Identification of Polynuclear Aromatic Hydrocarbons in a Complex Matrix with Diode Array Detection

LC

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Introduction

The analysis of polynuclear aromatic hydrocarbons (PAHs) is a typical environmental requirement. The HPLC methods used to analyze these compounds have several things in common. First, they require UV absorbance detection at 254 nm with a fluorescence detector added to achieve greater sensitivity. Second, an additional method of analysis, such as GC/MS, is usually recommended when the compounds cannot easily be identified by the HPLC methods. One of the limitations to single wavelength UV absorbance and fluorescence detectors is the lack of qualitative information, other than retention time. A diode array detector can simultaneously provide qualitative and quantitative information, such that a second method of analysis may not be required. Spectral libraries are quickly built from standards, reflecting actual run conditions and accurately confirming compound identity.

To make the rapid, accurate analysis of PAHs possible, the selectivity of some HPLC columns has been optimized for this analysis. These columns usually guarantee the separation of the PAHs monitored in drinking water in the European Community and the U.S. (Table 1). The rapid separation of all 16 compounds requires gradient HPLC conditions, which are provided by the column manufacturer. The six PAHs listed in the EC guidelines can be separated using an isocratic HPLC method. Combining these specialty PAH columns with the diode array detector provides the best equipment for a reliable separation and confirm compound identity in one quick analysis.

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 states for drinking water.

Experimental

Two specialty PAH columns were compared to a Varian MicroPak SP-C18-5 column. The physical characteristics of the three columns are listed in Table 2. Standard samples were analyzed on each column using a Polychrom diode array detector at 254 nm. An apex spectrum from each PAH was used to build the spectral library. A different library was built for each column, allowing retention time comparison in the library search. Wavelength conditions for peak detection (PolyView peak sensing) were optimized for accurate library searches.

Table 2. Column Descriptions

Shandon Hypersil Green PAH (P/N 01-900017-00):	100 mm x 4.6 mm 5 µm, 120Å Carbon loading (13.0-14.0%) Surface Area (175 m^2/g) Spherical
Vydac 201TP5415:	150 mm x 4.6 mm 5 µm, 300Å Carbon loading (8-9%) Surface area (90 m^2/g) Spheroidal
Varian MicroPak SP-C18-5 (P/N 03-912042-42):	150 mm x 4.0 mm 4.5 µm, 80Å Carbon loading (12.5-13.5%) Surface area 200 m^2/g Spherical

Once the best standard conditions were obtained, a coal tar extract containing 11 of the 16 PAHs was analyzed. This sample tested the capability of the diode array detector to identify PAHs in a complex matrix.

Results

The diode array detector can only be an acceptable substitute for the UV detector at 254 nm if the detection limits required by the method are still obtainable. Table 3 shows the detection limits obtained with UV, fluorescence, and the diode array detector (DAD) in comparison to the requirements of the EPA waste water method 610. The DAD compares well to the UV detector and meets the required detection limits for almost every compound. Therefore, it was worth pursuing the use of the diode array detector for compound identification.

Table 3. Detection Limits Comparison

Compound	UV (µg/L)	DAD (µg/L)	Fluo[1] (µg/L)	EPA 610 (µg/L)
1. Naphthalene	0.27	0.80	*	1.8
2. Acenaphthylene	0.27	0.81	**	2.3
3. Acenaphthalene	0.31	0.76	*	1.8
4. Fluorene	0.020	0.040	*	0.21
5. Phenanthrene	0.0062	0.008	0.003	0.64
6. Anthracene	0.0083	0.027	0.0012	0.66
7. Fluoranthene	0.010	0.028	0.01	0.21
8. Pyrene	0.010	0.040	0.0083	0.27
9. Benzo(a)anthracene	0.0062	0.015	0.0011	0.013
10. Chrysene	0.0042	0.014	0.0021	0.15
11. Benzo(b)fluoranthene	0.0045	0.017	0.00075	0.018
12. Benzo(k)fluoranthene	0.0056	0.011	0.00012	0.017
13. Benzo(a)pyrene	0.0050	0.020	0.00079	0.023
14. Dibenzo(a,h)anthracene	0.016	0.040	0.00094	0.030
15. Benzo(g,h,i)perylene	0.012	0.040	0.0016	0.076
16. Indeno(1,2,3-cd)pyrene	0.0093	0.020	0.0018	0.043

*Not determined by fluorescence, S/N=3.
**Acenaphthylene is present, but does not fluoresce.
[1] *See LC Application Note #7*

Figure 1 shows the standard resolved on the three different columns. In each chromatogram the elution order is from Table 3. While the MicroPak SP-C18-5 column was able to resolve the small impurity eluting after Pyrene, it could not resolve several of the pairs of the PAHs, including acenaphthalene and fluorene and benzo(a)anthracene and chrysene. These pairs could be well resolved on either of the Specialty columns. With each specialty column the separation was obtained using the recommended conditions. This makes it easy for the chromatographer to start operating successfully with no methods development.

The coal tar extract chromatogram is shown in Figure 2. There are many small matrix components which are not larger than the PAH components. Therefore, the peak detection (PolyView peak sensing) is optimized when wavelength ranges characteristic of the strongest UV absorbance bands of the PAHs are used, as in Figure 3. PolyView's flexibility to program either a range of wavelengths or a single wavelength allows more accurate peak detection. The chromatogram also contains very large peaks in the front and much smaller ones later in the chromatogram. This makes S/N peak sense programming a valuable aid in integrating all of the small peaks. This is combined in the peak sense program in Figure 3. The library search was applied to a time window in which PAHs are expected to elute (Data File Time Range, Figure 4). Relative retention time ranges were also narrowed to allow specific identification of the PAHs. Only the wavelengths at which the PAHs absorb were included in the search wavelength range. All of this information is determined from the standard runs from which the library was built.

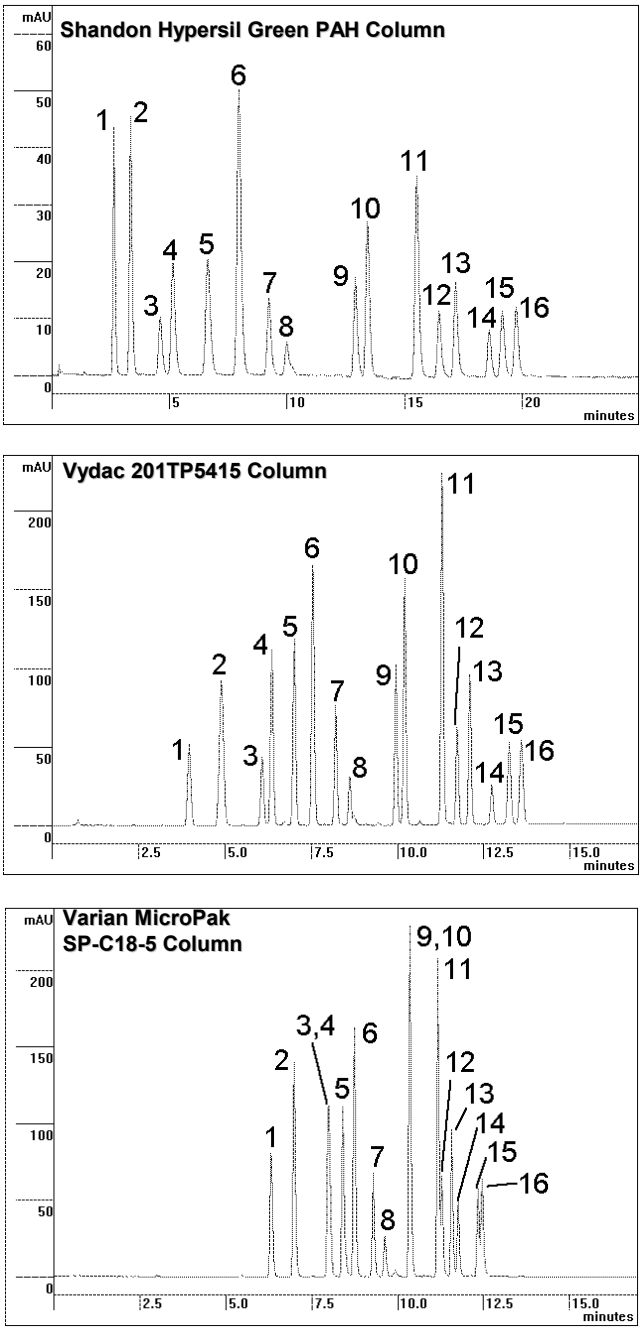


Figure 1. Comparison Separation of PAH Standards on 3 Columns

The results of PolyView's automated library search are shown in Figure 4. The accuracy of the match is demonstrated by the very high similarity values (0.993 or greater) and the very low dissimilarity values (0.12 or below). An example of the spectral match from fluoranthene to the library is shown in Figure 5 (next page). In addition to the PAHs accurately and correctly identified, none of the other peaks were incorrectly identified as these PAHs. One advantage of using the diode array detector was the accurate identification of both benzo(b)fluoranthene and benzo(k)fluoranthene. These isomers are not distinguished by a benchtop mass spectrometer.

Conclusions

This combination allows the requirements of many methods for PAH analysis to be met, including USEPA 610, with a minimum of effort. The diode array detector provides confirmation of peak identity for the PAHs separated in a complex matrix. When combined with a specialty PAH column the separation is easy to achieve.

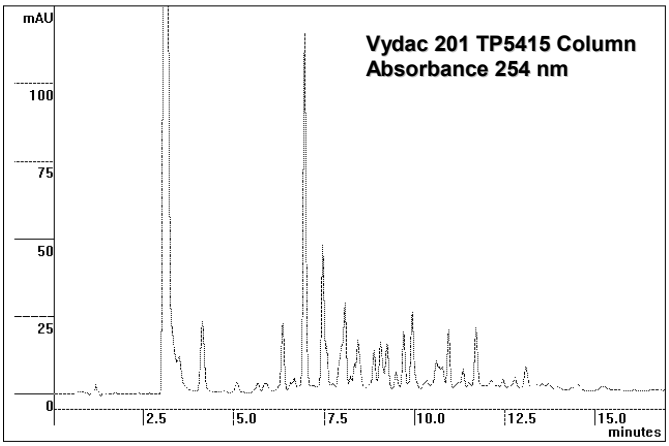


Figure 2. Chromatogram of Coal Tar Extract (NIST Reference Materials 1547)

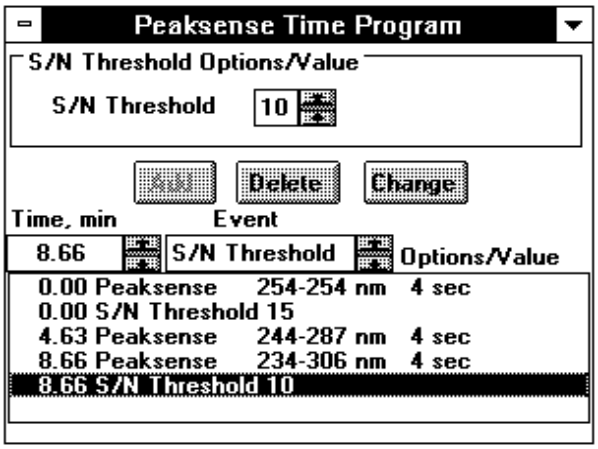


Figure 3. Peak Detection Program

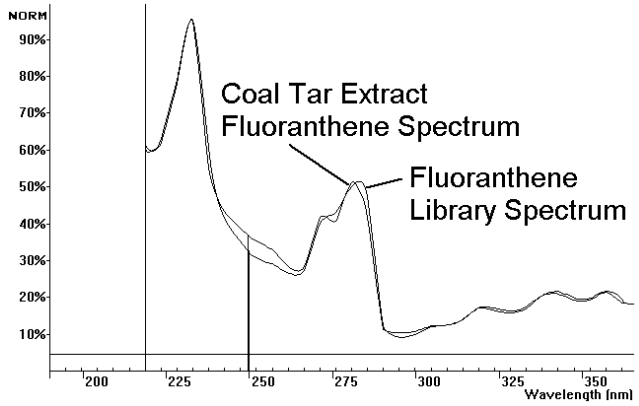
Scan Rate:10.851 Hz Bunch:4 Data Rate:2.713 Hz
Search Parameters:PuP Interval: ±2.00 nm, Wavelength Range: 220-339
Data File Time Range:3.800 - 10.600 min
Library Relative Time Range:0.950 - 1.050
Minimum Peak Height:10.000

Target Apex (min)	Best Possible Match	Sim	Dissim	t_R (min)	Lib	Entry #
4.123	Naphthalene	0.99995	0.00995	4.190	B	16
6.949	Phenanthrene	0.99990	0.01396	6.930	B	4
7.452	Anthracene	0.99986	0.01656	7.416	B	5
8.067	Fluoranthene	0.99986	0.01702	8.005	B	6
8.429	Pyrene	0.99993	0.01218	8.356	B	7
9.664	Benzo(a)anthracene	0.99999	0.00375	9.554	B	8
9.898	Chrysene	0.99846	0.05545	9.775	B	9

Search Parameters:PuP Interval: ±10.00 nm, Wavelength Range: 210-335 nm
Data File Time Range:10.800 - 15.000 min
Library Relative Time Range:0.000 - 2.000
Minimum Peak Height:0.000 mAU

10.905	Benzo(b)fluoranthene	0.99327	0.11580	10.764	B	10
11.305	Benzo(k)fluoranthene	0.99726	0.07400	11.151	B	11
11.661	Benzo(a)pyrene	0.99925	0.03872	11.514	B	12
12.742	Benzo(g,h,i)perylene	0.99503	0.09958	12.552	B	14
13.043	Indeno(1,2,3-c,d)pyrene	0.99705	0.07670	12.810	B	15

Figure 4. Coal Tar Extract - Library Search Report



Purity Parameters

249.48 nm

249.74 nm

Figure 5. Fluoranthene Spectral Overlay