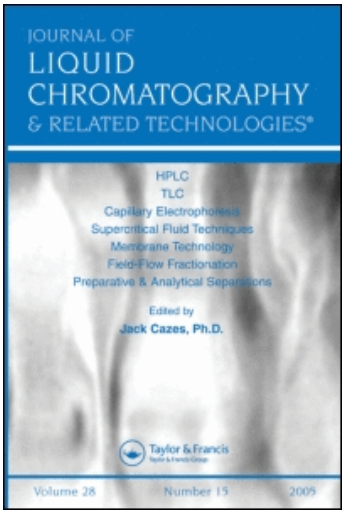

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LIQUID CHROMATOGRAPHIC ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS WITH DIODE ARRAY DETECTION

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ABSTRACT

The potential of using a high-sensitivity diode array detector for analysis of polynuclear aromatic hydrocarbons (PAHs) is examined. Separation of 16 priority pollutant PAHs is accomplished in 13 minutes with a 5-μm polymeric C18 column. Detection limits of about 1 ng are demonstrated for most PAHs. Wavelength programming enhances the detection specificity while spectral overlay confirms the presence of trace levels of PAHs in complex environmental samples. The sensitivity of this diode array detector is compared to that of a programmable fluorescence detector. The analysis of an urban particulate extract and a contaminated soil sample demonstrates the utility and versatility of this detection approach.

INTRODUCTION

Polynuclear aromatic hydrocarbons (PAHs) are important pollutants because of their carcinogenicity and their widespread occurrence. These compounds are pyrosynthesized during incomplete combustion and constitute trace fractions in tobacco tar and air particulate matter. Their presence in the environment, although in minute quantities, is significant because of their implication in the etiology of human lung cancer (1). Sixteen PAHs are in

the US Environmental Protection Agency "Consent Decree" priority pollutants list. The recommended analytical procedure is documented in method 610 in the Federal Register (2).

The use of variable wavelength (3), rapid-scanning (4), and diode array UV absorbance detectors (5), and fluorescence detectors (6) for PAH analysis has been described in the literature. While a fluorescence detector remains the most sensitive and selective LC detector (7), recent advances have significantly enhanced the sensitivity of UV diode array detectors (8). The performance and application of such a second-generation diode array detector (LC-235) in PAH analysis are documented in this study.

EXPERIMENTAL

Equipment

The LC system consisted of a Series 410 LC pump, a Rheodyne 7125 injection valve, a model LC-235 diode array detector, and a GP-100 printer/plotter. The LC-235 is a stand-alone, high-sensitivity diode array detector designed exclusively for HPLC. Details of its design concepts and performance characteristics are documented elsewhere (9). For sensitivity comparison with a programmable fluorescence detector, a Model LS-4 dual monochromator fluorescence detector was used.

LC Operating Conditions

The operating conditions of the LC pump and detectors are summarized below.

Column:	PerkinElmer HS/5 HCODS column (4.6 mm X 125 mm)
Mobile phase:	50% ACN for 1 min, then programmed to 100% ACN in 8 min, then purged with ACN for 3 min
Flow rate:	2.5 mL/min
LC-235:	280 nm for 5 min then 365 nm for 8 min
LS-4:	280 nm (excitation) and 340 nm (emission) for 5 min 305 nm (excitation) and 430 nm (emission) for 8 min, slit widths 10 nm

Procedure

The performance of the LC-235 was assessed using a certified National Bureau of Standards PAH Standard (1647). Both the chromatographic and spectrophotometric performance were evaluated. Procedures for sample collection of urban particulates via high-volume sampler, soxhlet extraction of the soiled filters with cyclohexane, and thin-layer chromatography sub-fractionation were published elsewhere (10,11). The particulate sample was collected in Newark while the contaminated soil sample was from the former site of a coal gasification plant in Jersey City.

RESULTS AND DISCUSSION

1. Detection Specificity.

The wavelength selectivity of the 16 priority pollutant PAHs is illustrated in Figure 1. Three chromatograms of a 5-μL injection of the NBS standard were recorded at monitoring wavelengths of 255 nm, 280 nm, and 365 nm, respectively. Separation was accomplished on an HCODS column filled with a 5-μm polymeric C18 bonded-phase material which exhibits unique selectivity effects for isomeric PAHs (2,12-13).

The effect of monitoring wavelength are summarized below:

- o 254 nm : Excellent sensitivity for all PAHs but poor selectivity since most aromatic compounds absorb at this wavelength
- o 280 nm : Excellent sensitivity and selectivity for all PAHs except for anthracene
- o 365 nm : Very poor sensitivity for 2 to 3-ring PAHs, moderate sensitivity for the higher PAHs and excellent selectivity for the 5-ring PAHs which are the most biologically active

These data suggest the use of 280 nm as a monitoring wavelength for PAHs. Although some sensitivity for anthracene is sacrificed at this wavelength (See Table 2), this might not be a problem because of a lack of biological activity of this

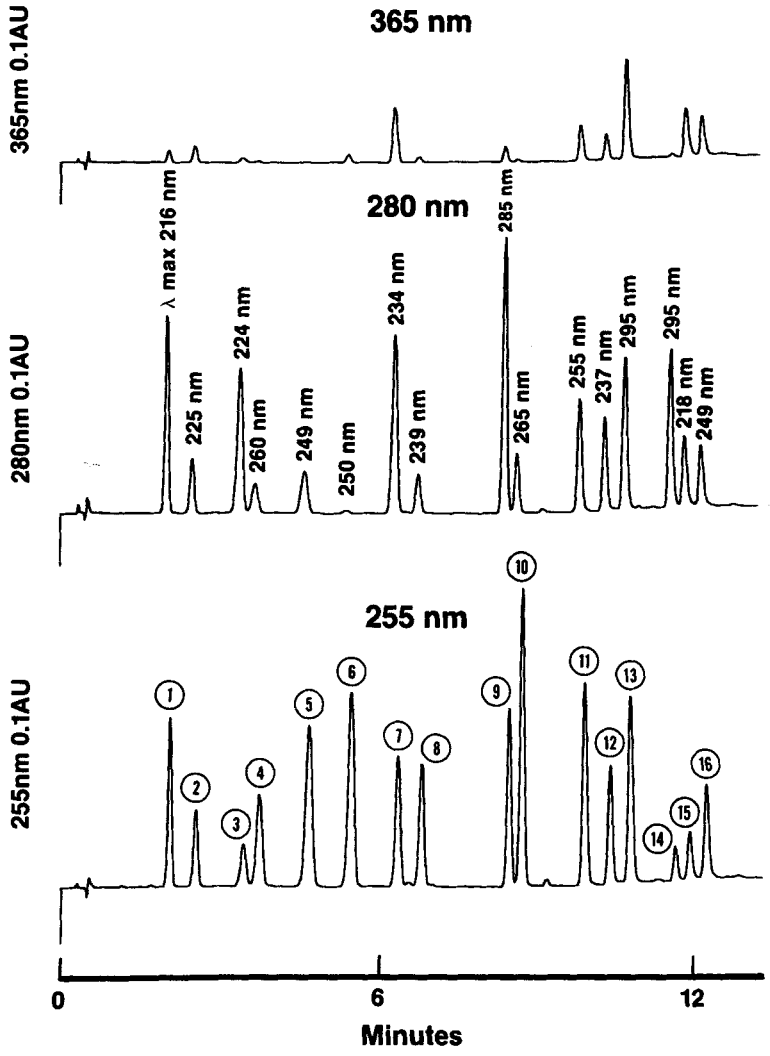


Fig. 1. The effect of monitoring wavelength in PAH analysis. See Table 2 for peak identification.

compound. To increase the detection specificity of higher PAHs in complex samples, the monitoring wavelength might be switched to 365 nm after the elution of phenanthrene as illustrated in Figure 9.

2. Peak Confirmation and Purity Assessment.

Chromatographic techniques suffer from a fundamental weakness of offering limited qualitative information. Retention time alone does not have sufficient discriminating power for unknown samples since peaks with identical retention times may or may not be the same compound. This problem is severe for trace analysis of complex samples. The LC-235 offers a number of convenient and powerful techniques for assessing peak identity and purity. These are listed in Table 1. Their use is demonstrated in Figures 2 and 3.

Figure 3 shows the dual channel plot of the first 8 PAHs in the NBS standard separated with a Pecosphere-CR 3X3, 33 mm X 4.6 mm i.d. C18 column using an acetonitrile/water gradient listed in the experimental section. Because this column does not exhibit column selectivity for isomeric PAHs, coelutions occur for several PAH pairs. Using the AUTO mode on the LC-235, each peak apex on the bottom chromatogram is annotated with 4 numbers

TABLE 1

Techniques for Assessing Peak Identity and Purity on the LC-235

- Maximum absorbance wavelength (λmax)
- Absorbance ratio plot
- Purity index
- Overlay of upslope and downslope spectra
- Overlay of sample and standard spectra

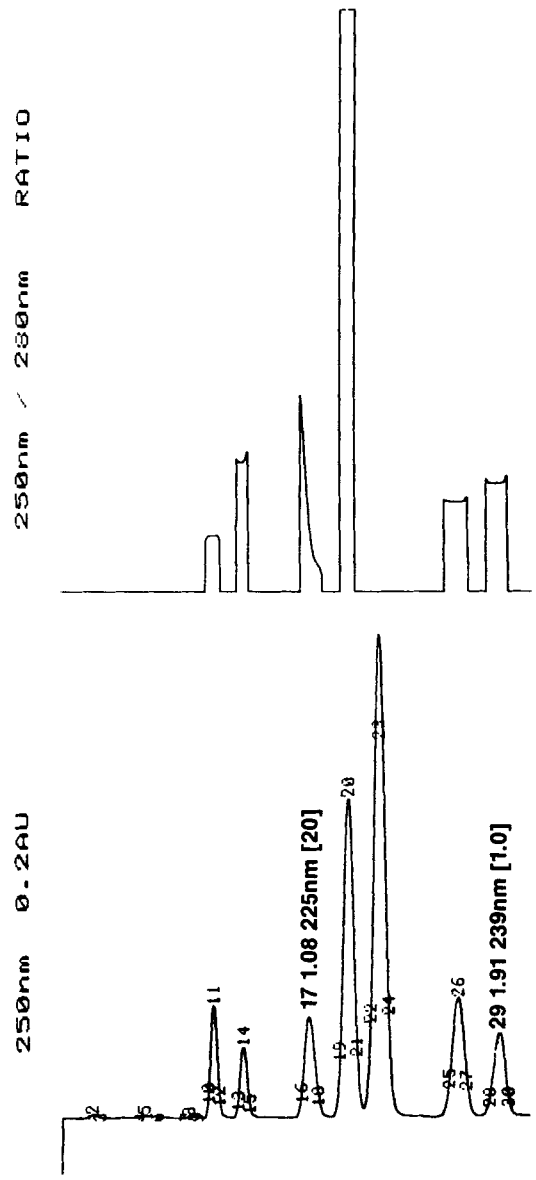
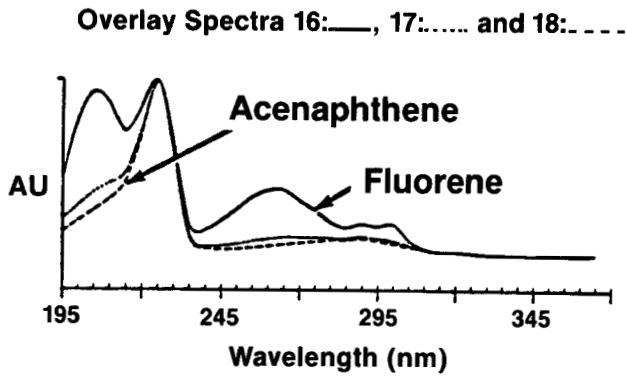


Fig. 2. Dual-channel plot on the LC-235 showing the separation of the first 8 PAHs in the NBS standard 1647 using a 3-cm long C18 column which does not resolve PAH isomers. Channel A (lower chromatogram) shows the absorbance at 250 nm using the AUTO mode. Channel B shows the absorbance ratio plot (ratiogram) at 250 nm / 280 nm.



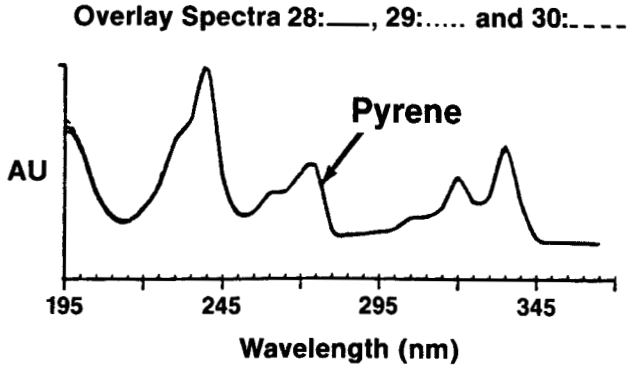


Fig. 3a. Overlay of the upslope, apex and downslope spectra of third peak in Fig. 2 (acenaphthene and fluorene). Fig. 3b. Overlaid spectra of the last peak in Fig. 2 (pyrene) confirming peak purity. The overlay spectra numbers correspond to peak annotations in Figure 2.

indicating spectra number, retention time (min), maximum absorbance wavelength, and peak purity index, respectively. Coelution occurred for acenaphthene and fluorene at 1.08 minutes. The top plot is an absorbance ratio plot (ratiogram) at Abs 250 nm / Abs 280 nm. The use of each spectral technique mentioned in Table 1 is explained briefly below.

o Maximum absorbance wavelength (λmax)

The maximum absorbance wavelength is a distinctive characteristic for compounds with a chromophore. In the LC-235 this parameter is accurate to +/- 1 nm and is annotated post-run in the AUTO mode without any user intervention. The max of each PAH is listed in the middle chromatogram of Fig. 1.

o Absorbance ratio plot (ratiogram)

The absorbance ratio of two wavelengths is a characteristic fixed value for a pure compound and remains unchanged during peak elution. The absorbance ratio plot can be selected as a second channel on the LC-235. As shown in Figure 2, flat-top ratiograms indicate purity while ratiograms with sloping tops indicate coelution (peak 3 of Figure 2).

o Purity index

The purity index is a numerical discriminator calculated from the absorbance ratios of two spectra under consideration, using a numerical algorithm developed by Poile and Conlon (9). While an absorbance ratio plot compares 2 wavelengths, the purity index compares the absorbance at every pixel. The LC-235 generates the purity index automatically by comparing the upslope and downslope spectra. Purity indices (PI) close to 1.0 indicate peak purity (last peak of Figure 3, pyrene, PI = 1.0) while indices above 1.5 may indicate coelution (3rd peak of Figure 2, acenaphthene and fluorene, PI = 20). The purity index method is a simple but effective means to indicate peak purity without running a standard sample.

o Overlay of upslope and downslope spectra

The overlay of normalized upslope, apex, and downslope spectra provide clear indication of a suspect coelution (Fig. 3a)

and elution order of the two merged components. For pure peaks, the overlaid spectra should be almost identical and superimpose perfectly as in Fig. 3b.

o Overlay of sample and standard spectra

The exact matching of the UV spectrum of an unknown peak to that of the standard with the same retention time offers strong confirmation of solute identity. This technique is illustrated in the sample analysis section.

While each spectral technique might be used individually, their combined use eliminates pitfalls and increases the confidence in the assessment of peak identity or purity (9).

3. Detector Sensitivity

Figure 4 shows a high-sensitivity analysis from a 1-μL injection of the NBS 1647 PAH standard. The amount injected ranged from 4 to 22 ng. The detection limits at 280 nm are better than 1 ng for most PAH. A comparison of detection limits between the LC-235 and LS-4 is given in Table 2. The operating conditions of the LS-4 are listed in the experimental section. The ratios of detection limits (UV/FL) under the specified operating conditions are found to range from 1 to 250.

While a programmable fluorescence detection offers better sensitivity and specificity for most PAHs, the increased performance of the second generation diode array detector proves to be adequate for many environmental samples as detailed in the latter section.

4. Spectral Information

The rapid scanning capability of the diode array detector allows the generation of all 16 PAH spectra shown in Figures 5 and 6 in a single analysis. These spectra are highly structured

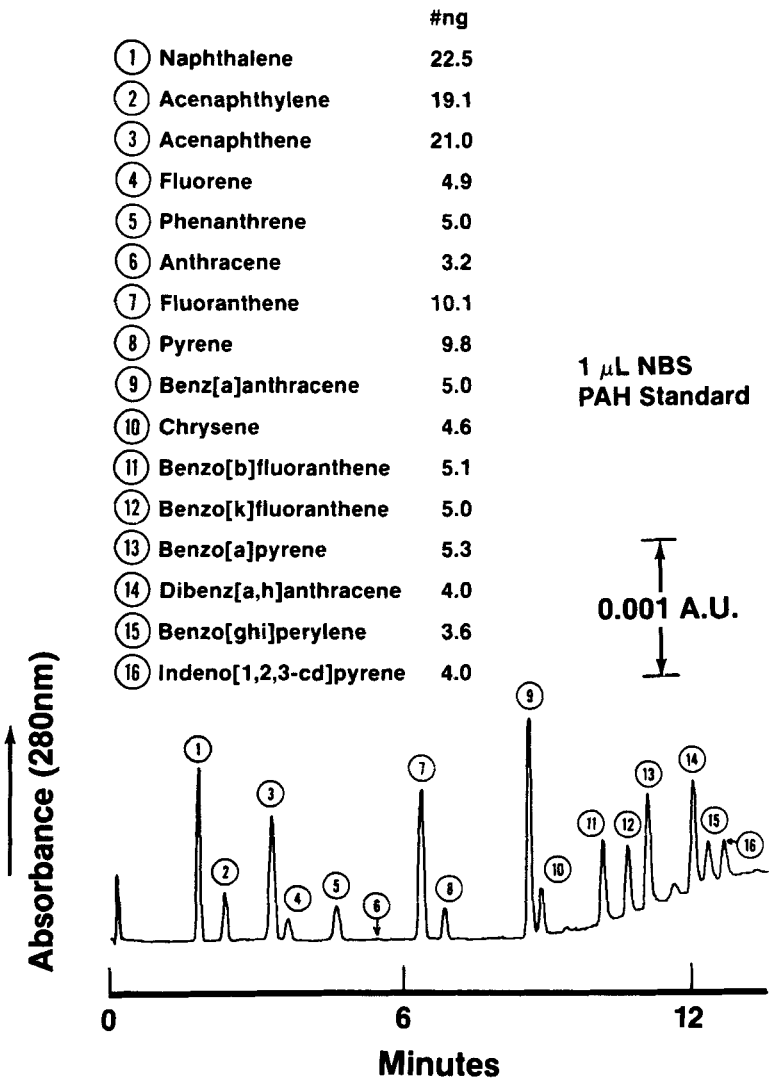


Fig. 4. A high-sensitivity PAH analysis of a 1-μL injection of the NBS standard using 280 nm detection. The amount injected ranged from 4 to 22 ng. See Table 2 for peak identification.

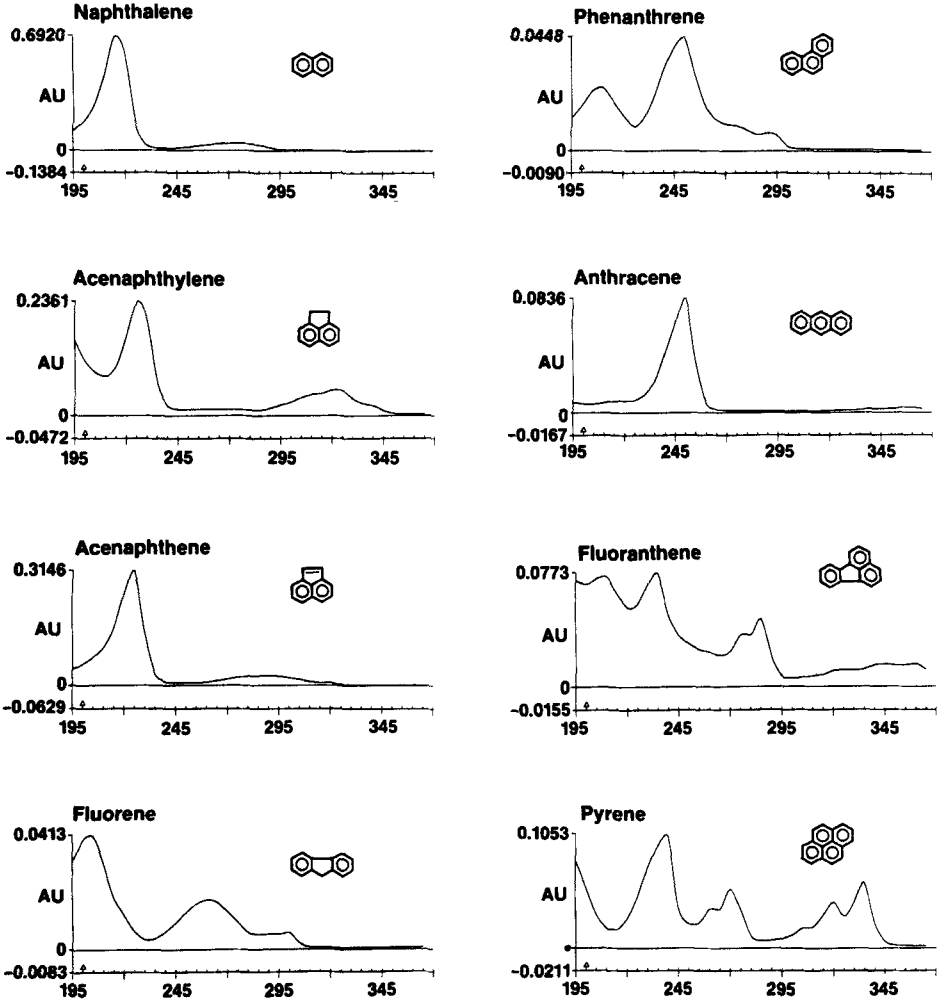


Fig. 5. UV spectra obtained on-the-fly for the first 8 individual PAH in the NBS standards.

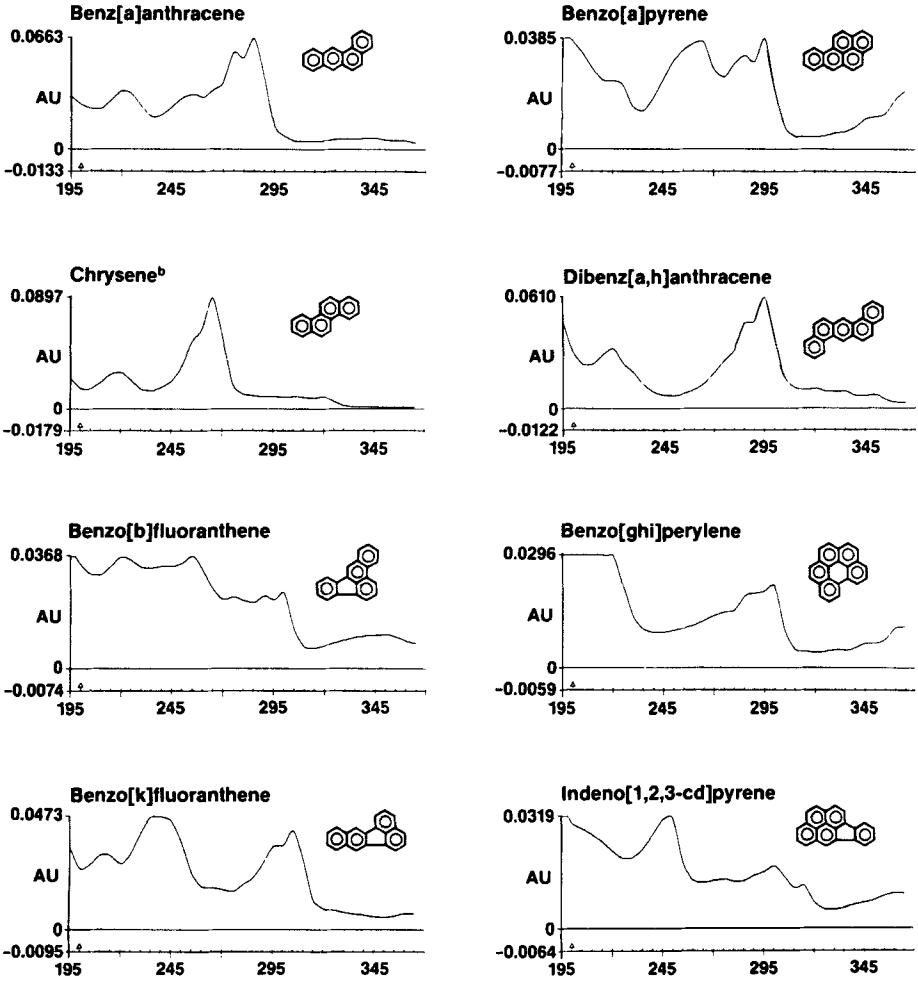


Fig. 6. UV spectra obtained on-the-fly for the last 8 individual PAH in the NBS standards.

TABLE 2

Comparison of Sensitivity: UV vs. Fluorescence

PAHs	Detection limit (ng) UV (280 nm)	Fluorescence	FL/UV Ratio
1. Naphthalene	0.5	0.03	16
2. Acenaphthylene	2	-	-
3. Acenaphthene	1	0.015	60
4. Fluorene	1	0.025	40
5. Phenanthrene	0.5	0.05	10
6. Anthracene	5	0.5	10
7. Fluoranthene	0.5	0.2	2
8. Pyrene	2	0.2	10
9. Benz(a)anthracene	0.1	0.1	1
10. Chrysene	0.5	0.2	2
11. Benzo(b)fluoranthene	0.5	0.02	25
12. Benzo(k)fluoranthene	0.5	0.002	250
13. Benzo(a)pyrene	0.3	0.01	30
14. Dibenz(a,h)anthracene	0.3	0.05	6
15. Benzo(ghi)perylene	0.5	0.05	10
16. Indeno(1,2,3-cd)pyrene	1	0.3	3

(signal/noise ratio of 5:1)

NOTE: See experimental section for operating conditions for the fluorescence detector.

and distinctive because of the aromaticity of the PAH structures. In general, the spectra show increased absorption at higher wavelengths with the higher PAHs (6). Additionally, the λmax of alkylated PAHs tends to shift towards longer wavelengths by 3-4 nm (5).

Figure 7 shows a high-sensitivity UV spectrum of 1.2 ng of benzo(a)pyrene at 0.001 absorbance unit. The dual polychromator optics and the externally scanned diode format are responsible for the enhanced detector and spectral sensitivity. In contrast, most self-scanned diode array detectors give noisy spectra below 10 milliabsorbance because of the noise contribution from the multiplexers (9).

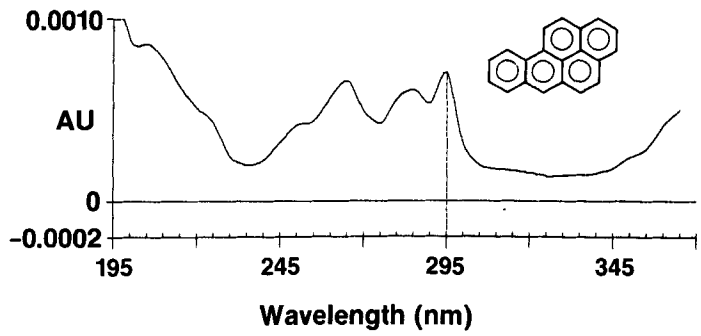


Fig. 7. A high-sensitivity UV spectrum of 1.2 ng of benzo(a)pyrene at 0.001 full scale absorbance unit.

Figure 8 shows an absorbance profile map, a global representation of the separation at all wavelengths. The circles locate the max of the spectra while the circle size approximates the actual absorbance values. This map facilitates the selection of monitoring wavelengths for optimized sensitivity or specificity. For instance, the map indicates that 335 nm is an optimum wavelength for selective detection of pyrene.

5. Analyzing Complex Environmental Samples

The analysis of two environmental samples demonstrates the utility of the LC-235 in PAH analysis. Figure 9 shows a chromatogram of an air particulate extract in which all identified PAH peaks are confirmed by retention time and UV spectrum. The monitoring wavelength is changed from 280 nm to 365 nm after the elution of phenanthrene to improve the selectivity for the higher PAHs and to decrease the unresolved "envelope" under the 5-ring PAHs (14).

Figure 10 shows a similar chromatogram of the PAH sub-fraction of a contaminated soil sample. Figure 11 (left) shows

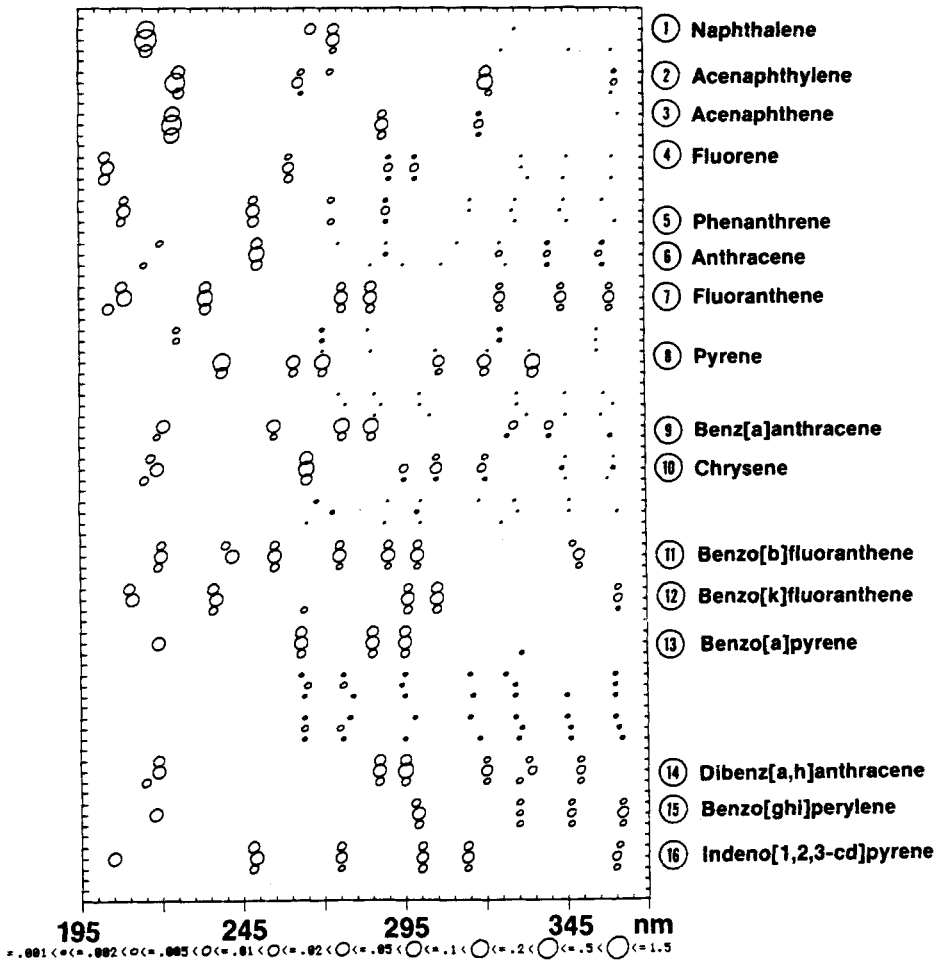


Fig. 8. The absorbance profile map for the 16 priority pollutant PAHs.

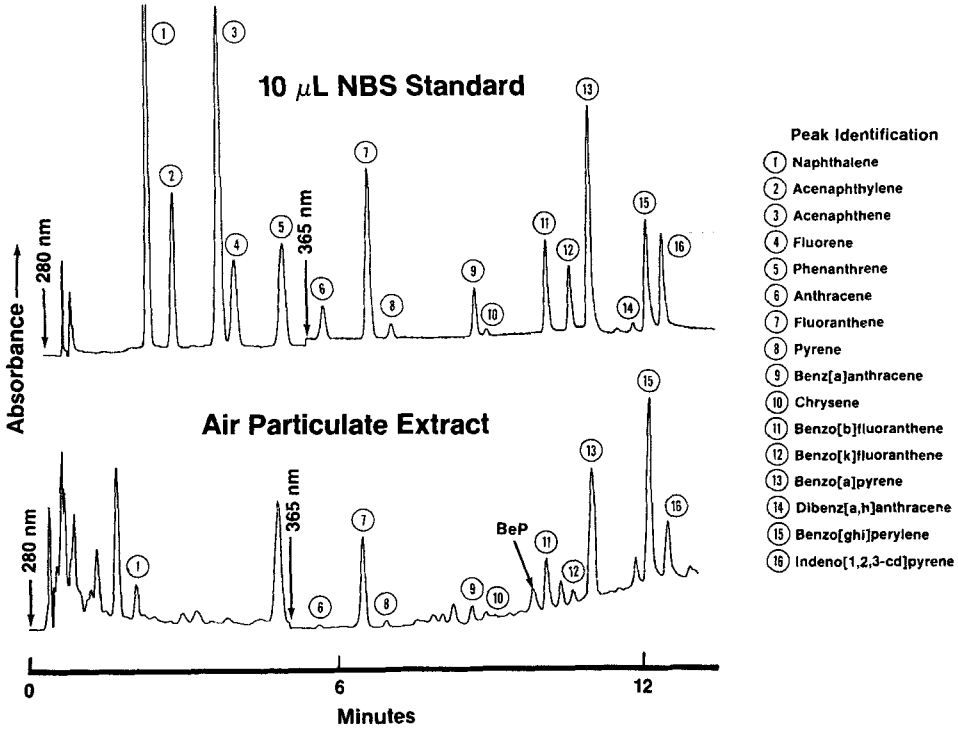


Fig. 9. Chromatogram of the PAH fraction isolated from a cyclohexane extract of an urban suspended particulate sample. See Table 2 for peak identification.

the overlay spectra of PAHs isolated from the air particulate extract vs. a standard, indicating excellent matches for both fluoranthene and pyrene, and the presence of interferences in the benzo(a)pyrene peak. Figure 11 (right) shows the overlay spectra of benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene isolated from the soil sample vs. that of the genuine standards. Calculated PAH concentrations in both samples are listed in Table 3.

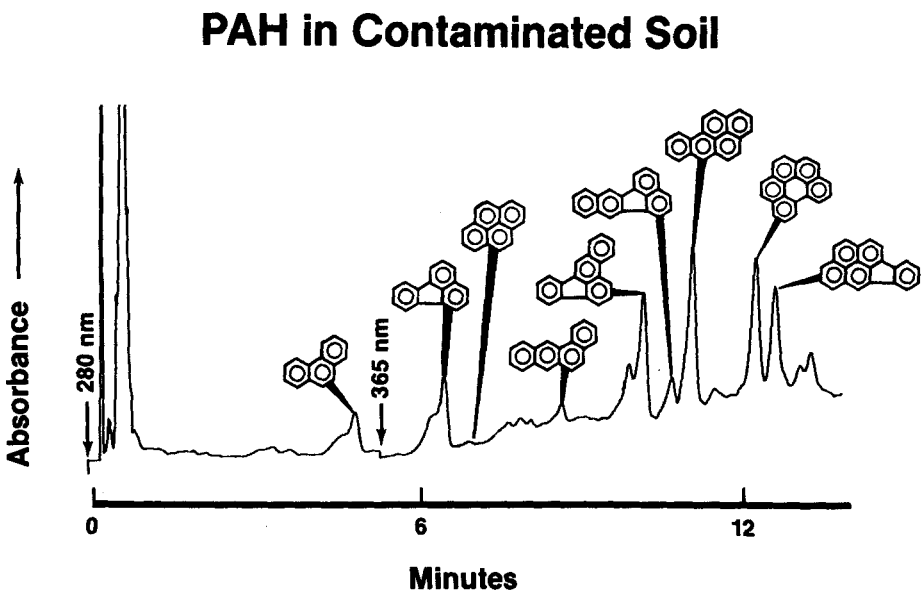


Fig. 10. Chromatogram of the PAH fraction of a contaminated soil sample from the site of a coal gasification plant.

TABLE 3

Quantitation of Environmental Samples

	Newark air sample	Contaminated soil
PAHs	µg/M	µg/g
Naphthalene	0.1	-
Phenanthrene	-	0.04
Anthracene	0.03	-
Fluoranthene	0.6	0.2
Pyrene	0.6	0.1
Benz(a)anthracene	0.2	0.07
Chrysene	0.3	-
Benzo(b)fluoranthene	0.4	0.2
Benzo(k)fluoranthene	0.1	0.1
Benzo(a)pyrene	0.4	0.1
Benzo(ghi)perylene	0.6	0.1
Indeno(1,2,3-cd)pyrene	0.3	0.1

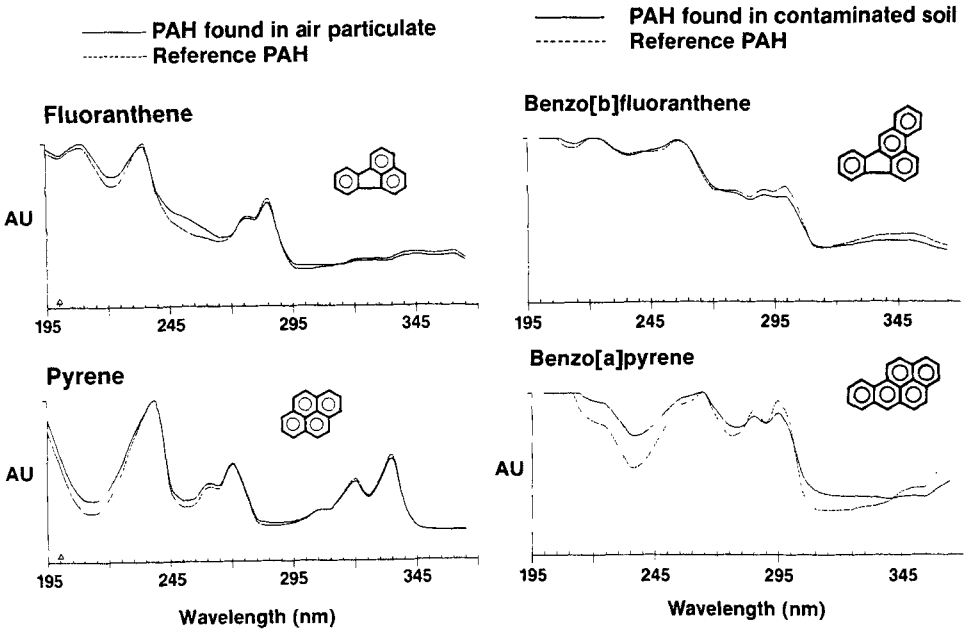


Fig. 11. Overlaid spectra of PAHs isolated from samples vs. standard.

CONCLUSIONS

This study clearly demonstrated the utility of the LC-235 for PAH analysis. Besides offering low nanogram detection limits for most PAHs, the rapid scanning capability assures identification while automated annotation of λmax and purity index aids in peak purity assessment. Wavelength programming optimizes sensitivity and specificity.

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