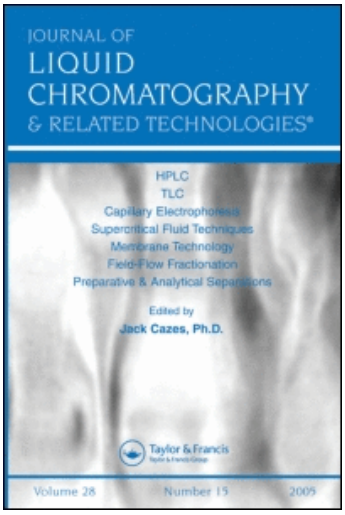

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Use of Fluorescence Emission Spectra for the Routine Identification of Polycyclic Aromatic Hydrocarbons in Liquid Chromatography

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USE OF FLUORESCENCE EMISSION SPECTRA FOR THE ROUTINE IDENTIFICATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN LIQUID CHROMATOGRAPHY

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ABSTRACT

Fluorescence detection provides a highly sensitive and selective method of detection for polycyclic aromatic hydrocarbons (PAHs). However, the acquisition of fluorescence spectral data for purposes of peak identification has hitherto only been reported using techniques which interrupt chromatographic runs or rely on complex additional instrumentation. The application and limitations of acquiring fluorescence spectra using a commercially available detector during routine chromatography are discussed.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are produced by incomplete combustion and are ubiquitous pollutants in many samples of environmental interest. HPLC is well established in the analysis of PAH.[1-4] When used in conjunction with fluorimetry, this technique affords a highly sensitive and selective method of detection since few other species in environmental samples exhibit significant fluorescence.

Selectivity can be further enhanced by the use of programmable wavelength detectors by which excitation and emission wavelengths can be optimized for individual PAHs. However, a typical sample matrix is a complex mixture of parent PAHs together with PAHs with various substituents. Alkyl derivatives may be particularly abundant in some samples possibly co-eluting with the parent PAH of interest. Where significant amounts of substituted PAHs are likely to be present, initial cleanup followed by multi-dimensional LC may be required whereby the sample is first fractionated using normal phase HPLC and the resultant fractions are then analyzed by reverse phase HPLC.[1-4] For practical purposes, this approach somewhat negates the advantages associated with fluorescence detection since it is often desirable to exploit the selectivity of the technique using relatively simple cleanup methods (solvent/solvent extraction or solid phase extraction (SPE)) prior to analysis. In this case, however, the risk of interfering PAH derivatives remains.

The use of fluorescence and UV absorption measurements in series is commonly used to identify PAH by their response ratios.[5] The acquisition of spectral data using a diode array detector can provide more specificity achieving a good match between standards and environmental samples.[6] However, absorption measurements lack the sensitivity and selectivity of fluorescence and numerous interfering species may remain following cleanup. The use of fluorescence spectra to confirm peak identity has been reported previously.[7] The technique employed relies on "stop/start" scanning where the flow is stopped during elution to enable scanning of the spectrum. The expedient of using this technique arises from the fact that, unlike diode array detection, scan times are long relative to peak elution time. Stop/start scanning performed on standards can be useful in determining suitable excitation and emission wavelengths for analysis of samples. It is also possible to stop a peak several stages during its elution to investigate possible coelution by observing the consistency of the spectrum obtained.[7] However, stop/start scanning is laborious, requires precise timing, and may alter the retention characteristics of downstream peaks limiting the number of PAHs which may be identified in a single run.

Video fluorimetry has also been reported as a means of obtaining fluorescence spectra of PAH during a chromatographic run.[8,9] Multiple excitation and emission spectra enable a two dimensional excitation/emission matrix (EEM) to be obtained over a range of 300 nm. However, this technique requires complex additional instrumentation and extensive data processing capacity.

The aim of this study was to develop a method of obtaining PAH spectra which combined the advantages of the stop/start scanning and video fluorimetry techniques and which would be useful in the confirmation of peak identity

Table 1

HPLC Conditions

Column (& guard)	Hypersil Green PAH (C_{18})	
Length	100 mm	
i.d.	4.6 mm	
Particle size	5μm	
Solvent gradient	50:50 CH_3CN/H_2O	5 mins
	50:50 CH_3CN/H_2O to 100% CH_3CN	25 mins
	100% CH_3CN	15 mins
Flow rate	1.2 mL/min	
Detection	Shimadzu RF551 programmable detector, (12 μL flow cell) Perkin Elmer LC75 UV at 254 nm	
Data handling	Jones Chromatography JCL 6000 chromatography software Spectral data downloaded to Excel 5.0 (Microsoft)	

Table 2

Wavelength Program

	$\lambda_{EX}/\lambda_{EM}$
Phenanthrene	250/365
Anthracene	245/401
Fluoranthene	281/465
Pyrene	269/378
Benz(a)anthracene	281/409
Chrysene	269/378
Benzo(b)fluoranthene	297/430
Benzo(k)fluoranthene	279/430
Benzo(a)pyrene	297/430
Dibenz(ah)anthracene	297/430
Benzo(ghi)perylene	297/430

during the routine analysis of PAH, particularly with regard to sample throughput. The feasibility of using the rapid scanning capability of a commercially available fluorescence detector to obtain emission spectra during a chromatographic run was therefore investigated.

EXPERIMENTAL

HPLC grade solvents were used throughout (BDH, Poole, UK). EPA PAH standards, (purity >98%) were obtained from Chemservice (West Chester PA). Standard solutions were made up in acetonitrile/water (60:40). Additional PAH (purity >97%) were obtained from Aldrich (Gillingham, Dorset, UK). These were dissolved in benzene as a primary solvent and solutions for analysis were made up in acetonitrile/water (60:40).

A marine sediment standard reference material (SRM1941a) was obtained from Promochem (St Albans, UK). The SRM was Soxhlet extracted for 12 hours in dichloromethane, reduced almost to dryness and then made up in hexane.

Cleanup was performed using solvent extraction in dimethylsulphoxide (DMSO) followed by back extraction from aqueous DMSO solution into pentane. This method is described fully elsewhere.[10]

Samples of incinerator bottom ash were collected from a clinical waste incinerator in amber colored bottles. These samples were sonicated and then refluxed in benzene for two hours. The benzene was evaporated almost to dryness and cleanup was performed as for the SRM sample.

The final sample solutions in pentane were evaporated almost to dryness, made up in acetonitrile/water (60:40), and filtered through a 0.2 μm PTFE syringe filter (Whatman, Maidstone, UK) prior to analysis. HPLC conditions and wavelength programs are shown in Tables 1 and 2.

PAH identification was based on a combination of retention time and spectral data. Since retention times in HPLC are prone to drifting, relative retention times (RRTs) were used as a more reliable means of identification.[7,11] A retention time index (RTI) based on RRTs standardized to the retention time of an unambiguously determined compound, benzo(a)pyrene (BaP), was used in conjunction with spectral scanning data in this study. $\lambda max_{(Em)}$ values for each scan were obtained through the data processing function of the detector.

RESULTS

The RTI values for standards were found to be highly reproducible. The RTI, standardised to BaP, together with coefficients of variation are shown in Table 3. Emission maxima for standards and samples were reproducible to ±1 nm in most cases. Values obtained are shown in Table 4.

Table 3

Retention Time Index *

	Standards (cv%)	SRM	Incinerator Ash
Phenanthrene	0.440 (1.4)	0.443	0.443
Anthracene	0.509 (0.9)	0.510	0.509
Fluoranthene	0570 (0.8)	0.567	0.570
Pyrene	0.613 (0.6)	0.615	0.612
Benz(a)anthracene	0.767 (0.3)	0.768	0.771
Chrysene	0.800 (0.1)	0.800	0.796
Benzo(b)fluoranthene	0.910 (0.1)	0.909	0.908
Benzo(k)fluoranthene	0.964 (0.2)	0.962	0.963
Benzo(a)pyrene	1.000	1.000	1.000
Dibenz(ah)anthracene	1.072 (0.1)	1.073	1.075
Benzo(ghi)perylene	1.101 (0.2)	1.102	1.102

* RTI, means RRT/RT BaP.

Table 4

Fluorescence Emission

	λmax (± 1)
Phenanthrene	365
Anthracene	380/401
Fluoranthene	465
Pyrene	378/388
Benz(a)anthracene	388/409
Chrysene	378
Benzo(b)fluoranthene	432
Benzo(k)fluoranthene	413/433
Benzo(a)pyrene	408/429
Dibenz(ah)anthracene	398/418
Benzo(ghi)perylene	418

Figures 1a and 1b show chromatograms for the SRM and incinerator ash samples indicating the selectivity possible with fluorescence detection. However, in order to identify all PAHs using the optimal, or near optimal, wavelengths shown in Table 2, it was necessary to conduct two runs due to

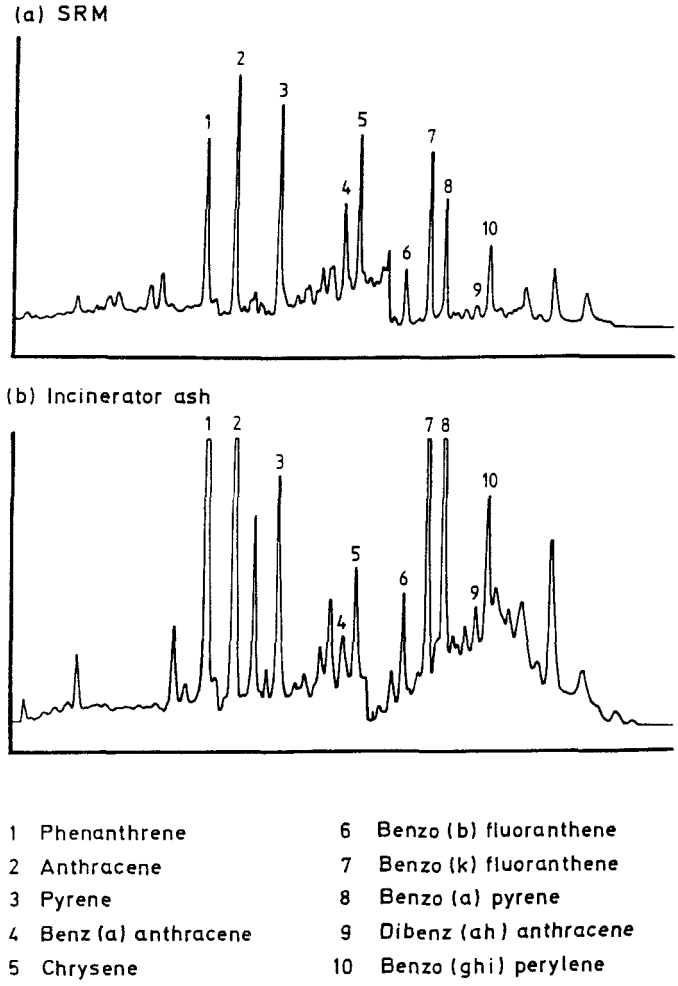
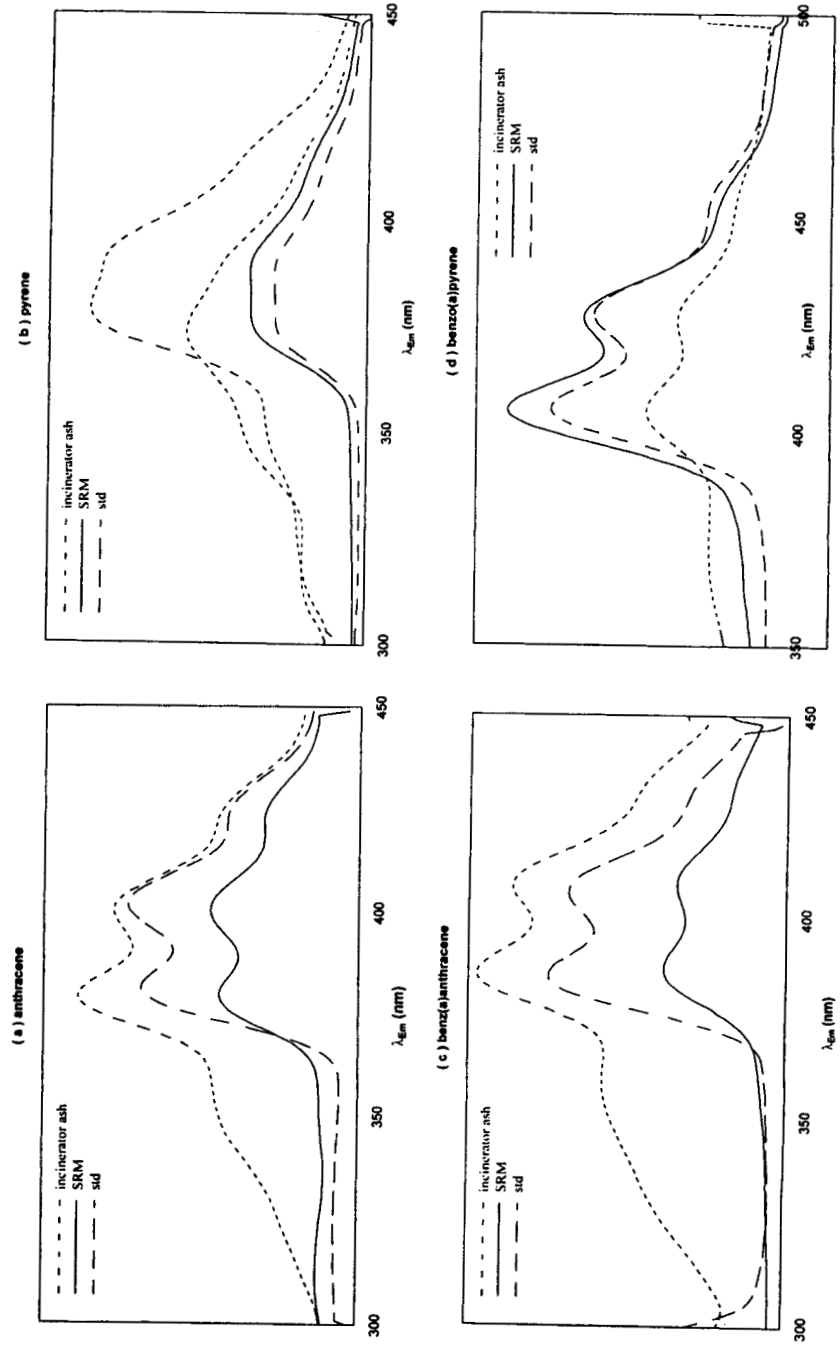


Figure 1. Chromatograms for SRM and incinerator ash samples.

major wavelength changes for closely eluting compounds. Figures 2a-2d are superimposed fluorescence emission spectra obtained from standards, SRM and incinerator ash samples for selected PAHs. Figure 3 shows the reproducibility of the relative magnitude of maxima for BaP at varying concentrations and times

Figure 2. (right). Fluorescence emission spectra for selected PAH in standards, SRM, and incinerator ash.



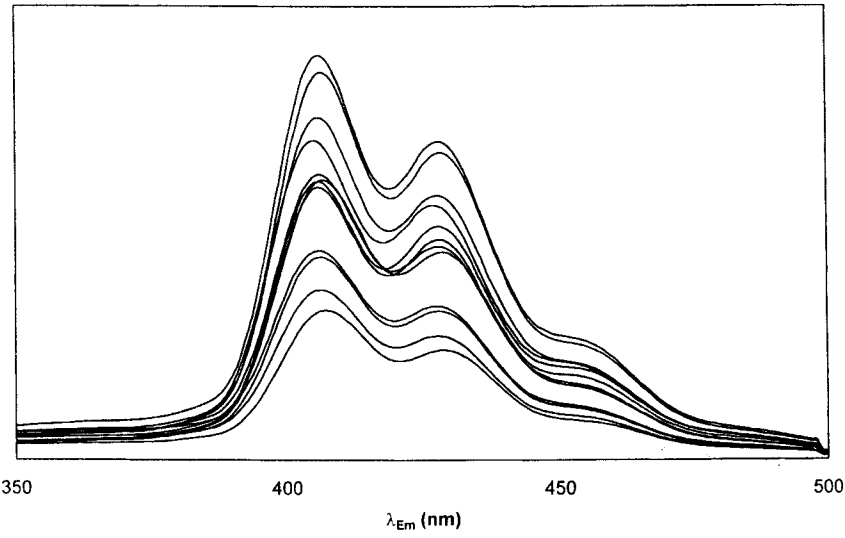


Figure 3. Reproducibility of benzo(a)pyrene emission spectra.

during peak elution. Figures 4 and 5 show fluorescence emission spectra for benzopyrenes and dibenzanthracenes illustrating the distinctive spectra of isomers of common PAH. Figure 6 shows the detection limits at which useful spectra can be obtained for BaP.

DISCUSSION

Figure 1a indicates the selectivity achievable for PAHs in the SRM sample; all the peaks are those for PAHs of interest confirmed by spectral scanning and RTI. The reproducibility of emission spectra for standards and the SRM was excellent (Figures 2a-2d). Maxima for the SRM were within ±1 nm those of the standards for all the PAHs listed in Table 4. In this chromatogram it was possible to identify all these PAHs, except fluoranthene, in a single run. Figure 1b shows the chromatogram for an incinerator ash sample. In this case, several additional peaks are evident.

As with the SRM sample, the emission spectra of most PAH of interest correspond to those for the standards ±1 nm. However, in some ash samples the peak for pyrene was found to be masked by a co-eluting compound producing a quite different spectrum with $\lambda max_{(Em)}$ =373 (Figure 2b).

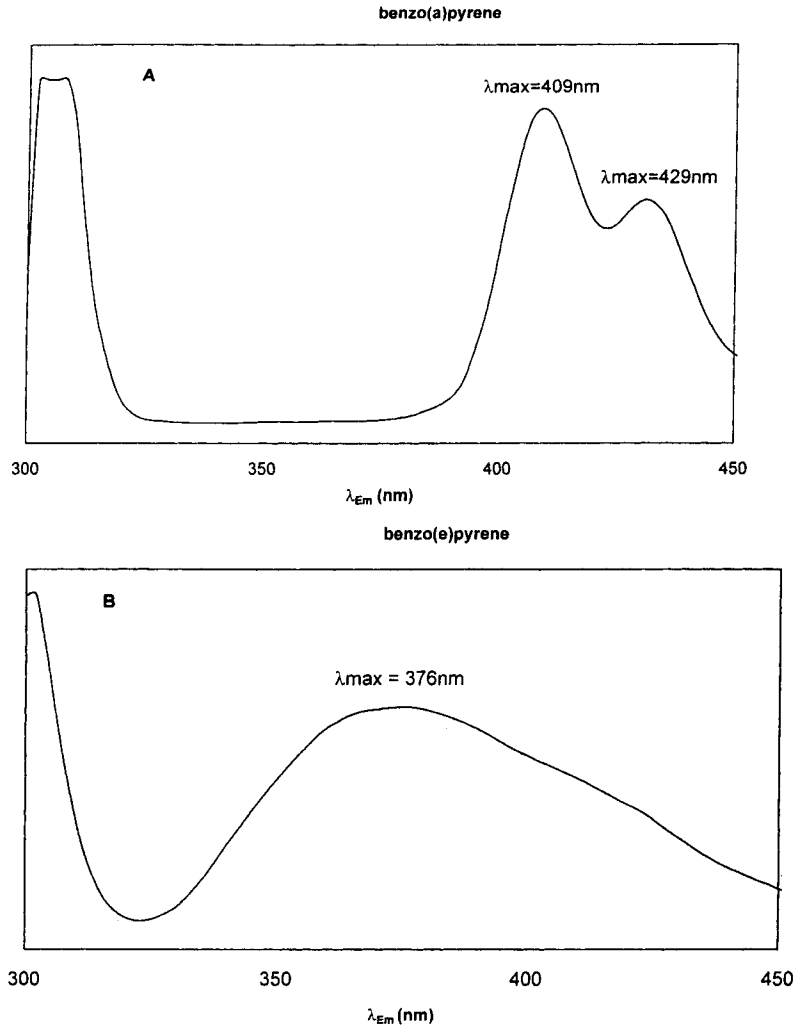


Figure 4. Fluorescence emission spectra for benzopyrene isomers.

Other additional peaks were present in the chromatogram but did not co-elute with PAHs of interest. Generally, it was noticeable that the profile of known and unknown PAHs was consistent for ash samples taken on different occasions. Although maxima were highly reproducible, some differences were noted in the magnitude of the spectral peaks between standards and samples.

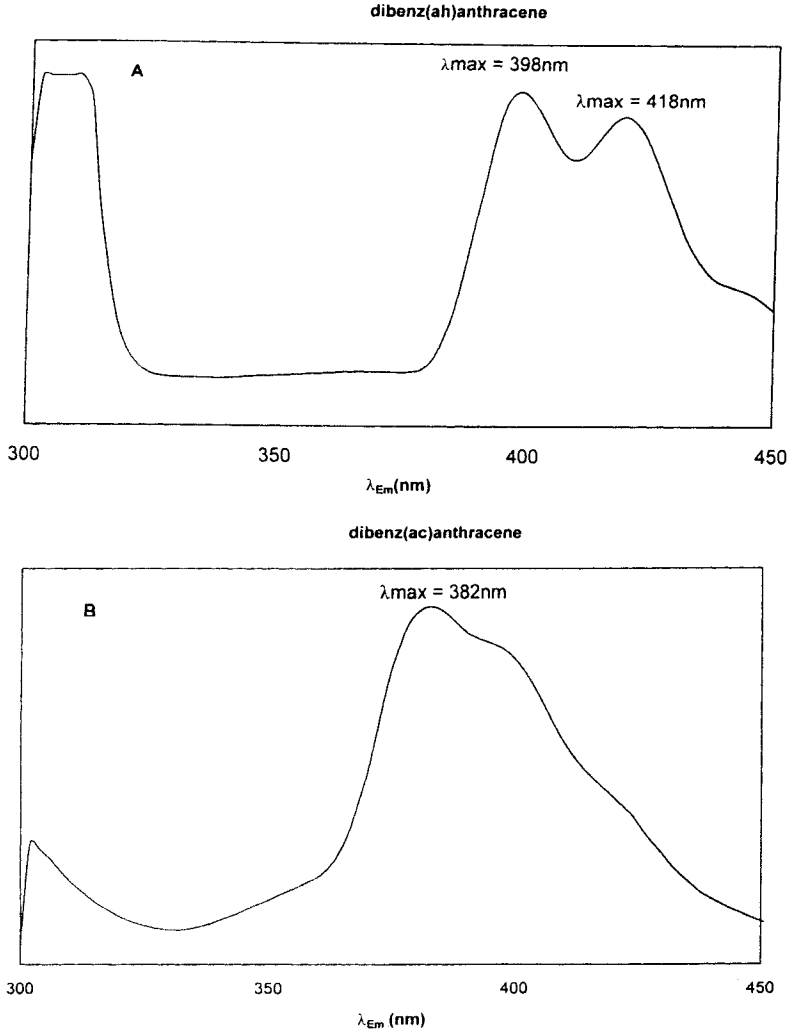


Figure 5. Fluorescence emission spectra for dibenzanthracene isomers.

Using the rapid scanning technique employed, the scan time (approximately 4 seconds) is still relatively long compared to elution time in comparison with other methods of acquiring spectra (e.g. diode array or video fluorimetry). It is therefore possible that variations in spectral shape could reflect the changing concentration (assumed to be Gaussian) as the analyte passes through the flow cell during the scan. The reproducibility of the relative

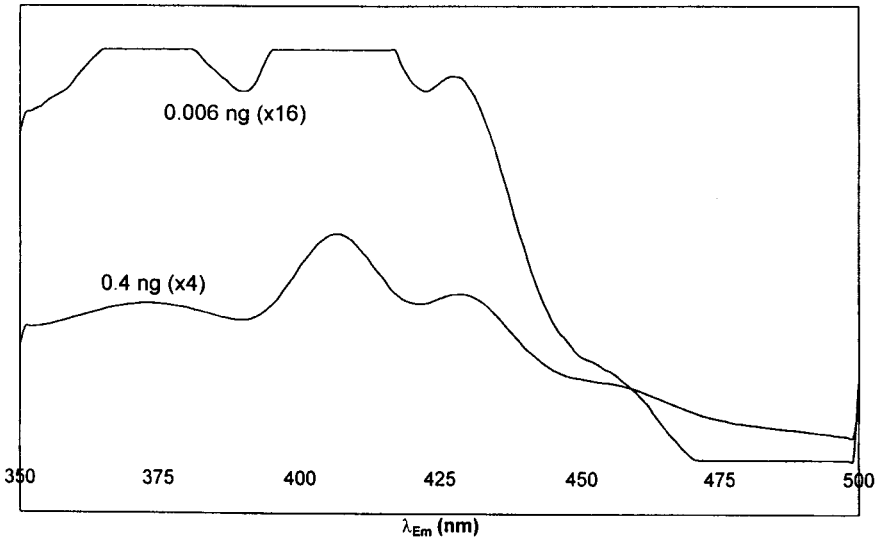


Figure 6. Detection limits for benzo(a)pyrene.

magnitude of the spectral peaks was investigated by multiple injections of BaP standards covering a range of concentrations. The resulting chromatographic peaks were scanned at various points during elution. Figure 3 shows that the shape of the spectra are highly reproducible, although a progressive loss of resolution is apparent at low concentrations or when the tail of the peak is scanned.

Differences in the spectral shape between standards and samples could therefore indicate minor co-elution or matrix effects in the samples. This is apparent, for example, in the spectrum for anthracene for the ash sample (Figure 2a). The peak at 380 nm is greater than the other major peak at 400 nm in contrast to the standard (and SRM) spectra suggesting some co-elution at 380 nm. Interference at λ_{Em}<350 nm is also noticeable which is common to many samples at λ_{Ex}<250 nm.

In order to correct for minor co-elution, an array of spectral detail, such as the excitation/emission matrix (EEM) obtainable in video fluorimetry[8,9] may be necessary, although some ambiguity may still remain. A practical approach to minimizing minor co-elution/matrix effects is to use the longer λ_{Em} for quantification where possible as these effects are always more apparent at shorter wavelengths. For anthracene, therefore, the selected λ_{Em} was 401 nm.

An interesting observation is the fact that isomeric PAH have highly distinctive emission spectra (Figures 4 and 5) which suggests that any co-elution of these isomers would not lead to misidentification. The choice of excitation wavelength is also significant in optimizing selectivity for isomers although excitation spectra, particularly for higher molecular weight compounds, are rather ambiguous for identification purposes.

The acquisition of spectra at low levels was investigated by scanning the BaP standard at progressively lower concentrations. At 0.4 ng the characteristic spectrum with maxima at 407/429 was obtained (Figure 6). However, at lower concentrations, solvent effects become apparent concealing the early part of the spectrum, although it may be possible to tentatively identify some compounds based on spectral peaks occurring on the shoulder of the solvent effect. In the case of BaP, a peak at 429 nm is just apparent. Peak identification at 0.4 ng compared favorably with the value reported for diode array detection of 1.2 ng.[6] However, it appears from the data presented here that it may not be possible to realize the full potential for peak identification offered by the sensitivity of fluorescence measurements due to the relatively high solvent background at very low PAH concentrations. When air sampling is carried out, background effects from residual impurities in polymeric resins may further limit the sensitivity of measurements at low concentrations.[12,13]

Although some drift in retention times was observed as expected, the precision of the RTI was found to be very reliable. It, therefore, appears from the sample types investigated that most PAHs in complex mixtures can be separated and identified using a combination of RTI and emission maxima. However, the ash sample suggests that the extent to which full PAH characterization of a given mixture can be achieved without more extensive preparatory chromatography may be somewhat matrix dependent. Some minor co-elution/matrix effects may remain but can be minimized by optimizing the selectivity available with fluorescence detection through careful wavelength selection. The technique could also be made more time effective by employing two fluorescence detectors working in parallel allowing simultaneous acquisition of quantitative and qualitative data.

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