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Comparison of Strategies for Extraction of High Molecular Weight Polycyclic Aromatic Hydrocarbons from Drinking Waters

M. S. GARCÍA-FALCÓN, M. PÉREZ-LAMELA, AND J. SIMAL-GÁNDARA*

Nutrition and Bromatology Group, Analytical and Food Chemistry Department, Faculty of Food Science and Technology, Ourense Campus, University of Vigo, 32004 Ourense, Spain

Simple, rapid, and inexpensive methods have been developed for the determination of polycyclic aromatic hydrocarbons (PAHs) in drinking waters without interferences from other chemical contaminants by use of two different extraction techniques and analysis by an optimized reverse-phase (RP) high-performance liquid chromatography followed by fluorescence detection (HPLC−FLD) method. The feasibility of SPE (solid-phase extraction) and SPME (solid-phase microextraction) for the determination of PAH in drinking water samples has been evaluated. Several parameters have been studied and optimized for both extraction procedures. The relationship between the nature of the fibers and the quantity of extracted compounds and the effects of organic solvent, salt addition, sampling temperature, and sampling time was studied for SPME. Acetonitrile percentage added to the sample, sample storage conditions (temperature and time), and type of organic elution solvent and elution volume were evaluated for SPE. The results show that both extraction procedures can be used to determine PAHs in drinking waters, but SPE gives better performance (recovery, precision, and quantification limits) for the determination of PAHs in drinking water at the levels established by the legislation.

KEYWORDS: PAHs; drinking waters; high-performance liquid chromatography followed by fluorescence detection (HPLC−FLD); solid-phase extraction (SPE); solid-phase microextraction (SPME)

INTRODUCTION

One of the most prominent groups of chemicals in smoke, soot and exhaust are polycyclic aromatic hydrocarbons (or PAHs), natural products of the incomplete combustion of carbon compounds (*1*−*3*). When released directly into the atmosphere through burning, PAHs may attach to small particles and be transported for considerable distances before returning to earth directly or in rainfall. Present in low concentrations virtually everywhere, PAHs occasionally reach elevated concentrations as the result of prolonged industrial activities involving burning or by releases from materials, such as creosote-based wood preservatives, which contain PAHs in high concentrations.

PAHs are a concern because some of them can cause cancers in humans and are harmful to fish and other aquatic life (*4*). Most of the information on the toxicology of PAHs was derived from experimental animals exposed to PAHs under controlled conditions. Studies related to effects on humans from exposure to PAHs, singly or collectively, are rare. Epidemiological studies have shown increased mortality due to lung cancer in humans exposed to coke-oven emissions, roofing-tar emissions, and cigarette smoke. Because of the complex nature of the mixtures, it is difficult to evaluate the contribution of any single PAH to the total carcinogenicity of these mixtures. Humans can be exposed to PAHs via air, water, and food. In comparing the inhalation and ingestion pathways in each home, some investigators found that potential intake could be similar in each medium (*5*).

PAHs are controlled under various laws, regulations, and agreements designed to protect the environment and human health. Due to the pervasive nature of PAHs and their sources of release, reducing exposure presents a significant challenge. On drinking waters, the recent Directive 98/83/EC (*6*) proposes the determination of four PAHs (benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, and indeno[1,2,3-*cd*]pyrene) and limits the maximum concentration to 100 ng/L for all of them and to 10 ng/L for the particular case of benzo[*a*]pyrene. It is imperative that the raw waters that may be used by the food and beverage processing industries be potable and meet drinking water quality criteria.

The determination of PAHs by means of HPLC−FLD (fluorescence detection) has been sufficiently verified (*7*, *8*). In this paper we compare two extraction systems (solid-phase extraction, SPE, and solid-phase microextraction, SPME), taking into account the time that each case requires, the analyzed sample volume, the recoveries and repeatabilities at the nanograms per liter level, and the limits of quantification. Most papers involving the generation of environmental PAH data for

* To whom correspondence should be addressed: e-mail jsimal@uvigo.es.

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waters involve either SPE or SPME, not both. The methods of analysis, especially the one based on SPE because of its more sensitive detection properties, were applied to real drinking waters from different origins in order to study the possible interferences in the various matrixes.

MATERIALS AND METHODS

Chemicals, Solvents, Solutions, and Small Apparatus. The nine PAHs studied (benzo[*b*]fluoranthene (B[*b*]F, 98%), benzo[*k*]fluoranthene (B[*k*]F, 98%), benzo[*a*]pyrene (B[*a*]P, 97%), benzo[*ghi*]perylene (B[*ghi*]P, 98%), indeno[1,2,3-*cd*]pyrene (I[1,2,3-*cd*]P, 98%), fluoranthene (Fl, 99%), benzo[*a*]anthracene (B[*a*]A, 98%), dibenzo[*ah*]anthracene (DB[*ah*]A, 97%), and benzo[*e*]pyrene (B[*e*]P, 99%)) were purchased from Aldrich and Supelco. The first five are indicators of drinking water quality, whereas it is important to monitor the rest in environmental (wild animals, soil, particulate matter in air, etc.) and food (vegetable oils, fried and smoked foods, etc.) samples. B[*e*]P closely elutes with B[*b*]F; to solve such a problem in the case of SPE, a second wavelength program was used with the intention of quantifying B[*e*]P without the interference of B[*b*]F, while spending this chance to increase sensitivity for B[*ghi*]P by using its optimum detection wavelengths. Acetonitrile, water, dichloromethane, and hexane of HPLC grade were supplied by Merck.

Independent 100 mg/L stock solutions of PAHs were prepared by dissolving about 0.01 g of the different PAHs in a small amount of hexane and diluting to 100 mL with the same solvent. From this solution, intermediary independent solutions of ca. 1−20 mg/L were prepared into hexane. From the intermediary independent solutions, a PAH mix solution at levels ranging from 25 to 350 μg/L was prepared into acetonitrile after evaporation of hexane. These solutions were stored in amber flasks at 4 °C and were then stable for at least 6 months. From the PAH mix solution, different calibration solutions in acetonitrile were prepared to construct 10-point calibration lines (in duplicate) for the PAHs (peak areas vs concentrations). All these solutions were preserved for at least 2 months in the same storage conditions as the other solutions.

Waters Sep-Pak C18 Plus (360 mg) cartridges were used as solid-phase extraction (SPE) minicolumns for purification and concentration. A Visiprep solid-phase extraction vacuum manifold was used to simultaneously process up to 24 SPE cartridges. The Visidry drying attachment was used to dry up to 24 SPE cartridges at one time and can be used with any inert gas supply. Nitrogen C-45 of analytical quality was supplied by Carburos Metálicos. Other small apparatus such as a rotary evaporator, an ultrasonic bath, and a vortex shaker were used.

Three SPME fibers were considered in this study: 100 μm poly(dimethylsiloxane) (100-PDMS), 85 μm polyacrylate (PA), and 65 μm poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB). The commercially available SPME device and fibers were purchased from Supelco (Bellefonte). Fibers were initially conditioned according to the manufacturer's instructions in order to remove contaminants and to stabilize the polymeric phase. For the SPME extraction, water samples were placed in 40-mL amber EPA vials (Wheaton) equipped with stir bars and sealed with poly(tetrafluoroethylene)- (PTFE-) faced silicone septum, and stirred with a magnetic stirrer (Raypa, Spain).

Liquid Chromatograph and Operating Conditions. All HPLC measurements were taken by use of a Thermo Separation Products (TSP) P2000 binary pump, equipped with a TSP AS1000 autosampler, a TSP SCM1000 vacuum membrane degasser, and a Jasco FP-1520 fluorescence detector. The chromatographic data were collected and processed by use of the Chrom-Card software. The optimized instrumental parameters for the chromatographic analysis of PAHs were as follows:

The injection loop was 50 μL.

The column was a 25 cm × 4.6 mm i.d. stainless steel analytical column packed with 5 μm Supelcosil LC-PAH (Supelco).

Elution conditions were a 32 min linear gradient elution from 80:20 acetonitrile/water to 97:03 acetonitrile/water, followed by a 5 min linear gradient to 100% acetonitrile. Flow rate was 1 mL/min throughout. Elution temperature was maintained at 33 °C.

Fluorescence detectors provide only response for fluorescent compounds such as the PAHs. This helps to remove interference from other components in the samples but also to increase sensitivity in detection for the fluorescent compounds, above all when the detector allows to change detection wavelengths along the elution time. The main wavelength program was as follows: 10 min λ_{ex} at 284 nm and λ_{em} at 464 nm, followed by 6 min λ_{ex} at 274 nm and λ_{em} at 414 nm, followed by 6 min λ_{ex} at 300 nm and λ_{em} at 446 nm, followed by 6 min λ_{ex} at 296 nm and λ_{em} at 406 nm, and followed by 9 min λ_{ex} at 300 nm and λ_{em} at 470 nm. To minimize the detection levels with the fluorescence detector, gain and slit were established at the maximum (1000 and 40, respectively). In such conditions, the instrument linearity range is shorter. This wavelength program allows the determination of eight PAHs: Fl, B[*a*]A, B[*b*]F, B[*k*]F, B[*a*]P, D[*ah*]A, B[*ghi*]P and I[*1,2,3-cd*]P. The secondary wavelength program involved changing 300 and 446 nm in such a time window by 270 and 380 nm, and keeping for the last 10 minutes 296 and 406 nm as detection wavelengths; this allows confirmation of PAH identification in such windows. This program is used to quantify one more PAH, B[*e*]P, without the interference of B[*b*]F by changing wavelengths in the time window, while spending this chance to use other wavelengths the last 10 min with the aim of increasing sensitivity by a factor of 3 for B[*ghi*]P.

Extraction Parameters Evaluated To Maximize PAHs Recovery and Sensitivity: (A) Solid-Phase Extraction. Samples of Milli-Q water (250 mL) were spiked with PAHs (2−30 ng/L), and then left for 12 h at 4 °C in amber glass bottles protected from light. These samples were employed to select SPE conditions providing high recovery and sensitivity for the selected PAH.

Loading Conditions. The percent of acetonitrile added to the water was optimized to prevent PAHs adsorption to the glass surface and yet guarantee the total retention of PAHs onto the C18 minicolumn. Acetonitrile/Milli-Q water spiked solutions at 0%, 5%, 20%, 30%, and 40% acetonitrile added were tested.

Elution Conditions. After the cartridge was dried completely, the next task was to select the solvent for the selective elution of PAHs. Acetonitrile, dichloromethane, and hexane were tested at different volumes.

(B) Solid-Phase Microextraction. Samples of Milli-Q water (40 mL) were spiked with PAH (15−250 ng/L) and then left for 12 h at 4 °C in amber glass bottles protected from light. These samples were employed to select SPME conditions providing high recovery and sensitivity for the selected PAH.

Selection of SPME Coating. Three SPME fiber coatings were evaluated to select the most appropriate. Fortified aqueous samples were analyzed in duplicate with each fiber. The extraction time was 30 min at room temperature for all fibers. The samples were magnetically stirred during the extraction process. The desorption process was produced at room temperature for 10 min with 120 μL of acetonitrile.

Effect of Acetonitrile Addition To Prevent Sorption to Glass. Fortified aqueous samples were analyzed as above in duplicate with the addition of different volumes of acetonitrile (0, 2, and 4 mL).

Effect of Salt Addition. Aqueous samples fortified with 2 mL of acetonitrile and with added PAHs as before were unsalted and salted with NaCl (1−3 g) and analyzed twice as above.

Effect of Temperature. The effect of temperature in PAH-spiked waters was evaluated in duplicate when aqueous samples fortified with added acetonitrile (2 mL) were extracted at 0, 25, and 60 °C by holding the sample in a water bath controlled at a constant temperature (to keep the temperature constant, a 0 °C ice was used in the water bath).

Sorption and Desorption Time Profiles. Duplicate aqueous samples fortified with added acetonitrile (2 mL) were analyzed at different extraction times (10, 20, 30, 40, 60, and 90 min) at 60 °C. Desorption profiles were obtained by plotting the detector response versus different desorption times (5−20 min).

Extraction Procedures. Once both extraction procedures were optimized, the final setup for water analysis in the search for PAHs was as follows.

Solid-Phase Extraction. The 360 mg C_{18} Sep-Pak cartridge was previously conditioned with 5 mL of acetonitrile followed by 10 mL of ultrapure water without allowing the cartridge to dry out. Aqueous sample (325 mL = 250 mL of water + 75 mL of acetonitrile) was

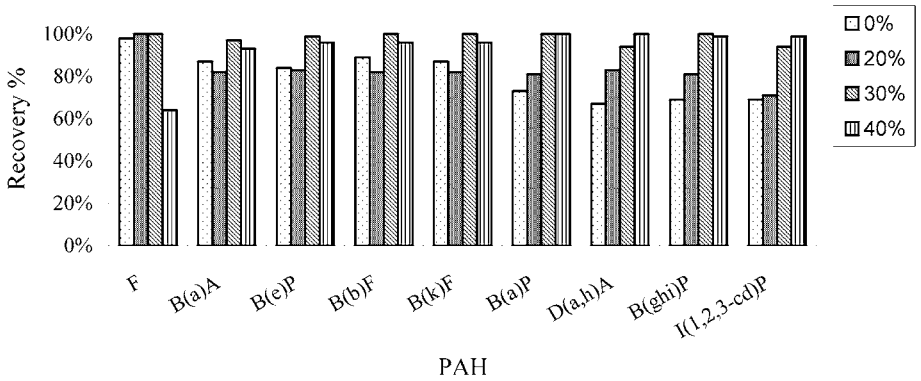

Figure 1. Influence of acetonitrile (0, 20, 30, and 40%) added to the water sample on PAH recovery obtained by SPE.

passed through the cartridge at the maximum rate allowed (approximately 25 mL/min). Twenty milliliters of acetonitrile/water (30/100) was used to wash all the glass material employed and subsequently passed through the cartridge. The cartridge was then dried by blowing N_2 for 20 min. Adsorbed PAHs were eluted by 5 mL of hexane; hexane was then rotary-evaporated to dryness, with the water-bath temperature kept below 60 °C to avoid PAH losses, and the residue was redissolved, finally, with 0.5 mL of acetonitrile. Homogenization of the final extract was achieved with ultrasound and vortex agitation. Calibration was performed by direct injection into the HPLC column of appropriate standard solutions.

Direct Solid-Phase Microextraction. For direct SPME, vials with aqueous samples (40 mL) were filled completely with ultrapure water to remove any remaining headspace in the amber EPA glass. The holder needle was inserted through the septum and the 100-PDMS fiber was directly immersed in the sample solution for 40 min under magnetic stirring at 60 °C. The extracted analytes were immediately transferred to a glass vial with a 150 μL insert containing acetonitrile (120 μL), where the desorption process was produced at room temperature for 5 min. Calibration was performed by direct injection into the HPLC column of desorbed extracts obtained from the SPME fiber used in the analysis of standard spiked waters.

Tap Water Samples. Samples of tap water with acetonitrile added ($n = 7$) were spiked with PAHs at levels 10 times lower than limits established in regulations (*6*). Samples were kept for 3 h at room and refrigeration temperature, exposed to and protected from day light, and without and with the addition of sodium thiosulfate as preservative according to U.S. EPA (*9*) to evaluate the stability of PAHs in tap water under those conditions.

Twenty samples of tap water from different locations in the region of Galicia (NW Spain) were collected.

Samples for SPE Extraction. Samples (500 mL) were collected in amber glass bottles, and 150 mL of acetonitrile was added to prevent PAH adsorption on the walls of the bottle and to facilitate subsequent PAH elution from C18 cartridge with low volumes of *n*-hexane.

Samples for SPME Extraction. Samples (40 mL) were collected in 40-mL amber glass EPA bottles capped with a PTFE-faced silicone septum, and 2 mL of acetonitrile was added to prevent PAH adsorption on the walls and to fill the vials completely, removing any remaining headspace.

To each sample, sodium thiosulfate was added until a concentration of 100 mg/L was reached to prevent oxidation of PAHs by free residual chlorine, dissolved oxygen, and other oxidants in water. These aqueous samples were kept under refrigeration for 2 days maximum until extraction and subsequent analysis.

RESULTS AND DISCUSSION

Stability of PAHs in Tap Water. Samples kept at room temperature, exposed to daylight, and without any kind of preservative showed a significant reduction of their PAH content, especially for B[*a*]P that reached a reduction of 40% ± 16% and the other higher molecular weight PAHs. When those spiked samples were protected from daylight and sodium thiosulfate was used, but the temperature was kept at 25 °C, B[*a*]P recovery was improved (90%) but precision was still poor (20%). A good recovery ± precision was obtained by reducing preservation temperature at 4 °C (95−104% ± <9% for all PAHs). Preservation is a key item for the stability of PAHs in drinking waters, since free chlorine and dissolved oxygen in the samples causes PAH reduction on storage.

SPE Procedure Performance: (A) Optimization. SPE C18 columns (a simple, automatable, and quantitative procedure) have been used satisfactorily for the isolation and concentration of PAHs from different matrixes: water (*9*−*15*), foods (*16*), and liquid smoke flavorings (*17*, *18*), replacing to the traditional liquid−liquid extraction (*19*). The loading step of our proposed SPE procedure is very rapid (15 min in total; 325 mL are passed through at a rate of 25 mL/min), whereas other procedures published are 4−6 times slower (*9*, *11*, *12*, *14*, *20*) because the sample volumes used are around 1−1.5 L.

Experiments were performed to optimize the different steps of SPE extraction (loading, washing, and elution) of PAHs from drinking waters. To avoid the sorption problems often encountered during PAH sampling and storage, it is necessary to develop a method that increases their solubility. Normally this is achieved by adding organic solvents to the sample (*9*, *10*, *13*, *20*−*22*) or surfactants (*11*, *13*). In this work PAH sorption problems were avoided by blending water with acetonitrile. This solvent also facilitates movement of PAHs along the cartridge and the subsequent elution with low volumes of hexane, avoiding the use of dichloromethane (carcinogenic solvent). Acetonitrile/water solutions were tested with PAH-spiked waters (**Figure 1**), and the best recoveries were obtained at 30% acetonitrile (at lower percentages, recovery was only 70% for those of higher molecular weight; at higher percentages, recovery was only 60% for those of lower molecular weight). These results were based on the increasing hydrophobic properties of these compounds with increasing molecular weight. When the percentage of acetonitrile was set below 30, the recoveries for the high molecular weight PAHs (HMW-PAHs) decreased because of their low water solubilities, leading to irreversible adsorption of these compounds onto glass surfaces during sample processing. In contrast, when the percent of acetonitrile added to the water was higher than 30, the observed recovery yields a decrease in the percentage recoveries observed for the low molecular weight PAHs (LMW-PAHs). This was due to the higher sample eluotrophic strength at high modifier concentrations, which resulted in lower breakthrough volumes for the LMW-PAHs. Therefore, an organic modifier strength of 30% was chosen as a good compromise. By using acetonitrile to prevent sorption of PAH to glass walls, it is not necessary to

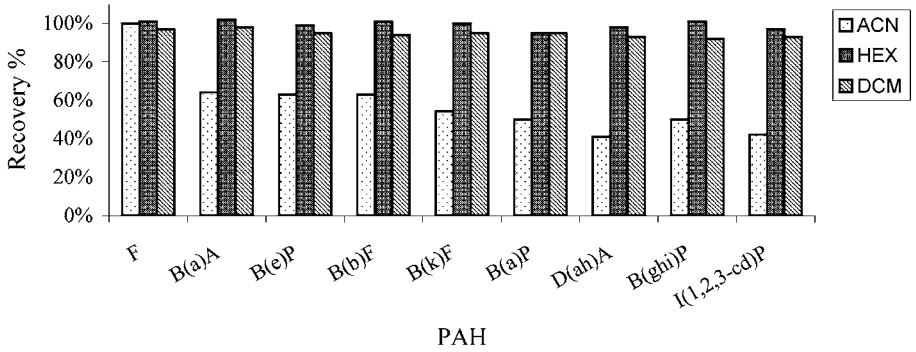

Figure 2. Influence of elution solvent (acetonitrile, ACN; hexane, HEX; and dichloromethane, DCM) on PAH recovery obtained by SPE.

use a surfactant at 1−2 times the critical micellar concentration (cmc) values, which produces clogging problems with the C18 cartridge.

The next step after drying the cartridge was to evaluate solvents for the selective elution of PAHs (**Figure 2**). In the scientific literature surveyed, many solvents were used: acetonitrile (*13*), dichloromethane (*9*, *10*, *12*, *20*), and hexane. All were tested with 10-mL elution volumes and 40−100% recovery was obtained with acetonitrile, 93−98% with dichloromethane, and 95−104% with hexane. The latter was selected since dichloromethane is carcinogenic, and also because hexane is less polar and so less likely to pull interferences off cartridges. It was proved that 4 mL of hexane was enough for a quantitative elution of all PAHs. SPE cartridge drying before elution is critical because moisture will affect hexane elution efficiency since hexane water solubility is very low.

(B) Characterization. The recovery ± repeatability of PAHs from tap water was measured by the analysis of six samples of tap water fortified with a PAH solution in acetonitrile. These samples were left at 4 °C overnight protected from light and preserved with sodium thiosulfate. Samples were then quantified against PAH standard solutions injected directly into the HPLC column (concentration factor = 500) to estimate absolute recovery percentage ± RSD (see **Table 1A**). There are no matrix effects affecting results as is proved by the excellent results obtained. Limits of detection (LOD) and quantitation (LOQ) were evaluated on the basis of the noise obtained with the analysis of seven unfortified tap water samples. LOD and LOQ were defined as the concentrations of the analyte that produced signal-to-noise ratios of 3 and 10, respectively (*23*), and were then tested experimentally (**Table 1A**).

SPME Procedure Performance. (A) Optimization. Recently, a new solvent-free technique (SPME) has been satisfactorily used for the extraction of PAHs from water (*24*−*28*). Since PAHs are nonpolar analytes with low affinity toward aqueous matrixes and since drinking water is not a complex matrix, immersion or direct SPME sampling was selected as extraction mode rather than headspace SPME. To develop a direct SPME procedure for the analysis of PAHs in waters, several parameters related to the extraction and desorption processes were evaluated. It is important to take into account a few precautions to prevent PAH losses via oxidation by air and light during extraction. The main strategy consists of using amber glass vials filled to the top with the water sample to remove any remaining headspace volume and to avoid the formation of air bubbles around the fiber.

Selection of SPME Coating. Three SPME fiber coatings were evaluated to select the most appropriate. The most suitable coating was the nonpolar 100-PDMS. PDMS fiber can be used repeatedly for 60 extraction cycles, providing reproducible and consistent results.

Table 1. Recovery, Repeatability, Linear Dynamic Ranges, Determination Coefficients (r^2), and Limits of Detection (LOD) and Quantification (LOQ) of the Techniques for Determining PAHs in Tap Waters

(A) Solid-Phase Extraction

PAHs	absolute recovery[a] spiking level (ng/L)	%	±% RSD	instrument linearity[b] range (μg/L)	r^2	LOD[a] (ng/L)	LOQ[a] (ng/L)
Fl	8.7	102	4	0.2–8.0	0.9994	0.2	0.5
B[*a*]A	2.2	97	4	0.15–3.0	0.9995	0.1	0.3
B[*e*]P	30.0	98	4	0.7–35	0.9995	0.7	1.5
B[*b*]F	8.6	101	2	0.5–6.0	0.9992	0.4	1
B[*k*]F	2.0	99	1	0.7–1.0	0.9997	0.05	0.15
B[*a*]P	4.0	96	2	0.15–4.0	0.9994	0.1	0.3
D[*ah*]A	8.0	96	1	0.3–5.0	0.9997	0.2	0.6
B[*ghi*]P	23.0	99	2	0.5–20	0.9994	0.6	1.0
I[1,2,3-*cd*]P	28.0	95	3	0.7–35	0.9997	0.7	1.5

(B) Solid-Phase Microextraction

PAHs	absolute recovery[a] spiking level (ng/L)	%	±% RSD	method linearity[c] range (ng/L)	r^2	LOD[a] (ng/L)	LOQ[a] (ng/L)
Fl	70	5	4	20–175	0.9968	6	20
B[*a*]A	18	5	3	10–440	0.9905	3	10
B[*e*]P	140	8	6	80–600	0.9990	27	80
B[*b*]F	70	7	6	40–175	0.9925	13	40
B[*k*]F	18	7	6	10–440	0.9957	3	10
B[*a*]P	35	7	5	20–90	0.9999	6	20
D[*ah*]A	70	8	8	40–175	0.9996	13	40
B[*ghi*]P	140	7	6	80–350	0.9989	27	80
I[1,2,3-*cd*]P	185	7	7	100–600	0.9985	37	100

[a] $n = 7$ determinations. [b] $n = 10$ in duplicate determinations. [c] $n = 5$ in duplicate determinations.

Effect of Acetonitrile Addition To Prevent Sorption to Glass. Different volumes of acetonitrile (0, 2, and 4 mL; **Figure 3**) were tested. At levels below 2 mL, their extraction yield decreases, because their solubility in the aqueous solution is more reduced, especially for the high molecular weight PAHs. At higher volumes than 2 mL, their extraction yield decreases too, since their partioning increases in favor of the aqueous mix. Higher areas were registered when 2 mL of acetonitrile was added. By using 2 mL of acetonitrile, it is not necessary to use a surfactant, which increases partitioning in favor of the aqueous mix and reduces extraction.

Effect of Salt Addition. The addition of salt decreases the extraction PAH yield, because their solubility in the aqueous sample is strongly reduced, especially for high molecular weight PAHs. Therefore since they are repulsed to the water/headspace interface (the so-called oil effect), they cannot interact with the fiber completely plunged into the aqueous matrix. This effect

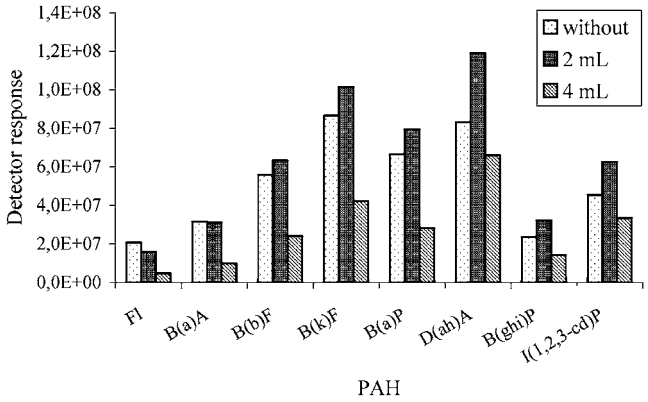

Figure 3. Effect of acetonitrile volume (0, 2, or 4 mL of acetonitrile in a 40 mL sample) on the PAH extraction efficiency by direct SPME.

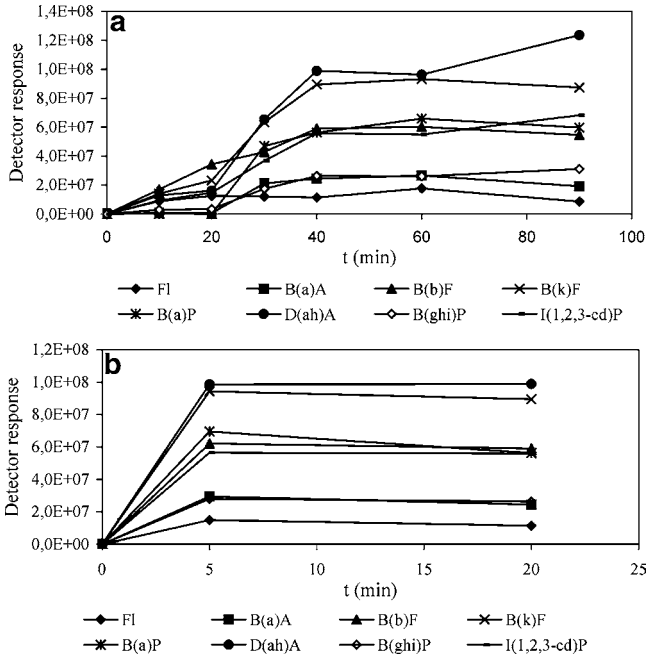

Figure 4. Sorption (**a**) and desorption (**b**) time profiles for PAHs by direct SPME.

was also found by other authors (*27*, *28*). Higher areas were registered when no NaCl was added.

Effect of Temperature. It was observed that areas increase with temperature, especially for the high molecular weight PAHs, since the oil effect is minimized with the temperature increase while the increase in temperature can also increase the extraction rate of PAHs from water to the fiber surface. This effect was also found by other authors (*25*, *28*). Further experiments were performed at 60 °C; we have also seen that the effect of acetonitrile addition does not depend on the temperature selected.

Sorption and Desorption Time Profiles. The extraction time profiles of this fiber were obtained by plotting the PAH response vs the extraction times evaluated (**Figure 4a**) for each PAH. The extraction time profile shows that all PAHs reached the maximum extraction yield in 40 min; this equilibrium time is especially necessary for those of higher molecular weight, in good agreement with other authors (*27*, *28*). Further experiments were performed for this time. The desorption time is also an important parameter to ensure that PAHs are completely desorbed from the fiber to reach the highest sensitivity and to avoid carryover. Desorption profiles were obtained by plotting the detector response versus different desorption times. Desorption profiles showed that a 5-min period was sufficient to desorb them (**Figure 4b**). When chromatographic analysis was completed, the fiber was immediately desorbed again at these conditions to determine carryover; no peaks were registered. Magnetic or ultrasonic stirring were then not necessary for desorption.

(B) Characterization. The linearity of the method was evaluated by regressing PAH peak areas, separately, versus the analyte concentration for standard fortified aqueous samples after the optimized SPME procedure was applied to all standards. The calibration line was found to have good linearity (**Table 1B**). The recovery ± repeatability of SPME/HPLC/FLD for PAHs from water was measured by the analysis of six spiked samples of tap water fortified with a PAH solution in acetonitrile. These samples were left overnight at 4 °C, protected from light and preserved with sodium thiosulfate. Samples were then quantified with PAH standard solutions injected directly into the HPLC column to estimate absolute recovery percentages ± RSD for PAHs. The absolute recovery with SPME was low but quite constant (RSD < 8%). To correct the effect of such a low constant recovery with calibration, PAH-spiked water at different levels was then submitted to the complete sample treatment and analysis, in the same way as real water samples. The low constant slope of the calibration line obtained with the PAH-spiked water allows then to correct for the low constant recovery.

Limits of detection (LOD) and quantitation (LOQ) were evaluated on the basis of the noise obtained with the analysis of seven unfortified tap water samples. LOD and LOQ were defined as the concentrations of the analyte that produced signal-to-noise ratios of 3 and 10, respectively (*23*), and were then tested experimentally (**Table 1B**).

Comparison of SPME and SPE Performance. Absolute recovery was found to be maximum for SPE (ca. 100% for all PAHs); meanwhile, it was low for SPME (ca. 8% for all PAHs). Precision expressed as relative standard deviation (RSD) was satisfactory for both extraction techniques, ranging from 1% to 8%, although SPME precision is slightly worse since SPME experiments were carried out under nonequilibrium conditions. SPE has lower detection limits than SPME. SPME yielded limits of detection very close to the European drinking water regulations and did not allow determination of PAHs at the European values established. The concentration factor for SPE procedure was 500 (250 mL of water to 0.5 mL of acetonitrile), while the concentration factor for SPME was 333 (40 mL of water to 0.12 mL ofacetonitrile), which makes a factor of 1.5 in favor of SPE. Since recovery percentages were 12.5 times higher for SPE (ca. 100% vs ca. 8%), the difference in method sensitivity between both extraction techniques is around 20 times higher for SPE.

HPLC Procedure Performance: (A) Optimization. The mobile phase acetonitrile−HPLC water was recommended by the HPLC column manufacturer. The unacceptable drifting chromatographic baseline was solved with the use of HPLC-grade water. The separation was optimized to be used in a quality control department of water distribution companies; the utilization of mobile-phase components such as acetonitrile and water, compatible with atmospheric pressure ionization techniques of mass spectrometric detection, allows the employment of such techniques in the case of the need of a confirmation procedure for the identification of the separated peaks as PAHs. The gradient conditions described in the Experimental Section

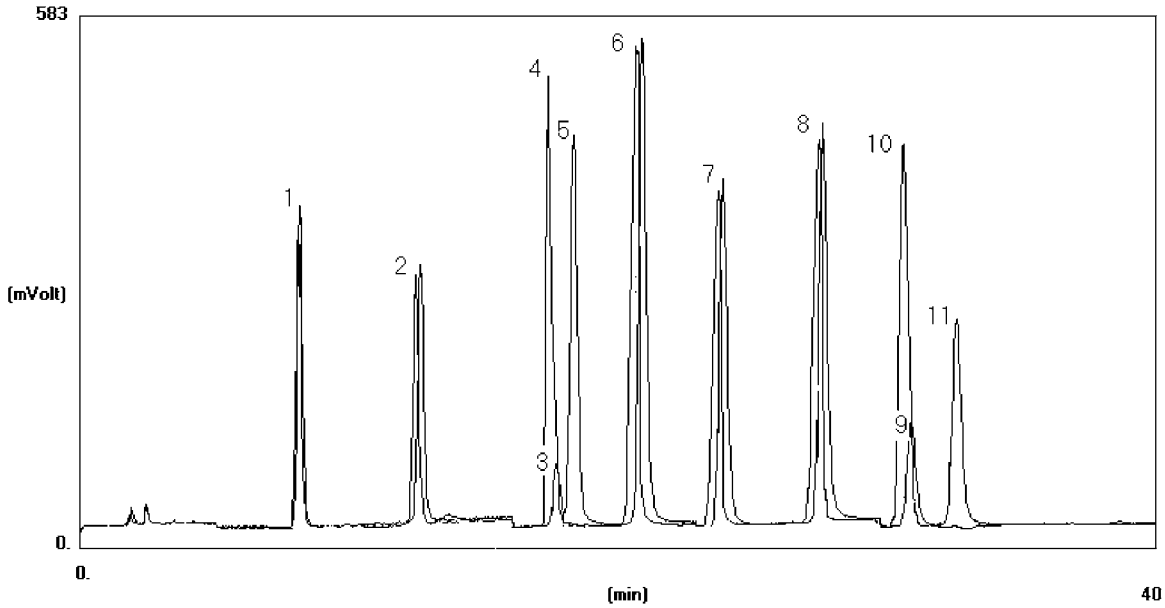

Figure 5. HPLC–FLD chromatograms registered for PAHs (0.05–0.75 ng injected) at the optimized and validated conditions: (1) Fl (λ_{exc} 284 nm, λ_{em} 464 nm); (2) B[*a*]A (λ_{exc} 274 nm, λ_{em} 414 nm); (3) B[*e*]P (λ_{exc} 300 nm, λ_{em} 446 nm); (4) B[*e*]P (λ_{exc} 270 nm, λ_{em} 380 nm); (5) B[*b*]F (λ_{exc} 300 nm, λ_{em} 446 nm); (6) B[*k*]F (λ_{exc} 300 nm, λ_{em} 446 nm); (7) B[*a*]P (λ_{exc} 296 nm, λ_{em} 406 nm); (8) DB[*ah*]A (λ_{exc} 296 nm, λ_{em} 406 nm); (9) B[*ghi*]P (λ_{exc} 300 nm, λ_{em} 470 nm); (10) B[*ghi*]P (λ_{exc} 296 nm, λ_{em} 406 nm); and (11) I[1,2,3-*cd*]P (λ_{exc} 300 nm, λ_{em} 470 nm).

allowed the correct resolution of all target compounds; a column heater programmed at constant temperature was necessary to obtain reproducible PAH retention times, which is of paramount importance when a defined wavelength program is used to optimize sensitivity for each PAH. The injection volume of 50 μL was the best compromise between sensitivity and resolution.

(B) Characterization. The PAH peaks were well separated except for B[*e*]P and B[*b*]F (**Figure 5**) and had acceptable symmetry under the gradient conditions developed for this multiresidue method. To solve the close coelution problem posed by these two PAHs, wavelengths for excitation and emission were selected at their elution retention time window of 16−22 min that are selective for B[*b*]F (300 and 446 nm). A second wavelength program was used with the intention of quantifying B[*e*]P without the interference of B[*b*]F (by changing wavelengths in such a time window to 270 and 380 nm), while spending this second chance to use other wavelengths the last 10 min (296 and 406 nm), with the aim of increasing sensitivity by a factor of 3 for B[*ghi*]P by use of its optimum detection wavelengths.

Analysis of Drinking Waters from Galicia (NW Spain) in the Search of PAHs. Twenty drinking water samples of different small inland and coastal cities of Galicia were evaluated with the method based on SPE/HPLC−FLD. In only one sample, Fl was found at a level of 10 ng/L; the rest of the samples contained Fl below the quantification limits. The analyzed samples fulfill the drinking water quality EU criteria. Similar results were found by other authors (*10*, *12*, *25*). The widespread presence of Fl in the water samples studied can be attributed to the pipeline coatings in contact with drinking water (*29*, *30*). Because Fl is not so toxic as those PAHs with higher molecular weight (*31*), its control in waters is not important. Since 1998, Directive 98/83/EC (*6*), relative to the quality of waters intended for human consumption, has removed Fl from the list of PAHs to control as indicators of drinking water quality.

CONCLUSIONS

The proposed methods help to cover some of the most important research and development needs in the area of PAHs in drinking waters, such as improving the minimum detectable concentrations for some PAHs, providing water quality data to assess the state of the aqueous environment with respects to PAHs, and determining human exposure levels from water sources. SPE is an equilibrium analysis and needs solvents and large sample volumes, whereas SPME is a dynamic analysis and needs no solvent and small sample volumes. LODs of PAHs in waters analyzed by SPE are lower than than those analyzed by SPME.

The present study has shown that the SPME method is not suitable for monitoring PAHs in drinking waters to the levels established by legislation. The 100-PDMS fiber is proposed for extracting them. Repeatability is on the same order as SPE, but not absolute recovery and concentration factor, and in consequence detection and quantification limits; no matrix effects interfere in the quantitation process. Although SPME uses lower volumes of organic solvents, SPE can be automatable and calibration can be performed by use of aqueous standards injected directly into the column because absolute recovery for PAHs is 100%.

The optimized SPE procedure is then proposed as an extraction method for determination of PAHs in waters; it is reliable (linear, with high recovery and precision and low detection and quantification levels for high molecular weight PAHs), simple, fast, uses lower volumes of solvents than liquid−liquid extraction, suffers no interference from the matrix, and allows automatization for processing a high number of samples.

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