Analysis of Polynuclear Aromatic Hydrocarbons (PAHs) in Water with ZORBAX Eclipse PAH Column

Application

Environmental

Authors

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Abstract

Polynuclear aromatic hydrocarbons (PAHs) are carcinogenic compounds that are commonly found in the environment as a result of incomplete combustion of tobacco, tar, and fuels. Many regulatory methods exist for the analysis of these compounds in all kinds of samples, such as air, water, soil, and food. To analyze the trace PAHs in water, a highly sensitive method is needed. This work describes a total HPLC methodology for PAH analysis in water using recently developed ZORBAX Eclipse PAH columns and the AccuBond ODS C18 Solid Phase Extraction (SPE) cartridge for extracting the water. Eclipse PAH's optimized C18 bonded phase delivers a high level of reproducibility, making it ideal for routine analysis.

Introduction

Polynuclear aromatic hydrocarbons (PAHs) are ubiquitous in the environment. Both naturally occurring and manmade PAHs appear to have similar origins. More recent work has demonstrated the carcinogenic, mutagenic, and teratogenic behavior of many of the PAHs. In view of this, PAHs are considered compounds of concern by every environmental organization, and to protect human health, their concentration in water is strictly regulated [1].

The term "polycyclic aromatic hydrocarbons" is also used to refer to these compounds. Now the abbreviation PAHs has been widely used for this class of compounds. The PAHs selected for control are those of the minimal list of 16 PAHs designated as "priority pollutants" and regulated by the U.S. Environmental Protection Agency (EPA), which requires that the 16 compounds be quantified in waste water (610[2]), drinking water (550.1[3]), and solid waste (8310[4]), not only identified.

Another regulatory method issued by the National Institute for Occupational Safety and Health (NIOSH) in the U.S. includes 17 PAHs in its method 5506[5]. Table 1 lists the 17 compounds and some of their properties.

Table 1[5]. Formulas and Physical Properties of NIOSH Method 5506 PAHs

	Compound (by M.W.)	Formula	Weight	Melting point (°C)	Boiling point (°C)	Aqueous solubility mg/L (25 °C)
1	Naphthalene	$C_{10}H_8$	128.17	80.2	218	30
2	Acenaphthylene	$C_{12}H_8$	152.20	92.5	280	3.9
3	Acenaphthene	$C_{12}H_{10}$	154.21	93.4	279	3.9
4	Fluorene	$C_{13}H_{10}$	166.22	115	295	2
5	Anthracene	$C_{14}H_{10}$	178.23	215	340	0.07
6	Phenanthrene	$C_{14}H_{10}$	178.23	99.2	340	1.2
7	Fluoranthene	$C_{16}H_{10}$	202.26	108	384	0.26
8	Pyrene	$C_{16}H_{10}$	202.26	151	404	0.13
9	Benz[a]anthracene	$C_{18}H_{12}$	228.29	167	435	0.01
10	Chrysene	$C_{18}H_{12}$	228.29	258	448	0.002
11	Benzo[b]fluoranthene	$C_{20}H_{12}$	252.32	168	–	0.014
12	Benzo[k]fluoranthene	$C_{20}H_{12}$	252.32	217	480	0.008
13	Benzo[a]pyrene	$C_{20}H_{12}$	252.32	177	495	0.004
14	Benzo[e]pyrene	$C_{20}H_{12}$	252.32	178	311	–
15	Benzo[ghi]perylene	$C_{22}H_{12}$	276.34	278	–	–
16	Indeno[1,2,3-cd]pyrene	$C_{22}H_{12}$	276.34	164	–	0.0005
17	Dibenz[a,h]anthracene	$C_{22}H_{14}$	278.35	270	524	0.00026

– Not Available

Experimental

Instrument

The results obtained in this application were all performed on an Agilent 1200SL Rapid Resolution HPLC system consisting of the following components:

G1312B	Binary pump SL
G1379B	Micro degasser
G1367C	High-performance well plate autosampler (WPS) configured with 54 × 2 mL sample tray
G1316B	Thermostatted column compartment SL (TCC)
G1315C	Diode array detector SL (DAD) with standard flow cell
G1321A	Fluorescence detector (FLD)

All tubing used throughout the instrument is 0.17 mm id.

Other instruments, such as 1100 binary systems and possibly 1100 and 1200 quaternary pump-based systems, might also run this method, though these were not evaluated here. With other instruments, slight adjustments in the gradient may be required.

HPLC Conditions

Column: ZORBAX Eclipse PAH, 4.6 mm × 150 mm, 3.5 µm (Agilent p/n 959963-918)

Mobile phase: A Water; B Acetonitrile

Gradient timetable:

Time (min)	%B
0.0	50
2.0	50
22.0	100

Stop time 28 min, Post run 5 min.

Flow: 1.5 mL/min

DAD: PW 0.1 min; slit 4 nM; Standard Flow Cell

Signal A: 254, 4 nm; Ref off.

FLD:

Timetable: PMT=12

Time (min)	Ex(nm)	Em(nm)
0	220	330
7	210	330
9.6	250	363
10.7	250	405
12	250	460
13	270	400
20.2	270	415
24.3	250	490

Standard solution

PAH standards of the EPA 610 mixture and a single standard of benzo[e]pyrene were obtained from SUPELCO (Bellefonte, PA, USA). Prepare the stock solution by mixing 0.9 mL EPA 610 mix together with 0.1 mL 1.25 mg/mL benzo(e)pyrene. Table 2 shows the concentrations of the stock

PAHs solution. The calibration standards were prepared through dilution of the stock solution with acetonitrile. Five points were used to make the calibration curve.

Table 2. Concentration of PAH Standards in Stock Solvents

	Compound (by M.W.)	Concentration (µg/mL)
1	Naphthalene	900
2	Acenaphthylene	1800
3	Acenaphthene	900
4	Fluorene	180.1
5	Anthracene	90.2
6	Phenanthrene	90.1
7	Fluoranthene	180
8	Pyrene	89.9
9	Benz[a]anthracene	89.9
10	Chrysene	90.2
11	Benzo[b]fluoranthene	180
12	Benzo[k]fluoranthene	89.9
13	Benzo[a]pyrene	90.1
14	Benzo[e]pyrene	125
15	Benzo[ghi]perylene	179.9
16	Indeno[1,2,3-cd]pyrene	90.1
17	Dibenz[a,h]anthracene	179.9

Sample Preparation

To analyze the trace levels of PAHs in water, 1 L water was extracted using an AccuBond ODS C18 SPE cartridge (Agilent p/n 188-1356). The cartridge (0.5 g) was conditioned by sequentially rinsing with four 10-mL aliquots of dichloromethane (DCM), methanol, and two 10-mL aliquots of water (HPLC grade).

The 1-L water sample was then pulled through the SPE cartridge at a flow rate of 2.5 mL/min using an automated solid phase extractor. Then the cartridge was washed with 10 mL of HPLC water. The SPE cartridge was then dried by drawing nitrogen for about 10 min. PAH sample was then eluted from the cartridge with two 5-mL portions of DCM and added together. The eluate was evaporated with a stream of N_2 to a volume of 1 mL, and 3.0 mL of acetonitrile was added and concentrated to a final volume of 1.0 mL [3].

Results and Discussion

Separation Options

In this application we demonstrate that all 17 PAHs can be baseline separated on a ZORBAX Eclipse PAH column. The chromatograms for the 17 PAHs obtained on this column are shown in Figures 1 and 2 for DAD and FLD respectively. The mixture includes five groups of geometric isomers (first is 5 and 6; second is 7 and 8; third is 9 and 10; fourth is 11, 12, 13, and 14; fifth is 16 and 17). Some of them cannot be separated or baseline resolved on other reversed phase PAH columns. The Eclipse PAH column is a unique bonded phase column designed specially for separating these isomers. Resolution factors of the most closely spaced peaks, such as peaks 3 and 4 and peaks 11 and 12 are all larger than 2.0.

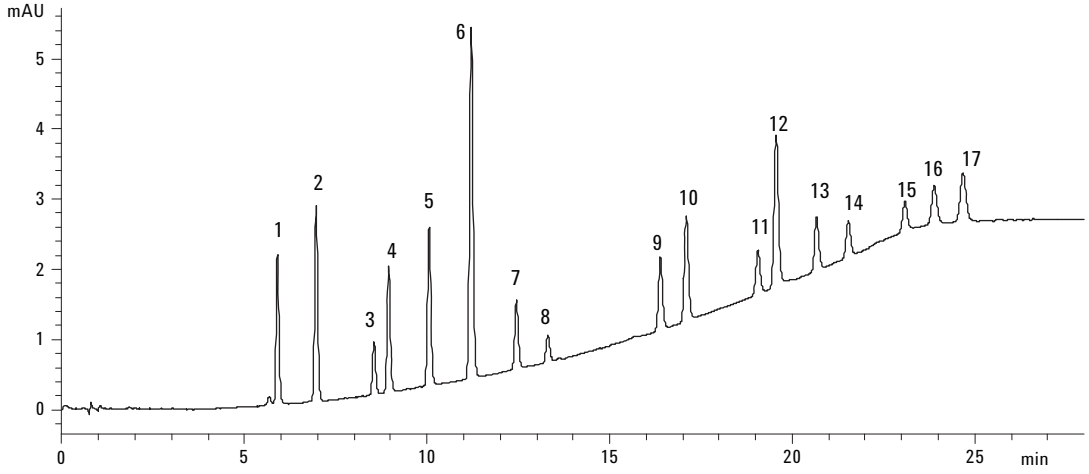

Figure 1. Seventeen PAHs separated on Eclipse PAH, 4.6 mm × 150 mm, 3.5 µm (p/n 959963-918), DAD-254 nm.

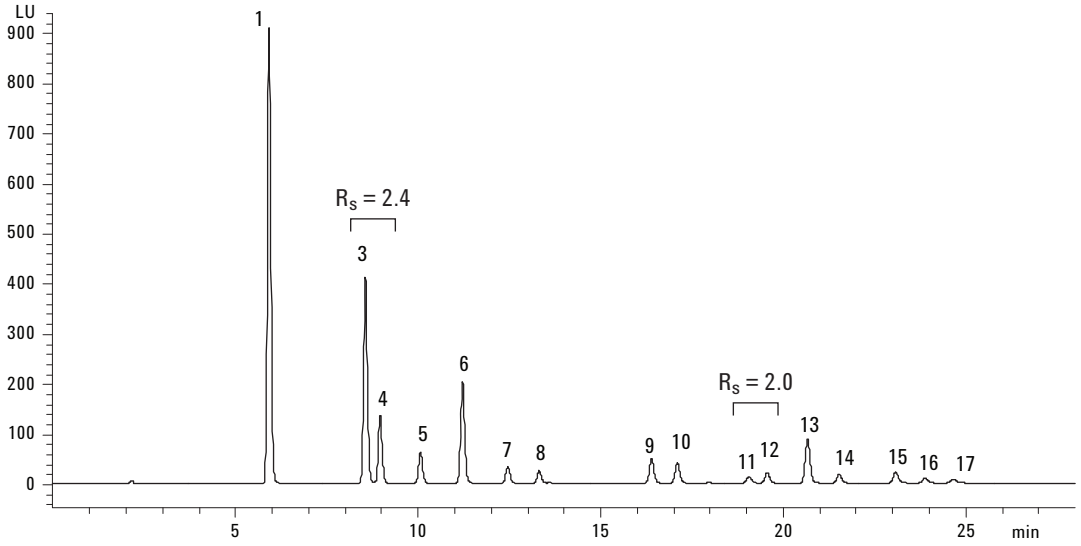

Figure 2. Seventeen PAHs separated on Eclipse PAH, 4.6 mm × 150 mm, 3.5 µm (p/n 959963-918), FLD program.

In this method, we use both the DAD and FLD as detectors. Fluorescence detection is recommended for trace analysis of PAHs with maximum sensitivity. All the PAHs have their own optimum excitation and emission wavelengths. To obtain the highest sensitivity, a FLD program that gave a low picogram limit of detection (LOD) was used. Compared to UV absorbance, the analyte signal-to-noise ratio is about 100-fold better, and the LOD therefore a factor of about 100 lower. The data of LOD by FLD and DAD, which were calculated with a signal-to-noise ratio of 3, are shown in Table 3.

Table 3. Theoretical LOD of All 17 PAHs by FLD and DAD (S/N = 3)

	Compound (by eluted order)	LOD (FLD) (pg)	LOD (DAD) (pg)
1	Naphthalene	1.93	196.6
2	Acenaphthylene	–	521.1
3	Acenaphthene	2.64	936.3
4	Fluorene	2.13	774.5
5	Phenanthrene	0.54	31.5
6	Anthracene	0.14	16.0
7	Fluoranthene	0.62	150.0
8	Pyrene	0.24	189.8
9	Benzo(a)anthracene	0.69	56.5
10	Chrysene	0.80	41.9
11	Benzo(e)pyrene	2.18	133.2
12	Benzo(b)fluoranthene	2.35	55.8
13	Benzo(k)fluoranthene	0.17	72.6
14	Benzo(a)pyrene	0.38	117.0
15	Dibenzo(a,h)anthracene	2.11	202.1
16	Benzo(g,h,i)perylene	1.89	206.5
17	Indeno(1,2,3-c,d)pyrene	1.69	87.0

For highly contaminated samples UV-visible absorbance diode-array detection offers additional analytical tools such as peak-identity and peak purity confirmation. In addition, acenaphthylene does not fluoresce, so for this compound UV detection is needed. UV-254 nm is used in many regulatory methods for all the PAHs, but it is not the ideal wavelength for all the compounds. In some papers, a DAD wavelength switching program was used to get optimized signal-to-noise response [6].

Reproducibility and Linearity

Stable retention times (RTs) are important for correct identification of analytes in complex environmental matrices. When using a time-programmable fluorescence detector, stable RTs are also very important to set wavelength switching during an analysis. Precision of peak areas is important for obtaining reliable quantitative data.

Table 4 demonstrates typical RT precision of better than 0.1 % obtained over 10 PAH runs using an Eclipse PAH column. Peak area precision by FLD and DAD is below 2% relative standard deviation (RSD).

The quality of the Eclipse PAH column as well as the reliability of the 1200 SL instrument provides these high-quality results. The new Eclipse PAH columns are based on the same silica used in other Eclipse Plus columns. This is a new high-purity silica treated for the best peak shape and delivering the highest efficiencies and reproducibility for all sample types, including the PAH samples.

Table 4. Peak Area Reproducibility and Retention Time Reproducibility on Eclipse PAH, 4.6 mm × 150 mm, 3.5-µm Column

	Compound (by eluted order)	DAD area RSD (%) (N = 10)	FLD area RSD (%) (N = 10)	DAD RT RSD (%) (N = 10)	FLD RT RSD (%) (N = 10)
1	Naphthalene	0.39	0.70	0.04	0.07
2	Acenaphthylene	0.44	–	0.06	–
3	Acenaphthene	0.56	0.84	0.05	0.05
4	Fluorene	0.61	0.77	0.05	0.05
5	Phenanthrene	0.47	0.45	0.04	0.11
6	Anthracene	0.57	0.27	0.04	0.04
7	Fluoranthene	0.67	0.45	0.03	0.04
8	Pyrene	1.44	0.27	0.03	0.02
9	Benzo(a)anthracene	0.48	0.36	0.09	0.02
10	Chrysene	0.41	0.30	0.02	0.02
11	Benzo(e)pyrene	1.15	0.29	0.02	0.02
12	Benzo(b)fluoranthene	0.42	0.41	0.01	0.02
13	Benzo(k)fluoranthene	0.74	0.47	0.01	0.02
14	Benzo(a)pyrene	1.41	0.98	0.009	0.02
15	Dibenzo(a,h)anthracene	1.02	0.39	0.007	0.01
16	Benzo(g,h,i)perylene	1.12	0.89	0.01	0.01
17	Indeno(1,2,3-c,d)pyrene	0.75	0.39	0.008	0.01

Note that the coefficients of linearity for both by DAD and FLD shown in Table 5 are also excellent for all the PAHs, being very close to 1.00. Figure 3 shows the linearity plot for benzo(a)pyrene, a range of 0.011 ng to 0.18 ng gave correlation coefficients of 0.99990, which gives a good linearity and range for low level PAH quantification in drinking water.

Table 5. Coefficients of Linearity on Eclipse PAH Column by DAD and FLD

	Compound (by eluted order)	DAD coefficients of linearity (r2)	Range of linearity (ng)	FLD coefficients of linearity (r2)	Range of linearity (ng)
1	Naphthalene	0.9999	1.76 – 56.25	0.9999	0.11-1.76
2	Acenaphthylene	0.9999	3.52 – 112.5	–	–
3	Acenaphthene	0.9999	1.76 – 56.25	0.9998	0.22 – 3.52
4	Fluorene	0.9999	3.52 – 112.5	0.9993	0.11 – 1.76
5	Phenanthrene	0.9999	0.18 – 5.63	0.9998	0.22 – 3.52
6	Anthracene	0.9999	0.18 – 5.64	0.9998	0.011 – 0.176
7	Fluoranthene	0.9999	0.35 – 11.25	0.9999	0.011 – 0.176
8	Pyrene	0.9990	0.35 – 11.25	0.9999	0.022 – 0.352
9	Benzo(a)anthracene	0.9998	0.18 – 5.62	0.9999	0.011 – 0.176
10	Chrysene	0.9999	0.18 – 5.64	0.9999	0.011 – 0.176
11	Benzo(e)pyrene	0.9993	0.49 – 15.62	0.9999	0.011 – 0.176
12	Benzo(b)fluoranthene	0.9999	0.35 – 11.25	0.9999	0.015 – 0.244
13	Benzo(k)fluoranthene	0.9994	0.35 – 11.25	0.9999	0.022 – 0.352
14	Benzo(a)pyrene	0.9994	1.41 – 45.0	0.9999	0.011 – 0.176
15	Dibenzo(a,h)anthracene	0.9993	0.70 – 22.5	0.9999	0.022 – 0.352
16	Benzo(g,h,i)perylene	0.9990	0.70 – 22.5	0.9996	0.022 – 0.352
17	Indeno(1,2,3-c,d)pyrene	0.9994	0.35 – 11.25	0.9996	0.011 – 0.176

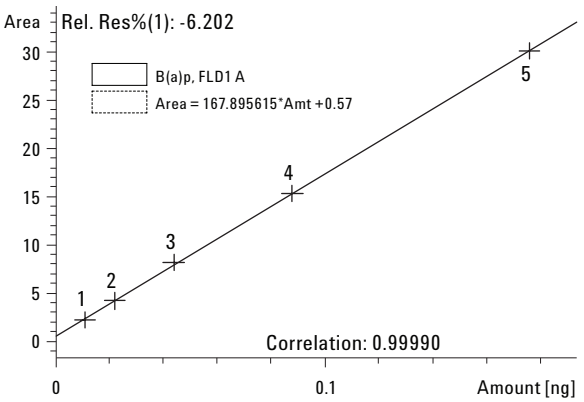

Figure 3. Linearity plot for benzo(a)pyrene by FLD program.

Recovery

Table 6 shows recovery results based on a solution spiked with 10 µl of the stock solution in 1 L HPLC water. The recovery samples were made according to the procedure described under Sample Preparation. Considering the low solubility of PAHs in water, especially the later eluting compounds, an organic solvent modifier, such as methanol, isopropanol, tetrahydrofuran, or a mixture of these, is commonly used in STD-spiked water In this method, 20% methanol was used as an organic solvent modifier. The recovery data were better than 85% for compounds 2, 3, 4, 5, 6, and 7, but not as high for compounds 1, 10, 11, 12, 13, 15, 16, and 17, which were between 35 and 49%. The compound naphthalene is volatile, which leads to the lower recovery when concentrating by a flow of nitrogen and the lower recovery of the later-eluted compounds due to its low solubility, even with 20% methanol added to the solution at the spiked concentration.

To enhance the recovery of volatile naphthalene, a tube heater concentration technique was used to condense and solvent exchange the ODS-C18 DCM eluant into acetonitrile [1].

Real samples

The chromatograms of a variety of water samples, including reagent water, drinking water, tap water, and river water, are shown in Figure 4. Each water sample was spiked with 40 µL of a 1/640 stock solution at a level of 0.225 ppb for benzo(a)pyrene. All the spiked standards were detected and well separated. In the previous study, we used a shorter gradient time of 18 min and all the standards were also baseline separated, but there was an interfering peak between 13 and 14. To separate this interfering peak, the gradient time was increased to 22 min. During our study, no target PAHs were found under this condition in the HPLC purity water, so this water was regarded as blank water. The top chromatogram is reagent water (HPLC purity) spiked with standards. We found trace naphthalene in some tap water samples. In some river water samples, the compounds naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, and benzo(a)anthracene were detected at low levels by FLD.

Table 6. Recovery Data for All 17 PAHs (n = 3)

	Compound (by eluted order)	FLD recovery (%)	RSD (%)	DAD recovery (%)	RSD (%)
1	Naphthalene	42.6	1.48	44.0	5.9
2	Acenaphthylene	–	–	97.1	5.3
3	Acenaphthene	97.2	4.38	95.7	4.4
4	Fluorene	100.6	5.5	100.4	6.1
5	Phenanthrene	95.4	5.11	101.6	5.6
6	Anthracene	88.3	3.73	90.9	4.7
7	Fluoranthene	94.1	3.60	92.4	4.5
8	Pyrene	88.4	4.30	83.5	2.4
9	Benzo(a)anthracene	61.6	1.74	60.3	5.8
10	Chrysene	48.0	2.99	48.9	6.1
11	Benzo(e)pyrene	40.1	4.97	40.4	12.8
12	Benzo(b)fluoranthene	48.7	2.06	48.8	3.4
13	Benzo(k)fluoranthene	42.0	3.81	45.8	1.7
14	Benzo(a)pyrene	58.8	3.59	57.6	10.0
15	Dibenzo(a,h)anthracene	39.1	6.36	35.2	11.1
16	Benzo(g,h,i)perylene	39.2	2.34	45.6	4.8
17	Indeno(1,2,3-c,d)pyrene	46.7	1.57	39.8	6.8

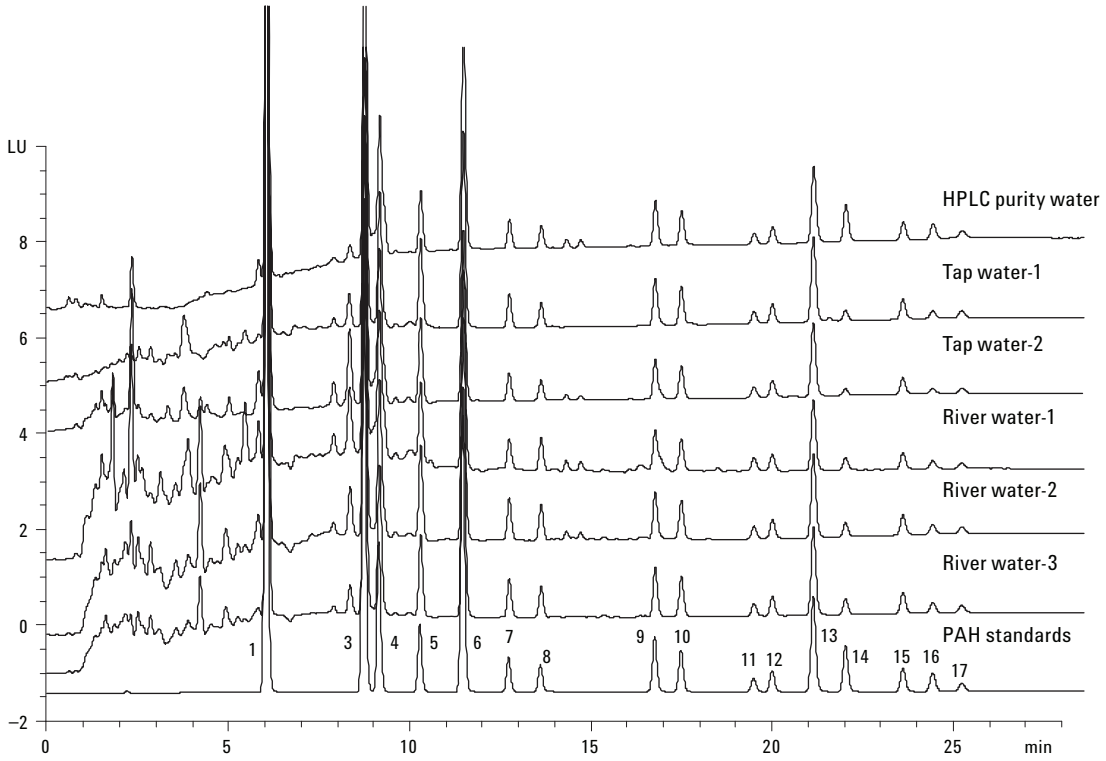

Figure 4. Variety of water samples spiked with PAH standards on Eclipse PAH column.

Conclusions

Using the new ZORBAX Eclipse PAH column, all 17 PAHs are baseline separated and resolution factors of the most closely spaced analytes are larger than 2.0. In the river and drinking water samples, all spiked PAHs can be separated from interfering substance. The linearity is excellent; the average peak area reproducibility is below 1.5% and the average retention time reproducibility is below 0.1%. The recovery is also good using AccuBond ODS C18. Better results can likely be achieved by further optimizing the sample preparation process, but we didn't study that in this paper. An FLD program was used to achieve low picogram LOD. The FLD settings in this application gave the best signal-to-noise ratio for all PAHs except acenaphthylene, which has no fluorescence.

Performance of the Eclipse PAH column, including high efficiency and unique selectivity for the PAHs, contributed to the high sensitivity of trace PAH analysis in real water samples.

References

1. Harry Prest, "Solid-Phase Extraction and Retention-Time Locked GC/MS Analysis of Selected Polycyclic Aromatic Hydrocarbons (PAHs)" Agilent Technologies publication, 5988-7150EN (2002).
2. United States Environmental Protection Agency, "Determination of Polycyclic Aromatic Hydrocarbons in Municipal and Industrial Wastewater," *Method 610*, **1982**, EPA Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, Ohio 45268.
3. United States Environmental Protection Agency, "Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Solid Extration and HPLC with Coupled Ultraviolet and Fluorescence Detection," *Method 550.1*, **1990**, EPA Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, Ohio 45268.

4. United States Environmental Protection Agency, "Determination of Polycyclic Aromatic Hydrocarbons in Ground Water and Wastes," *Method 8310*, **1986**, EPA Environmental Monitoring Systems Laboratory, Office of Research and Development, Cincinnati, Ohio 45268.

5. National Institute for Occupational Safety and Health Manual of Analytical Methods (NMAM), Fourth Edition, Polynuclear Aromatic Hydrocarbons by HPLC, *Method 5506*, Issue 3, 1998, Washington DC 20201.

6. Sun, Determination of 16 Polycyclic Aromatic Hydrocarbons by Liquid Chromatography with Solid Phase Extraction (II), *J. of University of Science and Technology of Suzhou*, 19(4): 43-48 (2006).

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