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Determination of Polycyclic Aromatic Hydrocarbons in Coffee Brew Using Solid-Phase Extraction

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The presence of polycyclic aromatic hydrocarbons (PAHs) in coffee has been reported and is suspected to be due to the degradation of coffee compounds during the roasting step. Due to the high toxicity of these compounds, among which benzo[*a*]pyrene is known to be the most carcinogenic, their presence in the coffee, especially the coffee brew that is directly ingested by the consumer, is of prime importance. However, due to the low solubility of these compounds, their concentrations are expected to be rather low. As a consequence, reliable and sensitive analytical methods are required. The aim of this study was to develop a reliable and fast analytical procedure to determine these organic micropollutants in coffee brew samples. PAHs were retained on a 0.5 g polystyrene−divinylbenzene cartridge before being eluted by a mixture of methanol/tetrahydrofuran (10:90 v/v), concentrated, and directly analyzed by reversed-phase high-performance liquid chromatography coupled to a fluorescence detector. Application to the determination of PAHs in several coffee brew samples is also given, with mean estimated concentrations in the range of 0−100 ng L^{-1} for suspected benzo[*b*]fluoranthene and benzo[*a*]pyrene, whereas no fluoranthene could be detected. Tentative identification was made on the basis of UV spectra. However, identification of the suspected traces of PAHs could not be achieved due to matrix effects, so that the presence of coeluting compounds may not be excluded.

KEYWORDS: Coffee; fluorescence detection; liquid chromatography; PAHs; polycyclic aromatic hydrocarbons; roasting; solid-phase extraction

INTRODUCTION

Polynuclear aromatic hydrocarbons (PAHs) are well-known environmental pollutants, which may be formed during the combustion of carbonaceous materials at high temperature. Owing to their mutagenic and carcinogenic potential (*1*), they have been determined in several matrices, particularly waters, soils, and sediments. Of prime concern is also their occurrence in numerous foods, especially the highly carcinogenic benzo[*a*]pyrene as this compound has been reported in several foods (*2*−*14*). Hence, a total PAH intake through foods in Italy was estimated at ∼3 μg/day, with an intake of carcinogenic PAHs near 1.4 μg/day, which is quite high compared to the estimated intake through respiration (near 0.37 and 0.13 ng/day for total and carcinogenic PAHs, respectively) (*10*). In fact, PAH presence in food may come from external contamination of the initial food matrix (mainly atmospheric deposition, root uptake, or contact with hydrocarbon-based materials) and/or from degradation of some food material during food processes involving elevated temperatures (especially drying, cooking, frying, roasting, and smoking) (*11*). In particular, their presence in coffee has been attributed to their formation during the roasting step. Despite quite low PAH concentrations (micrograms per kilogram) in foods, this is one of the major factors contributing to human cancer (*15*). Therefore, it is of prime importance to develop rapid and reliable analytical methods for their accurate determination in food items in order to estimate the total amount of human exposure to PAHs.

Concentrations of benzo[*a*]pyrene ranging from 0.01 to 1.2 μg kg^{-1} have been reported in several solid coffee samples (*10*, *16*). However, as solid coffee is not directly ingested by the consumer, it is more pertinent to estimate the PAH concentration in the coffee brew samples. This task represents quite a challenge, as the expected concentrations are rather low, due to the low solubility of PAHs. As a matter of fact, previous studies reported that only ∼0.8-1% of benzo[*a*]pyrene initially present in solid coffee samples was found in coffee brew samples, leading to concentrations ranging from 0.3 to 7 ng L^{-1} (*16*, *17*). However, when other studies are taken into account, the transfer percentage ranges from 0.6 to 26%, with a mean value around 5% (*18*). This percentage was found to depend on the infusion strength, with values of 4.6 and 2.6% for 50 and 100 g L^{-1}, respectively, which suggests that saturation may have occurred, as in both cases similar PAH

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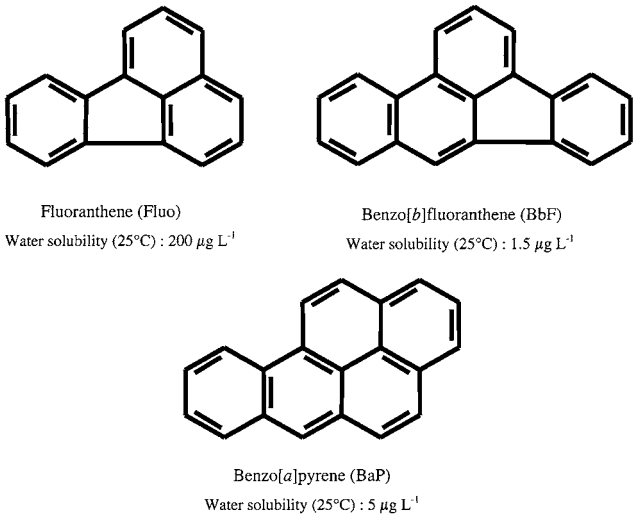

Figure 1. Chemical structure and water solubility of the investigated PAHs.

concentrations (0.3−10 ng L^{-1}) were found in the coffee brew (*18*). Most of the methods proposed for the PAH determination in coffee brew use large solvent volumes due to a liquid−liquid extraction step. In addition, they may be not quantitative. At the present time, only one study reports a low-solvent volume method (*19*). It involves solid-phase extraction on a C_{18}-silica cartridge, followed by reversed phase high-performance liquid chromatography with fluorometric detection. Detection limits ranging from 0.021 to 0.41 μg L^{-1} could be attained using this method. Applying this analytical method to several coffee brew samples gave higher PAH concentrations than the previous study, ranging from 0.29 to 10.9 ng L^{-1}, with concentrations of benzo[*a*]pyrene near 3 ng L^{-1}. However, nonquantitative recoveries of PAHs were reported. Consequently, the development of a more quantitative analytical method would be of great interest, as a means of giving more reliable and sensitive results. This study was therefore undertaken to find better solid-phase extraction conditions, with a view to develop a quantitative analytical method for the determination of traces of PAHs in coffee brew samples. Three model compounds were considered: fluoranthene, benzo[*b*]fluoranthene, and benzo[*a*]pyrene (their structures along with their water solubilities are given in **Figure 1**). Recoveries were estimated on the basis of spiked coffee brew samples as well as nonspiked samples to take into account the native PAHs present in the coffee brew samples. Finally, the method was applied to the determination of traces of PAHs in several commercial coffee brew samples.

MATERIALS AND METHODS

All experiments were done in triplicate, unless otherwise specified in the text, enabling mean values and relative standard deviations (RSDs) to be determined.

Reagents and Chemicals. The reagents in this study were all used in the form purchased without additional purification or alteration. Individual standard solutions (10 mg L^{-1} in acetonitrile) of the following PAHs were obtained from CIL Cluzeau (Paris, France): fluoranthene (Fluo), benzo[*b*]fluoranthene (BbF), and benzo[*a*]pyrene (BaP). A PAH mix solution was also used (Supelco, Saint-Quentin Fallavier, France) containing the 16 EPA PAHs (10 mg L^{-1} in acetonitrile): naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, benzo[*g,h,i*]perylene, and indeno[1,2,3-*c,d*]pyrene. HPLC grade acetonitrile was supplied by Prolabo (Briare, France). PAH purities were guaranteed between 97 and 99.7%. Other purities were stated to be >99%. Deionized water was produced with a Milli-Q system from Millipore (Saint-Quentin-en-Yvelines, France). Stock standard solutions were prepared by diluting the PAH solutions in an appropriate volume of methanol (MeOH)/tetrahydrofuran (THF) 10:90 (0.4, 1, 2, 2.5, 5, 8, 10, 20, 25, 35, 50, or 100 μg L^{-1}). All solutions were stored at 4 °C in the dark.

Coffee Samples. Coffee samples were obtained from commercial milled coffees, available at the supermarket. The coffee brew samples were obtained using an electric coffee maker equipped with a paper filter. In all cases, 50 g of commercial milled coffee was treated by passing 300 mL of Milli-Q water as performed in another study (*19*). Afterward, 50 mL (or 30 mL for some experiments as specified in the text) was taken and mixed with 7 mL of methanol, as the addition of an organic solvent is well-known to minimize possible adsorption of the PAHs onto the glass flask as well as onto the cartridge walls (*20*, *21*). This sample was then passed through the solid-phase extraction cartridge as detailed below. In most of the experiments both nonspiked and spiked coffee brew sample were extracted using solid-phase extraction (SPE), to estimate traces of PAHs initially present in the coffee (native PAHs) in order to avoid overestimation of spiked PAH recoveries. In addition, blank experiments have been performed using 300 mL of Milli-Q water and an empty paper filter in the electric coffee maker and analyzed after the SPE step to ensure no traces of PAH were present in the blank.

For spiking, 100 μL of individual PAH standard solutions at 1 ng μL^{-1} in methanol was added to the 50 mL sample, leading to a spiking level of 2 μg L^{-1} for each PAH. Once the entire 50 mL sample had been passed through the SPE cartridge, the glass flask that contained the 50 mL sample was further rinsed with 8 mL of methanol and concentrated to near 2 mL under a gentle stream of nitrogen (the exact volume being measured upon weighing), and this extract was analyzed by HPLC-FLD to estimate the percentage of PAHs adsorbed on the glass walls. The presence of methanol in the 50 mL sample allowed minimization of adsorption effects. However, some adsorption remained for the less soluble benzo[*b*]fluoranthene and benzo[*a*]pyrene in spiked coffee brew samples, with mean fraction adsorbed around 1.5−8% in all of the experiments.

Solid-Phase Extraction. SPE was performed using disposable SPE cartridges containing a hydrophobic sorbent. The tested sorbents were C_{18}-silica (ENVI-18, 0.5 g, supplied by Supelco, Saint-Quentin Fallavier, France) and polystyrene−divinylbenzene (PS-DVB) copolymer (BondElut PPL supplied by Varian, France; 0.2 and 0.5 g). A Visiprep vacuum manifold system (Supelco) was used. All cartridges were conditioned with 5 mL of MeOH, 5 mL of MeOH/THF 50:50 (v/v), and 5 mL of water. PAHs were eluted using fractions of 2 mL of the eluting solvent unless specified in the text. Each fraction was further filtered through nylon A-Luer filters (13 mm, 0.45 μm, supplied by CIL Cluzeau, France) and then injected into the HPLC-FLD system. For lowering the limits of detection of the method, some extracts were analyzed after concentration to ~0.5 mL under a gentle stream of nitrogen. For quantification, the final volume of each extract was determined by weighing.

Liquid Chromatography−Fluorescence Detection. Extracts were analyzed using HPLC coupled to fluorometric detection (FLD). The HPLC system consisted of a Varian 9010 high-pressure gradient pump, a Rheodyne model 7125 injection valve equipped with a 20 μL loop, a Thermo Separation Science fluorometric detector (FL3000), and a computer. Data analysis was performed using the TurboChrom TC4 Navigator. A Supelcosil LC-PAH column (250 × 4.6 mm i.d., 5 μm particle size, Supelco) was used, along with a precolumn (containing C_{18}-silica). Separation was performed using the following gradient: acetonitrile/water (60:40, v/v) for 5 min, followed by a 25 min ramp to 100% acetonitrile, this solvent being further maintained for 15 min. The total flow rate was 1.5 mL min^{-1}. Detection was performed at selected excitation and emission wavelengths, respectively 230−410 nm for fluoranthene and 250−420 nm for benzo[*b*]fluoranthene and benzo[*a*]pyrene. For coffee extracts, due to the high level of contaminants for low retention times, excitation and emission wavelengths were fixed, respectively, at 320 and 510 nm for the first 10 min to avoid saturation of the detector. External calibration was performed using standard solutions of PAHs in MeOH/THF 10:90 (v/v) in the range of

Table 1. Effect of Matrix-Interfering Compounds, of Coffee Brew Volume Percolated, and of Eluting Solvent[a]

	elution, MeOH/THF 50:50; coffee brew, 50 mL; blended LO lot P5 07:37		elution, MeOH/THF 20:80; coffee brew, 50 mL; blended LO lot P5		elution, MeOH/THF 20:80; water, 50 mL		elution, MeOH/THF 10:90; coffee brew, 50 mL; blended LO lot P5 12:26		elution, MeOH/THF 10:90; coffee brew, 30 mL; blended LO lot P6 18:26	
	recovery (%)	*RSD (%)*	recovery (%)	*RSD (%)*	recovery (%)	*RSD (%)*	recovery (%)	*RSD (%)*	recovery (%)	*RSD (%)*
					Adsorption					
Fluo	**nd**	*nd*	**nd**	*nd*	**nd**	*nd*	**nd**	*nd*	**nd**	*nd*
BbF	**2.8**	*91.3*	**4.6**	*14.8*	**16.1**	*8.8*	**8.0**	12.7	**1.3**	19.2
BaP	**5.1**	*19.8*	**4.9**	*7.0*	**16.2**	*6.3*	**7.4**	12.1	**1.1**	17.2
					SPE 1					
fraction 1										
Fluo	67.8	*3.1*	75.6	*4.3*	74.9	*5.9*	85.9	*6.9*	107.7	*7.8*
BbF	61.2	*3.0*	71.0	*0.6*	76.9	*2.4*	81.4	*7.6*	71.8	*4.9*
BaP	55.6	*6.2*	59.3	*13.7*	74.0	*0.7*	84.4	*2.8*	74.7	*2.6*
fraction 2										
Fluo	4.8	*106.3*	nd	*nd*	11.8	*23.9*	nd	*nd*	nd	*nd*
BbF	8.1	*22.6*	4.0	*28.1*	11.7	*14.0*	2.3	*9.4*	4.8	*23.9*
BaP	11.9	*18.7*	5.8	*8.6*	13.8	*15.6*	5.0	*12.5*	5.3	*25.0*
total SPE 1										
Fluo	**72.6**	*9.5*	**75.6**	*4.3*	**86.7**	*1.8*	**85.9**	*6.9*	**107.7**	*7.8*
BbF	**69.7**	*1.3*	**74.9**	*1.7*	**88.6**	*1.4*	**83.7**	*7.1*	**76.6**	*4.6*
BaP	**67.5**	*6.3*	**65.1**	*11.8*	**87.8**	*2.0*	**89.4**	*2.1*	**80.0**	*2.1*
					SPE 2					
fraction 1										
Fluo	nd	*nd*	nd	*nd*	np	*np*	nd	*nd*	nd	*nd*
BbF	13.8	*8.6*	12.7	*9.2*	np	*np*	12.7	*2.7*	20.8	*2.8*
BaP	10.0	*32.3*	9.9	*12.3*	np	*np*	14.3	*8.0*	20.6	*5.9*
fraction 2										
Fluo	nd	*nd*	np	*np*	np	*np*	np	*np*	np	*np*
BbF	4.1	*0.6*	np	*np*	np	*np*	np	*np*	np	*np*
BaP	5.9	*7.5*	np	*np*	np	*np*	np	*np*	np	*np*
total SPE										
Fluo	**72.6**	*9.5*	**75.6**	*4.3*	**86.7**	*1.5*	**85.9**	*6.9*	**107.7**	*7.8*
BbF	**87.6**	*1.5*	**87.7**	*0.7*	**88.6**	*1.2*	**96.4**	*6.3*	**97.4**	*3.9*
BaP	**83.4**	*7.9*	**75.0**	*11.2*	**87.8**	*1.6*	**103.7**	*1.4*	**100.7**	*1.7*

[a] Experimental conditions: spiked coffee brew or water (30 or 50 mL); PS-DVB, 0.2 g; elution with MeOH/THF 50:50, 20:80, or 10:90 (v/v) (fractions of 2 mL). HPLC conditions: Supelcosil LC-PAH column (250 × 4.6 mm i.d., 5 μm); gradient, acetonitrile/water (60:40, v/v) for 5 min, 25 min ramp to 100% acetonitrile, acetonitrile for 15 min; total flow rate, 1.5 mL min^{-1}; detection, fluorescence. np, experiment not performed; nd, not detected.

0.4−100 μg L^{-1}. Standard solutions were analyzed daily to check the fluorometer sensitivity.

For identification of possible traces of PAHs in coffee brew samples, some extracts were also analyzed using an HPLC system coupled with a diode array UV−visible detector (HPLC-DAD). For that purpose, extracts were concentrated to ∼0.5 mL under a gentle stream of nitrogen, due to the lower sensitivity of the UV detector as compared to the fluorometric detector. The system used consisted of a Waters 1525 high-pressure gradient pump, a Rheodyne injection valve equipped with a 20 μL loop, a Waters 2996 DAD detector, and a computer. Data analysis was performed using the Millenium software. A Supelcosil LC-PAH column (150 × 3.0 mm i.d., C_{18}-silica, 5 μm particle size, Supelco) was used, along with a precolumn (filled with C_{18}-silica). Separation was performed using the following gradient: acetonitrile/water (40:60 v/v) for 4 min, followed by an 11 min ramp to attain 100% acetonitrile, this solvent being further maintained for 10 min. The total flow rate was 0.8 mL min^{-1}.

RESULTS AND DISCUSSION

To find the suitable conditions for efficient recoveries of PAHs after solid-phase extraction, experiments were conducted on spiked coffee brew samples. The aqueous character of the sample requires the use of reversed-phase sorbent for efficient retention of the organic compounds. Both C_{18}-silica and PS-DVB copolymer were tested as hydrophobic sorbents for the retention of PAHs from coffee brew. Once retained, the PAHs were eluted with different eluting solvents. To estimate the eluting solvent volume required for desorbing the PAHs, successive fractions of 2 mL were recovered in each case and separately analyzed. When only traces of PAHs were found in a fraction, no additional fraction was performed. In that way, as shown below, two successive fractions were obtained for C_{18}-silica (0.5 g) and PS-DVB (0.2 g), whereas three successive fractions were required for PS-DVB (0.5 g).

Due to possible adsorption of PAHs on recipient walls for spiked aqueous samples, for each experiment the glass flask that contained the spiked aqueous sample (coffee brew or water) was rinsed with methanol, to estimate the percentage of PAH adsorbed. The final recoveries for the SPE step were then estimated by taking into account the real content of spiked PAHs that passed through the cartridge.

Improvement of SPE Conditions for Quantitative Recoveries of PAHs on PS-DVB Copolymer. *Choice of Elution Solvent.* The PS-DVB copolymer was selected as a suitable reversed-phase sorbent, due to its hydrophobic character and additional $\pi-\pi$ interactions between the PAHs and the sorbent, leading to increased retention on this sorbent as compared to C_{18}-silica (*20*, *21*). Experiments were first conducted with cartridges containing 0.2 g of PS-DVB, to minimize the required eluting solvent volumes. Several MeOH/THF mixtures were investigated for eluting the retained PAHs: 50:50, 20:80, and 10:90 (v/v). Results are presented in **Table 1**. Best results were obtained with MeOH/THF 10:90 (v/v), with mean recoveries ranging from 83.7 to 89.4% after one SPE step, as compared to between 65.1 and 75.6% for MeOH/THF 20:80 (v/v) and

Table 2. Influence of the Nature and Mass of Solid Sorbent[a]

	PS-DVB, 0.5 g; water, 50 mL		PS-DVB, 0.5 g; coffee brew, 50 mL; blended LO lot P6 18-09-04[b]		PS-DVB, 0.2 g; coffee brew, 50 mL; blended LO lot P5 12:26		C_{18}-silica, 0.5 g; coffee brew, 50 mL; blended LO lot P5 31-12-03	
	mean recovery (%)	RSD (%)	mean recovery (%)	RSD (%)	mean recovery (%)	RSD (%)	mean recovery (%)	RSD (%)
				Adsorption				
Fluo	**8.8**	*4.7*	nd	*nd*	nd	*nd*	nd	*nd*
BbF	**22.8**	*3.7*	**3.1**	*1.9*	**8.0**	*12.7*	**3.6**	*12.5*
BaP	**20.7**	*13.7*	**2.6**	*9.6*	**7.4**	*12.1*	**2.3**	*36.0*
				Fraction 1				
Fluo	67.7	*13.8*	79.4	*4.0*	85.9	*6.9*	97.6	*10.7*
BbF	68.5	*11.7*	71.2	*6.3*	81.4	*7.6*	86.7	*15.5*
BaP	57.3	*10.9*	74.2	*1.9*	84.4	*2.8*	95.6	*19.1*
				Fraction 2				
Fluo	18.9	*34.1*	17.2	*17.6*	nd	*nd*	nd	*nd*
BbF	20.5	*30.1*	14.7	*4.2*	2.3	*9.4*	6.7	*8.8*
BaP	21.7	*26.2*	17.3	*0.6*	5.0	*12.5*	9.1	*22.9*
				Fraction 3				
Fluo	nd	*nd*	nd	*nd*	np	*np*	np	*np*
BbF	3.1	*12.2*	2.4	*5.6*	np	*np*	np	*np*
BaP	3.2	*8.1*	3.8	*68.1*	np	*np*	np	*np*
				Total Fractions				
Fluo	**86.6**	*3.6*	**96.7**	*6.0*	**85.9**	*6.9*	**97.6**	*10.7*
BbF	**92.1**	*2.0*	**88.4**	*5.1*	**83.7**	*7.1*	**93.5**	*14.9*
BaP	**82.3**	*1.4*	**95.4**	*1.3*	**89.4**	*2.1*	**104.7**	*17.8*

[a] Experimental conditions: spiked coffee brew or water (50 mL); elution with MeOH/THF 10:90 (v/v) (fractions of 2 mL). HPLC conditions: Supelcosil LC-PAH column (250 × 4.6 mm i.d., 5 μm); gradient, acetonitrile/water (60:40, v/v) for 5 min, 25 min ramp to 100% acetonitrile, acetonitrile for 15 min; total flow rate, 1.5 mL min^{-1}; detection, fluorescence. np, experiment not performed; nd, not detected. [b] For this coffee, nonspiked coffee brew was not analyzed.

between 67.5 and 72.6% for MeOH/THF 50:50 (v/v). This was indeed expected, as increasing the percentage of THF results in increasing the eluent strength on reversed phase as this solvent is more eluent than MeOH ($\epsilon^\circ = 3.7$ and 1.0, respectively, on C_{18}-silica), which should enhance PAH desorption. In addition, the mixture polarity is decreased (polarity $P' = 4.2$ and 6.6 for THF and MeOH, respectively), which should favor PAH solubilization. As a consequence, MeOH/THF 10:90 (v/v) was used as the eluting solvent in further experiments.

Choice of Suitable Sorbent Mass. Due to the low recoveries obtained after the second elution fraction, the incomplete recoveries were expected to be due to partial saturation of the sorbent bed by coffee material, thereby leading to losses of PAHs. To validate this assumption, the sample that had been passed through a first SPE cartridge was further submitted to another SPE. As shown in **Table 1**, significant recoveries were found in the first elution fraction, ranging from 9.9 to 14.3% depending on the elution solvent. However, recoveries remained nonquantitative for the less retained compound, fluoranthene. Therefore, additional experiments were performed with a reduced sample volume (i.e., 30 mL instead of 50 mL). As indicated in **Table 1**, quantitative recoveries could thus be achieved for the three investigated PAHs. Besides, it is interesting to note that quantitative recovery of fluoranthene could be achieved in the first fraction. Indeed, it seems that this compound is less retained than the other two on the PS-DVB copolymer and that it is eluted out of the cartridge when the sample volume is excessive. This is in agreement with the lower hydrophobic character of this compound as compared to the other two investigated PAHs. However, reducing the sample volume is not desirable, as this will reduce the overall limits of detection and quantification of the method. In addition, two successive SPE steps were still required, as nearly 20% of benzo[*b*]fluoranthene and benzo[*a*]pyrene passed through the first cartridge.

Consequently, the use of cartridges containing 0.5 g of PS-DVB was preferred to enable the performance of one single-step SPE. As shown in **Table 2**, acceptable recoveries could thus be obtained after one SPE step, with recoveries ranging from 88.4 to 96.7% for a spiked coffee brew sample. However, as expected, a larger volume of eluting solvent was required (i.e., 3 × 2 mL instead of 2 × 2 mL) as compared to the use of 0.2 g of PS-DVB, as traces of benzo[*b*]fluoranthene and benzo-[*a*]pyrene were found in the third fraction.

Comparison with SPE on C_{18}-Silica. As retention of PAHs on C_{18}-silica is slightly lower than that on PS-DVB, experiments were performed on 0.5 g C_{18}-silica cartridges to avoid saturation of the sorbent bed by the coffee material. As indicated in **Table 2**, quantitative recoveries of the three tested PAHs could be achieved. Besides, due to their lower retention on this sorbent, PAHs were mainly recovered in the first fraction. However, a poor repeatability was unexpectedly found, with RSDs ranging from 10 to 20%.

For comparison, additional experiments were then performed on C_{18}-silica cartridges, using the conditions reported in a previous study (*19*). For that purpose the cartridges were conditioned with 3 mL of eluting solvent, 2 × 3 mL of methanol, and 3 mL of water. Retained PAHs were further eluted with 3 mL of eluting solvent. Diethyl ether was tested at first, as proposed in the method of Kayali et al. (*19*), even though this solvent is not usually recommended for reversed-phase SPE. The obtained extracts were concentrated to dryness under a gentle stream of nitrogen, and the solid residue was redissolved in 0.5 mL of methanol (the solvent was changed because diethyl ether is not miscible with water present in the HPLC mobile phase). With a spiked coffee brew sample of 100 mL (i.e., 93 mL of coffee brew plus 7 mL of methanol as suggested in ref *19*), unacceptable recoveries were found, values ranging from 18 to 27% depending on the PAH. Similar values were obtained with spiked water samples under the same conditions, indicating

Table 3. Influence of a Rinsing Step before Elution[a]

	coffee, Colombia lot 03-259; water rinsing		coffee, Colombia lot 03-259; MeOH rinsing		coffee, Ethiopia		
					water rinsing	MeOH/water 50:50 rinsing	MeOH rinsing
	mean recovery (%)	RSD (%)	mean recovery (%)	RSD (%)	recovery[b] (%)	recovery[b] (%)	recovery[b] (%)
				Adsorption			
Fluo	nd	*nd*	nd	*nd*	nd	nd	nd
BbF	**3.6**	*18.9*	**2.1**	*1.4*	**3.5**	**3.9**	**2.9**
BaP	**3.9**	*13.8*	**2.5**	*3.6*	**4.2**	**4.5**	**3.7**
				Fraction 1			
Fluo	61.6	*5.2*	67.7	*5.0*	57.3	56.0	63.4
BbF	72.1	*5.8*	66.0	*2.6*	62.3	57.5	61.8
BaP	69.8	*6.0*	64.1	*1.9*	63.1	56.5	66.4
				Fraction 2			
Fluo	13.0	*20.2*	10.2	*6.9*	9.9	11.9	8.4
BbF	13.7	*13.7*	8.1	*10.4*	16.1	12.5	9.4
BaP	15.7	*11.9*	11.4	*2.8*	19.1	15.8	8.8
				Fraction 3			
Fluo	nd	*nd*	nd	*nd*	nd	nd	nd
BbF	3.3	*39.2*	2.8	*33.5*	2.7	2.2	2.4
BaP	4.5	*30.3*	6.0	*86.1*	3.7	3.4	2.4
				Total Fractions			
Fluo	**74.6**	*4.3*	**77.8**	*3.4*	**67.2**	**67.9**	**71.9**
BbF	**89.1**	*2.2*	**77.7**	*2.9*	**81.1**	**72.2**	**73.6**
BaP	**90.1**	*1.3*	**81.9**	*5.7*	**85.8**	**75.7**	**77.6**

[a] Experimental conditions: PS-DVB, 0.5 g; spiked coffee brew (50 mL); rinsing (5 mL); elution with MeOH/THF 10:90 (v/v) (fractions of 2 mL). HPLC conditions: Supelcosil LC-PAH column (250 × 4.6 mm i.d., 5 μm); gradient, acetonitrile/water (60:40, v/v) for 5 min, 25 min ramp to 100% acetonitrile, acetonitrile for 15 min; total flow rate, 1.5 mL min^{-1}; detection, fluorescence. nd, not detected. [b] For this coffee, only one experiment was conducted for each rinsing solvent.

Table 4. Application to Several Spiked Coffee Brew Samples[a]

	coffee 1a, Arabica				coffee 2, Arabica					
	blended LO lot P6 18-09-04[b]; extract, 2 mL		blended LO lot P6 18-09-04[b]; concentrated, 0.5 mL		Colombia lot 03073 10/2003; extract, 2 mL		Colombia lot 03073 12/2003; extract, 2 mL		Colombia lot 03073; concentrated, 0.5 mL	
	mean recovery (%)	RSD (%)	mean recovery (%)	RSD (%)	mean recovery (%)	RSD (%)	mean recovery (%)	RSD (%)	mean recovery (%)	RSD (%)
					Adsorption					
Fluo	nd	*nd*	nd	*nd*	nd	*nd*	nd	*nd*	**2.2**	*10.5*
BbF	**3.1**	*1.9*	**1.7**	*27.5*	**3.0**	*17.3*	**4.1**	*20.2*	**2.8**	*16.3*
BaP	**2.6**	*9.6*	**1.5**	*32.8*	**2.9**	*23.0*	**4.9**	*22.9*	**2.8**	*15.9*
					Fraction 1					
Fluo	79.4	*4.0*	70.0	*5.5*	62.7	*13.4*	65.0	*23.8*	95.5	*5.0*
BbF	71.2	*6.3*	65.9	*5.6*	64.3	*2.1*	73.8	*10.8*	63.0	*30.8*
BaP	74.2	*1.9*	69.1	*3.0*	65.5	*4.3*	78.7	*8.7*	73.3	*32.6*
					Fraction 2					
Fluo	17.2	*17.6*	14.0	*30.8*	28.9	*1.5*	16.5	*9.1*	4.6	*90.4*
BbF	14.7	*4.2*	17.0	*35.0*	23.6	*21.2*	18.4	*11.0*	0.6	*428.1*
BaP	17.3	*0.6*	19.9	*28.4*	30.3	*18.7*	21.0	*4.7*	3.0	*71.5*
					Fraction 3					
Fluo	nd	*nd*	nd	*nd*	nd	*nd*	nd	*nd*	nd	*nd*
BbF	2.4	*5.6*	nd	*nd*	2.9	*25.6*	3.8	*26.3*	2.4	*48.6*
BaP	3.8	*68.1*	1.3	*42.3*	3.9	*35.9*	5.5	*14.8*	3.2	*30.9*
					Total Fractions					
Fluo	**96.7**	*6.0*	**84.0**	*8.5*	**91.6**	*9.6*	**81.5**	*17.3*	**105.6**	*1.0*
BbF	**88.4**	*5.1*	**82.9**	*9.9*	**90.7**	*5.7*	**96.1**	*5.9*	**79.5**	*19.9*
BaP	**95.4**	*1.3*	**90.2**	*8.2*	**99.8**	*4.7*	**105.2**	*5.1*	**92.8**	*22.5*

[a] Experimental conditions: spiked coffee brew (50 mL); PS-DVB, 0.5 g; elution with MeOH/THF 10:90 (v/v) 3 × 2 mL. HPLC conditions: Supelcosil LC-PAH column (250 × 4.6 mm i.d., 5 μm); gradient, acetonitrile/water (60:40, v/v) for 5 min, 25 min ramp to 100% acetonitrile, acetonitrile for 15 min; total flow rate, 1.5 mL min^{-1}; detection, fluorescence. [b] For this coffee, nonspiked coffee brew was not analyzed.

no particular matrix effect in that case. Increasing the elution volume (e.g., 3 × 3 mL diethyl ether) slightly increased the recoveries, but overall they remained below 40%. Besides, adding a step of washing the cartridge with water before elution of the PAHs did not improve the recoveries. These poor recoveries were quite surprising, as Kayali et al. reported recoveries ranging from 11 to 111% depending on the concentration tested and the PAH considered. However, in the study of Kayali et al., recoveries are directly based on spiked coffee brew samples, without taking into account the native PAHs

Table 5. Application to the Determination of PAHs in Several Coffee Brew Samples[a]

	coffee 1b, Arabica blended LO lot P5 31-12-03 07:37; $n = 2$[c]; extract 2 mL		coffee 1c, Arabica blended LO lot P6 18:26; $n = 3$; extract 2 mL		coffee 1d,[b] Arabica blended LO lot P5 12:26; $n = 2$; extract 2 mL		coffee 2, Arabica Colombia; $n = 3$; extract 2 mL		coffee 3, Arabica Ethiopia; $n = 3$; extract 2 mL	
	mean concn (ng L^{-1})	RSD (%)	mean concn (ng L^{-1})	RSD (%)	mean concn (ng L^{-1})	RSD (%)	mean concn (ng L^{-1})	RSD (%)	mean concn (ng L^{-1})	RSD (%)
					Fraction 1					
Fluo	nd	*nd*	nd	*nd*	nd	*nd*	nd	*nd*	nd	*nd*
suspected BbF	38.4	*31.7*	24.0	*29.8*	75.8	*44.0*	nd	*nd*	nd	*nd*
suspected BaP	65.3	*54.4*	34.1	*54.6*	nd	*nd*	nd	*nd*	nd	*nd*
					Fraction 2					
Fluo	nd	*nd*	nd	*nd*	nd	*nd*	nd	*nd*	nd	*nd*
suspected BbF	28.2	*23.1*	11.1	*16.3*	13.9	*5.1*	nd	*nd*	nd	*nd*
suspected BaP	30.4	*37.2*	8.9	*91.1*	nd	*nd*	nd	*nd*	nd	*nd*
					Total Fractions					
Fluo	**nd**	*nd*	**nd**	*nd*	**nd**	*nd*	**nd**	*nd*	**nd**	*nd*
suspected BbF	**66.6**	*28.0*	**35.1**	*20.1*	**89.7**	*38.0*	**nd**	*nd*	**nd**	*nd*
suspected BaP	**95.7**	*48.9*	**42.9**	*61.6*	**nd**	*nd*	**nd**	*nd*	**nd**	*nd*

[a] Experimental conditions: nonspiked coffee brew (50 mL); PS-DVB, 0.5 g; elution with MeOH/THF 10:90 (v/v) 2 × 2 mL. HPLC conditions: Supelcosil LC-PAH column (250 × 4.6 mm i.d., 5 μm); gradient, acetonitrile/water (60:40, v/v) for 5 min, 25 min ramp to 100% acetonitrile, acetonitrile for 15 min; total flow rate, 1.5 mL min^{-1}; detection, fluorescence, except [b] PS-DVB, 0.2 g; elution with MeOH/THF 10:90 (v/v) 2 × 2 mL. [c] Number of samples analyzed.

initially present in the coffee brew, so that their recoveries may be overestimated. Besides, with their highest concentration tested (still below our spiking concentration), they found a mean recovery of 49%, with only 25% recovery for benzo[*a*]pyrene, which is quite unsatisfactory.

Consequently, we tested other eluting solvents to improve recoveries: acetone, acetonitrile, acetonitrile/THF 75:25 (v/v), and methanol/THF 75:25 (v/v). These solvents offer the advantage of being miscible with the HPLC mobile phase, so that simple concentration of the extracts to nearly 0.5 mL (under a gentle stream of nitrogen) was performed before injection into the HPLC system. Acetone was found to be nonselective, with highly colored extracts obtained. In fact, this nonselectivity can be attributed to its elevated elution strength on reversed-phase sorbents ($\epsilon^\circ = 8.8$ on C_{18}-silica). As no cleanup step was desirable after the SPE to reduce the overall analysis time as well as to avoid possible losses or contaminations, this solvent was not further used. With regard to the other solvents tested, quite similar results were obtained, but still with nonquantitative recoveries (data not shown). Therefore, it appears that the conditions found in our study, namely, 50 mL of sample and MeOH/THF 10:90 (v/v) as the elution solvent, are best suited for quantitative recoveries of traces of PAHs in coffee brew using 0.5 g C_{18}-silica cartridges (mean recoveries from 93.5 to 104.7%).

Nevertheless, our results (see **Table 2**) clearly show that PS-DVB should be preferred to C_{18}-silica to achieve acceptable repeatability (RSDs ranging from 1.3 to 6.0% for 0.5 g of PS-DVB and from 10.7 to 17.8% for 0.5 g of C_{18}-silica).

Influence of Coffee Matrix. *Recoveries from Spiked Water Samples.* As coffee brew samples were brown, with numerous materials extracted from the milled coffee (especially hydrophobic compounds), the effect of these interfering compounds was studied. It was previously shown that partial losses were found after a single SPE on 0.2 g PS-DVB cartridges, possibly due to saturation of the sorbent bed by coffee material. Therefore, similar experiments were performed with Milli-Q water passing through the cartridge and initially spiked with PAHs as for the coffee brew. Results are presented in **Tables 1** and **2** for the 0.2 g PS-DVB and 0.5 g PS-DVB cartridges, respectively.

First, it is important to note that, whereas in the presence of methanol added to the coffee brew minimization of adsorption onto glass walls could be achieved (<8%), this was not the case for the water samples. Despite the similar addition of methanol, significant adsorption was found, ranging from 0 to 8.8% for fluoranthene and from 16.1 to 22.8% for the other two compounds. In fact, this could be attributed to a better solubility of PAHs in coffee brew as compared to water, possibly due to the presence of coffee material such as caffeine, which has been reported to complex PAHs (*17*, *22*). In practice, these adsorption effects were taken into account to estimate the real SPE recoveries.

Second, recoveries observed after the first SPE step on 0.2 g of PS-DVB were higher for the spiked water (from 86.7 to 88.6%) than for the spiked coffee brew (from 65.1 to 75.6%) using the same elution solvent MeOH/THF 20:80 (see **Table 1**). This result is consistent with partial saturation of the sorbent bed by the coffee material, leading to a lower retention of PAHs on the sorbent. This clearly shows that 0.5 g of PS-DVB should be preferred to 0.2 g of PS-DVB for that application. With spiked water passed through the 0.5 g PS-DVB cartridge, mean recoveries were 86.6 ± 7.8, 92.1 ± 4.6, and 82.3 ± 2.9% for fluoranthene, benzo[*b*]fluoranthene, and benzo[*a*]pyrene, respectively (see **Table 2**). Near 5−10% losses were observed, possibly due to incomplete elution of the retained compounds, as this is consistent with the highest lost for the more retained PAH, benzo[*a*]pyrene. With spiked coffee brew, mean recoveries were 96.7 ± 14.4, 88.4 ± 11.2, and 95.4 ± 3.1% for fluoranthene, benzo[*b*]fluoranthene, and benzo[*a*]pyrene, respectively, showing quite similar results for the first two compounds and enhanced recovery for benzo[*a*]pyrene. In fact, recoveries were higher in the first fraction as compared to spiked water. A possible explanation is that the solid sorbent is partially saturated with coffee material, leading to the PAHs being more easily eluted from the cartridge. Another hypothesis is that PAHs associate with coffee material and are thus more eluted with coffee material in the first fraction.

Addition of a Rinsing Step before Elution. The effect of adding a rinsing step before elution of the PAHs has been tested, with the objective of reducing matrix material coextracted in the final extract while keeping quantitative recoveries for PAHs. Water,

Table 6. Performance of the Proposed Method for the Determination of PAHs[a]

PAH	instrument linearity range[b] (μg L^{-1})	regression curve[c]	r^2	analytical LOD[d] (μg L^{-1})	analytical LOQ[d] (μg L^{-1})	method LOD[e] (ng L^{-1})	method LOQ[e] (ng L^{-1})
Fluo	2.5–50	$y = 34297x + 68645$	0.9981	2.49	8.3	9.96	33.2
BbF	0.4–50	$y = 165129x - 15240$	0.9993	0.33	1.11	1.32	4.44
BaP	0.4–50	$y = 276881x - 21141$	0.9993	0.19	0.63	0.76	2.52

[a] HPLC conditions: Supelcosil LC-PAH column (250 × 4.6 mm i.d., 5 μm); gradient, acetonitrile/water (60:40, v/v) for 5 min, 25 min ramp to 100% acetonitrile, acetonitrile for 15 min; total flow rate, 1.5 mL min^{-1}; detection, fluorescence. [b] Based on ($n = 12$) determinations in the range of 0.4–50 μg L^{-1} (0.4, 1, 2, 2.5, 3.5, 5, 8, 10, 20, 25, 35, 50 μg L^{-1}). [c] y = peak area; x = concentration (μg L^{-1}). [d] S/N = 3 for LOD and 10 for LOQ, based on the analysis of blanks ($n = 7$). [e] Estimated LOD and LOQ for the whole method, assuming a coffee brew volume of 50 mL and the final concentration of the extract to 0.2 mL.

water/MeOH 50:50 (v/v), and MeOH have been tested. Results are presented in **Table 3**. Our results give evidence of partial elution of the PAHs during the rinsing step, especially for the less retained compound fluoranthene; therefore, no rinsing step was further performed.

Recoveries from Several Spiked Coffee Brew Samples. Coffees from different origins (blended coffee, Colombia, Ethiopia) and differents lots have been spiked and SPE extracted using the selected conditions, with a view of testing the reproducibility of the method. As shown in **Table 4**, satisfactory recoveries were obtained, ranging from 81.5 to 105.2%. To improve the reproducibility and the accurracy, it should be preferred to recombine the three 2 mL fractions and then analyze the final extract after concentration. This would avoid the analysis of traces of PAHs in the third fraction and reduce the error on the final result. Also, this would lead to only one analysis, thus reducing the time for obtaining results. However, in our study we analyzed separately the three fractions for a better understanding of PAH behavior during the SPE step.

To enhance sensitivity, concentration of the final extract to ~0.2−0.5 mL is also feasible. As shown in **Table 4**, satisfactory recoveries could still be obtained despite possible slight losses of PAHs during the solvent evaporation, especially the most volatile compounds.

Application to Several Coffee Samples. Finally, the SPE method has been used for the determination of PAHs in nonspiked coffee brew samples. Our experiments (see **Table 5**) clearly show a high variability of the PAH content of the coffee samples. Hence, no traces of the PAHs could be detected in the coffees from Colombia and Ethiopia, whereas traces of PAHs were suspected in the blended coffee. Typical chromatograms obtained using the HPLC-FLD system are presented in **Figure 2**. No fluoranthene could be detected, whereas this compound has previously been reported in coffee brew samples at concentrations ranging from 0.74 to 2.27 ng L^{-1} (*19*). In fact, this could be attributed to the lack of sensitivity of our method for this compound as indicated in **Table 6**, leading to an overall limit of detection (LOD) of the proposed method near 10 ng L^{-1} in the coffee brew sample. On the other hand, for benzo[*b*]fluoranthene and benzo[*a*]pyrene, acceptable LODs were estimated for the method, with estimated values of 1.32 and 0.76 ng L^{-1}, respectively (see **Table 6**). Traces of benzo[*b*]-fluoranthene and benzo[*a*]pyrene were suspected in the analyzed coffee brew samples, with suspected concentrations in the range of 0−95.7 ng L^{-1}, as compared to 0−10 ng L^{-1} frequently reported in other studies. The nonquantitative recoveries of the methods used in other studies may partially explain these differences. Besides, traces were near the limits of quantification (LOQ) of the method, so that the accurracy of our results is poor. Larger sample volumes (such as 100 mL) may be considered to lower the LOQs, but in this case caution must be taken to avoid losses during the SPE step. The replacement of the 20 μL injection loop by a 50 μL injection loop in the HPLC system could also contribute to the achievement of lower LOQs.

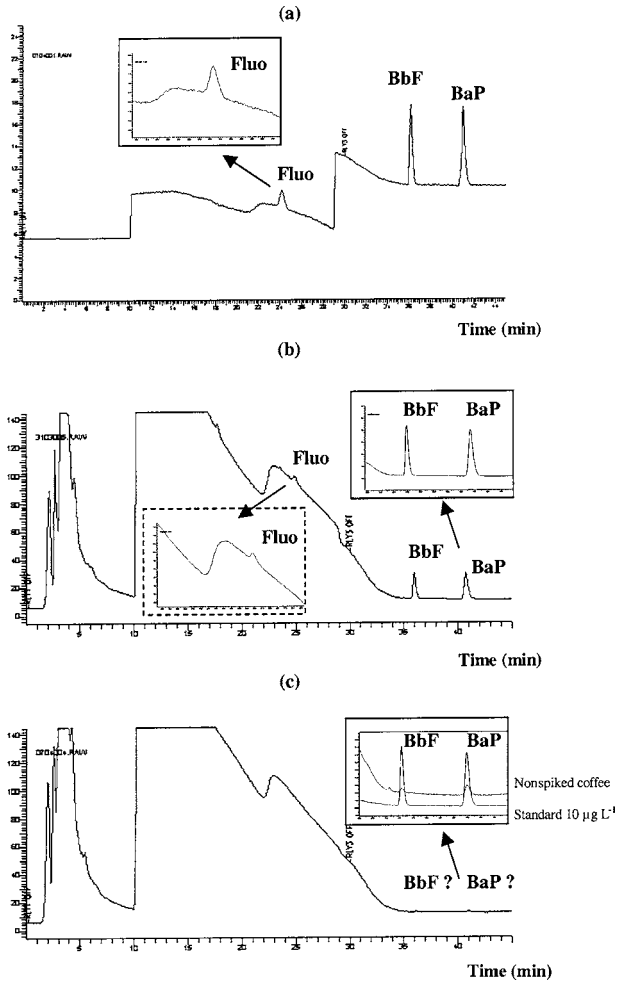

Figure 2. Example of typical chromatograms obtained: (**a**) standard three PAHs, 10 μg L^{-1}; (**b**) spiked (2 μg L^{-1}) coffee brew; (**c**) nonspiked coffee brew. HPLC conditions: Supelcosil LC-PAH column (250 × 4.6 mm i.d., 5 μm); gradient, acetonitrile/water (60:40, v/v) for 5 min, 25 min ramp to 100% acetonitrile, acetonitrile for 15 min; total flow rate, 1.5 mL min^{-1}; detection, fluorescence (0–10 min, 320/510 nm; 10–29 min, 230/410 nm; 29–55 min, 250/420 nm).

Also, we assumed that traces of PAHs in our samples may have been overestimated due to coeluting compounds. Therefore, we tried to identify the PAHs by comparing the UV spectra obtained after HPLC-DAD analysis of the coffee brew samples and PAH standards. Typical chromatograms and UV spectra are given in **Figure 3**. Despite concentration of the final extract to ~0.2−0.5 mL, the peaks found were near the LOD. Comparison of the UV spectra of the observed peaks with those of the PAH standard revealed no similarity, thus avoiding

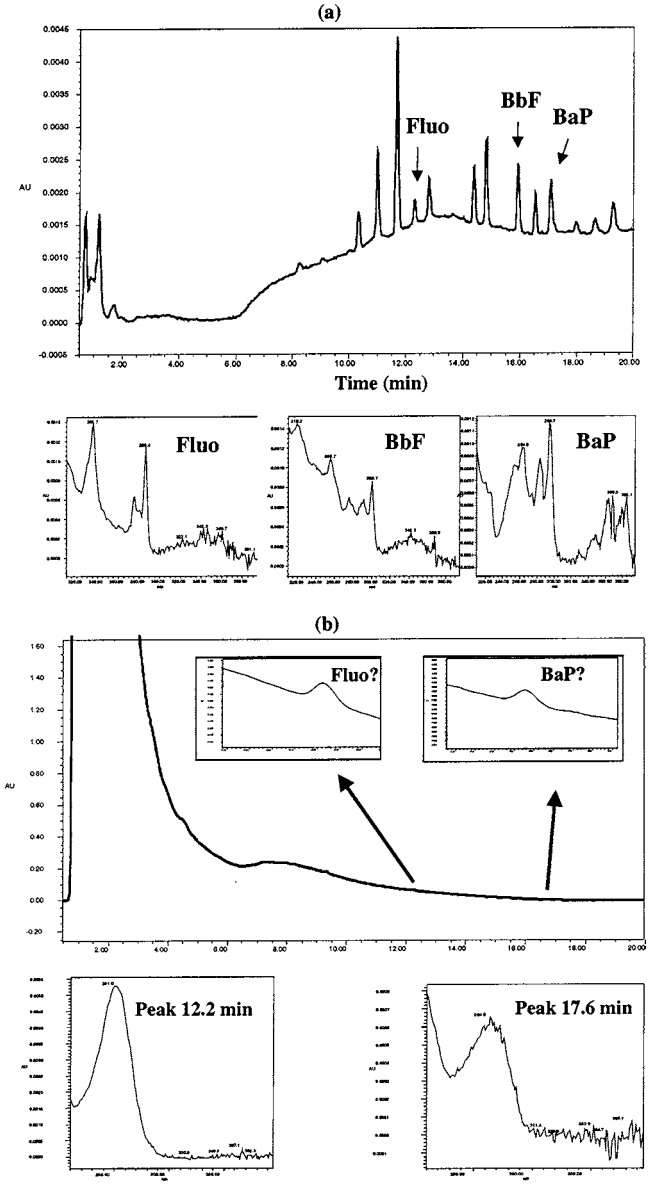

Figure 3. Chromatograms (254 nm) of a 16 PAH standard and a nonspiked coffee brew and UV spectra of selected PAHs: (**a**) standard 16 PAHs, 25 μg L^{-1}; (**b**) nonspiked coffee brew (lot P5 31-12-03 07:37). HPLC conditions: Supelcosil LC-PAH column (150 × 3.0 mm i.d., 5 μm); gradient, acetonitrile/water (40:60 v/v) for 4 min, 11 min ramp to 100% acetonitrile, acetonitrile for 10 min; flow rate, 0.8 mL min^{-1}; detection, UV (254 nm).

identification of the PAHs, even though addition of spiked PAHs to these extracts confirmed the peak attributions. In fact, we could observe that the coffee matrix had a strong influence on the PAH spectra, as the UV spectra of PAH spiked directly to the extract were not similar to those of the standard. Further experiments need to be conducted to better study the effect of coffee matrix, to possibly enable further PAH identification in the extracts.

From **Table 5** it is interesting to note the great differences obtained for lots of the same origin. This points out the necessity of regular analysis to determine the PAH content of the coffee brews. Also, further studies need to be conducted to see if these variations could be attributed to the roasting step conditions (such as temperature and time).

Conclusion. A simple and rapid solid-phase extraction method has been developed and applied to the determination of PAHs in coffee brew, namely, fluoranthene, benzo[*b*]-fluoranthene, and benzo[*a*]pyrene. This method involves preconcentration of the compounds on 0.5 g PS-DVB cartridges, elution with methanol/THF 10:90 (v/v), concentration of the final extract, and HPLC-FLD analysis. Acceptable recoveries (from 79.5 to 105.6%) were obtained from several spiked coffee brew samples, with satisfactory repeatability, and as low as 0.76 ng L^{-1} of benzo[*a*]pyrene could be detected in coffee brew. In the case of nonspiked coffee brew samples, traces of PAHs were suspected and estimated, but identification based on UV spectra was hindered due to matrix effects.

SAFETY

Due to the high toxicity of PAHs as well as the use of organic solvents, special attention must be taken during all experiments. Gloves must be used, and experiments must be conducted under a fume hood.

ABBREVIATIONS USED

ACN, acetonitrile; BaP, benzo[*a*]pyrene; BbF, benzo[*b*]fluoranthene; FLD, fluorometric detection; Fluo, fluoranthene; HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MeOH, methanol; PAH, polycyclic aromatic hydrocarbon; PS-DVB, polystyrene−divinylbenzene; RSD, relative standard deviation; SPE, solid-phase extraction; THF, tetrahydrofuran.

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