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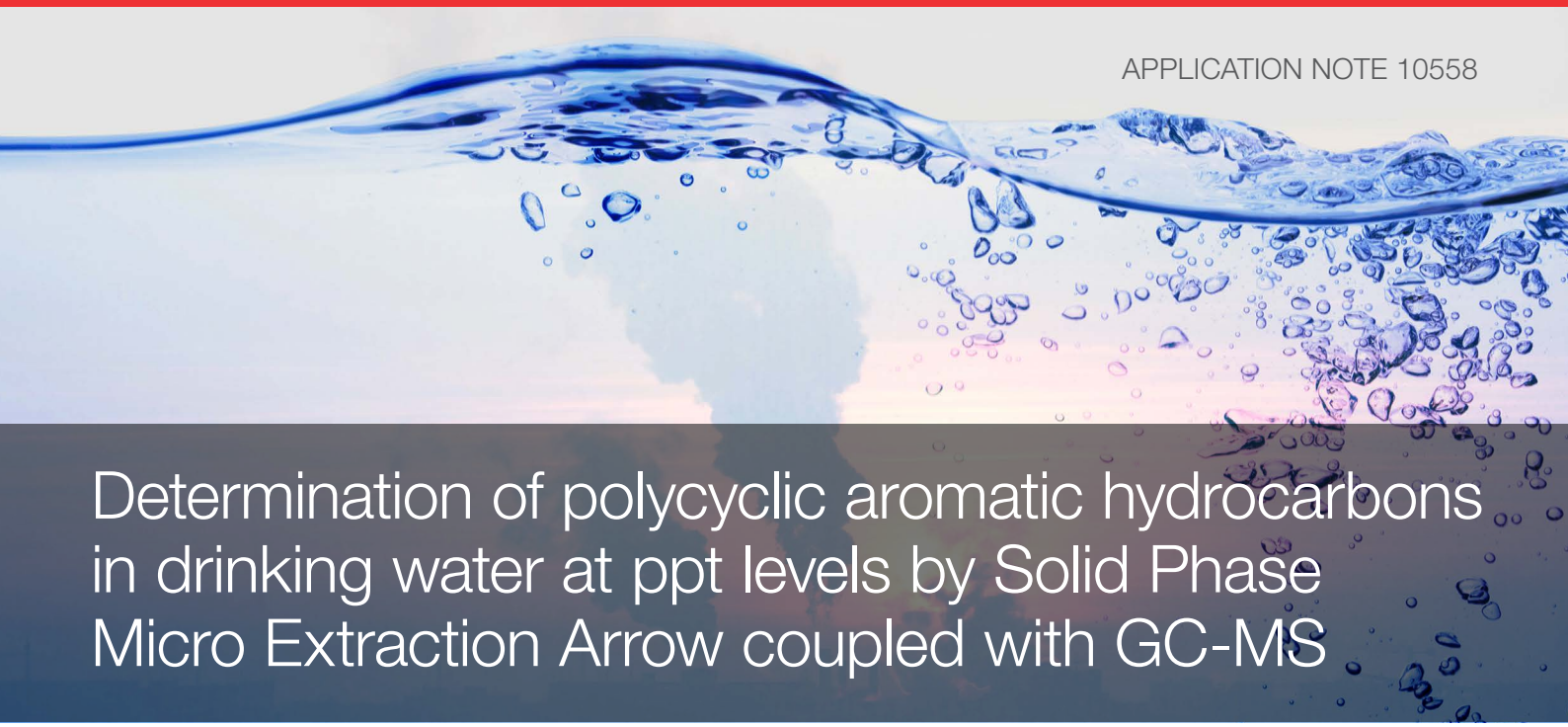
APPLICATION NOTE 10558



Determination of polycyclic aromatic hydrocarbons in drinking water at ppt levels by Solid Phase Micro Extraction Arrow coupled with GC-MS

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Keywords
Solid Phase Micro Extraction, SPME, SPME Arrow, Polycyclic aromatic hydrocarbons, PAH, Gas chromatography-mass spectrometry, GC-MS, Drinking water

Goal

To demonstrate a fully automatized method for extraction and quantification of low level PAHs from drinking water using Thermo Scientific™ SPME Arrow technology.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are considered priority environmental contaminants as they are ubiquitous persistent organic pollutants (POPs). They originate from incomplete combustion of natural deposits (such as oil, coal, tar, wood, and petroleum) and artificial sources (such as fuels, vehicle emissions, rubber, plastics, and cigarettes).

Environmental protection agencies strictly regulate the presence of PAHs in air, water, soil, industrial, and food because this group of chemicals varies in structure and toxicity. The analysis of drinking water for the presence of PAHs is important as coal tar is normally used to protect distribution pipes from corrosion and therefore can be considered the main source of PAHs in drinking water. Different maximum concentration limits are set by local regulatory agencies.[1,2]

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PAHs are present at very low levels (ppb and sub-ppb levels), so extraction of these compounds from matrices that are often complex is time-consuming, expensive, and a potential source of error and cross contamination. Solid phase extraction (SPE) and liquid-liquid extraction (LLE) are the most common techniques used for isolating PAHs from matrices. SPE cartridges are expensive and not reusable, while LLE requires a large volume of organic solvents for extraction procedures. Alternative techniques such as stir bar sorptive extraction (SBSE) and solid phase micro extraction (SPME) minimize sample preparation, eliminate solvent consumption, and reduce analysis cost.

SPME is an innovative solvent-free technique introduced in 1989 by Pawliszyn and co-workers[5] that combines sample extraction and concentration in a single step. It consists of a fiber coated by an organic phase that acts by extracting and concentrating the analytes present using selective absorptive/adsorptive processes. A steel tube protects the fiber during the transfer processes and when the device is not in use, thus minimizing the risk of contamination from ambient air. The fiber can be exposed in the vapor phase above the liquid or solid matrix (headspace) or directly immersed in the liquid sample (direct immersion) so that the analytes can be extracted from the sample matrix and concentrated on the fiber coating. After the equilibrium between the coating phase and the matrix has been reached, the fiber is removed from the sample, inserted into a GC SSL injector, and the analytes are transferred directly into the analytical column following thermal desorption. Reduced fiber phase capacity and mechanical robustness are the main limitations to wider adoption of this technology for intensive usage.

SBSE was introduced in 1999 by Baltussen and co-workers.[4] It consists of a magnetic stirring rod coated with a sorptive layer. Extraction is based on the same SPME partition theory: after exposure to samples, the stir bar is manually removed, rinsed with deionized water, and dried prior to inserting it into a desorber tube. The stir bar is thermally desorbed and the analytes are transferred into the chromatographic system. Due to its larger amount of sorptive phase, higher sensitivities can be obtained, but longer extraction times are required compared to classic SPME fiber. Furthermore, SBSE has the disadvantage of a very low grade of automation since some manual tasks are necessary prior to thermal desorption.

SPME Arrow fibers (Figure 1) overcome the limitation of both classic SPME and SBSE, combining superior sensitivity, improved extraction efficiency, and higher mechanical robustness with a fully automated workflow. The name of the SPME Arrow comes from the shape of the fiber tip that resembles that of an arrow. This particular tip geometry facilitates the smooth penetration of the GC septum, eliminating coring issues and prolonging its lifetime.

In this study, a method for the determination of the 16 EPA regulated PAHs in drinking water using a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and ISQ™ Series Single Quadrupole GC-MS coupled with a TriPlus™ RSH™ autosampler equipped with the SPME Arrow technology has been developed.

Experimental

Sample preparation

Samples were prepared by placing 15 mL of drinking water into 20 mL screw cap headspace vials and spiking each with 50 ng/L deuterated internal standard mix before sealing the vial.

Reagents

Assessment of linearity was made using a serial dilution of a 16 PAH standard solution (Restek® SV Calibration Mix #5, P/N 31011) at 2000 mg/L.

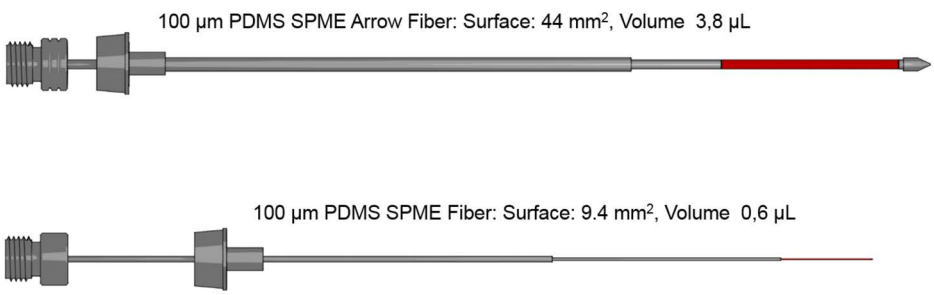

Figure 1. Design of SPME Arrow (above) in comparison with classical SPME (below).

From the concentrated standard solution, three stock solutions were serially diluted to 0.1, 1.0, and 10.0 µg/L in methanol (Chromasolv®, purity ≥ 99,9%).

Deuterated internal standards, acenaphthene-d_{10}, chrysene-d_{12} and benzo[a]pyrene-d_{12} were purchased from Neochema GmbH, Germany. The internal standard mix solution was diluted in acetone (Chromasolv, purity ≥ 99,9%) at 10 µg/L and stored at -4 °C until use.

Different aliquots of the stock solutions were spiked ranging from 1 ng/L to 500 ng/L into 100 mL MilliQ® water for assessing method linearity and repeatability. Calibration levels were prepared at 1, 2.5, 5, 10, 25, 50, 100, and 500 ng/L. Each calibration standard was prepared and run in triplicate, aliquoting 15 mL of 100 mL diluted stock solution added with 50 ng/L internal standard mix into three 20 mL headspace vials to avoid sample depletion due to repeated extractions.

Extraction

A polydimethylsiloxane (PDMS) coating was chosen because, as reported in literature,[3] it is more suitable for the analysis of non-polar compounds such as PAHs. A 100 µm PDMS SPME Arrow fiber (20 mm length, 3.8 µL volume) was selected for PAH extraction by direct immersion. The fiber was conditioned for 30 minutes at 250 °C prior to the first use.

Samples were stored at room temperature and pre-equilibrated at 35 °C at 500 rpm for 15 minutes prior to extraction. Simultaneously, the fiber was conditioned at 250 °C under a nitrogen stream (pressure regulator was set at 2 bars). The extraction step was carried out at the same temperature of pre-equilibration but with a 1500 rpm stirring speed. Compound diffusion is limited by the presence of the aqueous layer around the surface of the fiber coating. The higher stirring speed was optimized, reducing the thickness of the aqueous boundary layer to improve the analyte diffusion to the fiber. After the 30 minute extraction, the fiber was exposed into the hottest part of the injector port (70 mm injection depth) and thermally desorbed in splitless mode at 280 °C for 5 minutes. At the end of the desorption step, the fiber was cleaned up for 15 minutes at 250 °C to reduce the risk of carry-over.

Optimized TriPlus RSH autosampler - SPME Arrow extraction parameters are listed in Table 1.

Table 1. TriPlus RSH autosampler - SPME Arrow extraction parameters.

Extraction Parameters	
Incubation Temperature:	35 °C
Incubation Time:	15 min
Incubation Speed	500 rpm
Extraction Temperature:	35 °C
Extraction Time:	30 min
Stirring Speed:	1500 rpm
Fiber Conditioning Temperature:	250 °C
Fiber Conditioning Time:	15 min
Fiber Depth in Vial:	55 mm
Fiber Depth in Injector:	70 mm

GC/MS analysis

A TriPlus RSH autosampler was used for SPME Arrow extraction and sample introduction into a Thermo Scientific™ TRACE™ 1310 gas chromatograph equipped with an Thermo Scientific™ Instant Connect split/splitless (SSL) injector and coupled with a Thermo Scientific™ ISQ™ LT mass spectrometer operated in selected ion monitoring (SIM) mode using electron ionization (EI). Chromatographic separation was achieved using a Thermo Scientific™ TG-5 SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N 26098-1420). Additional details on instrument parameters are listed in Tables 2 and 3.

Data were acquired and processed using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS).

Table 2. GC/MS system setup parameters.

TRACE 1310 GC System Parameters	
Inlet:	280 °C
Liner:	Arrow liner, 1.8 mm ID (P/N 453A0415)
Inlet Module and Mode:	SSL, splitless
Splitless Time:	8 min
Purge Flow:	5 mL/min, stopped during desorption
Carrier Gas:	He, 1.2 mL/min
Oven Temperature Program	
Temperature:	35 °C
Hold Time:	5 min
Rate:	40 °C/min
Temperature 2:	150 °C
Rate:	20 °C/min
Temperature 3:	250 °C
Rate:	10 °C/min
Temperature 4:	305 °C
Hold Time:	15 min
ISQ-LT MS System Parameters	
Transfer Line Temperature:	310 °C
Source Temperature:	350 °C
Ionization Mode:	EI
Electron Energy:	70 eV
Acquisition Mode:	SIM

Table 3. Monitored *m/z* for quantifier and qualifier ions.

Component	Quantification Ion (*m/z*)	Confirmation Ion 1 (*m/z*)	Confirmation Ion 2 (*m/z*)
Naphthalene	128	129	127
Acenaphtylene	152	151	150
Acenaphthene	153	154	152
Fluorene	166	165	167
Phenantrene	178	176	179
Anthracene	178	176	179
Fluoranthene	202	200	203
Pyrene	202	200	203
Benzo[a]anthracene	228	226	229
Chrysene	228	226	229
Benzo[b]fluoranthene	252	253	250
Benzo[k]fluoranthene	252	253	250
Benzo[a]pyrene	252	250	253
Indeno[1,2,3-cd]pyrene	276	277	274
Dibenzo[a,h]anthracene	278	279	276
Benzo[ghi]perylene	276	277	274

Results and discussion

Results obtained with the optimized parameters described in the previous section are discussed.

Linearity

Fiber extraction linearity was assessed across an 8-point calibration curve ranging from 1-500 ng/L. The 100 µm PDMS SPME Arrow fiber shows good linearity in extracting all 16 regulated PAHs; the coefficient of determination (R^2) was ≥ 0.995 for all components with an average value of R^2 = 0.998 as reported in Table 4.

Typical calibration curves for benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno (1,2,3-cd) pyrene, and benzo(ghi)perylene are shown in Figure 2.

Table 4. Linearity, recovery, log $k_{o/w}$, and %RSD results for 16 regulated PAHs.

Compound	R^2	MDL (ng/L)	Recovery (%)	Log $K_{o/w}$	RSD (%)	Carry-over (%)
Naphthalene	0.999	2.86	18	2.84	6.52	0.59
Acenaphthylene	0.995	2.32	22	3.32	3.75	0.06
Acenaphthene	0.997	1.92	31	2.94	3.92	0.01
Fluorene	0.999	1.31	54	3.26	3.72	0.02
Phenanthrene	0.997	2.17	90	3.99	5.35	0.05
Anthracene	0.997	1.64	95	3.99	5.30	0.04
Fluoranthene	0.998	2.17	94	4.49	4.87	0.04
Pyrene	0.998	1.99	91	4.58	4.40	0.03
Benzo[a]anthracene	1.000	1.15	96	4.77	5.36	0.07
Chrysene	1.000	1.38	94	5.15	5.34	0.08
Benzo[b]fluoranthene	1.000	1.23	75	5.16	4.65	0.12
Benzo[k]fluoranthene	1.000	1.28	60	5.33	5.49	0.19
Benzo[a]pyrene	1.000	1.06	79	5.27	4.91	0.10
Indeno[1,2,3-cd]pyrene	1.000	0.71	38	6.23	7.76	0.23
Dibenzo[a,h]anthracene	0.997	0.58	70	6.20	12.22	0.30
Benzo[ghi]perylene	0.997	0.92	42	6.63	5.15	0.30

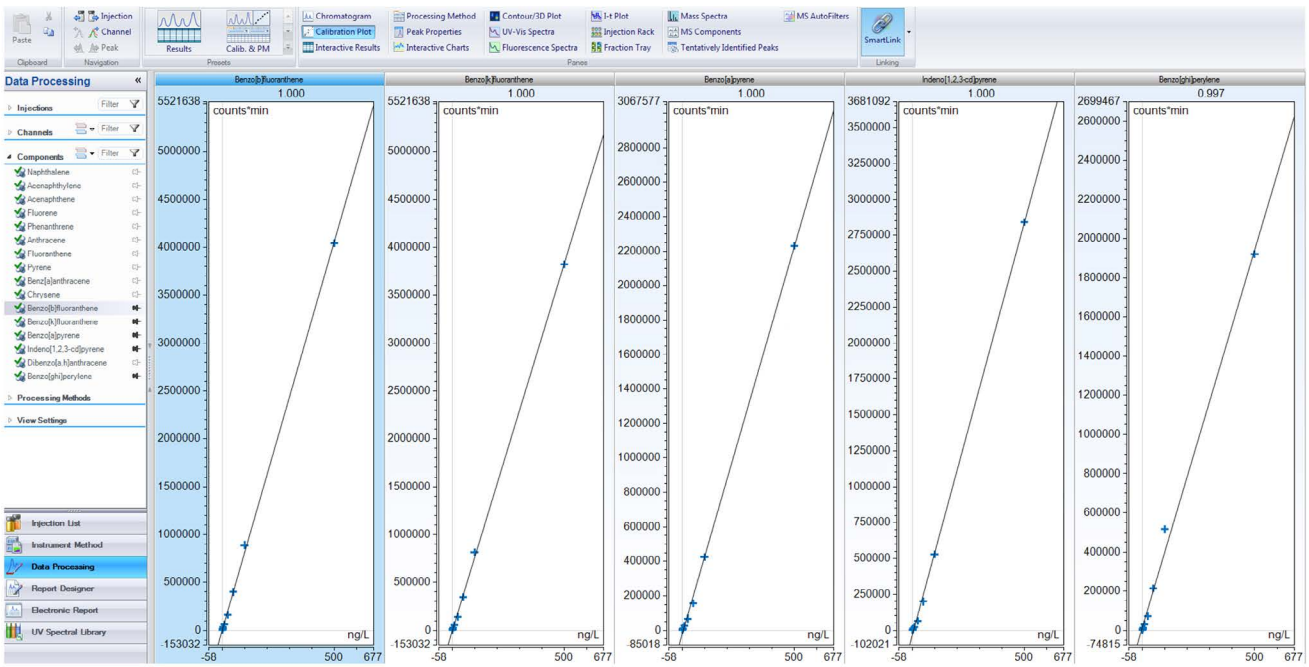

Figure 2. Chromeleon CDS results browser showing 8-point calibration curve obtained over a concentration range of 1–500 ng/L for benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene.

Recovery

Recovery was calculated by comparing the peak areas obtained by extracting 15 mL of a 10 ng/L PAH standard solution with those obtained by injecting 1 µL of a 1.5 mg/L PAH standard solution. In both cases, the analyte absolute amount was 150 pg.

According to literature[3,4] more volatile compounds show lower recovery compared to higher mass molecules as shown in Figure 3. This trend depends on the polarity of the components and on their partitioning between the PDMS phase and water. The octanol/water partitioning coefficient ($K_{o/w}$) is expressed as the ratio of the equilibrium concentrations of a chemical in octanol and in water; thus, it can be considered a good approximation of the PDMS/water distribution coefficient. Less apolar components with a log $K_{o/w}$ < 3 show lower recoveries compared to more apolar compounds with 4 < log $K_{o/w}$ < 5 due to the unfavorable ratio between PDMS and the aqueous phase. Highly hydrophobic compounds with log $K_{o/w}$ > 6 show very low recoveries as well. Furthermore, literature[3,4] documents their strong trend to adsorb onto the glass wall of the extraction vials and in some parts of the chromatographic system.

Recovery results and log $K_{o/w}$ for 16 investigated PAHs are reported in Table 4. The recoveries range from 18% to 96%, depending on the $K_{o/w}$ of each compound, and are consistent with data reported in literature.

Extraction efficiency

Optimized extraction conditions were applied to assess a comparison between the 100 µm PDMS classic SPME fiber and the 100 µm PDMS SPME Arrow fiber in extracting 100 ng/L PAHs from water to determine the effect of a wider sorption surface. Larger phase volume provided by SPME Arrow allows to enhance the average extraction yield for all the components up to five times as shown in Figure 4.

Method detection limit

The method detection limit (MDL) is the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The MDL was calculated by extracting nine vials spiked with 15 mL of 5 ng/L PAHs standard solution and taking into account the one-tailed Students *t*-test values for the corresponding nine degrees of freedom at 99% confidence.

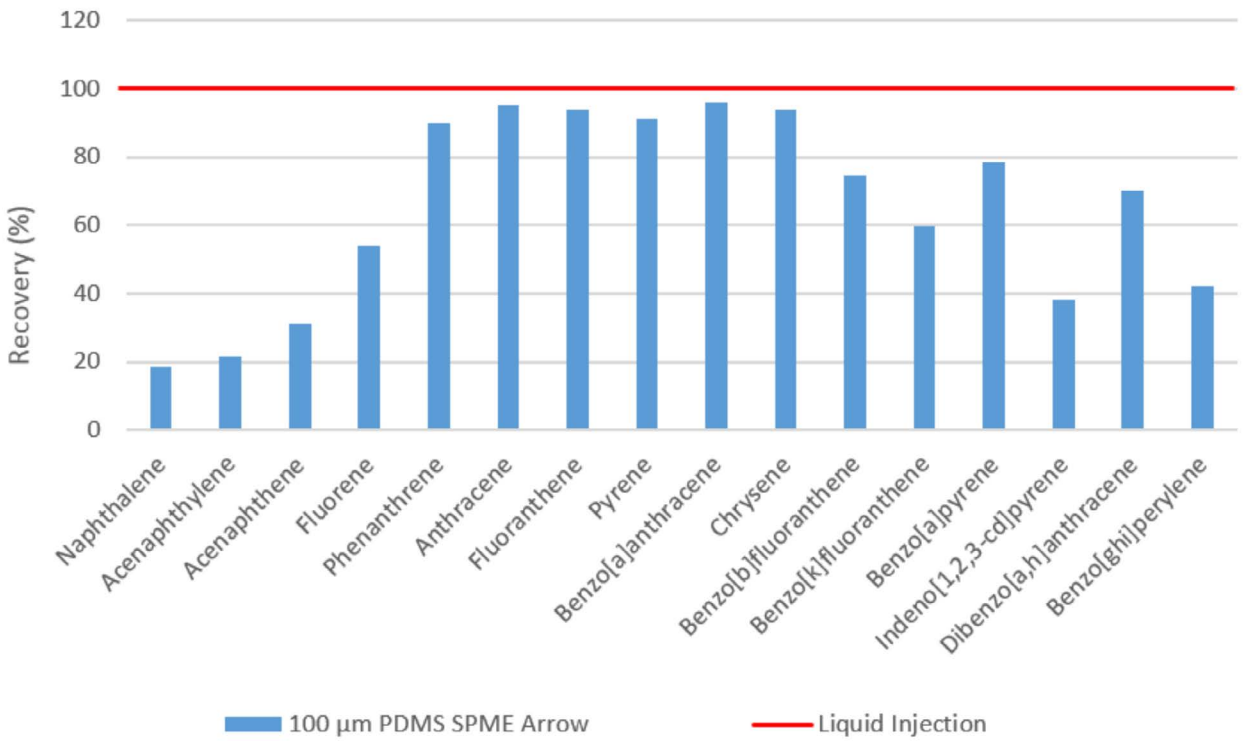

Figure 3. Recovery results (in %) obtained for 150 pg analyte absolute amount with SPME Arrow fiber and liquid injection.

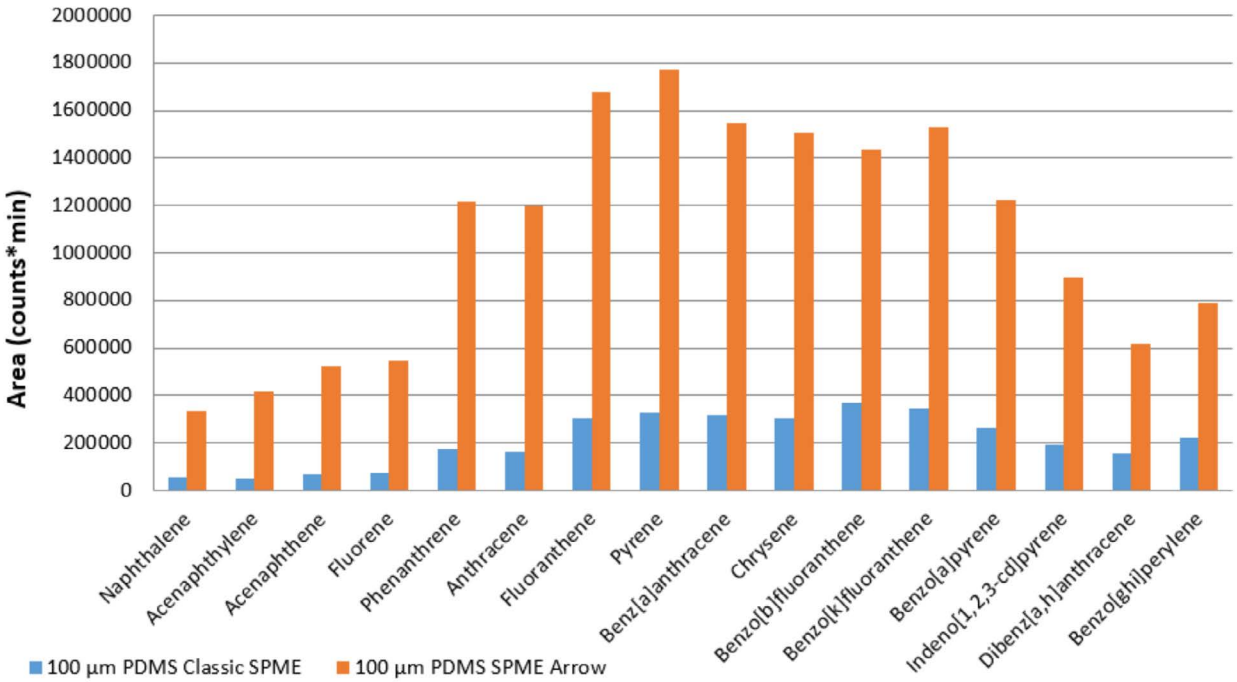

Figure 4. Extraction efficiency (peak area counts) in extracting 100 ng/L of 16 EPA regulated PAHs from water using a 100 µm PDMS SPME fiber and 100 µm PDMS SPME Arrow fiber.

The range of detection for the 16 EPA regulated PAHs was 0.5–3.0 ng/L (data shown in Table 4). As expected, more volatile compounds show higher MDLs since lower amounts are extracted from the fiber as a result of their PDMS-water partitioning coefficient. For benzo[a]pyrene (the most toxic of the 16 regulated PAHs) a 1 ng/L MDL was achieved.

An example of compound detection and identification is shown in Figure 5. Benzo[a]pyrene at the calculated 1 ng/L MDL is identified based on expected retention time, quantifier and qualifier ions. Both qualifier ions are confirmed at such low concentration level; the match (forward match = 834) against the NIST library adds confidence to compound identification.

Calculated carry-over

To assess the carry-over, a 6-point calibration curve ranging from 1 to 500 ng/L was run in triplicate followed by a fiber blank. The fiber blank was obtained by desorbing the fiber directly into the injector port at 280 °C for 5 minutes. Carry-over was < 0.60% for all components as reported in Table 4. Data was successively confirmed by acquiring a fiber blank after each extraction of the highest calibration point at 500 ng/L.

Repeatability

Peak area repeatability was tested on 10 consecutive extractions at 10 ng/L PAH standard solution from different vials. %RSDs ranged from 3.0 to 12.2%. These data are consistent and the values obtained are significantly lower than % RSD ranges reported in literature[5,6] at such low concentration levels for the 100 µm PDMS classic SPME fiber.

Robustness

The arrow-shaped tip allows a smoother penetration of vials and injector septa compared to the classic SPME square tip. Furthermore, a larger fiber diameter ensures higher resistance to mechanical stress: more than 100 extractions can be performed without any loss of performance or any mechanical failure. Up to 150 injections can be performed without septum replacement using Thermo Scientific™ TR-Green (P/N 313G3230) or Marathon Septa (P/N 313P3233).

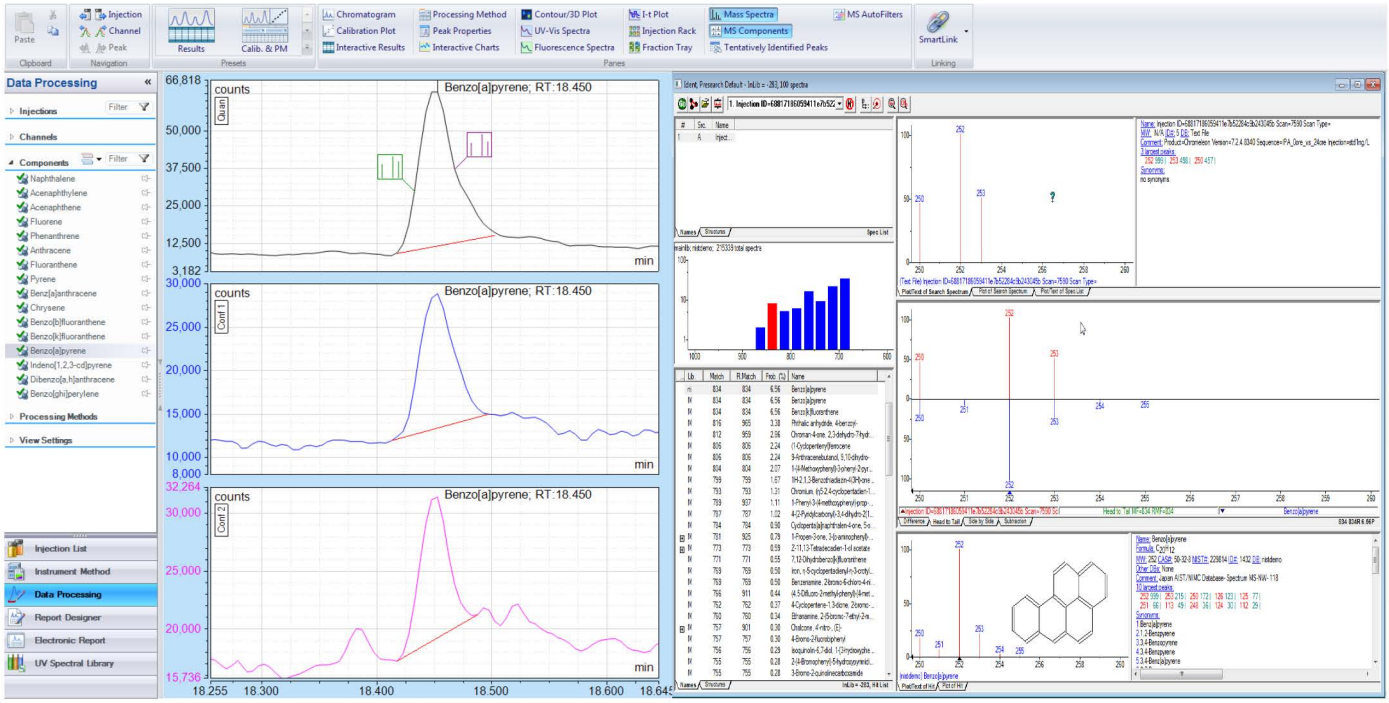

Figure 5. Chromeleon CDS results browser showing benzo[a]pyrene identification and confirmation at 1 ng/L MDL value.

Detection and quantification of PAHs in real drinking water samples

The optimized method was applied to the analysis of tap water to assess the performance on real samples. A locally sourced tap water sample was aliquoted and spiked with the internal standard deuterated PAHs mix following the procedure described above. An example of quantitative analysis is shown in Figure 6 for benzo[a] pyrene. The sample must be considered negative according to European Council Directive[7] (2015/1787/UE) on the Quality of Water Intended for Human Consumption. Acceptance limits for PAHs are set at 100 ng/L expressed as the sum of benzo[b]fuoranthene, benzo[k]fuoranthene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene. Limits for benzo[a]pyrene are set at 10 ng/L.

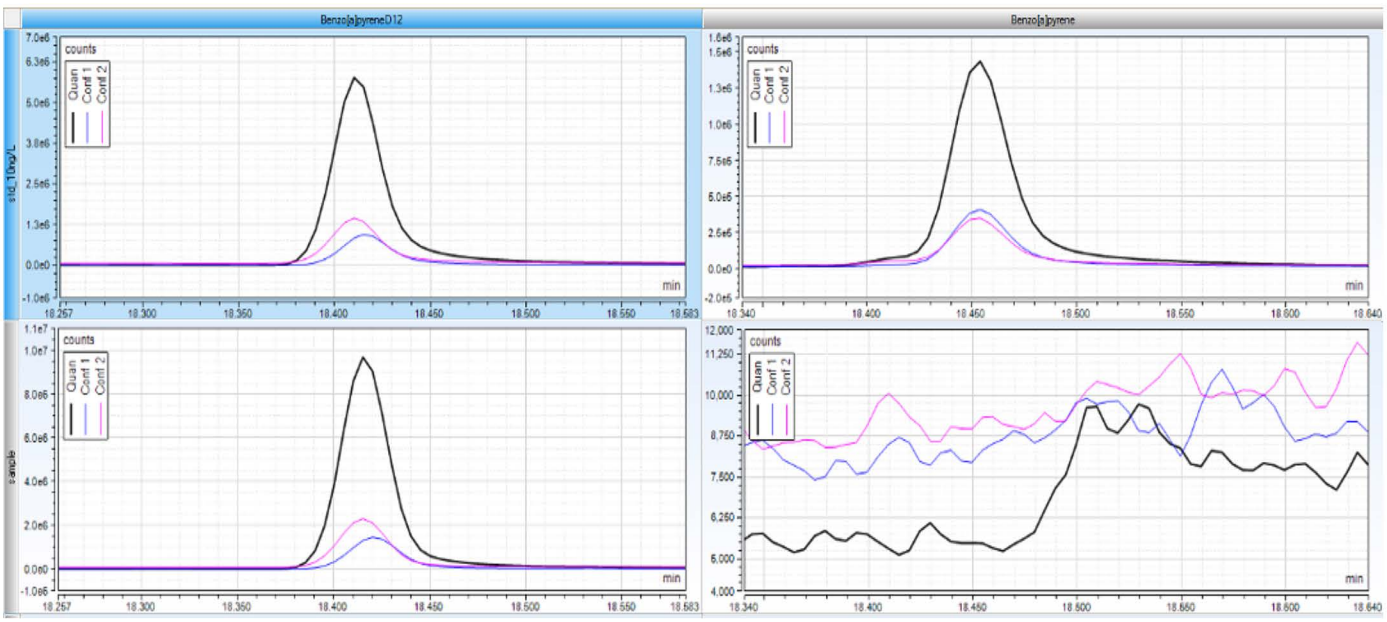

Figure 6. Chromeleon CDS browser showing the comparison between a standard solution and a real drinking water sample (deuterated ISTD on the left, benzo[a]pyrene on the right). According to European Legislation on Water Intended for Human Consumption, the sample is negative for benzo[a]pyrene.

Conclusion

- Trace PAHs levels can be detected down to 1 ng/L with minimal sample preparation.
- Outstanding compound linearity (average $R^2 > 0.995$) can be achieved over the concentration range 1–500 ng/L.
- Excellent peak area repeatability (%RSDs < 15) was also obtained with lower values compared to the classic SPME fiber.
- The larger sorption surface provided by the SPME Arrow fiber provides a significant increase in the extraction efficiency for more volatile and polar compounds with partition coefficients $K_{o/w} < 3$.
- The innovative SPME Arrow fibers design ensures robustness and reliability towards mechanical stress even under fast stirring speed.

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