Sensitive Determination of Polycyclic Aromatic Hydrocarbons in Tap Water by Online Solid Phase Extraction and UHPLC

Application Note

Environmental – Water Analysis

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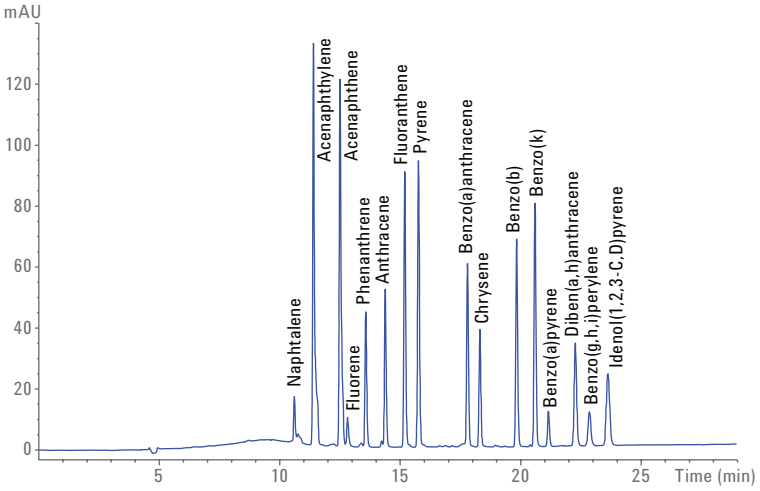
Abstract

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants with a high carcinogenetic and mutagenic potential, and can be found in different kinds of environmental and drinking waters worldwide. A sensitive and rapid determination of these substances is essential. In this Application Note, an automated and fast online solid-phase extraction (SPE) method was developed using the Agilent 1200 Infinity Series online SPE solution with an Agilent 1260 Infinity autosampler. In just 30 minutes, 16 PAHs were separated and detected in spiked drinking water according to EPA methods 550, 550.1, and 610.



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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment and formed by the incomplete burning and combustion of hydrocarbons such as woods, gases, fuel for automobiles, and cigarettes. They are usually found as a mixture by adsorption onto airborne particles. These particles are part of rain drops and snow, and can be found in sediment, surface water, and drinking water. Another major source of PAHs in drinking water is leaching from linings of water storage tanks and distribution lines[1].

Seven PAHs are classified as probable human carcinogens by the Environmental Protection Agency (US EPA): benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene. Benzo[a]pyrene is known as one of the most dangerous carcinogens, and is used as an indicator for the presence of PAH. The EPA, for example, uses mathematical models to estimate the probability of a person developing cancer from ingesting water containing a specified concentration of a chemical. For benzo[a]pyrene, the EPA has calculated an oral cancer slope factor of 7.3 (mg/kg/d)[2].

Consequently, the determination of PAHs in environmental and drinking water is highly important. According to EPA (method 550, 550.1 and 610) and the European Union legislation, 16 parent PAHs should be monitored[3,4,5,6]. These methods are based on offline solid-phase extraction (SPE) or liquid-liquid extraction (LLE), and have run times greater than 60 minutes. Both offline SPE and LLE require high sample volumes (1 L), and are labor intensive and time-consuming. Additionally, high amounts of organic solvents are needed, which may result in human health effects, and are of environmental concern.

An effective alternative is online SPE due to cost, time, and solvent savings. The sample preparation and disposable waste can be reduced to a minimum, and water samples of less than 2 mL are sufficient for the highly sensitive determination of PAHs in drinking water. The overall run time in this work, which included the enrichment process and analytical run, was just 30 minutes.

This Application Note shows and describes the sensitive, fast, and reproducible online SPE analysis of 16 PAHs spiked in drinking water. Good recovery rates, linearity, and precision for retention time and area are shown. The UHPLC method included both diode array and fluorescence detection modes, and was developed on an Agilent 1200 Infinity Series online SPE system, based on an Agilent 1260 Infinity LC with an Agilent 1260 Infinity autosampler for more sample capacity (2 × 18, 6-mL vials).

Experimental

Instrumentation

An Agilent 1200 Infinity Series Online SPE system comprising the following modules was used for the experiments.

- Agilent 1260 Infinity autosampler with sample cooler (G7129A with option #100)
- Agilent 1260 Infinity binary pump with degasser (G1312B and G1322A)
- Agilent 1260 Infinity quaternary pump (G1311B)
- Agilent 1290 Infinity thermostatted column compartment (G1316B)
- Agilent 1290 Infinity Flexible Cube (G4227A)
- Agilent 1260 Infinity diode array detector (G4212B)
- Agilent 1290 Infinity fluorescence detector (G1321B)

Software

Agilent OpenLab CDS ChemStation Edition for LC and LC/MS systems, Rev. C.01.07 [22]

Instrumentation setup

The online SPE Solution is based on the 1290 Infinity Flexible Cube that houses re-usable SPE cartridges and up to two valves. The two valves enable the choice of switching between direct injection and online SPE methods without replumbing the system. The conventional setup uses the built-in piston pump for loading and cleaning the cartridges. The integrated single-piston pump has a maximum pressure of 60 bar. As two analytical columns were used as enrichment columns and, therefore, the backpressure was too high, the system was modified by the addition of a 1260 Infinity quaternary pump for loading and cleaning the enrichment column. The built-in pump of the 1290 Infinity Flexible Cube was bypassed (Figure 1).

Another addition to the system is the 1260 Infinity autosampler instead of the 1260 Infinity standard autosampler that was previously used. The 1260 Infinity autosampler shortens injection cycles to 18 seconds, lowers carryover with an automated needle wash station, and enlarges sample capacity to 36 × 6-mL vials or 132 × 2-mL vials. The 1260 Infinity autosampler can be equipped with an integrated cooler. With a 900-µL loop, analytical head, and a multidraw seat capillary of 1,500 µL (G1313-98308), the 1260 Infinity autosampler can inject up to 1,800 µL.

The setup with two valves installed enables direct injection and online SPE methods without replumbing the system. For direct injection methods, the left valve has to be in position 1 to 2 to directly connect the binary pump to the autosampler. In this mode, the right valve is disabled, and no analytical flow passes the enrichment columns.

Otherwise, position 1 to 10 on the left valve enables the online SPE enrichment process and subsequent chromatographic separation on the analytical column and detection of compounds. Figure 1 shows the schematic flow chart of this setup. The sample is alternatively loaded to one of the enrichment columns by the quaternary pump, while the binary pump equilibrates the analytical column. After switching the right valve, the loaded enrichment column is connected to the binary pump, and the compounds will be eluted by the gradient and detected by the UV and fluorescence detectors. At the same time, the quaternary pump cleans and re-equilibrates the second enrichment column for the next run.

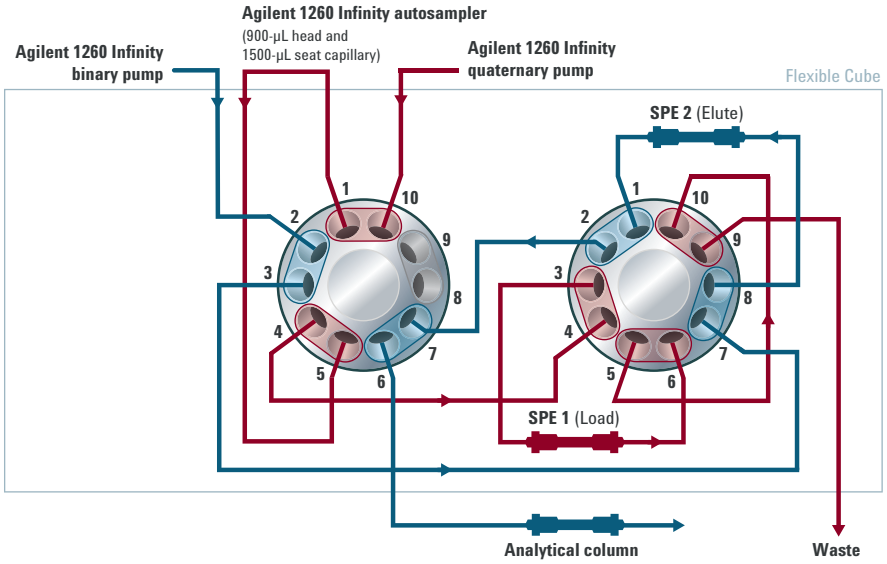

Figure 1. Schematic of online SPE enrichment process. Loading and cleaning is done by the Agilent 1260 Infinity quaternary pump while the Agilent 1260 Infinity binary pump delivers the elution gradient.

Chromatographic conditions

Parameter	Value
Analytical column	Agilent ZORBAX Eclipse PAH 4.6 × 150 mm, 3.5 µm (p/n 959961-918)
Column temperature	25 °C
Gradient	A) Water, B) Acetonitrile 0 minutes, 40 %B at 1.3 mL/min 5 minutes, 40 %B 20 minutes, 100 %B 30 minutes, 100 %B 30.1 minutes, 40 %B Posttime 6 minutes
Injection volume	1,800 µL (online SPE), needle wash 3 seconds with acetonitrile
Autosampler temperature	18 °C
Online SPE enrichment column	Polaris 5 C18-A, 3.0 × 50 mm (p/n A2000050X030)
Online SPE enrichment method	A) Water, B) Acetonitrile 0 minutes, 20 %B at 1.3 mL/min 3 minutes, 20 %B 4 minutes at 0 mL/min 5 minutes, 100 %B at 1 mL/min 9 minutes, 100 %B 9.1 minutes, 20 %B at 1 mL/min 15 minutes, 20 %B 15.1 minutes, 20 %B at 0 mL/min
Diode array detector	230 nm, bandwidth 4 nm, reference 400 nm, reference band width 100 nm, 10 Hz
Fluorescence detector	Multisignal acquisition, set at λ_{ex} = 260 nm and λ_{em} = 350 nm (FLD A), 330 nm (FLD B), 440 nm (FLD C), 500 nm (FLD D), 18.51 Hz, PMT 13

Chemicals

All solvents used were LC grade. Acetonitrile was purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with an LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak).

Sample

A PAH mixture of 16 compounds according to EPA 550 (p/n 8500-6035) was purchased from Agilent Technologies. Drinking water was from Waldbronn, Germany, and was spiked with PAHs in different concentrations. For the calibration in drinking water, seven standards were prepared by diluting a stock solution of 500 µg/mL to 10, 5, 1, 0.5, 0.1, 0.05, and 0.01 µg/L.

Results and Discussion

A suite of 16 PAHs was spiked at different levels in drinking water. Fifteen of these PAHs can be detected by fluorescence, which offers a better sensitivity than UV. Acenaphthylene has no fluorescence activity, and requires UV detection. At first, the chromatographic separation was optimized with direct injection methods and UV detection, then later on transferred to online SPE and UV/fluorescence detection.

For the determination of method precision for all PAHs and online SPE, seven consecutive runs with 1 µg/L standard were injected. Table 1 shows the retention time (RT) detection conditions as well as RT and area precision for all PAHs. RT reproducibility (RSD) was less than 0.09 %, and area reproducibility less than 6.0 %, and, thus, shows good precision for the online SPE method. Figure 2 shows a chromatogram with UV and different FLD wavelengths for the detection of 16 PAHs in drinking water.

Table 1. Retention time (RT), detection conditions, and RT and area reproducibility for 16 PAHs.

Elution order	Compound	Retention time (min)	Detection (nm)	RT RSD (%) (n = 7)	Area RSD (%) (n = 7)
1	Naphthalene	10.6	UV 230/Ref 400, Ex 260, Em 330	0.028	3.8
2	Acenaphthylene	11.4	UV 230/Ref 400	0.025	0.9
3	Acenaphtene	12.5	Ex 260/Em 330	0.029	1.0
4	Fluorene	12.8	Ex 260/Em 330	0.031	0.5
5	Phenantrene	13.5	Ex 260/Em 350	0.035	0.9
6	Anthracene	14.3	Ex 260/Em 440	0.040	0.6
7	Fluoranthene	15.1	Ex260/Em 440	0.050	0.3
8	Pyrene	15.7	Ex 260/Em 440	0.050	6.0
9	Benzo(a)anthracene	17.7	Ex 260/Em 440	0.070	2.3
10	Chrysene	18.2	Ex 260/Em 350	0.071	0.8
11	Benzo(b)fluoranthene	19.7	Ex 260/Em 440	0.080	1.3
12	Benzo(k)fluoranthene	20.5	Ex 260/Em 440	0.090	1.1
13	Benzo(a)pyrene	22.1	Ex 260/Em 440	0.080	2.5
14	Dibenzo(a,h)anthracene	22.2	Ex 260/Em 440	0.011	2.0
15	Benzo(g,h,i)perylene	22.8	Ex 260/Em 440	0.013	1.4
16	Indeno(1,2,3-cd)pyrene	23.5	Ex 260/Em 500	0.015	3.9

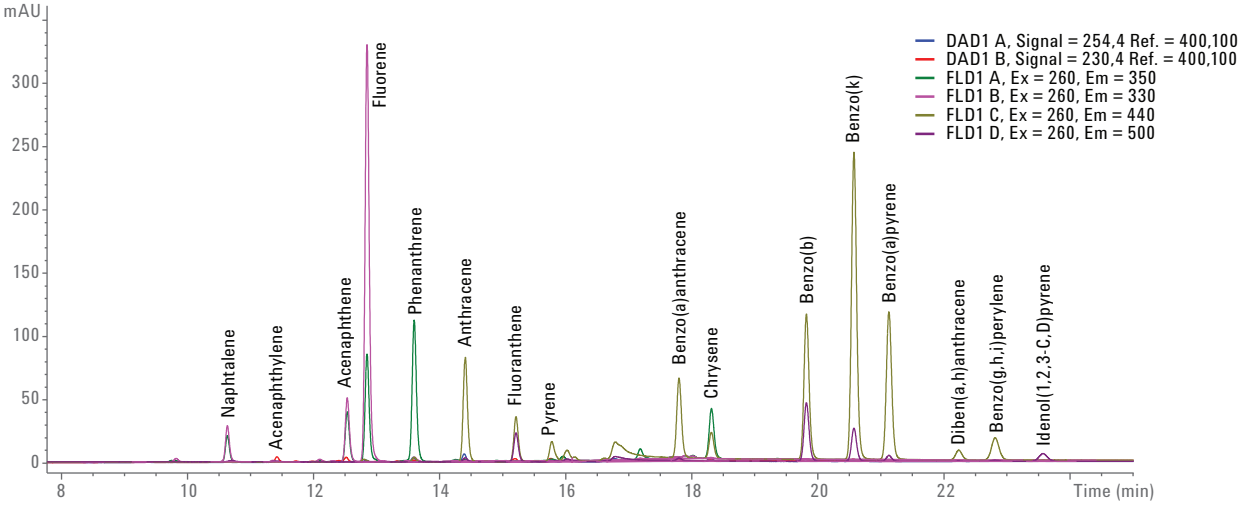

Figure 2. Overlay of chromatograms with UV and different FLD wavelength for the detection of 16 PAHs in drinking water (1 µg/L).

Calibration linearity was investigated with replicates for each concentration level. In Table 2, different linearity ranges are shown for all PAHs. Linearity was good across the ranges, giving values of 0.999 or better. Limits of detection (LODs) and limits of quantification (LOQs) were evaluated from the concentration of PAHs required to give at least a signal-to-noise ratio (S/N) of 3 and 10, respectively.

LODs were between 0.2 and 23 ng/L. LOQs for all compounds were between 1 and 38 ng/L (except for acenaphthylene, with 77 ng/L because of lower sensitivity of UV detection).

Figure 3 clearly shows the calibration curves of four PAHs (anthracene, benzo(a)anthracene, benzo(a)pyrene, and benzo(b)fl uoranthene).

The EPA has set a maximum contaminant level (MCL) for benzo(a)pyrene in drinking water at 0.2 µg/L. This MCL can be easily achieved. The online SPE system is 100 times more sensitive with an injection volume of 1,800 µL.

Table 2. Linearity range, correlation coefficient, LOD, and LOQ are shown for 16 PAHs in drinking water.

Elution order	Compound	Linearity range (µg/L)	R^2	LOD (S/N = 3) (µg/L)	LOQ (S/N = 10) (µg/L)
1	Naphthalene	0.01–10	0.99834	0.0009	0.0031
2	Acenaphthylene	0.5–10	0.99992	0.0233	0.0775
3	Acenaphtene	0.01–5	0.99970	0.0018	0.0061
4	Fluorene	0.01–1	0.99932	0.0003	0.0009
5	Phenantrene	0.01–1	0.99932	0.0004	0.0013
6	Anthracene	0.01–1	0.99982	0.0009	0.0032
7	Fluoranthene	0.01–10	0.99995	0.0018	0.0058
8	Pyrene	0.05–10	0.99991	0.0028	0.0092
9	Benzo(a)anthracene	0.05–5	0.99991	0.0008	0.0028
10	Chrysene	0.01–10	0.99915	0.0039	0.0129
11	Benzo(b)fluoranthene	0.01–1	0.99996	0.0016	0.0053
12	Benzo(k)fluoranthene	0.01–1	0.99985	0.0008	0.0025
13	Benzo(a)pyrene	0.01–0.5	0.99985	0.0022	0.0074
14	Dibenzo(a,h)anthracene	0.01–10	0.99874	0.0019	0.0062
15	Benzo(g,h,i)perylene	0.05–10	0.99835	0.0109	0.0362
16	Indeno(1,2,3-cd)pyrene	0.05–10	0.99989	0.0114	0.0380

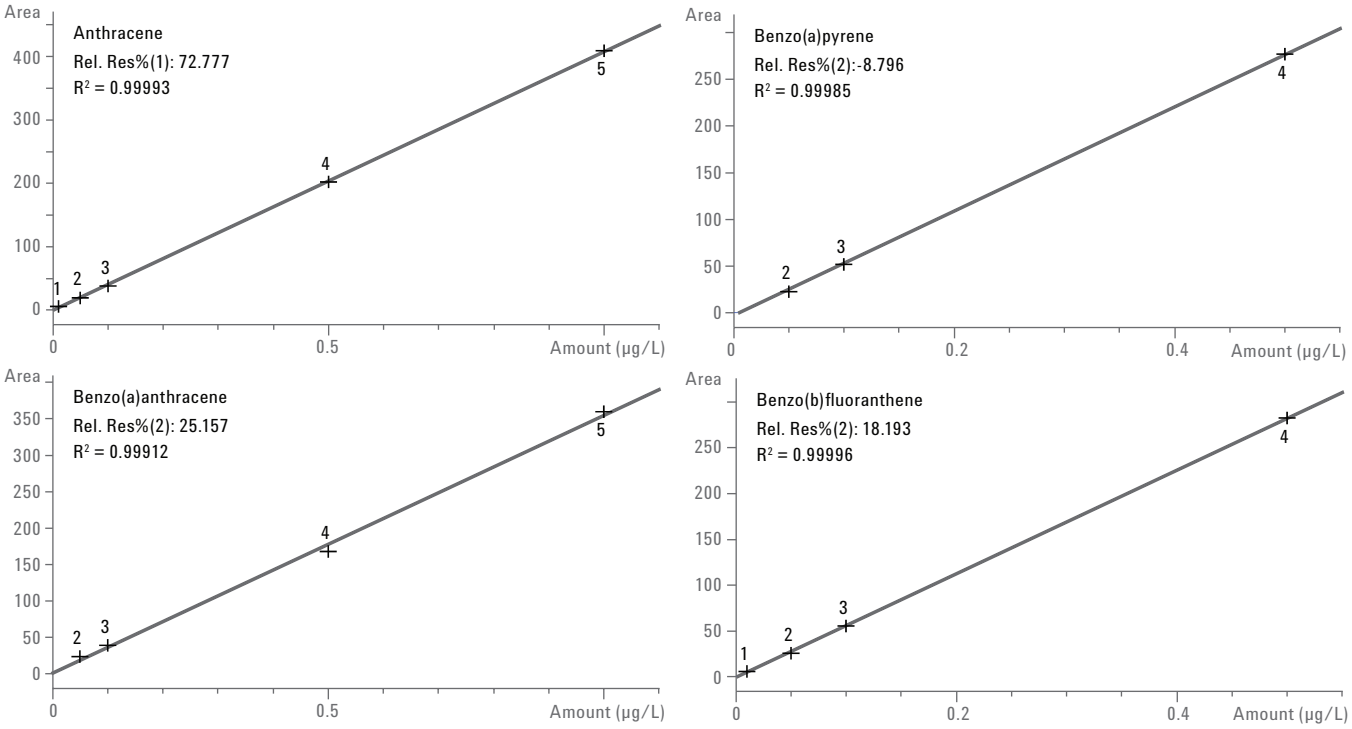

Figure 3. Calibration curves of anthracene, benzo(a)anthracene, benzo(a)pyrene, and benzo(b)fluoranthene in concentration ranges between 0.01 to 10 µg/L for the online SPE method and an injection volume of 1,800 µL.

To determine the recovery of the trapping process on the SPE cartridges, the same volume (5 µL) of a 250 µg/L solution was injected directly onto the analytical column and afterwards onto the SPE trapping columns. Seven replicates of each method were averaged and compared. The recovery range was between 90 and 110 % (Figure 4), with the exception of benzo(a)pyrene and indeno(1,2,3-cd)pyrene, where the recovery was 140 and 73 %, respectively. This demonstrates that the online SPE method is perfectly suited for the analysis of PAHs in drinking water, because no analyte is flushed out and lost during the enrichment process.

Conclusion

The analysis of PAHs in water can be fully automated with online solid-phase extraction with a combination of UV and fluorescence detection. No sample pretreatment is necessary, and the overall run time of 16 PAHs is just 30 minutes. Excellent recoveries were determined for all analytes. Detection limits were between 0.2 and 23 ng/L, with an 1,800 µL injection, and quantification limits were in the range of 1 to 38 ng/L. Reproducibility for two alternating enrichment columns was less than 6 % RSD with excellent correlation (typically $R^2 = 0.999$), which demonstrates that online SPE is a perfect alternative to time-consuming and expensive offline SPE.

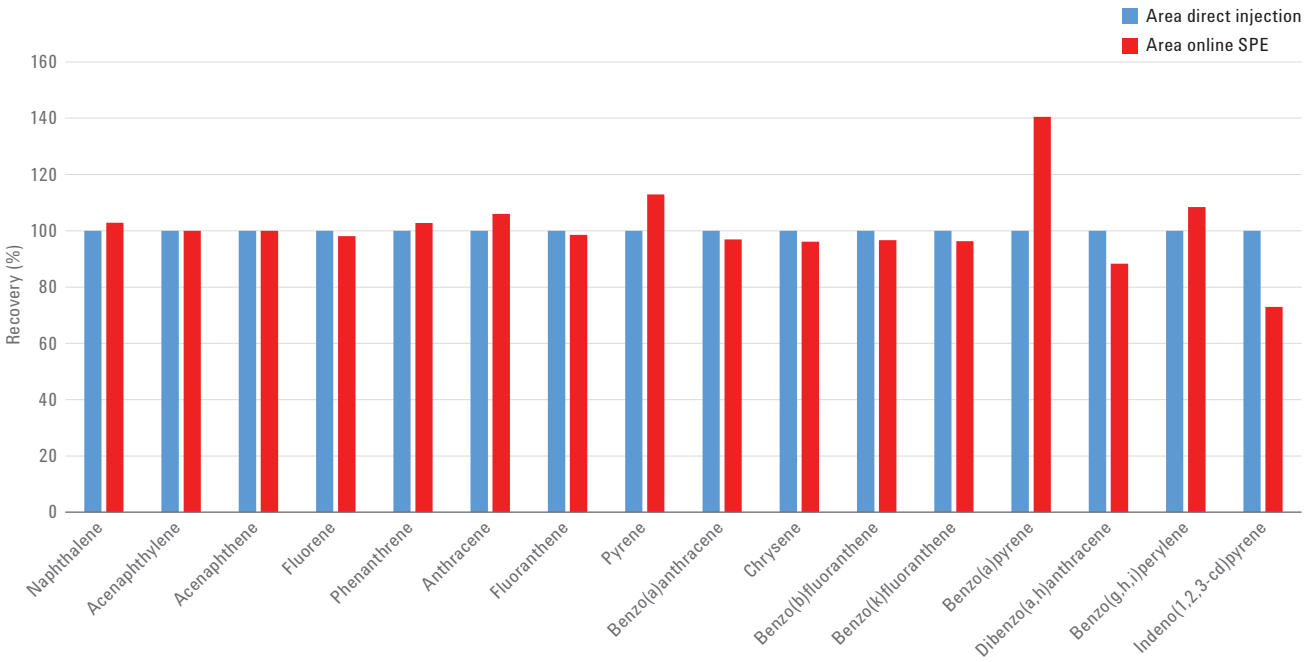

Figure 4. Recovery of the online SPE method in comparison to the direct injection method.

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