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Application Note 95

Polycyclic Aromatic Hydrocarbon Determination by Reversed-Phase High-Performance Liquid Chromatography

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are prevalent in the environment. They either occur naturally in fossil fuel products (e.g., coal and oil), or among the effluents of combustion processes commonly used for heating, incineration, and electric power generation. PAHs are classified as carcinogenic compounds and consequently are monitored worldwide in a wide range of environmental matrices, including drinking water, waste water, furnace emissions, soil, and hazardous waste extracts.

Methods for the determination of PAHs in the environment include gas chromatography with flame ionization detection (GC/FID) and high-performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection. While GC/FID is the more sensitive technique, it is subject to background interferences from other carbonaceous sources. HPLC is the preferred method of analysis, because it provides the necessary sensitivity in combination with higher specificity.

This application note presents two different reversed-phase high-performance liquid chromatography (RP-HPLC) methods for the determination of PAHs in extracted environmental samples.

Sample Preparation and Preservation

Samples taken for PAH analysis should be stored in glass containers, protected from light and refrigerated until extracted. PAHs are typically extracted using dichloromethane and then exchanged into acetonitrile prior to analysis. Aqueous samples may also be extracted using cartridge or disk extraction devices. Solid samples may be extracted using supercritical fluid extraction (SFE) methods. Extracts should be stored in glass, protected from light, and refrigerated prior to analysis by RP-HPLC.

Summary of Analytical Methods

After sample extraction, a portion of the extract (e.g., 25 µL) is injected onto a reversed-phase HPLC column and the PAHs eluted using a water/acetonitrile gradient. The PAHs are detected using UV absorbance and/or fluorescence detection. Method A, as described in Table 1, is designed to determine 16 PAHs using both UV and fluorescence detection and is consistent with U.S. EPA Methods 550,[1] 810,[2] and 8310,[3] as well as other regulatory methods worldwide.

Method B is designed to determine six PAHs using isocratic elution and wavelength-programmed fluorescence detection. These six PAHs were established by the World Health Organization as indicators of the presence of other PAHs. This method is consistent with the Association Française de Normalization (AFNOR) Method T91M[4] and is similar to other international methods used for the analysis of these six compounds.

The HPLC column specified in this application note is tested by the manufacturer for reliable separation of the 16 PAHs specified. Other columns that are quality controlled for the same analytes may also be suitable for this application.

EQUIPMENT

Dionex DX 500 HPLC system consisting of:

GP40 Gradient Pump

AD20 Absorbance Detector (Method A only)

Jasco® FP-920 Fluorescence Detector (Easton, Maryland, USA)

Jasco L-39 Emission Filter (for Method A only)

LC10 Liquid Chromatography Module (suitable for Method A only), or LC30 Chromatography Oven Eluent Organizer

PeakNet Chromatography Workstation with a UI20 Interface

REAGENTS

Acetonitrile, Fisher Optima™ Grade or equivalent

Deionized Water, 17.8 MΩ-cm

Table 1 Experimental conditions

Method A: Gradient Separation of 16 PAHs with UV and Fluorescence Detection

Column:	SUPELCOSIL™ LC-PAH, 250 mm x 4.6 mm x 5 µm
Guard:	Supelguard™ LC-PAH
Temperature:	Ambient
Mobile Phase:	A: Deionized water B: Acetonitrile

Gradient:

Time (min)	A (%)	B (%)
0	60	40
5	60	4
30	0	100
45	0	100

Flow Rate:	1.5 mL/min
Inj. Volume:	25 µL
UV Detection:	254 nm
Fluorescence:	Excitation: 280 nm
Emission:	> 390 nm (cut-off filter)

Method B: Isocratic Separation of 6 Representative PAHs with Programmed Fluorescence Detection

Column:	SUPELCOSIL LC-PAH, 250 mm x 4.6 mm x 5 µm
Guard:	Supelguard LC-PAH
Temperature:	35 °C
Mobile Phase:	75% Acetonitrile, 25% water
Flow Rate:	1.5 mL/min
Inj. Volume:	100 µL

Fluorescence:

Compound	Excitation (nm)	Emission (nm)
Fluoranthene	365	462
Benzo(*b*)fluoranthene	302	452
Benzo(*k*)fluoranthene	302	431
Benzo(*a*)pyrene	297	405
Benzo(*g,h,i*)perylene	302	419
Indeno(*1,2,3-cd*)pyrene	300	500

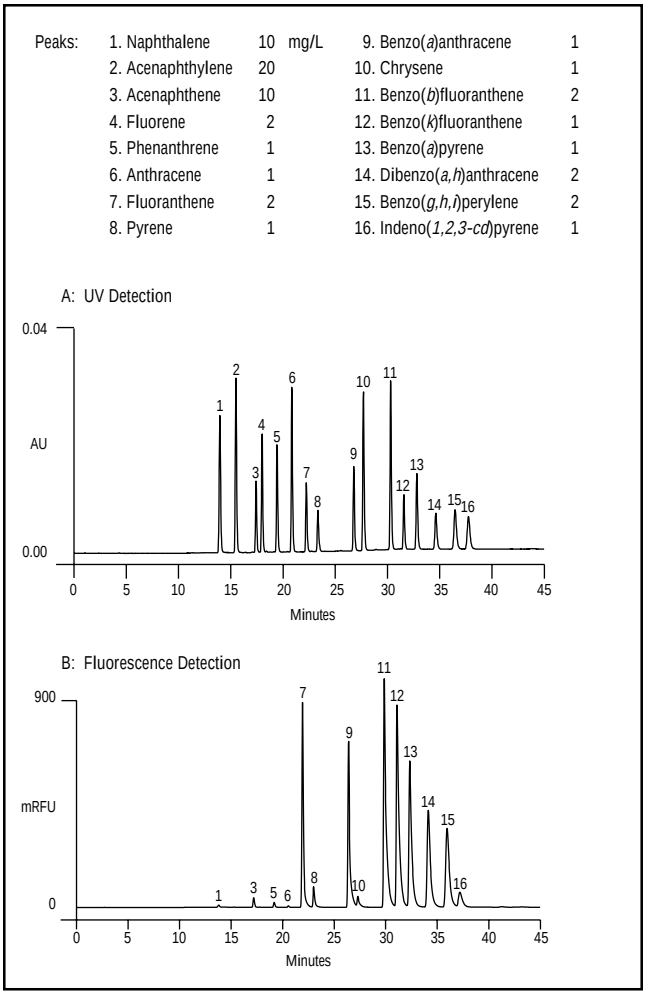

Figure 1 *Standard mixture of 16 PAHs separated by Method A.*

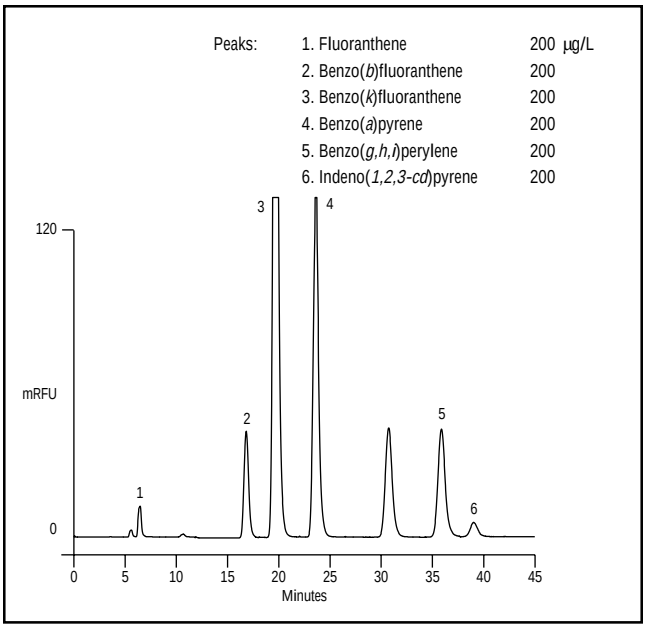

Figure 2 *Standard mixture of 16 PAHs separated by Method B. The unidentified peak, dibenzo(*a,h*)anthracene, is normally not determined using this method.*

CALIBRATION STANDARDS PREPARATION

Stock Solutions

Standard mix solutions are available commercially (e.g., Supelco catalog number 4-8905), or they can be prepared from individual pure compounds as follows:

Accurately weigh 100 mg of the desired PAH into a 100-mL volumetric flask. Add 2–3 mL acetonitrile to dissolve. Dilute to volume with acetonitrile to obtain a 1000-mg/L stock solution. If stored correctly (in amber glassware at 4 °C), stock solutions have a shelf-life of approximately six months.

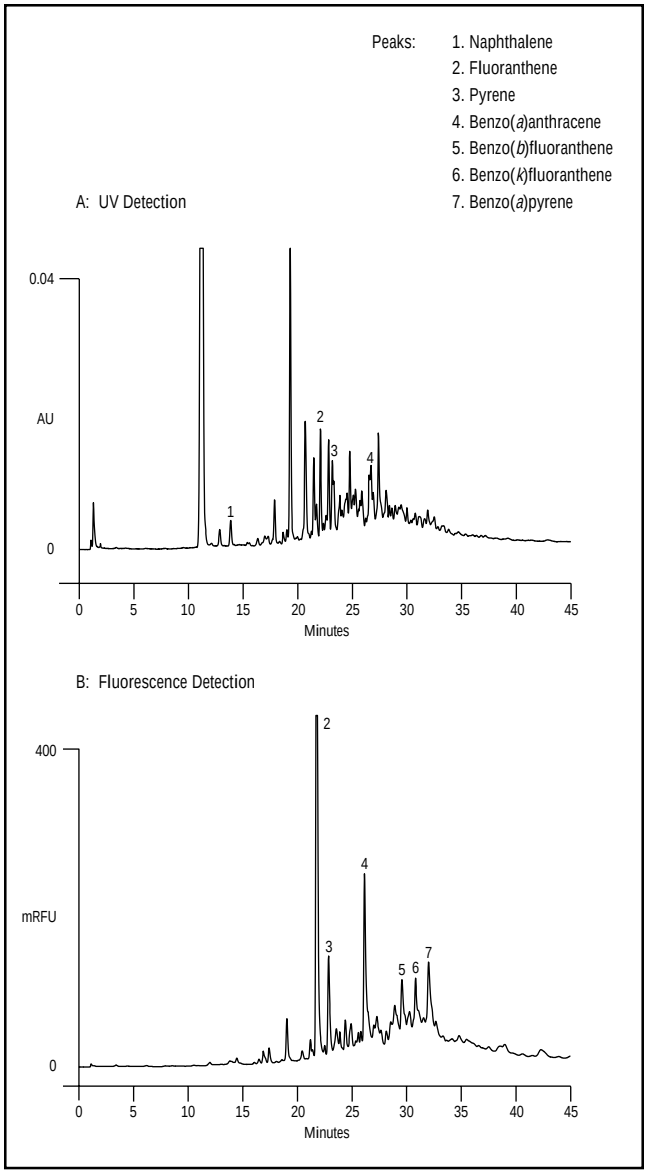

Figure 3 *Extract of PAH-contaminated soil analyzed by Method A.*

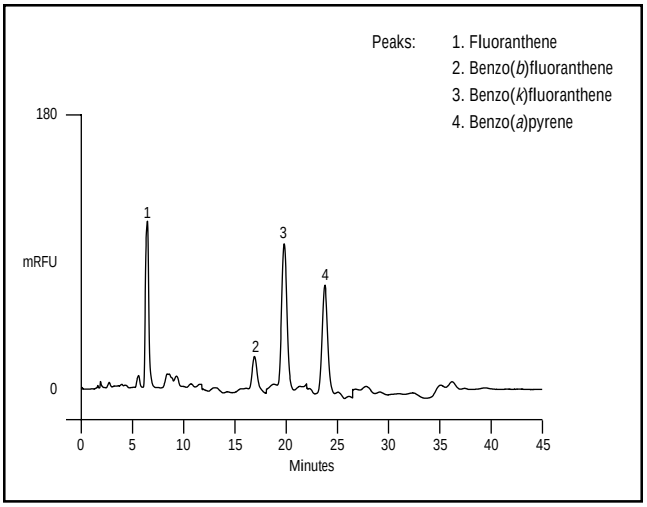

Figure 4 *Extract of PAH-contaminated soil analyzed by Method B.*

Standard Solutions

Make appropriate dilutions in acetonitrile to bracket the expected concentration level of the analytes.

DISCUSSION AND RESULTS

Figure 1a shows the result of a 16-PAH standard analyzed by Method A using an absorbance detector. Figure 1b shows the same analysis using a fluorescence detector. Note that the four early eluting PAHs are best quantified using UV detection. Fluorescence detection, on the other hand, gives greater sensitivity for the remaining 12 PAHs.

Method B uses only fluorescence detection and relies on the specificity that is gained by using wavelength programming. Figure 2 is a typical standard chromatogram that is generated using the program outlined in Table 1.

Detection in Complex Matrices

An extract of PAH-contaminated soil analyzed by Method A is shown in Figures 3a and 3b. Note that naphthalene, which is the first PAH to elute, is only detectable by UV. On the other hand, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, and benzo(*a*)pyrene are virtually absent in the UV chromatogram but easily quantifiable with fluorescence detection.

The specificity of the fluorescence detector for fluoranthene, pyrene, and benzo(*a*)anthracene is also demonstrated in chromatograms 3a and 3b. The matrix interferences, which make quantification of these analytes difficult by UV, are minimized with fluorescence.

The same soil extract sample used in Figures 3a and 3b was run using Method B (see Figure 4). For this example, the specificity enhancement is dramatic due to the use of wavelength programming. Fluoranthene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, and benzo(*a*)pyrene were all easily quantified, while the interferences blended into the baseline.

METHOD DETECTION LIMITS

Method A

Method detection limits shown in Table 2 are for the concentration of analyte injected on-column. To determine detection limits for untreated sample, divide by the appropriate concentration factor. For example, EPA Method 550 specifies starting with 1 L of sample and concentrating to a volume of 0.5 mL before performing the HPLC analysis. In this case, divide the numbers in the following table by 2000 to determine detection limits for the original samples.

Peaks 1–4 are quantified using data from the absorbance detector. The 12 remaining compounds are quantified using fluorescence data. All detection limits meet or exceed those specified in U.S. EPA Methods 550, 810, and 8310.

Table 2 Method detection limits for Method A

Compound	MDL[a] (µg/L)
Naphthalene	59
Acenaphthylene	25
Acenaphthene	84
Fluorene	15
Phenanthrene	8.0
Anthracene	12
Fluoranthene	0.54
Pyrene	4.1
Benzo(*a*)anthracene	0.31
Chrysene	6.6
Benzo(*b*)fluoranthene	0.61
Benzo(*k*)fluoranthene	0.19
Benzo(*a*)pyrene	0.28
Dibenzo(*a,h*)anthracene	2.5
Benzo(*g,h,i*)perylene	2.5
Indeno(*1,2,3-cd*)pyrene	2.5

[a] M.D.L. = (s.d.) x $(t_s)_{99\%}$, where (t_s) is for a 99% single-sided student's *t* test distribution.

Method B

Detection limits shown in Table 3 are for the directly injected material. Divide by the appropriate concentration factor to obtain sample detection limits.

Table 3 Method detection limits for Method B

Compound	MDL[a] (µg/L)
Fluoranthene	3.0
Benzo(*b*)fluoranthene	1.5
Benzo(*k*)fluoranthene	0.48
Benzo(*a*)pyrene	1.4
Benzo(*g,h,i*)perylene	3.0
Indeno(*1,2,3-cd*)pyrene	5.4

[a] M.D.L. = (s.d.) x $(t_s)_{99\%}$, where (t_s) is for a 99% single-sided student's *t* test distribution.

PRECAUTIONS

Whenever wavelength programming is used, temperature control is recommended to ensure stable retention times. Temperature control is especially important for gradients, but can also be important in isocratic methods, such as Method B. Figure 2 shows a 16 PAH-standard run using Method B. Peaks 5 and 6 elute closely together, with a wavelength change occurring between them. A change in retention times could cause quantification errors.

However, Method B can be used at room temperature with good results. Figure 5 shows an isocractic separation of the 16 PAH-standard run at room temperature, using a 90% acetonitrile, 10% water mobile phase. All other method conditions remain the same, and method detection limits are very close to those listed in Table 3.

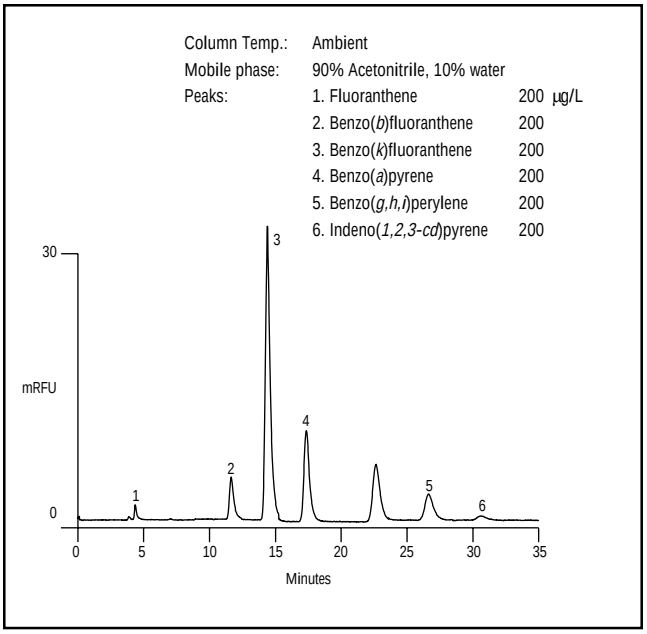

Figure 5 *Standard mixture of 16 PAHs separated by a variation of Method B.*

REFERENCES

1. U.S. Environmental Protection Agency. *U.S. EPA Method 550,*"Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Liquid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection," J.W. Hodgeson, Environmental Monitoring Systems Laboratory, Cincinnati, OH, July 1990.
2. U.S. Environmental Protection Agency. *U.S. EPA Method 8310*, "Polynuclear Aromatic Hydrocarbons in Drinking Water Using HPLC," September 1986.
3. U.S. Environmental Protection Agency. *U.S. EPA Method 610,*"Polynuclear Aromatic Hydrocarbons," Environmental Monitoring and Support Laboratory, Cincinnati, OH, July 1982.
4. Association of Normalization (AFNOR), NFT 90–115, "Quantitation of 6 Polycyclic Aromatic Hydrocarbons," September 1988.

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