

Sensitive and Rapid Determination of Polycyclic Aromatic Hydrocarbons in Tap Water

Chen Jing,¹ Dai Zhenyu,¹ Xu Qun,¹ Liang Lina,¹ and Jeffrey Rohrer²

¹Thermo Fisher Scientific, Shanghai, People's Republic of China;

²Thermo Fisher Scientific, Sunnyvale, CA, USA

Key Words

Hypersil Green PAH Column, Acclaim PA2 Column, HPLC, On-Line SPE, Water Analysis

Goal

To develop an efficient high-performance liquid chromatography (HPLC) method for sensitive and rapid determination of 20 polycyclic aromatic hydrocarbons (PAHs) in environmental waters using on-line solid-phase extraction (SPE) for sample preparation instead of the liquid-liquid extraction and off-line SPE specified in the U.S. Environmental Protection Agency (EPA) Methods 550 and 550.1, respectively

Introduction

Polycyclic aromatic hydrocarbons are a group of chemicals formed from the incomplete combustion of organic matter. Due to their potential carcinogenic and mutagenic properties, most countries have regulations limiting the concentrations of a variety of PAHs in drinking water, food additives, cosmetics, workplaces, and factory emissions.

Dionex (now part of Thermo Scientific) Application Notes (ANs) 196 and 213 provide on-line SPE HPLC methods to quantify low concentrations of PAHs in oil and water samples, respectively.^{1,2} The costs for the SPE cartridge, labor, time, and reagents are significantly reduced using these methods, and the results are more consistent. This is because on-line SPE eliminates manual processes such as rotary evaporation and nitrogen-assisted evaporation in the routine liquid-liquid extraction and off-line SPE steps described in EPA Methods 550, 550.1, and 610.³⁻⁵ However, because the run times of the two reported on-line SPE HPLC methods both exceed 60 min in this study, the analytical column used in the aforementioned ANs was replaced to shorten analysis time.

Equipment, Software, and Consumables

- Thermo Scientific™ Dionex™ UltiMate™ 3000 Dual Rapid Separation LC (RSLC) system, including:
 - DGP-3600RS Dual Gradient Rapid Separation Pump (P/N 5040.0066)
 - SRD-3600 Integrated Solvent and Degasser Rack (P/N 5035.9230)



- WPS-3000TRS Rapid Separation Wellplate Sampler, Thermostatted (P/N 5840.0020) with a 1000 μ L sample loop (P/N 6820.2429) and a 1000 μ L syringe (P/N 6822.0005)
- TCC-3000SD Standard (P/N 5730.0010) or TCC-3000RS Rapid Separation (P/N 5730.0000) Thermostatted Column Compartment, equipped with one 2–6p valve
- DAD-3000RS Rapid Separation Diode Array Detector (P/N 5082.0020)
- FLD-3400RS Rapid Separation Fluorescence Detector (P/N 5078.0025)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.1 or above
- Thermo Scientific™ Target2™ Nylon Syringe Filters, 0.45 μ m, 30 mm (P/N F2500-1)

Reagents and Standards

- Deionized (DI) water, 18.2 M Ω -cm resistivity
- Methanol (CH₃OH), 99.8%, HPLC Grade (Fisher Scientific P/N AC610090040)
- Acetonitrile (CH₃CN), HPLC Grade (Fisher Scientific P/N AC610010040)
- EPA 610 PAH Solution (1000 mg/L for each, in methylene chloride) containing 18 PAH standards: benzo[k]fluoranthene, acenaphthene, acenaphthylene, anthracene, fluorene, naphthalene, phenanthrene, benzo[a]anthracene, benzo[a]pyrene, chrysene, fluoranthene, indeno[1,2,3-cd]pyrene, pyrene, benzo[b]fluoranthene, benzo[ghi]perylene, dibenz[a,h]anthracene, benzo[e]pyrene, and benzo(j)fluoranthene (O2si Smart Solutions P/N 110064-01-5PAK)
- 1-Methylnaphthalene (AccuStandard P/N H-001N)
- 2-Methylnaphthalene (AccuStandard P/N H-002N)

Conditions (Applicable to Figures 1, 2, and 6)

On-Line SPE

Column:	Thermo Scientific™ Acclaim™ PolarAdvantage II (PA2), 3 μ m Analytical, 4.6 \times 50 mm (P/N 063189)
Mobile Phase:	A. Water B. Acetonitrile
Gradient:	Table 1
Flow Rate:	0.4 and 0.6 mL/min (Table 1)
Inj. Volume	1 mL on the On-Line SPE column

Separation

Column:	Thermo Scientific™ Hypersil™ Green PAH Column, 3 μ m, 3.0 \times 150 mm (P/N 31105-153030)
Mobile Phase:	A. Water B. Acetonitrile
Gradient:	Table 1
Flow Rate:	0.8 mL/min
Temperature:	30 $^{\circ}$ C
Detection:	UV, 254 nm; Fluorescence at different excitation and emission (Ex/Em) wavelengths for each PAH (Table 2)

Note: The PAHs have good fluorescent responses, except for acenaphthylene (Figure 1, Peak 2). Because their maximum fluorescent responses occur at different Ex/Em wavelengths, it is necessary to change the Ex/Em wavelengths to acquire the best detection sensitivity. These changes are dictated by the individual PAH retention times. Table 2 shows the program for wavelength changes. Although naphthalene (Figure 1, Peak 1) has fluorescent response, EPA method 550.1 requires it to be determined using UV detection, together with acenaphthylene (Figure 1, Peak 2). Figures 1 and 2 show the chromatograms of all 20 PAHs with UV and fluorescence detection, respectively, under the conditions specified above.

Peaks:

- | | |
|------------------------|----------------------------|
| 1. Naphthalene | 11. Benzo(a)anthracene |
| 2. Acenaphthylene | 12. Chrysene |
| 3. 1-Methylnaphthalene | 13. Benzo(j)fluoranthene |
| 4. 2-Methylnaphthalene | 14. Benzo(e)pyrene |
| 5. Acenaphthene | 15. Benzo(b)fluoranthene |
| 6. Fluorene | 16. Benzo(a)pyrene |
| 7. Phenanthrene | 17. Benzo(k)fluoranthene |
| 8. Anthracene | 18. Dibenz(a,h)anthracene |
| 9. Fluoranthene | 19. Benzo(ghi)perylene |
| 10. Pyrene | 20. Indeno(1,2,3-cd)pyrene |

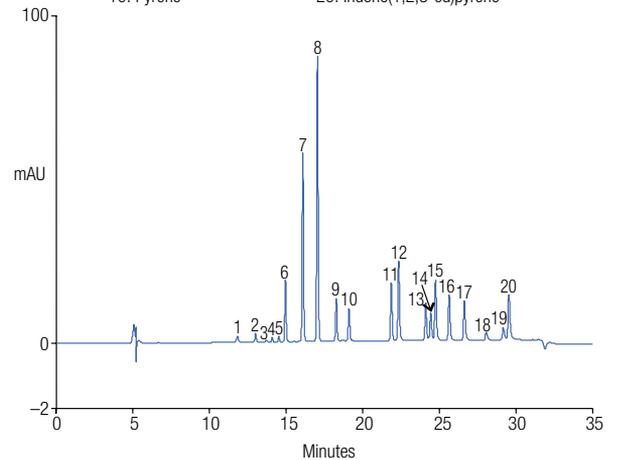


Figure 1. All 20 PAHs (50 μ g/L for each PAH) detected at UV 254 nm.

Peaks:

- | | |
|------------------------|----------------------------|
| 1. Naphthalene | 11. Benzo(a)anthracene |
| 2. Acenaphthylene | 12. Chrysene |
| 3. 1-Methylnaphthalene | 13. Benzo(j)fluoranthene |
| 4. 2-Methylnaphthalene | 14. Benzo(e)pyrene |
| 5. Acenaphthene | 15. Benzo(b)fluoranthene |
| 6. Fluorene | 16. Benzo(a)pyrene |
| 7. Phenanthrene | 17. Benzo(k)fluoranthene |
| 8. Anthracene | 18. Dibenz(a,h)anthracene |
| 9. Fluoranthene | 19. Benzo(ghi)perylene |
| 10. Pyrene | 20. Indeno(1,2,3-cd)pyrene |

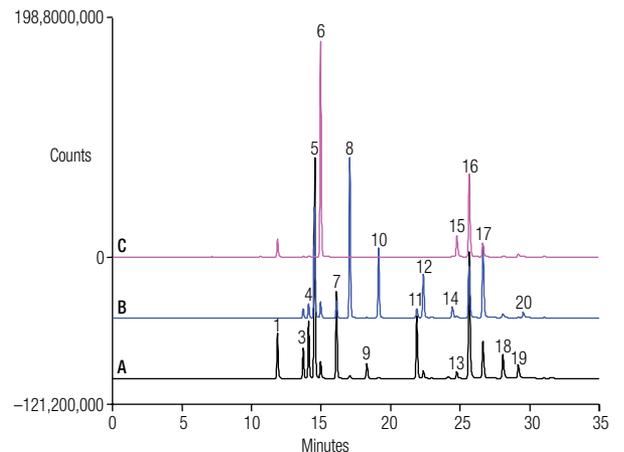


Figure 2. All 20 PAHs (50 μ g/L for each PAH) detected by fluorescence detection using programmed wavelength switching in three parallel channels: (A) Emission_1, (B) Emission_2, (C) Emission_3. Note: Acenaphthylene (Peak 2) is not shown here because there was no fluorescent response for acenaphthylene.

Table 1. Gradient programs for loading and analytical pumps.

Loading Pump				Analytical Pump			
Time (min)	Flow Rate (mL/min)	% A (H ₂ O)	% B (CH ₃ CN)	Time (min)	Flow Rate (mL/min)	% A (H ₂ O)	% B (CH ₃ CN)
0	0.6	95	5	0	0.8	60	40
4.0	0.6	95	5	5	0.8	60	40
4.5	0.4	0	100	30	0.8	0	100
25	0.4	0	100	30.5	0.8	60	40
25.5	0.6	95	5	35	0.8	60	40
35	0.6	95	5	—	—	—	—

Table 2. Ex/Em maximums for each PAH and programmed wavelength switching times.

Time (min)	Fluorescence Detection Channel	Ex/Em Wavelengths (nm)	PAH	Peak No.
0.0	Emission_1	219/330	Naphthalene	1
13.45	Emission_1	225/333	1-Methylnaphthalene	3
			2-Methylnaphthalene	4
	Emission_2	235/332	Acenaphthene	5
15.50	Emission_3	263/310	Fluorene	6
	Emission_1	247/364	Phenanthrene	7
17.80	Emission_2	247/401	Anthracene	8
	Emission_1	281/453	Fluoranthene	9
20.50	Emission_2	236/389	Pyrene	10
	Emission_1	281/391	Benzo(a)anthracene	11
23.50	Emission_2	264/381	Chrysene	12
	Emission_1	240/510	Benzo(j)fluoranthene	13
	Emission_2	283/394	Benzo(e)pyrene	14
25.40	Emission_3	249/443	Benzo(b)fluoranthene	15
	Emission_1	243/412	Benzo(k)fluoranthene	16
27.50	Emission_2	260/408	Benzo(a)pyrene	17
	Emission_1	290/398	Dibenz(a,h)anthracene	18
28.70	Emission_1	292/415	Benzo(ghi)perylene	19
	Emission_2	246/503	Indeno(1,2,3-cd)pyrene	20

Preparation of Standard Solutions

In addition to the 16 PAHs specified in EPA Methods 550, 550.1, and 610 (Figure 3), the target analytes in the experiments described here include four other PAHs: benzo[e]pyrene, benzo(j)fluoranthene, 1-methylnaphthalene, and 2-methylnaphthalene (Figure 4). The EPA 610 PAH Solution product contains benzo[e]pyrene and benzo(j)fluoranthene, in addition to the 16 specified PAHs.

Stock Solutions of 1-Methylnaphthalene and 2-Methylnaphthalene

In a 100 mL volumetric flask, dissolve 100 mg of 1-methylnaphthalene in 2 mL of acetonitrile and dilute to the mark with methanol. The final concentration of 1-methylnaphthalene will be 1000 mg/L. Prepare a 1000 mg/L stock solution of 2-methylnaphthalene in the same manner.

Stock Standard Mixes 1 and 2

Add 100 μ L of EPA 610 PAH Solution (containing 18 PAH standards, 1000 mg/L each), 100 μ L of 1-Methylnaphthalene Stock Solution, and 100 μ L of 2-Methylnaphthalene Stock Solution to a 10 mL volumetric flask, then dilute to the mark with methanol. The final concentration of each PAH will be 10 mg/L. This is Stock Standard Solution Mix 1.

Add 1 mL of Stock Standard Mix 1 to a 10 mL volumetric flask and dilute to the mark with methanol. This is Stock Standard Solution Mix 2. The final concentration of each PAH in Stock Standard Mix 2 will be 1.0 mg/L.

Use these mixed standard stock solutions to prepare working mixed standard solutions for calibration.

Working Mixed Standard Solutions for Calibration

For calibration, prepare nine working standard solutions with different concentrations by diluting the proper amount of either Stock Standard Mix 1 or 2 with DI water. The volumes of each solution needed to make the calibration standards are shown in Table 3. The calibration standards with concentrations of 100 and 10 μ g/L are also used as stock standards for the preparation of the working mixed standard solutions with lower concentrations.

Table 3. Preparation of calibration standards.

Stock Standard of PAHs Calibration Mixture	Volume of Stock Standard of PAHs Calibration Mixture (μ L)	Volume of Water (mL)	Final Volume of Calibration Standard (mL)	Final Conc of Calibration Standard (μ g/L)
Stock Standard Mix 1 (10 mg/L)	100	9.9	10.0	100
	50	9.95		50
Stock Standard Mix 2 (1 mg/L)	100	9.9		10
	50	9.95		5.0
Calibration Standard with Conc of 100 μ g/L	100	9.9		1.0
	50	9.95		0.5
Calibration Standard with Conc of 10 μ g/L	100	9.9		0.1
	50	9.95		0.05
	10	9.99		0.01

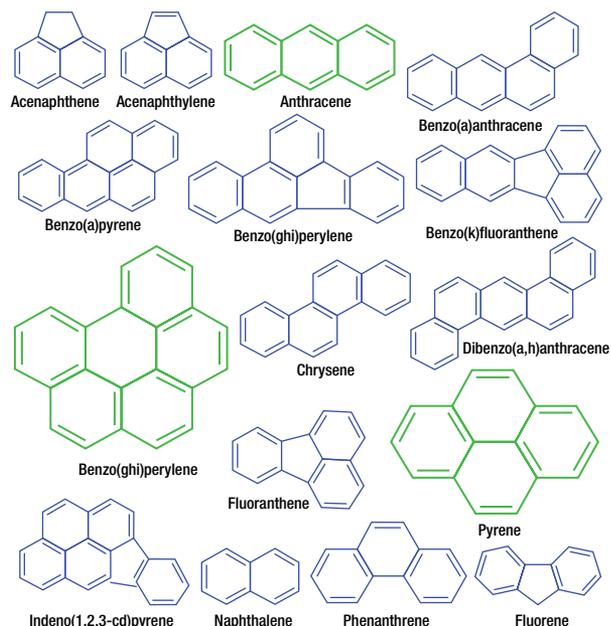


Figure 3. Structures of the 16 PAHs specified in EPA Methods 550, 550.1, and 610.

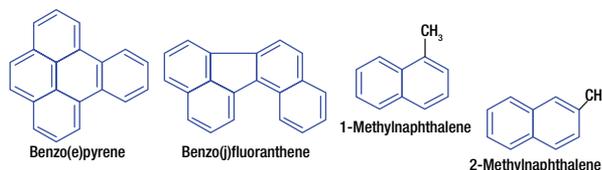


Figure 4. Structures of four additional PAHs not specified in EPA Methods 550, 550.1, and 610.

Preparation of Water Samples

Tap water samples were collected at the Thermo Scientific Shanghai HPLC Applications Laboratory located in the Pudong District, Shanghai, People's Republic of China.

Filter water samples using nylon syringe filters prior to injection.

Add 200 μL of Stock Standard Mix 2 (1.0 mg/L of each PAH) and 39.8 mL of each filtered water sample to a conical flask with plug. The concentration of each PAH in the spiked water sample will be 5 $\mu\text{g/L}$.

Add 200 μL of the calibration standard with a concentration of 10 $\mu\text{g/L}$ of each PAH and 39.8 mL of each filtered water sample to a conical flask with plug. The concentration of each PAH in the spiked water sample will be 0.05 $\mu\text{g/L}$.

Results and Discussion

Evaluation of On-Line SPE

Figure 5 shows a typical flow schematic of an on-line SPE system that is directly coupled to the HPLC column using one 6-port (2–6p) valve. The filtered sample is directly injected onto the system and delivered to the SPE column for enrichment (1_2 position) using one pump of the dual-pump module (labeled For On-Line SPE); the analytical column is simultaneously equilibrated with the second analytical pump (labeled For Separation) of the dual-pump module. After the analytes are bound to the SPE column and impurities are washed out, the SPE column is switched into the analytical flow path to elute the bound analytes (6_1 position); then the analytes are separated on the analytical column and detected by the UV and fluorescence detectors. This method is easily accomplished using an UltiMate 3000 Dual RSLC system.

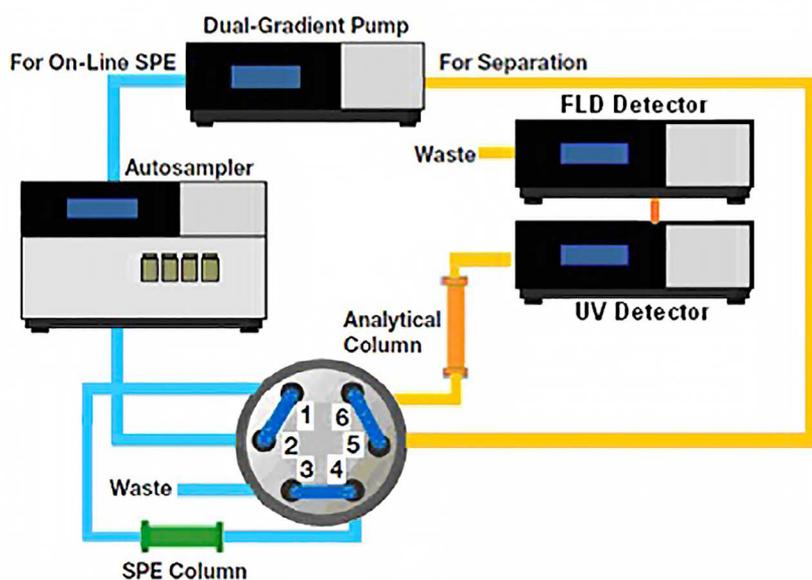


Figure 5. Flow schematic of on-line SPE.

Selection of On-Line SPE and Analytical Columns

As discussed in the previous work (AN 213),² the Acclaim PA2 column is a good choice as a SPE column for on-line SPE preconcentration of a large volume of 100% aqueous sample, which is why it was chosen for this work. The Hypersil Green PAH column that features specially tailored alkyl-bonded silica with high carbon loading was chosen as the analytical column. This column was designed specifically for the separation of PAHs and optimized for the published EPA methods.³⁻⁵ The column resolves benzo[e]pyrene, benzo[j]fluoranthene, and benzo[b]fluoranthene, which have similar structures (Figure 1). As shown in Figures 1 and 2, using the combination of Acclaim PA2 and Hypersil Green PAH columns under the specified conditions, on-line SPE followed by HPLC can be accomplished in 35 min with baseline separation of 20 PAHs.

Determination of Ex/Em Maximums for PAHs

All 20 PAHs except acenaphthylene can be detected using a fluorescence detector, which usually offers much higher sensitivity than UV detection. As previously discussed, each PAH has its own Ex/Em maximum, and thus programmed fluorescence wavelength switching (switching to the Ex/Em maximum wavelength of each individual PAH when the PAH peak passes through the fluorescence detector) is necessary to obtain the best sensitivity for each PAH. To determine the appropriate fluorescence Ex/Em wavelength for each PAH, a PAH standard mixture was injected and the diode array detector used to obtain the maximum of UV absorption (UVmax) of each PAH. This experiment showed that all UVmax of PAHs were close to 220, 240, or 280 nm. So, initially 220, 240, and 280 nm were used as the excitation wavelengths to perform emission scans for each PAH in order to determine its emission maximum. Excitation scans were performed using resultant emission maximums for all PAHs.⁶ The determined Ex/Em maximums for each PAH are shown in Table 2.

Optimization of Detection Parameters

Unfortunately, practical problems—as when two peaks elute close to one another—sometimes prevent switching to the appropriate Ex/Em wavelength for each PAH peak. When wavelength switching is programmed during the elution of a peak or even at the shoulder of a peak, the detector can be saturated and the analysis that follows can be disrupted. To resolve this problem, when two nearby peaks need to use different wavelengths, three parallel fluorescence monitoring channels are used to perform the analysis (one channel for each of the nearby peaks).

For example, as shown in Figure 2, benzo(j)fluoranthene (Peak 13), benzo(e)pyrene (Peak 14), and benzo(b)fluoranthene (Peak 15) elute in a small retention time window; therefore, three parallel fluorescence monitoring channels (Emmissions_1, _2, and _3) are used for their determination. For the same reason, 1-methylnaphthalene (Peak 3), 2-methylnaphthalene (Peak 4), acenaphthene (Peak 5), and fluorene (Peak 6) are also monitored using three parallel fluorescence monitoring channels. Table 2 lists the parallel fluorescence monitoring channels used for all 20 PAHs.

Reproducibility, Linearity, and Detection Limits

Method precision was estimated using fluorescence detection by making seven consecutive 1000 μL injections of a calibration standard with a concentration of 1 $\mu\text{g/L}$ of each PAH. Method precision using UV detection was measured in the same manner, but with a calibration standard having a concentration of 10 $\mu\text{g/L}$ of each PAH. Retention time reproducibilities (RSD) are all ≤ 0.16 and peak area reproducibilities (RSD) are all ≤ 1.3 , thus demonstrating good short-term precision for this on-line SPE HPLC method.

Calibration linearity of 20 PAHs (using UV detection for naphthalene and acenaphthylene while using fluorescence detection for the other 18 PAHs) was investigated by making three consecutive 1000 μL injections of a mixed standard prepared at nine different concentrations (i.e., 27 total injections). The external standard method was used to establish the calibration curve and quantify the analytes in the tap water samples. Different linearity ranges were observed for the PAHs when plotting concentration versus peak area. Detailed calibration data calculated by Chromeleon CDS software are shown in Table 4. The method detection limit (MDL) of each PAH for UV or fluorescence detection was calculated using the single-sided Student's *t* test method (at the 99% confidence limit). Eight consecutive injections of three reagent water (DI water) samples mixed with 0.1, 1.0, and 10 $\mu\text{g/L}$ of the PAH standard mixtures were used to determine the standard deviation values for calculating MDLs. The calculated MDLs are listed in Table 4.

Analyte	Detection	Regression Equation	r ²	Linearity Range (µg/L)	Concn of PAH Standard Mixtures in Reagent Water (µg/L) ^a	MDL (µg/L) ^b
Naphthalene ^b	UV	A = 0.0025c + 0.0136	0.9970	1.5~100	10	0.47
Acenaphthylene ^b		A = 0.0034c + 0.0062	0.9989	1.5~100	10	0.72
1-Methylnaphthalene	Fluorescence	A = 7492.48c	0.9910	0.10~50	0.1	0.031
2-Methylnaphthalene		A = 10516.8c	0.9982	0.10~50	0.1	0.031
Acenaphthene		A = 110163c	0.9972	0.05~50	0.1	0.028
Fluorene		A = 266343c	0.9966	0.05~50	0.1	0.016
Phenanthrene		A = 138211c	0.9966	0.05~50	0.1	0.011
Anthracene		A = 237397c	0.9973	0.05~50	0.1	0.010
Fluoranthene		A = 32560.4c	0.9971	0.05~50	0.1	0.017
Pyrene		A = 148615c	0.9965	0.05~50	0.1	0.012
Benzo(a)anthracene		A = 100842c	0.9980	0.10~50	0.1	0.020
Chrysene		A = 74195.7c	0.9986	0.10~50	0.1	0.024
Benzo(j)fluoranthene		A = 2405.81c	0.9992	0.5~100	1.0	0.156
Benzo(e)pyrene		A = 13930.5c	0.9996	0.5~100	1.0	0.161
Benzo(b)fluoranthene		A = 33121.4c	0.9954	0.1~50	0.1	0.034
Benzo(k)fluoranthene		A = 213065c	0.9962	0.1~50	0.1	0.023
Benzo(a)pyrene		A = 122278c	0.9969	0.1~50	0.1	0.035
Dibenz(a,h)anthracene		A = 54922.1c	0.9997	0.1~50	1.0	0.048
Benzo(ghi)perylene		A = 22558.1c	0.9998	0.1~100	1.0	0.137
Indeno(1,2,3-cd)pyrene		A = 8615.10c	0.9979	0.1~100	1.0	0.131

^aUsed for the determination of the standard deviation value for calculating MDLs

^bThe single-sided Student's *t* test method (at the 99% confidence limit) was used for estimating MDL, where the standard deviation of the peak area of eight injections of tap water sample spiked with mixed PAHs standard is multiplied by 3.5 (at *n* = 8) to yield the MDL.

Analysis of Tap Water Samples

No target analytes were found in the tap water samples. Figure 6 shows chromatograms of a tap water sample detected with fluorescence using three parallel channels. Method recovery was investigated by determining the recoveries in a tap water sample spiked at two concentrations (0.05 and 5 µg/L of each PAH). The recovery range was from 80 to 120%, demonstrating that this on-line SPE HPLC method combined with UV and fluorescence detections provides good selectivity and suitability for the determination of PAHs in water samples.

Conclusion

This work describes an on-line SPE HPLC method with UV absorbance and fluorescence detections for rapid and sensitive determination of 20 PAHs in tap water. The determination is performed on an UltiMate 3000 Dual RSLC system controlled by Chromeleon CDS software and combined with a Hypersil Green PAH analytical column. The reduced MDLs for UV and fluorescence detection enabled by on-line SPE using the Acclaim PA2 column provide a convenient method for determining these compounds in drinking and environmental waters using HPLC.

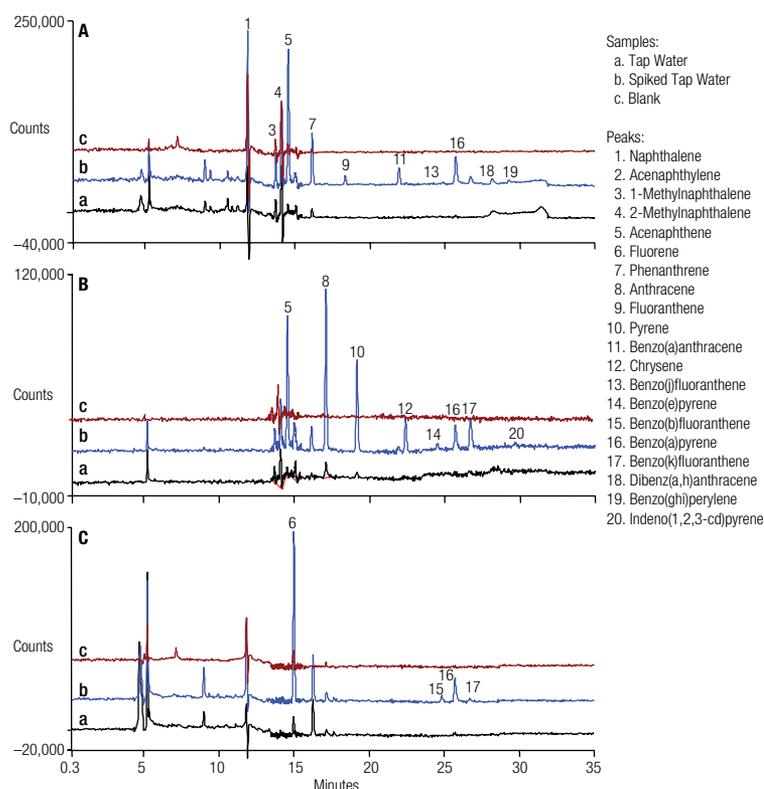


Figure 6. A tap water sample detected by fluorescence using programmed wavelength switching in three parallel channels: (A) Emission_1, (B) Emission_2, (C) Emission_3.

References

1. Dionex (now part of Thermo Scientific) Application Note 196: Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Edible Oils by Donor-Acceptor Complex Chromatography (DACC)-HPLC with Fluorescence Detection. Sunnyvale, CA, 2008. [Online] www.dionex.com/en-us/webdocs/66759-AN196_LC_PAH_EdibleOils_29Apr08_LPN1998.pdf (accessed Feb. 11, 2014).
2. Dionex (now part of Thermo Scientific) Application Note 213: Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Tap Water Using On-Line Solid-Phase Extraction Followed by HPLC with UV and Fluorescence Detections. Sunnyvale, CA, 2009. [Online] www.dionex.com/en-us/webdocs/69875-AN213-HPLC-PAHs-Water-06Jan09-LPN2128.pdf (accessed Feb. 11, 2014).
3. *Method 550: Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Liquid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection*; U.S. Environmental Protection Agency: Cincinnati, OH, 1990.
4. *Method 550.1: Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Solid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection*; U.S. Environmental Protection Agency: Cincinnati, OH, 1990.
5. *Method 610: Polynuclear Aromatic Hydrocarbons*; U.S. Environmental Protection Agency: Cincinnati, OH, 1982.
6. Dionex (now part of Thermo Scientific) Technique Note 92: Combining Fluorescence Detection with UHPLC: An Overview of the Technical Requirements. Sunnyvale, CA, 2011. [Online] www.dionex.com/en-us/webdocs/110810-TN92-UHPLC-Fluorescence-Detection-05Jul2011-LPN2716-01.pdf (accessed Feb. 11, 2014).

www.thermofisher.com/chromatography

©2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

Africa +43 1 333 50 34 0	Denmark +45 70 23 62 60	Japan +81 6 6885 1213	Russia/CIS +43 1 333 50 34 0
Australia +61 3 9757 4300	Europe-Other +43 1 333 50 34 0	Korea +82 2 3420 8600	Singapore +65 6289 1190
Austria +43 810 282 206	Finland +358 9 3291 0200	Latin America +1 561 688 8700	Sweden +46 8 556 468 00
Belgium +32 53 73 42 41	France +33 1 60 92 48 00	Middle East +43 1 333 50 34 0	Switzerland +41 61 716 77 00
Brazil +55 11 3731 5140	Germany +49 6103 408 1014	Netherlands +31 76 579 55 55	Taiwan +886 2 8751 6655
Canada +1 800 530 8447	India +91 22 6742 9494	New Zealand +64 9 980 6700	UK/Ireland +44 1442 233555
China 800 810 5118 (free call domestic) 400 650 5118	Italy +39 02 950 591	Norway +46 8 556 468 00	USA +1 800 532 4752

AN70923_E 07/16S

Thermo
SCIENTIFIC

A Thermo Fisher Scientific Brand