

Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Tap Water Using On-Line Solid-Phase Extraction Followed by HPLC with UV and Fluorescence Detections

INTRODUCTION

Numerous polycyclic aromatic hydrocarbons (PAHs) are carcinogenic, making their presence in foods and the environment a health concern. Regulations around the world limit levels of a variety of PAHs in drinking water, food additives, cosmetics, workplaces, and factory emissions. PAHs have traditionally been separated using HPLC, but method detection limits (MDLs) of HPLC techniques employing direct injection of samples are too high for the detection of the low concentrations in real samples that are near the regulated limit. Therefore, the analytes in these samples require preconcentration before analysis.

The U.S. EPA prescribes liquid-liquid extraction¹ and liquid-solid extraction² (also called solid-phase extraction, SPE) methods for preconcentrating PAHs in drinking water samples. However, preparing an individual sample is time consuming for each of the two extraction methods, and a new SPE cartridge must be used for each sample when using the SPE method. The expense of using multiple SPE cartridges and the associated manual labor can be eliminated with online SPE combined with the subsequent HPLC analysis. This technique delivers a simple, rapid, and accurate means for determining

PAHs at low concentrations in water samples. For example, Zhou *et al*³ prepared a copper (II) isonicotinate

[Cu(4-C₅H₄N-COO)₂(H₂O)₄] coordination polymer as adsorbent for online SPE coupled with HPLC and UV detection for determining eight trace PAHs in environmental waters.

The Dionex UltiMate® 3000 ×2 Dual HPLC system has already been used to execute the online SPE method coupled with HPLC to determine phenols in drinking and bottled waters,⁴ and PAHs in edible oils.⁵ The Acclaim® Polar Advantage II (PA2) is a polar-embedded column designed for enhanced hydrolytic stability within a wide range of pH values (pH 1.5–10). It is compatible with 100% aqueous mobile phases, overcoming the limitations of conventional C8 and C18 reversed-phase columns. Thus, the Acclaim PA2 is a good choice as an SPE column for concentrating polar and non-polar components in large volume water samples (e.g., tap water, pH ~8) without adding any organic solvents.

This application note details an online SPE method followed by HPLC with fluorescence and UV detections on the UltiMate 3000 ×2 Dual HPLC system for determining the 16 PAHs specified in the US EPA Priority Pollutants List (structures shown in Figure 1) at the concentrations required by world regulatory agencies. PAHs from water samples are trapped on the Acclaim PA2 column, and then separated on a Supelcosil™ LC-PAH column. This automated method is a cost-effective and accurate way to determine PAHs in drinking water samples.

EQUIPMENT

UltiMate 3000 ×2 Dual system consisting of:

DPG-3600A pump with SRD-3600 Solvent Rack with degasser

WPS-3000TSL autosampler (with 2.5 mL injection loop (P/N 6820.2416) installed)

TCC-3100 thermostatted column compartment with one 2P-6P valve

VWD-3400RS Variable Wavelength Detector

RF2000 fluorescence detector

Chromeleon® 6.80 (SP4) Chromatography Workstation

Device configurations for the online SPE with analytical HPLC are as shown in Figure 2.

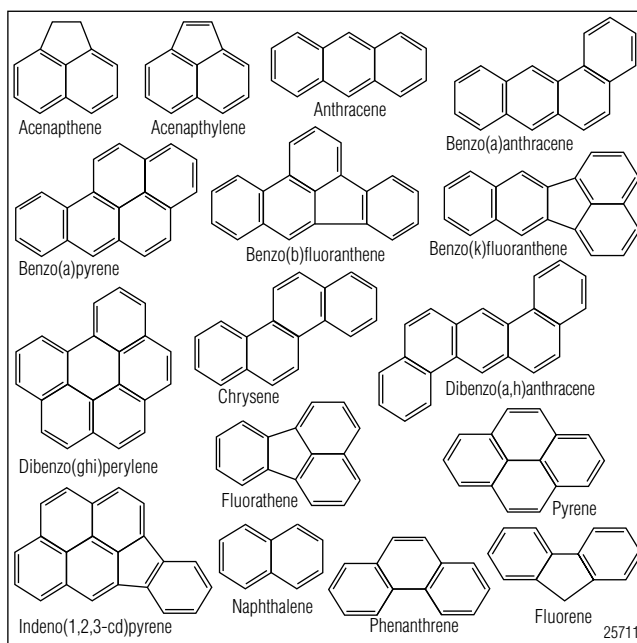


Figure 1. Structures of the 16 PAHs specified in U.S. EPA Method 550.1.

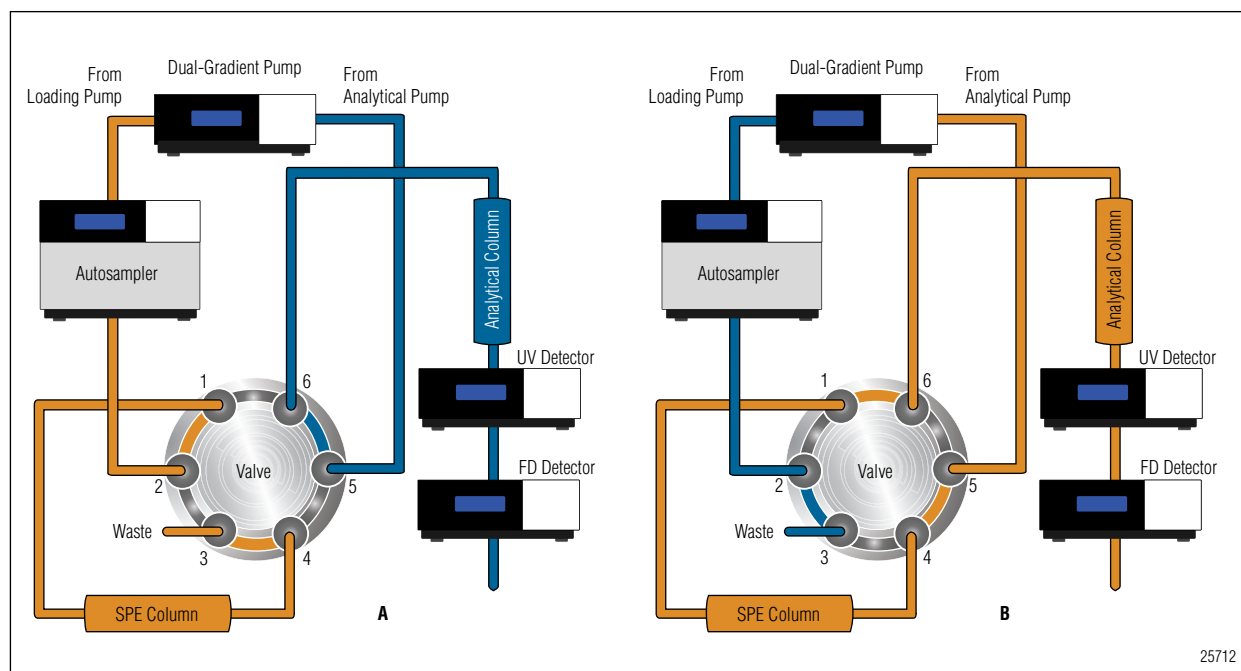


Figure 2. Flow scheme for on-line sample preparation and analysis. (A) The valve is positioned for injection of the sample on the online SPE column, and for equilibration of the online SPE column. (B) The online SPE column is switched into the analytical flow path, eluting the PAHs onto the analytical column for gradient separation followed by UV and fluorescence detections.

REAGENTS AND STANDARDS

Deionized water from a Milli-Q® Gradient A10

Acetonitrile (CH₃CN), HPLC grade (Fisher Scientific)

Methanol (CH₃OH), HPLC grade (Fisher Scientific)

Mix of PAHs standard, EPA Sample for Method 610, 550 and 550.1, (AccuStandard®) the concentration of each component is 2000 µg/mL for Acenaphthylene, 1000 µg/mL for Acenaphthene and Naphthalene, 200 µg/mL for Fluorene, Fluoranthene, Benzo(b) fluoranthene, Dibenzo(a,h)anthracene, and Benzo(g,h,i)perylene, 100 µg/mL for Anthracene, Benzo(a)anthracene, Benzo(a)pyrene, Benzo(k) fluoranthene, Chrysene, Indeno(1,2,3-cd)pyrene, Phenanthrene, and Pyrene

CONDITIONS

Analytical Column: Supelcosil LC-PAH columns,
4.6 × 250 mm
(Supelco Cat. # 58229)

Online SPE Column: Acclaim PA2, 3 µm,
4.6 × 50 mm (P/N 063189)

Mobile Phases: For both loading and analysis
pumps

A. Water

B. Acetonitrile

Injection Volume: 2.0 mL on the SPE column

Column

Temperature: 20 °C

Detection: UV (on 254 nm) and Fluorescence
(at various excitation and
emission wavelengths), in series.

Table 1 shows the gradient for on-line SPE using the loading pump, Table 2 the gradient for separation using the analysis pump, and Table 3 the valve switching program.

The PAHs have good fluorescent responses except for acenaphthylene. Because their fluorescent responses occur at different excitation and emission wavelengths, it is necessary to change these wavelengths based on individual PAH retention times. Table 4 shows the program for wavelength changes. UV detection is used to determine four compounds including acenaphthylene, naphthalene, acenaphthene, and fluorene according to EPA method 550.1 requirements.

Table 1. Gradient Program for On-line SPE

Time (min)	Flow rate (mL/min)	Solvent A (H ₂ O) (% vol.)	Solvent B (CH ₃ CN) (% vol.)	Curve
0	1.0	95	5	
8	1.0	95	5	5
8.5	0.5	0	100	5
54	0.5	0	100	5
54.5	1.0	95	5	5
65	1.0	95	5	5

Table 2. Gradient Program for Separation

Time (min)	Flow rate (mL/min)	Solvent A (H ₂ O) (% vol.)	Solvent B (CH ₃ CN) (% vol.)	Curve
0	1.0	60	40	
10	1.0	60	40	5
30	1.0	0	100	6
54	1.0	0	100	5
54.5	1.0	60	40	5
65	1.0	60	40	5

Table 3. Valve Switching Program

Time (min)	Position
0	1 – 2
8	6 – 1
54	1 – 2

Table 4. Wavelength Changes for RF2000 Fluorescence Detector

Time (min)	Ex / Em Wavelength (nm)	Gain
0	256 / 390	1
31.5	275 / 420	4
34	270 / 385	1
37	290 / 430	1
51	305 / 480	4
65	256 / 390	1

PREPARATION OF STANDARDS AND SAMPLES

Preparation of Stock and Working Standards

To prepare a mixed stock standard solution, add 5 μL of the mix of PAHs standard, using a 10 μL syringe, to a 10 mL vial, and then add 9995 μL CH_3CN -Methanol- H_2O (2:2:1, v/v), using a 5 mL pipette. The mixed stock standard solution is used to prepare working standards for calibration as described in Table 5.

SYSTEM SETUP

Description of the On-Line SPE-HPLC Method

The flow scheme, shown in Figure 2, couples the SPE directly with the analytical HPLC run, using a second gradient pump and one two-position, six-port (2P-6P) column-switching valve. Figure 2, diagram A shows the valve positions at the time of the injection. The filtered sample is injected directly onto the system, and delivered to the SPE column for enrichment. The analytical column is equilibrated with the second pump at the same time. 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 flush out the

bound analytes with CH_3CN /water (Figure 2, diagram B), and then the analytes are separated on the analytical column and detected by UV and fluorescence in series.

Selection of the Online SPE and Analytical Columns

The Acclaim PA2 is good choice for concentration of PAHs in water samples as explained in the introduction. The experiments showed that a Donor-Acceptor Complex Chromatography (DACC) column (Varian, ChromSpher Pi) also can be used as the SPE column in this method with similar performance under the same conditions.

The Supelcosil LC-PAH column was used for separation in this experiment, but another analytical PAH column such as the Phenomenex[®] Envirosep PP can be used for this application. If another PAH column is used, the separation conditions will need to be adjusted to account for the different column chemistry. Experiments showed that a longer analytical column is better for the online SPE application. This is because possible peak deterioration caused by the sample transfer from the online SPE column to the analytical column decreases with increasing SPE column length.

Table 5. Preparation of the Working Standards

Vial # (10 mL)	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	
Volume of mix of stock standard solution (μL)	20	50	100	200	500	
Volume of deionized water (μL)	9980	9950	9900	9800	9500	
Concentration of PAHs ($\mu\text{g/L}$)	Naphthalene	1.0	2.5	5.0	10	25
	Acenaphthylene	2.0	5.0	10	20	50
	Acenaphthene	1.0	2.5	5.0	10	25
	Fluorene	0.20	0.50	1.0	2.0	5.0
	Phenanthrene	0.10	0.25	0.50	1.0	2.5
	Anthracene	0.10	0.25	0.50	1.0	2.5
	Fluoranthene	0.20	0.50	1.00	2.0	5.0
	Pyrene	0.10	0.25	0.50	1.0	2.5
	Benzo(a)anthracene	0.10	0.25	0.50	1.0	2.5
	Chrysene	0.10	0.25	0.50	1.0	2.5
	Benzo(b)fluoranthene	0.20	0.50	1.00	2.0	5.0
	Benzo(k)fluoranthene	0.10	0.25	0.50	1.0	2.5
	Benzo(a)pyrene	0.10	0.25	0.50	1.0	2.5
	Dibenzo(a,h)anthracene	0.20	0.50	1.0	2.0	5.0
	Benzo(g,h,i)perylene	0.20	0.50	1.0	2.0	5.0
Indeno(1,2,3-cd)pyrene	0.10	0.25	0.50	1.0	2.5	

Conversion of WPS-3000TSL Autosampler for Online SPE

The WPS-3000TSL autosampler has 15 positions for 10 mL vials that can accommodate the 2 mL injection volume.

Because a 2 mL sample needs to be injected, the semipreparative version of WPS-3000 autosampler is required (S/N 5822.0028 with temperature control, or 5822.0018 without temperature control). It may be more convenient to install a 2500 μ L semipreparative sample loop (P/N 6820.2416) to the current analytical WPS-3000 autosampler for this application. The following parts, which belong to the WPS-3000SL Semipreparative Upgrade Kit (P/N 6822.2450), need to be installed in place of the parts used in common analytical version: a buffer loop (P/N 6820.2421), a needle for semipreparative sample loop (P/N 6820.2419), and a 2500 μ L syringe (P/N 6820.0006). There is no need to install the other parts of the Upgrade Kit.

SAMPLE PREPARATION

Tap water was collected at the Dionex Shanghai Applications Lab located in the Pudong District, Shanghai, China. Spiked tap water samples were

prepared by adding 400 μ L of the mixed stock standard solution to a 50 mL conical flask (with plug), then adding 39.6 mL of tap water filtered through a 0.45 μ m membrane (Millex-HN).

RESULTS AND DISCUSSION

Reproducibility, Detection Limits, and Linearity

Method reproducibility was estimated by making eight consecutive replicate injections of tap water spiked with the PAHs standard mix (Figure 3). Table 6 summarizes the retention time and peak area precision data. Calibration linearity for the determination of PAHs was investigated by making four replicate injections of a mixed standard of PAHs prepared at four different concentrations. The external standard method was used to calculate the calibration curve and for sample analysis. Table 7 reports the data from this determination as calculated by Chromeleon software. PAH method detection limits (MDLs) are also listed in Table 7, demonstrating that they can match the MDLs obtained in EPA 550.1.

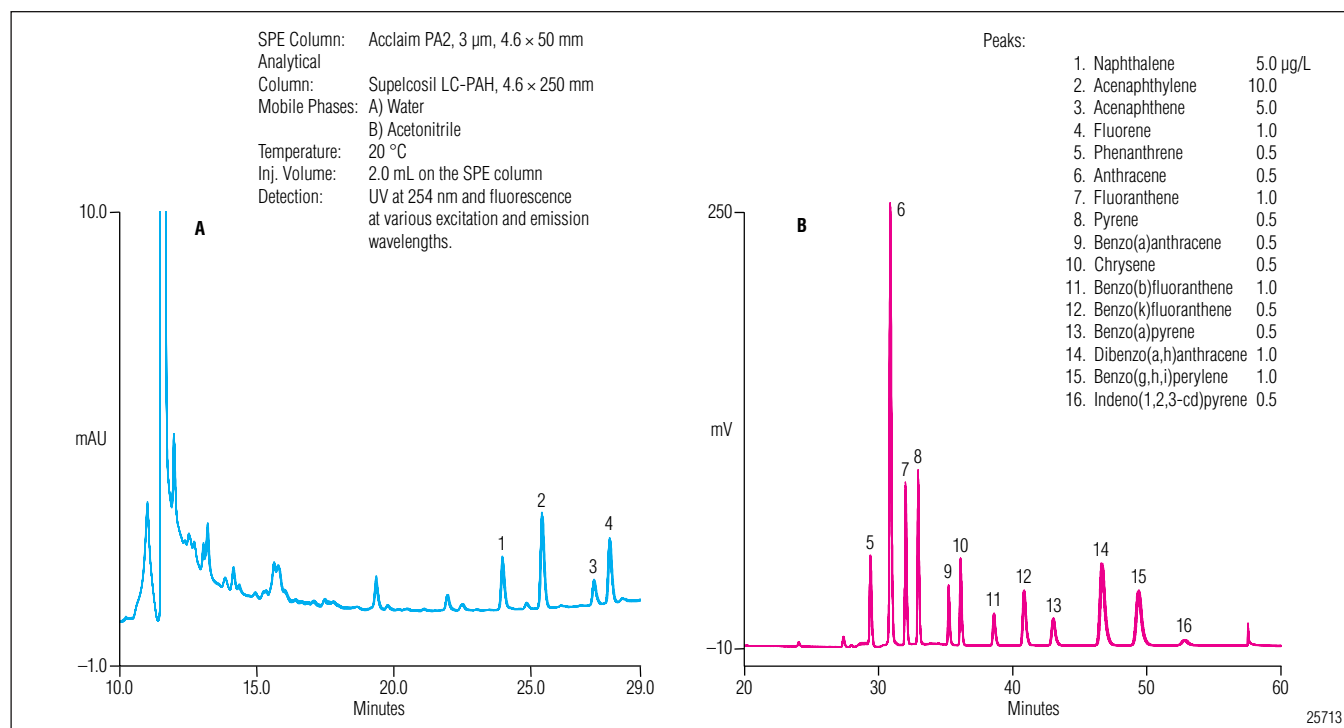


Figure 3. Overlay of chromatograms of eight consecutive injections of a tap water sample spiked with the PAHs standard mix, obtained by A) UV at 254 nm and B) Fluorescence at different wavelengths.

Table 6. Reproducibility of Retention Times and Peak Areas^a

PAH	RT RSD	Area RSD
Naphthalene	0.057	10.808
Acenaphthylene	0.049	4.093
Acenaphthene	0.051	6.211
Fluorene	0.049	3.535
Phenanthrene	0.048	7.861
Anthracene	0.046	1.792
Fluoranthene	0.040	2.754
Pyrene	0.034	3.591
Benzo(a)anthracene	0.033	1.635
Chrysene	0.039	2.015
Benzo(b)fluoranthene	0.052	1.013
Benzo(k)fluoranthene	0.067	2.018
Benzo(a)pyrene	0.074	1.593
Dibenzo(a,h)anthracene	0.106	2.266
Benzo(g,h,i)perylene	0.101	2.057
Indeno(1,2,3-cd)pyrene	0.132	5.777

^aEight consecutive injections of a tap water sample spiked with a mixed PAH standard.

Tap Water Sample Analysis

Figure 4 shows chromatograms of tap water and the tap water spiked with PAHs. The results are summarized in Table 8. Only Naphthalene (peak 1) was found in the tap water sample, and its concentration (0.46 µg/L) is below the calculated detection limit (1.17 µg/L for naphthalene). Recoveries of all PAHs in the spiked sample were acceptable.

Table 7. Calibration Data for the 16 PAHs

PAH	Equation	r (%)	Detection	MDL (µg/L)	MDL (µg/L), EPA method 550.1 required
Naphthalene	$A = 0.0500c - 0.0097$	99.50	UV	1.17	2.20
Acenaphthylene	$A = 0.0399c - 0.0092$	99.94	UV	1.08	1.41
Acenaphthene	$A = 0.0229c - 0.0041$	99.86	UV	0.84	2.04
Fluorene	$A = 0.2644c - 0.0103$	99.94	UV	0.11	0.126
Phenanthrene	$A = 17.17c - 1.035$	99.86	FL	0.15	0.15
Anthracene	$A = 71.17c - 4.346$	99.69	FL	0.08	0.14
Fluoranthene	$A = 20.72c - 0.959$	99.43	FL	0.09	0.009
Pyrene	$A = 44.77c - 10.50$	99.45	FL	0.26	0.126
Benzo(a)anthracene	$A = 11.34c - 0.7228$	99.43	FL	0.08	0.004
Chrysene	$A = 27.27c - 3.799$	99.52	FL	0.15	0.160
Benzo(b)fluoranthene	$A = 6.276c + 0.192$	99.64	FL	0.017	0.006
Benzo(k)fluoranthene	$A = 42.93c - 3.965$	99.91	FL	0.01	0.003
Benzo(a)pyrene	$A = 16.05c - 0.1821$	99.82	FL	0.022	0.016
Dibenzo(a,h)anthracene	$A = 23.29c - 0.480$	99.84	FL	0.025	0.035
Benzo(g,h,i)perylene	$A = 22.12c - 0.455$	99.89	FL	0.070	0.020
Indeno(1,2,3-cd)pyrene	$A = 6.184c - 0.1596$	99.92	FL	0.059	0.036

The single-sided Student's test method (at the 99% confidence limit) was used for estimating MDL, where the standard deviation (SD) of the peak area of eight injections of tap water sample spiked with mixed PAHs standard is multiplied by 3.50 (at $n = 8$) to yield the MDL.

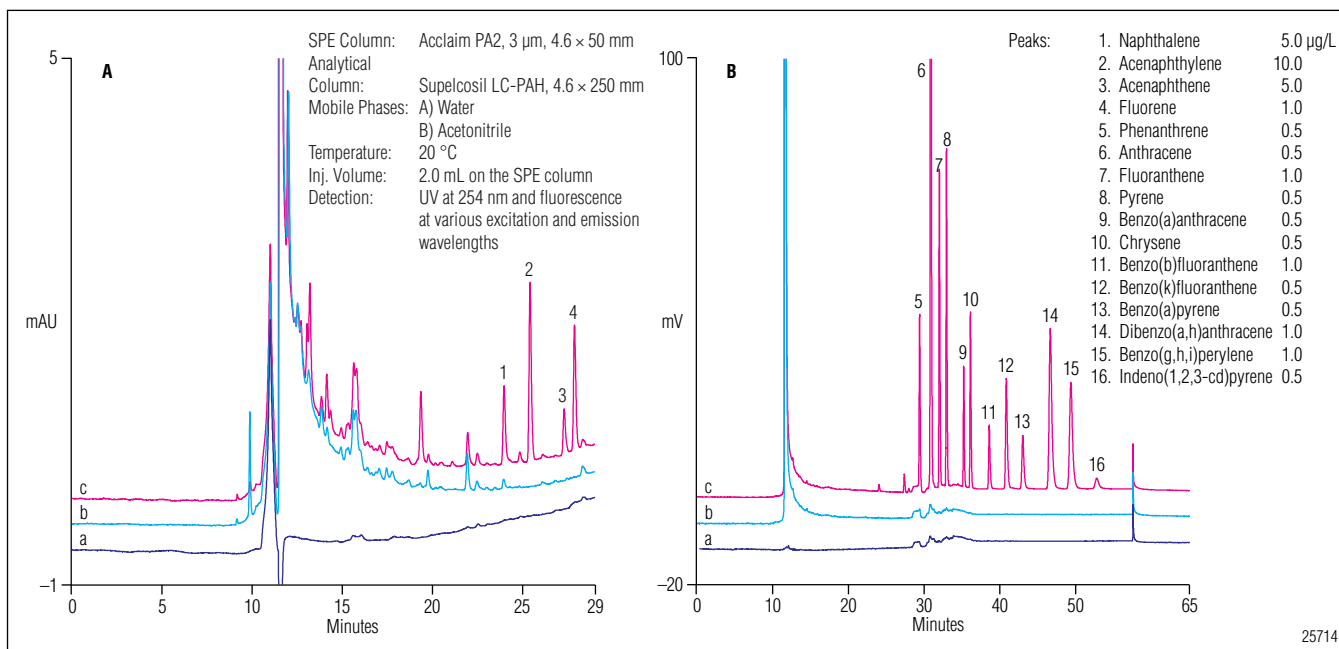


Figure 4 Chromatograms obtained by A) UV at 254 nm and B) FL at different wavelengths. Chromatograms of (a) blank, (b) tap water, and (c) tap water spiked with a PAH standard mixture.

CONCLUSION

This application note demonstrates that PAHs can be successfully determined in drinking water at concentrations that meet the detection limits specified in EPA Method 550.1² using an online SPE method with an UltiMate 3000 x2 dual HPLC system. This method saves analyst time and the expense of consumables compared to offline SPE.

PRECAUTIONS

Contaminants in solvents, reagents, glassware, and other sample processing hardware may cause method interferences, so glassware must be scrupulously cleaned. Use high-purity reagents and solvents to minimize interference problems. Fresh acetonitrile must be used.

A command for washing the 2.5 mL sample loop was added to the program to reduce carryover when the SPE column is on the analytical flow path, as follows:

```
20.000 WashSampleLoop Volume=2500.000
22.000 Wash
25.000 InjectValveToInject
```

It is advisable to add an on-line filter (2 µm) between the injector and switching valve to protect the SPE and analytical columns when running a large number of samples.

Table 8. Analytical Results for Tap Water Samples

PAH	Tap water		
	Detected (µg/L)	Added (µg/L)	Recovery (%)
Naphthalene	< MDL	5.0	72
Acenaphthylene	ND	10	85
Acenaphthene	ND	5.0	80
Fluorene	ND	1.0	90
Phenanthrene	ND	0.50	92
Anthracene	ND	0.50	106
Fluoranthene	ND	1.0	102
Pyrene	ND	0.50	99
Benzo(a)anthracene	ND	0.50	84
Chrysene	ND	0.50	76
Benzo(b)fluoranthene	ND	1.0	98
Benzo(k)fluoranthene	ND	0.50	104
Benzo(a)pyrene	ND	0.50	104
Dibenzo(a,h)anthracene	ND	1.0	90
Benzo(g,h,i)perylene	ND	1.0	76
Indeno(1,2,3-cd)pyrene	ND	0.50	96

One sample and one spiked sample were prepared, and three injections of each were made.

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