HPLC Detector Options for the Determination of Polynuclear Aromatic Hydrocarbons

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Maureen Joseph
Varian Chromatography Systems

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Introduction

The presence of polynuclear aromatic hydrocarbons (PAHs) is a common environmental concern. Some of the PAHs have been found to be carcinogenic and mutagenic. These compounds are typically introduced into the environment by both naturally occurring combustion processes, such as forest fires, and industrial combustion processes, such as the burning of fossil fuels. The PAHs then are carried into rivers, lakes, and other water sources.[1] Therefore waste water and other water sources are commonly tested for their presence. EPA method 610 for the determination of PAHs in municipal discharges is a commonly used method requiring the determination of the 16 priority pollutant PAHs that may be present in water. The European Community (EC) member states require the determination of six of the PAHs in drinking water. Some countries and states have additional recommendations and these may include the 16 PAHs in the EPA method and some additional PAHs. The list of frequently determined PAHs in Table 1 includes the 16 from EPA 610 and perylene.

Table 1. Commonly Determined PAHs

Acenaphthene	Dibenzo(a,h)anthracene
Acenaphthylene	Fluoranthene*
Anthracene	Fluorene
Benzo(a)anthracene	Indeno(1,2,3-cd)pyrene*
Benzo(a)pyrene*	Naphthalene
Benzo(b)fluoranthene*	Perylene
Benzo(g,h,i)perylene*	Phenanthrene
Benzo(k)fluoranthene*	Pyrene
Chrysene	

*PAHs regulated by the EC member states for drinking water

A common method for PAHs uses HPLC with UV detection at 254 nm because these compounds can be determined with good sensitivity under these conditions. This is easily done with a UV/Vis detector such as the Varian Star 9050. A second very popular option is fluorescence detection for some of the PAHs, as many of these compounds have a high natural fluorescence. Fluorescence detectors can be monochromator based allowing for wavelength programming to achieve optimum sensitivity. The appropriate choice of detector(s) depends upon the actual method being used and the detection limits required for the samples being studied. In general, methods for the analysis of samples with few matrix components, other than drinking water, may be analyzed by UV absorbance at 254 nm. More complex samples such as soil, may require fluorescence detection for added selectivity. Drinking water samples require the added sensitivity of fluorescence detection.

Experimental

This application note shows the analysis of PAH samples with two different detector options, UV and fluorescence. The Varian Star 9050 UV/Vis absorbance detector is used at 254 nm and with wavelength programming to enhance sensitivity and selectivity. The second detector used is the Varian 9070 dual monochromator fluorescence detector. An excitation and emission wavelength program is used with this detector to achieve the best sensitivity. The first column is a Vydac 201TP54, 250 mm x 4.6 mm, 5 µm particles. The separation of all 16 PAHs requires a gradient program. The conditions are:

Time (min)	Conditions	Flow
0	50% CH_3CN, 50% H_2O	1.5 mL/min
7.0	50% CH_3CN, 50% H_2O	1.5 mL/min
20.0	80% CH_3CN, 20% H_2O	1.5 mL/min
25.0	80% CH_3CN, 20% H_2O	1.5 mL/min
30.0	95% CH_3CN, 5% H_2O	1.5 mL/min

The second column is a Shandon Hypersil Green PAH column (P/N 01-900017-00), 100 mm x 4.6 mm, 5 µm particles. This column is designed for the separation of these PAHs and others. This separation is also a gradient program. The conditions are:

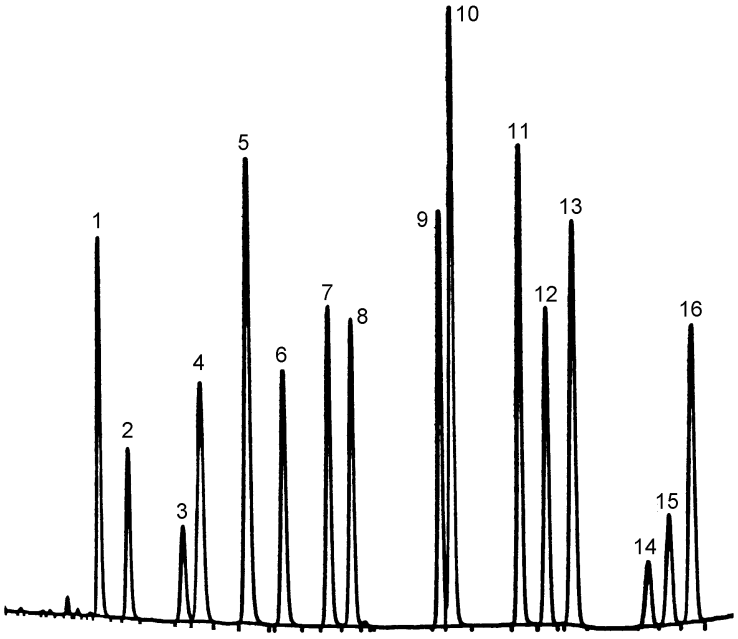
Time (min)	Conditions	Flow
0	50% CH_3CN, 50% H_2O	2.0 mL/min
5.0	50% CH_3CN, 50% H_2O	2.0 mL/min
25.0	100% CH_3CN	2.0 mL/min

All samples are 20 µL injections.

The NIST standard 1647 PAH mix is used to determine the optimum conditions and for spiking into water samples. The EC guidelines state requires that the total concentration of the 6 PAHs in drinking water not exceed 0.2 μg/L and benzo(a)pyrene may not exceed 0.01 μg/L. This requires approximate maximum concentrations of 0.03 μg/L for the remaining five PAHs.

Results

A sample chromatogram of the separation of 16 PAHs using the 9050 absorbance detector at 254 nm is shown in Figure 1. There is no wavelength programming for this run. This is the simplest way to operate the detector and achieve satisfactory detection limits (Table 2). These detection limits are based on extraction from a 1L water sample with concentration of the extract to 1 mL. The compounds are listed in elution order.



Compound	R/T	Amount on Column
Naphthalene	5.96	450 ng
Acenaphthylene	7.16	382 ng
Acenaphthene	9.37	420 ng
Fluorene	10.06	98 ng
Phenanthrene	11.95	101 ng
Anthracene	13.41	65 ng
Fluoranthene	15.25	202 ng
Pyrene	16.18	197 ng
Benzo(a)anthracene	19.77	101 ng
Chrysene	20.24	93 ng
Benzo(b)fluoranthene	23.00	102 ng
Benzo(k)fluoranthene	24.10	101 ng
Benzo(a)pyrene	25.18	106 ng
Dibenzo(a,h)anthracene	29.09	74 ng
Benzo(g,h,i)perylene	28.24	82 ng
Indeno(1,2,3-c,d)pyrene	30.03	82 ng

Figure 1. UV-254 nm Chromatogramm, Vydac Column

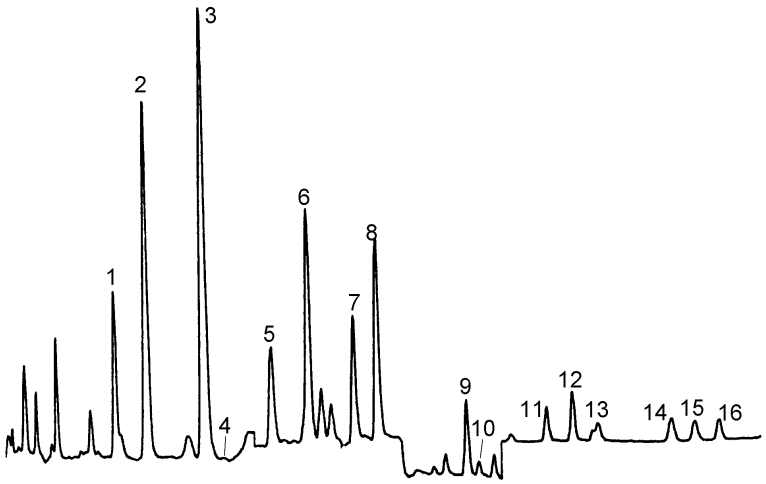
Table 2. UV and Fluorescence Detection Limits

Compound	UV 254 nm (μg/L)	Fluorescence (μg/L)	EPA 610 (μg/L)	EPA 550.1 (μg/L)
1. Naphthalene	0.27	**	1.8	2.20
2. Acenaphthylene	0.27	**	2.3	1.41
3. Acenaphthalene	0.31	**	1.8	2.04
4. Fluorene	0.020	**	0.21	0.126
5. Phenanthrene	0.0062	0.003	0.64	0.150
6. Anthracene	0.0083	0.0012	0.66	0.140
7. Fluoranthene*	0.010	0.010	0.21	0.009
8. Pyrene	0.010	0.0083	0.27	0.126
9. Benzo(a)anthracene	0.0062	0.0011	0.013	0.004
10. Chrysene	0.0042	0.0021	0.15	0.160
11. Benzo(b)fluoranthene*	0.0045	0.00075	0.018	0.006
12. Benzo(k)fluoranthene*	0.0056	0.00012	0.017	0.003
13. Benzo(a)pyrene*	0.0050	0.00079	0.023	0.016
14. Dibenzo(a,h)anthracene	0.016	0.00094	0.030	0.035
15. Benzo(g,h,i)perylene*	0.012	0.0016	0.076	0.020
16. Indeno(1,2,3-cd)pyrene*	0.0093	0.0018	0.043	0.036

*Indicates the EC 6, with detection limits estimated based on total of 0.2 μg/L
**Not normally determined by fluorescence

Figure 2 shows the PAHs extracted from a spiked water sample. In this case wavelength programming of the 9050 was used to optimize sensitivity and selectivity. By wavelength programming it is possible to reduce the number of matrix components that are visible in the chromatogram. The wavelength program used is listed in Table 3. Sensitivity is enhanced by wavelength programming at appropriate absorbance maximas. This can also enhance selectivity where these wavelengths discriminate against matrix components.

Fluorescence detection was also optimized with wavelength programming of both the excitation and emission wavelengths. The chromatogram in Figure 3 shows the PAHs detected with the Varian 9070 fluorescence detector following the wavelength program in Table 3. Acenaphthylene has the weakest fluorescence and must be determined by UV absorbance. Naphthalene, acenaphthene, and fluorene have a sufficiently strong UV absorbance to use this method of detection. Therefore, some PAH methods recommend placing the UV and the fluorescence detectors in series.



Compound	R/T
1. Naphthalene	6.13
2. Acenaphthylene	7.36
3. Acenaphthene	9.63
4. Fluorene	10.32
5. Phenanthrene	12.25
6. Anthracene	13.70
7. Fluoranthene	15.49
8. Pyrene	16.40
9. Benzo(a)anthracene	19.91
10. Chrysene	20.37
11. Benzo(b)fluoranthene	23.05
12. Benzo(k)fluoranthene	24.08
13. Benzo(a)pyrene	25.08
14. Dibenzo(a,h)anthracene	27.98
15. Benzo(g,h,i)perylene	28.89
16. Indeno(1,2,3-c,d)pyrene	29.85

Figure 2. Water Sample Spiked with Low Level Standard, Vydac Column

Table 3. Wavelength Programs for UV Absorbance and Fluorescence Detection

			Fluorescence	
Compound	UV Wavelength	Sensitivity Enhancement Factor	Excitation (nm)	Emission (nm)
1. Naphthalene	227	25.0	224	330
2. Acenaphthylene	227	8.5	not detected	
3. Acenaphthalene	227	35.0	234	320
4. Fluorene	227	1.0	224	320
5. Phenanthrene	250	1.2	252	370
6. Anthracene	250	1.7	252	402
7. Fluoranthene	235	1.6	252	402
8. Pyrene	235	1.0	238	398
9. Benzo(a)anthracene	280	1.0	238	398
10. Chrysene	280	1.8	238	398
11. Benzo(b)fluoranthene	297	1.0	268	398
12. Benzo(k)fluoranthene	297	1.2	268	398
13. Benzo(a)pyrene	297	1.1	268	398
14. Dibenzo(a,h)anthracene	297	12.0	234	420
15. Benzo(g,h,i)perylene	297	2.5	234	420
16. Indeno(1,2,3-cd)pyrene	297	1.0	300	466

Excitation and Emission Detector Bandwidth = 8 nm for 9070 fluorescence detector

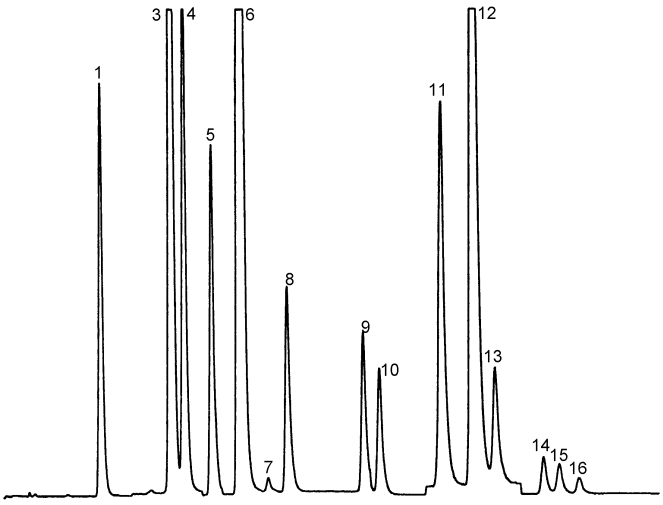
This allows even greater sensitivity and selectivity than with the UV detector alone. The detection limits achieved with the 9070 are listed in Table 2. The chromatogram in Figure 4 is the same spiked water sample as the one analyzed with the UV detector. This chromatogram shows all of the fluorescent PAHs and shows fewer matrix peaks. This simplification of the chromatogram is an excellent reason for adding the fluorescence detector in series with the UV detector, even when the UV detector has adequate sensitivity for the PAHs.

Conclusions

Both UV and fluorescence detectors can be used for the determination of PAHs. UV detectors are often used at 254 nm with no wavelength programming. This provides adequate sensitivity for the PAHs as defined by methods such as USEPA 610. But more sensitivity and selectivity can be achieved with fluorescence detectors when using wavelength programming. A fluorescence detector in series with a UV detector will provide the best sensitivity and selectivity. Very few compounds naturally fluoresce so it is easy to discriminate against matrix components with a fluorescence detector. Wavelength programming of the fluorescence detector can be done with a monochromator based fluorescence detector to result in the lowest detection limits possible. UV detection at 254 nm is both simple and sensitive, with enhanced selectivity when the UV detector is also wavelength programmed.

Reference

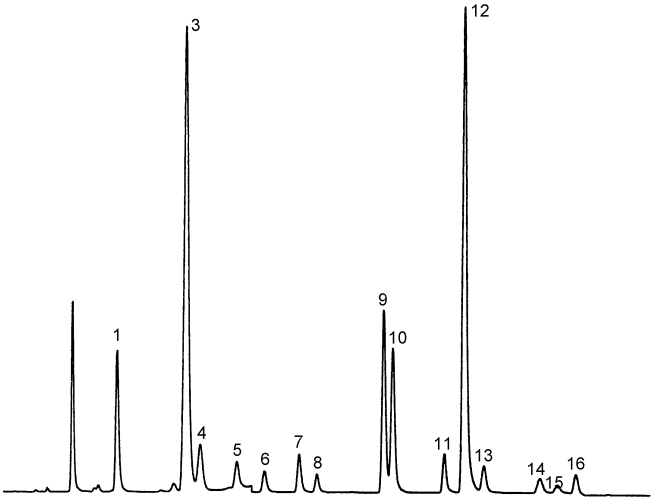
1. Furata, N.; Otsuki, A., Anal. Chem. 1983, *55*, 2407-2413.



Compound	R/T	Amount on Column
1. Naphthalene	3.97	16.0 ng
2. Acenaphthylene	N.D.	32.0 ng
3. Acenaphthene	6.87	16.0 ng
4. Fluorene	7.34	3.2 ng
5. Phenanthrene	8.53	1.6 ng
6. Anthracene	9.70	1.6 ng
7. Fluoranthene	10.85	3.2 ng
8. Pyrene	11.63	1.6 ng
9. Benzo(a)anthracene	14.76	1.6 ng
10. Chrysene	15.42	1.6 ng
11. Benzo(b)fluoranthene	17.98	3.2 ng
12. Benzo(k)fluoranthene	19.31	1.6 ng
13. Benzo(a)pyrene	20.19	1.6 ng
14. Dibenzo(a,h)anthracene	22.17	3.2 ng
15. Benzo(g,h,i)perylene	22.83	3.2 ng
16. Indeno(1,2,3-c,d)pyrene	23.64	1.6 ng

N.D=Not Detected

Figure 3. Chromatogram of PAHs with Fluorescence Detection, Shandon Hypersil Green PAH Column



Compound	R/T
1. Naphthalene	6.23
2. Acenaphthene	9.78
3. Fluorene	10.53
4. Phenanthrene	12.39
5. Anthracene	13.83
6. Fluoranthene	15.63
7. Pyrene	16.55
8. Benzo(a)anthracene	20.03
9. Chrysene	20.50
10. Benzo(b)fluoranthene	23.17
11. Benzo(k)fluoranthene	24.20
12. Benzo(a)pyrene	25.22
13. Dibenzo(a,h)anthracene	29.04
14. Benzo(g,h,i)perylene	28.13
15. Indeno(1,2,3-c,d)pyrene	29.99

Figure 4. Fluorescence Chromatogram of Water Spiked Sample, Vydac column

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These data represent typical results.
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