31
BkF	1.02	4.09	3.37	3.09	1.17	4.02	3.31	3.03	1.15	0.82	0.75	0.29	0.92	0.35	0.38

benzo[a]pyrene

RATIO	d/e	d/b	d/c	d/k	d/l	d/j	e/b	b/f	e/c	e/k	e/l	e/j	b/c	b/k	b/l	b/j	c/k	c/l	c/j	k/l	k/j
STD	0.92	1.47	0.61	0.82	1.19	1.03	1.60	0.67	0.90	1.30	1.12	0.42	0.56	0.81	0.70	1.34	1.94	1.68	1.45	1.25	0.86
BaP	0.73	1.11	0.43	0.54	0.77	0.55	1.53	0.59	0.74	1.06	0.76	0.39	0.48	0.69	0.49	1.24	1.79	1.27	1.44	1.02	0.71

[a] Codes for excitation and emission wavelengths in nm: a - 245/359; b - 245/391; c - 292/414; d - 232/423; e - 245/434; f - 244/480; g - 254/> 305; h - 263/371; i - 234/382; j - 266/399; k - 288/405; l - 245/443

[b] The first line gives data for the PAH standard mixture; second and succeeding lines refer to PAH/alkyl PAH in ETS.

[c] Abbreviations are given in the caption for Fig. 3

Table V. Dual detector fluorescence programs to quantitate PAHs & Alkyl-PAHs in ETS.

PAH/alkyl-PAH Class	Detector [a]	Wavelength change at min	Excitation Wavelength nm	Emission Wavelength nm
		Program I		
anthracene	1	0.0	244	391
chrysene		15.2	263	371
phenanthrene	2	0.0	245	359
benzo[b]fluoranthene		21.9	245	434
benzo[k]fluoranthene		21.9	245	433
		Program II		
pyrene	1	0.0	234	382
indeno[cd]pyrene		25.7	244	480
fluoranthene	2	0.0	244	480
benz[a]anthracene		16.4	292	414
benzo[a]pyrene		16.4	292	414
benzo[ghi]perylene		16.4	292	414

[a] Detector 1 follows the column; detector 2 follows detector 1.

Table VI. Dual detector fluorescence program for PAHs in SRM 1649.

PAH	Detector [a]	Wavelength change at min	Excitation Wavelength nm	Emission Wavelength nm
phenanthrene	1	0.0	250	370
pyrene		11.5	235	380
benz[a]anthracene		19.8	225	395
benz[e]pyrene		22.8	230	390
benz[a]pyrene		27.5	290	410
indeno[1,2,3-cd]pyrene		30.3	245	480
fluoranthene	2	0.0	230	450
chrysene		19.4	260	370
benzo[b]fluoranthene		22.9	230	430
benzo[k]fluoranthene		22.9	230	430
benzo[ghi]perylene		30.4	225	415
coronene		36.6	290	410

[a] Detector 1 follows the column. Detector 2 follows detector 1.

Table VII. Comparison of selected PAH concentrations measured in SRM 1649 with reference values.

PAH	Reference microg/g[a]	Measured microg/g	n	Coef Var % [b]	Rel Prec % [c]	Rel Prec % [d]
phenanthrene	4.5 ± 0.3	7.3 ± 0.6	5	8.59	10.7	24
fluoranthene	7.1 ± 0.5	6.5 ● 0.7	6	11.15	11.8	29
pyrene	6.3 ± 0.4	5.6 ● 1.0	6	17.80	18.8	46
benz[a]anthracene	2.6 ± 0.3	2.8 ± 0.1	6	4.10	4.1	10
chrysene	3.5 ± 0.1	3.4 ● 0.1	6	3.66	3.7	9
benzo[b]fluoranthene	6.2 ± 0.3	5.7 ± 0.3	6	4.99	5.3	13
benzo[k]fluoranthene	2.0 ± 0.1	2.2 ± 0.1	6	4.27	4.5	11
benzo[a]pyrene	2.9 ± 0.5	2.8 ● 0.2	6	7.76	8.2	20
benzo[ghi]perylene	4.5 ± 1.1	3.4 ± 0.2	6	4.92	5.3	13
indeno[cd]pyrene	3.3 ± 0.5	4.0 ± 0.1	6	3.40	3.7	9
			Avg	7.06	7.6	

[a] Certificate of Analysis, Standard Reference Materials 1649, Urban Dust/Organics; National Bureau of Standards (now NIST): Washington, D.C. 1982.
[b] Coefficient of Variation % = 100 x Standard Deviation / Mean
[c] Relative Precision % =100 x CI / Mean; CI =Confidence interval = t(0.05) x std dev / sqrt(n)
[d] Rel precision calculated without SQRT (n)

Table VIII. Limits of detection for selected PAHs in the standard mixtures, ETS and SRM 1649 samples.

PAH	Standard Mixture pg/microL	SRM 1649 microg/g	ETS microg/g
phenanthrene	0.19	0.06	0.05
fluoranthene	0.34	0.07	0.07
pyrene	0.14	0.02	0.06
benz[a]anthracene	0.38	0.09	0.02
chrysene	0.24	0.05	0.06
benzo[b]fluoranthene	0.27	0.05	0.09
benzo[k]fluoranthene	0.08	0.01	0.03
benzo[a]pyrene	0.16	0.03	0.03
benzo[ghi]perylene	0.44	0.09	- [a]
Average	0.23	0.05	0.05

[a] not identified

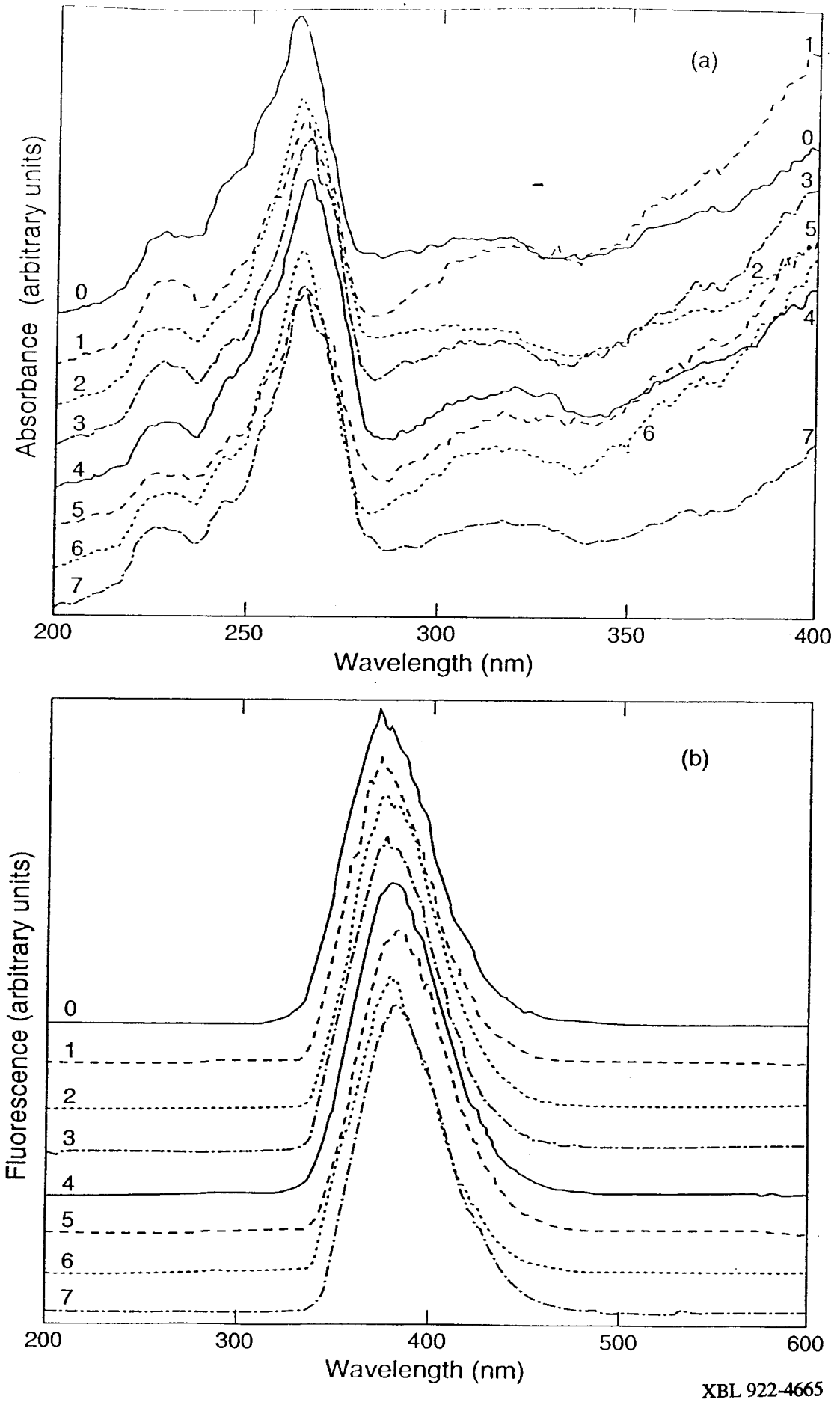

Figure 1. Excitation and emission spectra of chrysene and its alkyl-derivatives. Identification: 0= chrysene, 1= 1-methylchrysene, 2= 2-methylchrysene, 3= 3-methylchrysene, 4= 4-methylchrysene, 5= 5-methylchrysene, 6= 6-methylchrysene, 7= 6,12-dimethylchrysene. Absorbance and fluorescence are given in arbitrary units.

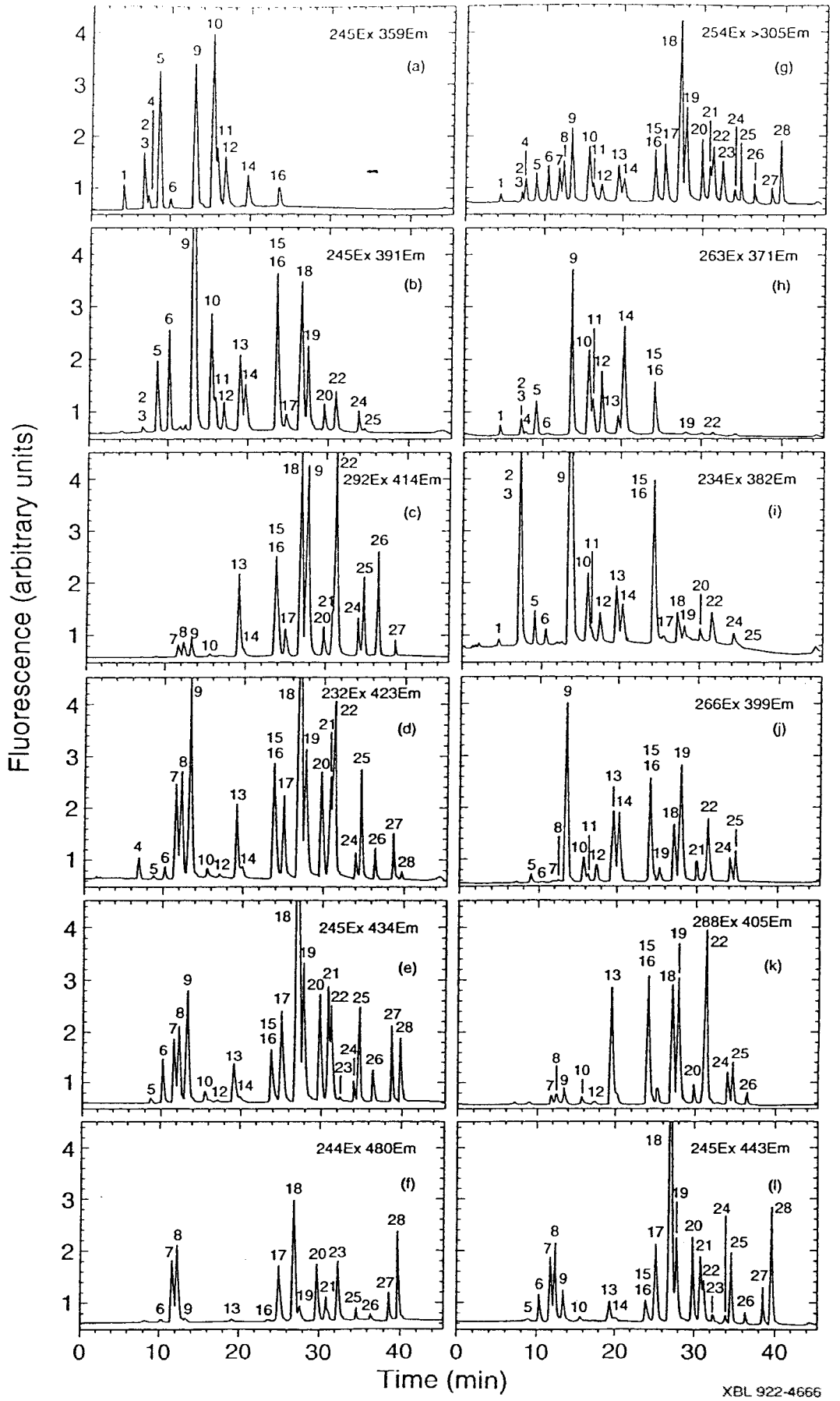

Figure 2. Standard mixture A at different fluorescence wavelength combinations. Peak identification: 1= naphthalene, 2= acenaphthene, 3= acenaphthylene, 4= fluorene, 5= phenanthrene, 6= anthracene, 7= fluoranthene-D_{10}, 8= fluoranthene, 9= pyrene, 10= 3,6-dimethylphenanthrene, 11= triphenylene, 12= 1,2-benzofluorene, 13= benz[a]anthracene, 14= chrysene, 15= 7,12-dimethylbenz[a]anthracene, 16= benzo[e]pyrene, 17= benzo[b]fluoranthene, 18= benzo[k]fluoranthene, 19= benzo[a]pyrene, 20= 10-methylbenzo[b]fluoranthene, 21= dibeno[al]pyrene, 22= benzo[ghi]perylene, 23= indeno[1,2,3-cd]pyrene, 24= dibenzo[ae]pyrene, 25= 3,6-dimethylbeno[a]pyrene, 26= coronene, 27= dibenzo[ai]pyrene, 28= dibenzo[ah]pyrene.

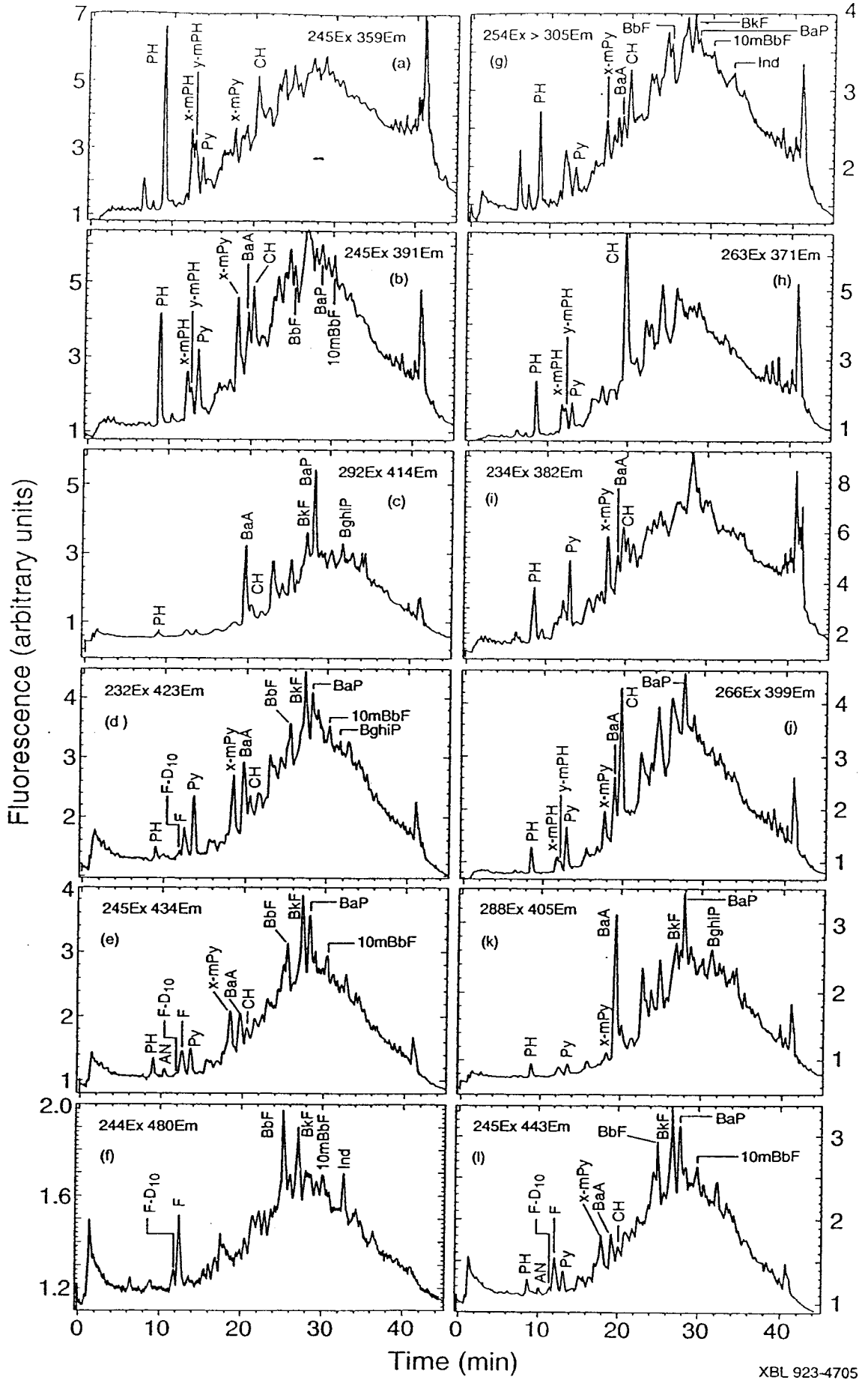

Figure 3. ETS extract at different fluorescence wavelength combinations. Peak identification: PH= phenanthrene, F= fluoranthene, BaA= benz[a]anthracene, CH= chrysene, BbF= benzo[b]fluoranthene, BkF= benzo[k]fluoranthene, BaP= benzo[a]pyrene, BghiP= benzo[ghi]perylene, IND= indeno[1,2,3-cd]pyrene], 10-mBbF= 10 methylbenzo[b]fluoranthene, x-mPH and y-mPH= suspected methylphenanthrenes, x-mPY= suspected methylpyrene.

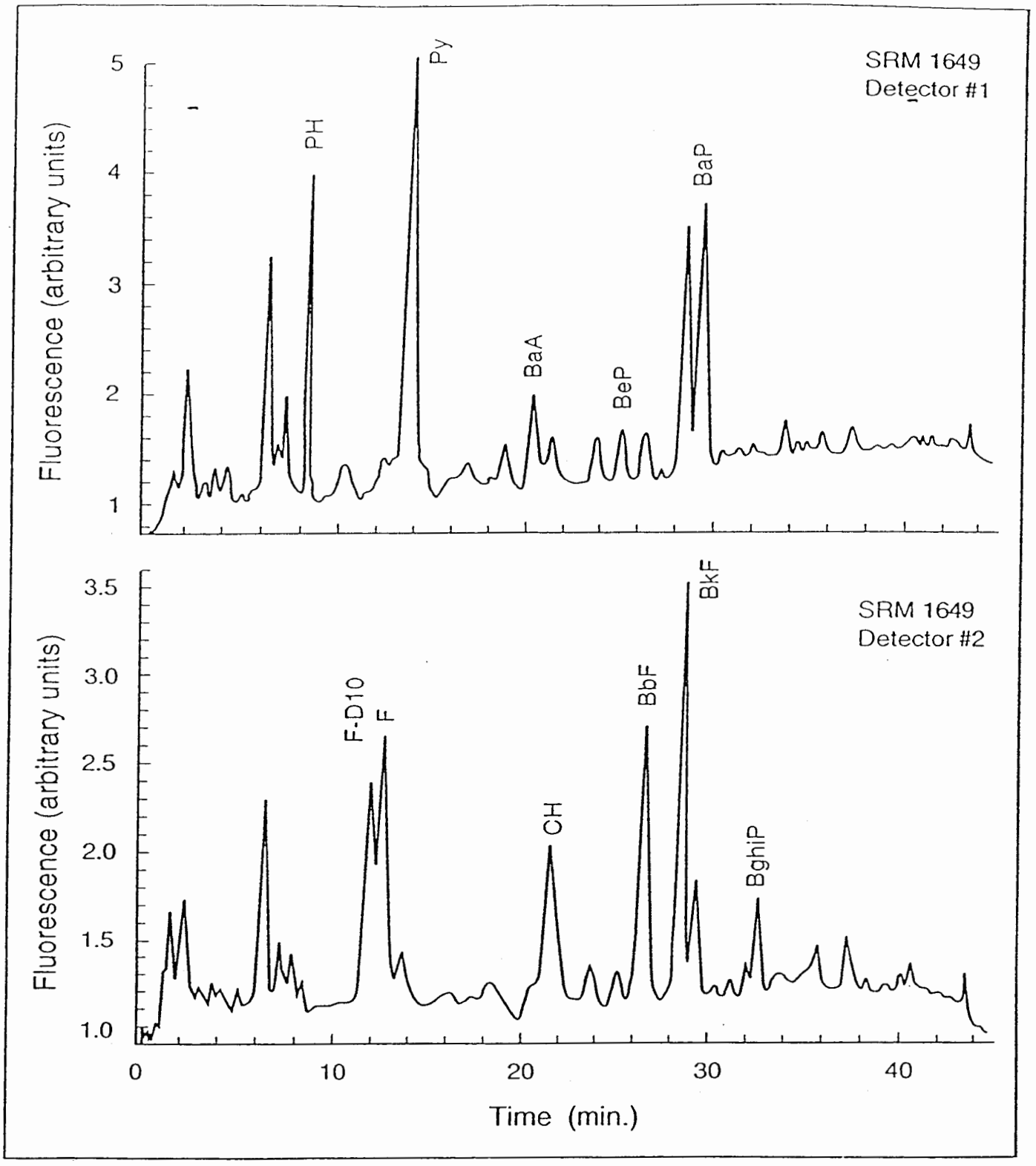

Figure 4. Analysis of PAHs in SRM 1649. Peak identification: PH= phenanthrene, F-D10= fluoranthene-D_{10} [internal standard], F= fluoranthene, BaA= benz[a]anthracene, CH= chrysene, BeP= benzo[e]pyrene, BbF= benzo[b]fluoranthene, BkF= benzo[k]fluoranthene, BaP= benzo[a]pyrene, BghiP= benzo[ghi]perylene, IND= indeno[1,2,3-cd]pyrene.

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