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Investigation of Sample Treatment Steps for the Analysis of Polycyclic Aromatic Hydrocarbons in Ground Coffee

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Sample treatment procedures were tested for the determination of polycyclic aromatic hydrocarbons (PAHs) in ground coffee. Pressurized liquid extraction (PLE), under different conditions, was combined with several cleanup methods, namely in situ purification, C_{18}-silica solid-phase extraction (SPE), silica SPE, acid digestion, and alkaline saponification. Soxhlet extraction and direct alkaline saponification were also tested. Best results were obtained using PLE with hexane/acetone 50:50 (v/v) under 150 °C. Alkaline saponification followed by cyclohexane extraction and silica SPE was required to eliminate interferent compounds. Finally, 11 PAHs could be quantified in ground coffee with limits of detection in the range of 0.11−0.18 μg kg^{-1}. Application to ground Arabica coffee lots from Colombia revealed the presence of several PAHs, giving an overall toxicity equivalence in the range of 0.16−0.87 μg kg^{-1}. PAH identification was performed using both high-performance liquid chromatography−diode array detection and gas chromatography coupled to mass spectrometry.

KEYWORDS: Coffee; polycyclic aromatic hydrocarbons; pressurized liquid extraction; saponification; solid-phase extraction

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants that may be formed during the incomplete combustion or pyrolysis of carbonaceous materials at high temperature. In particular, their presence in food matrices has been reported (*1*−*7*). Two ways of contamination can explain PAH content in foods: either contamination of the food sources, due to the presence of PAHs in the environment (via atmospheric deposition on leaves, for example), or formation of these compounds during food processing because of elevated temperatures. Hence, drying, cooking, frying, roasting, and smoking are food processes susceptible to lead to PAH generation in the food product. Due to their mutagenic and carcinogenic potentials, the presence of PAHs in food matrices is of great concern, especially the highest carcinogenic benzo[*a*]pyrene.

The presence of PAHs in coffee samples has been reported and may be attributed to either contamination of the initial green beans or formation of these compounds during the roasting step (*8*). Concentrations of benzo[*a*]pyrene in the range from <0.01 to 1.2 μg kg^{-1} have been usually reported in commercially ground and instant coffees, even though concentrations up to 22.7 μg kg^{-1} were found for highly roasted coffees (*8*−*13*). However, only a few published studies can be found, and each time different analytical procedures were used so that comparison of results is sometimes difficult. Reported analytical procedures are often tedious, involving classical solvent extraction (under shaking or in a Soxhlet apparatus), saponification (mostly alkaline saponification using potassium hydroxide in aqueous alcohol), liquid−liquid partitioning (usually with cyclohexane), and/or silica gel cleanup as indicated in **Table 1**. The principal problems associated with the determination of PAHs in coffee samples are the low detection levels and the diversity of potential interferents present that may hinder PAH determination (e.g., lipid content around 15% in ground coffee). Previous studies reported the necessity of an alkaline saponification stage to release PAH bound to food components (*16*) or to remove some matrix components (*17*, *18*). However, the degradation of some labile PAHs is suspected under strong alkaline conditions. Therefore, the performance of a saponification step for coffee samples is subject to question, especially as acetone extraction and alkaline digestion followed by cyclohexane extraction gave comparable results for the determination of benzo[*a*]pyrene in roasted coffee (*11*).

As most of these studies focused mainly on benzo[*a*]pyrene, additional studies are required for the other PAHs, especially those that are carcinogenic. Besides, usually PAHs were identified only on the basis of their chromatographic retention times, which is insufficient to ascertain their presence. Even though they could be identified in coffee brews by gas chromatography−mass spectrometry (GC-MS) (*19*), we previously reported problems for their identification in coffee brew samples based on UV spectra due to matrix effects (*20*).

This study investigates different sample treatment methods [namely, Soxhlet extraction, pressurized liquid extraction (PLE), saponification, solid-phase extraction (SPE), liquid−liquid

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Table 1. Survey of Analytical Procedures for PAH Determination in Ground and Instant Coffee Samples

PAH	extraction	saponification	cleanup	analysis	identification	recovery (%)	LOD (μg kg^{-1})	ref
B[a]P	none	KOH/MeOH	DMF/water washing silica gel petroleum ether elution	GC-MS (SIM)	based on retention times	ND[a]	0.1	*9*
F, Pyr, B[a]A, Chrys, B[e]P, B[b]F, B[k]F, B[a]P, DB[ah]A, B[ghi]P	none	KOH/EtOH (reflux, 40 min) cyclohexane extraction	DMF/water 9:1 washing silica gel (5 g) cyclohexane elution concentration	HPLC-FD	based on retention times	66.9–99.0	0.07–1.29	*14*
B[a]P	none	KOH/EtOH (reflux, 40 min) cyclohexane extraction	water washing (4 times) Na_2SO_4 drying concentration silica gel (5 g) cyclohexane elution evaporation to dryness THF redissolution	HPLC-FD	based on retention times	87.2–115	ND	*15*
B[a]P	Soxhlet (acetone, 6 h) evaporation to dryness	KOH/MeOH–water 9:1 (reflux, 30 min) cyclohexane extraction	water washing evaporation to dryness MeOH redissolution	HPLC-FD	based on retention times	78–97	0.1	*10*, *11*
B[a]P	ACN/water 50:50 dissolution molecular-imprinted polymer SPE	none	water washing ACN/water 50:50 washing CH_2Cl_2 elution	HPLC-FD	based on retention times	72.5	ND	*13*
B[b]F, B[k]F, B[a]P, B[ghi]P, I[cd]P, B[a]A, DB[ah]A	hexane extraction (shaking, 10 min) filtration	none	silica gel (0.69 g) hexane elution evaporation to dryness ACN redissolution	HPLC-FD	based on retention times	87–103	0.01–0.05	*12*

[a] Not determined.

partitioning (LLE)] for the determination of PAHs in ground coffee samples. The achievement of both satisfactory recoveries and clean extracts was considered for the selection of the sample treatment methods. Application of the retained analytical procedure to Arabica coffee samples was then performed.

MATERIALS AND METHODS

All experiments were done in duplicate or triplicate unless otherwise specified (n_{exp} being the number of experiments), enabling mean values and relative standard deviations (RSDs) to be determined. For the estimation of recoveries, both nonspiked and spiked coffee samples were extracted, to take into account the amount of native PAHs in the samples. Comparison of variances and mean recoveries were performed using statistical tests, namely, *F* tests for variances and two-sided paired *t* tests or two-sided Cochran tests for mean recoveries (*21*).

Reagents and Chemicals. Individual standard solutions (10 mg L^{-1} in acetonitrile) of the following PAHs were obtained from CIL Cluzeau (Paris, France): fluoranthene (F, purity = 98.0%), benzo[*b*]fluoranthene (B[b]F, purity = 99.5%), benzo[*a*]pyrene (B[a]P, purity = 99.5%). A PAH mix solution was also used (Supelco, Saint-Quentin Fallavier, France) containing the 16 EPA PAHs (10 mg L^{-1} in acetonitrile) with purities >96% as indicated: naphthalene (97.7%), acenaphthylene (99.9%), acenaphthene (Ace, 99.9%), fluorene (98.6%), phenanthrene (Phen, 99.9%), anthracene (Anthr, 99.8%), fluoranthene (F, 98.2%), pyrene (Pyr, 96.6%), benzo[*a*]anthracene (B[a]A, 97.9%), chrysene (Chrys, 98.7%), benzo[*b*]fluoranthene (B[b]F, 99.9%), benzo[*k*]fluoranthene (B[k]F, 99.5%), benzo[*a*]pyrene (B[a]P, 99.9%), dibenzo[*a*,*h*]anthracene (DB[ah]A, 99.6%), benzo[*g*,*h*,*i*]perylene (B[ghi]P, 99.1%), and indeno[1,2,3-*c*,*d*]pyrene (I[cd]P, 99.9%). HPLC-grade solvents were used, supplied by either Carlo-Erba (i.e., acetonitrile, methanol, anhydrous ethanol, tetrahydrofuran, cyclohexane) or Prolabo (i.e., acetone, dichloromethane, hexane). Deionized water was produced with a Milli-Q system from Millipore (Saint-Quentin-en-Yvelines, France). Anhydrous sodium sulfate was supplied by Merck (for analysis grade) and potassium hydroxide (KOH) by Prolabo (Rectapur grade). Stock standard solutions were prepared by diluting the PAH solutions in an appropriate volume of the same solvent as the extracts to obtain the desired concentrations in the range of 5−800 μg L^{-1}. All solutions were stored at 4 °C in the dark for up to 5 weeks.

Coffee Samples. Coffee samples were commercially ground coffees, available at the supermarket. They were all Arabica samples, either from several origins (blend) or from a particular region (Ethiopia or Colombia). For spiking, 50 ng of each PAH (50 μL of a stock solution at 1 mg L^{-1}) was added to the 5 g sample directly in the middle of the extraction cell before PLE (150 ng of each PAH to the 15 g sample in the case of direct saponification or Soxhlet extraction), leading to a spiking level of 10 μg kg^{-1} for each PAH, similar to the method used in a previous study (*15*).

Soxhlet Extraction. The Soxhlet extraction was the same as already reported for roasted coffee samples (*11*). Ground coffee (15 g) was placed in the cellulose extraction thimble and extracted with acetone (200 mL) for 6 h (30 min per cycle). The solvent was completely removed, and the dry residue was submitted to alkaline saponification, cyclohexane extraction, and silica SPE.

Pressurized Liquid Extraction. The PLE system was an ASE 100 (Dionex). Ground coffee (5 g) was mixed with 5 g of diatomaceous earth (Hydromatrix, Varian), and glass beads were added to fill the cells (cell volume = 34 mL). The extraction temperature (100, 120, or 150 °C) and the nature of the solvent [methanol, acetone, hexane, hexane/acetone 94:6 (v/v), hexane/acetone 50:50 (v/v)] have been tested. Other conditions were as follows unless otherwise specified in the text: 5 min of heating time, 5 min of static time per cycle, flush volume of 140%, purge time of 120 s, three static cycles.

Alkaline Saponification and Cyclohexane Extraction. For alkaline saponification of PLE extracts, 1.5 g of KOH was dissolved in 25 mL of ethanol and then added to the dry residue. Saponification was performed under reflux for 30 or 40 min depending on the experiment. After cooling, 100 mL of cyclohexane was added, and the mixture was heated under reflux for 5 min. After cooling, the cyclohexane phase was kept and mixed (for 5 min) with 100 mL of water. Then the two phases were kept overnight for efficient decantation. In the case of emulsions, 25 mL of ethanol was added to favor the phase separation. The aqueous phase was discarded and the organic layer again extracted twice with 100 mL of water. Then, anhydrous sodium sulfate (5−10 g) was added to dry the organic layer and removed from the liquid extract by filtration. The final extract was concentrated to near 1−2 mL.

For direct alkaline saponification of ground coffee samples, 15 g of coffee was weighed. Ethanolic KOH (1 mol L^{-1}, 200 mL) was added, along with a few pumice stones to regularize the boiling. After the mixture had been heated under reflux for 40 min and cooled, 200 mL of cyclohexane was added, and the mixture was heated under reflux for 5 min. After cooling, the cyclohexane phase was kept and mixed (for 5 min) with 200 mL of water. Then the two phases were kept overnight for efficient decantation. The aqueous phase was discarded

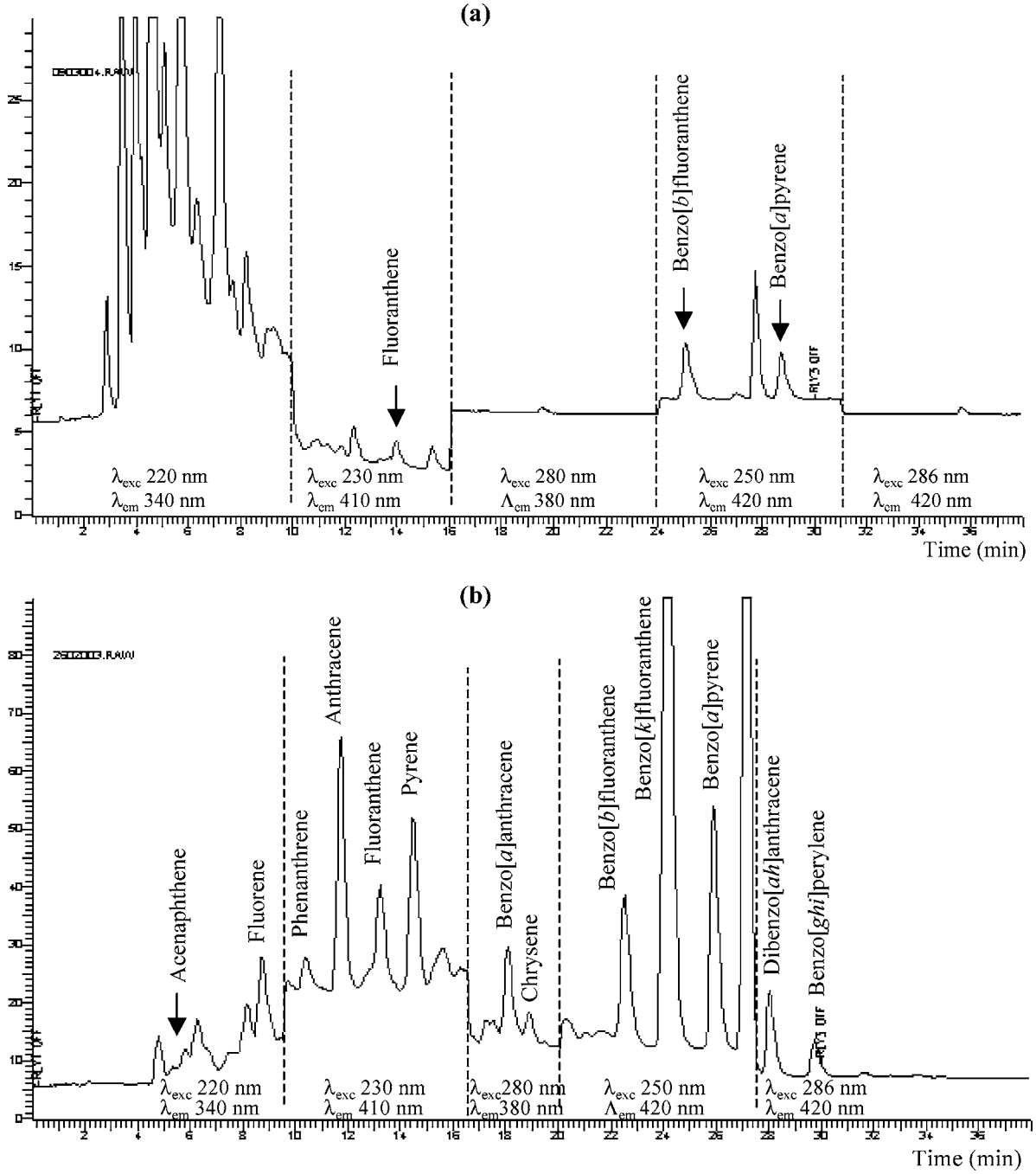

Figure 1. Chromatograms of spiked Arabica coffee samples showing the removal of interferent compounds upon saponification after PLE (differences in retention times between chromatograms are due to different column temperatures as detailed below): (**a**) no saponification (experiment 2bis) [PLE (150 °C, hexane)/silica SPE/HPLC-FD analysis, Colombia coffee (lot 03259) spiked with three PAHs (F, B[b]F, and B[a]P (column temperature not regulated in these experiments)]; (**b**) saponification (experiment 7) [PLE (150 °C, hexane–acetone 50:50)/saponification/cyclohexane extraction/silica SPE/HPLC-FD analysis, Colombia coffee (lot 04265) spiked with several PAHs (column temperature fixed at 35 °C in these experiments)].

and the organic layer again extracted twice with 200 mL of water. Then, anhydrous sodium sulfate (5−10 g) was added to dry the organic layer and then removed from the liquid extract by filtration, and the final extract was concentrated to near 1−2 mL.

Acid Digestion. To the PLE hexane extracts (around 10 mL) was added 9 mol L^{-1} sulfuric acid (five successive additions of 1 mL), and the mixture was shaken vigorously for 2 min. The two layers were separated, and the yellow-brown aqueous layer was discarded. The organic phase was then rinsed with 3 × 20 mL of Milli-Q water. After the obtained organic phase had been dried on anhydrous sodium sulfate, the extract was concentrated to near 1−2 mL before the SPE step.

Solid-Phase Extraction. SPE was performed using disposable SPE cartridges containing either silica (Supelclean LC-Si, 1 g, supplied by Supelco, Saint-Quentin Fallavier, France) or C_{18}-silica (ENVI-18, 0.5 g, supplied by Supelco) depending on the extract solvent (hexane/cyclohexane or acetonitrile, respectively). A Visiprep vacuum manifold system (Supelco) was used.

Silica cartridges were conditioned with 5 mL of the same solvent used for elution. Depending on the experiments, PAHs were eluted using fractions consisting of either 4 × 5 mL of cyclohexane or 2 × 2.5 mL of hexane followed by 2 × 2 mL of hexane/dichloromethane 50:50 (v/v). Each fraction was then evaporated to dryness under a gentle stream of nitrogen, and the dry residues were redissolved in 1 mL of acetonitrile (ACN) or 0.4 mL of tetrahydrofuran (THF).

C_{18}-silica cartridges were conditioned with 5 mL of methanol (MeOH), 5 mL of MeOH/THF 50:50 (v/v), and 5 mL of water. PAHs

Table 2. Description of the Different Experiments Carried out To Test the Cleanup Step after PLE Extraction of Ground Coffee Samples

expt no.	PLE conditions	extraction solvent	saponification	cleanup	final treatment	recovery (%)	comments[a]
1[b]	100 °C —flush 60% purge 100 s cycles 2 × 5 min	hexane	none	alumina (placed in the PLE cell: in situ purification)	evaporation to dryness ACN/water 60:40 redissolution	F: 82.6–91.8 B[b]F: 47.0–67.8 B[a]P: 74.0–89.5	PLE extract consisted of two distinct liquid phases
2[b]	120 °C —flush 100% purge 90 s cycles 2 × 5 min	hexane	none	concentration to 5 mL silica (SPE cartridge) hexane + hexane/CH_2Cl_2 50:50 elution	evaporation to dryness ACN redissolution	F: 97.2 B[b]F: 35.1 B[a]P: 32.0	losses occurred during 5 mL extract percolation through SPE silica evaporation to 2 mL before SPE is required
2bis[b]	150 °C all conditions as in expt 2	same as expt 2	same as expt 2	same as expt 2	same as expt 2	F: 90.3 B[b]F: 53.4 B[a]P: 26.8	same as expt 2
3[b]	100 °C –flush 140%, purge 120 s, cycles 3 × 5 min	hexane	none	sulfuric acid addition organic phase rinsed with water concentration to 2 mL silica (SPE cartridge) hexane elution	evaporation to dryness THF redissolution	not determined (performed on non-spiked coffee only)	organic phase is not easily separated from aqueous phase
4[b]	100 °C --flush 140% purge 120 s cycles 3 × 5 min	hexane/acetone 94:6	none	evaporation to dryness ACN redissolution 5 mL C_{18}-silica (SPE cartridge) ACN elution	concentration to 0.5 mL	F: 84.4 B[b]F: 65.9 B[a]P: 22.4	some losses are due to ACN entrainment during extract percolation through SPE C_{18}-silica
4bis[b]	same as expt 4	same as expt 4	same as expt 4	evaporation to dryness ACN/water 50:50 redis-solution 5 mL C_{18}-silica (SPE cartridge) MeOH/THF 10:90 elution	same as expt 4	F: 107.9 B[b]F: 103.7 B[a]P: 57.9	losses of B[a]P are due to insufficient PLE extraction

[a] Some additional experiments, in which spiking was performed just before the cleanup step, were performed to explain some observed losses. Losses of PAHs through the SPE cartridge were also confirmed by placing a second SPE cartridge underneath the first one. [b] In these experiments, coffee samples were spiked only with three model PAHs (fluoranthene, benzo[*b*]fluoranthene, and benzo[*a*]pyrene).

were eluted using fractions of 2 × 2.5 mL of acetonitrile or MeOH/THF 10:90 (v/v) depending on the experiment. They were further concentrated to near 0.5 mL under a gentle stream of nitrogen. The final volume of each extract was determined by weighing for a correct quantification of the PAH mass recovered.

Quantification of PAHs in the Final Extracts. PAH contents were quantified using high-performance liquid chromatography (HPLC) coupled to fluorometric detection (FD). 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., C_{18}-silica, 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 (λ_{exc}) and emission (λ_{em}) wavelengths as indicated in **Figure 1**. External calibration was performed in the range of 5−800 or 5−50 μg L^{-1} depending on the PAH concentrations in the samples. Identification of PAHs was based on peak retention times, by comparison with standards. At the beginning of this study, the HPLC column temperature was not regulated (ambient temperature), which resulted in slight daily variations of retention times, so that daily analysis of standards was crucial. Then, the column was placed in an oven (Waters column heater module connected to a Waters temperature control module) and its temperature regulated at 35 °C, enabling stability of retention times.

Identification of PAHs. Confirmation of the presence of suspected PAHs in coffee samples was performed using both HPLC coupled to diode array UV−visible detection (HPLC-DAD) and gas chromatography coupled to mass spectrometry (GC-MS/MS).

The HPLC system consisted of a Waters 1525 high-pressure gradient pump, a Rheodyne injection valve equipped with a 20 μL loop, an oven (column temperature fixed at 35 °C), 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 a 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}.

The GC-MS system was a Trace GC 2000 coupled to an ion-trap Polaris Q (Thermo Finnigan Corp., Austin, TX). Separation was performed on an Rtx-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Restek) with the following temperature program: 50 °C (for 1.2 min), increase to 180 °C at a 30 °C/min ramp, 180 °C maintained for 4 min, increase to 270 °C at a 25 °C/min ramp, 270 °C maintained for 14 min, increase to 300 °C at a 15 °C/min ramp, 300 °C maintained for 4 min (total analysis time = 33.1 min). Helium was used as the carrier gas at a constant flow rate (1 mL min^{-1}). The transfer line temperature was kept at 300 °C. Samples (1 μL) were injected in a programmed temperature vaporizer (PTV) injector under the following conditions: splitless injection at 60 °C (injection time = 0.2 min), increase to 290 °C at a 10 °C/s ramp, 290 °C maintained for 1 min, split valve opened after 1 min (split flow rate = 10 mL min^{-1}). A 5 mm i.d. Focusliner (Restek) was used as the injection chamber in the PTV injector. After each injection, a cleaning phase was programmed: increase to 300 °C at a 10 °C/s ramp, 300 °C maintained for 3 min, flow rate = 50 mL min^{-1}. The ion source was maintained at 250 °C, with the following tune-setting conditions: filament emission current of 250 mA at 70 eV and automatic gain control of 50 (arbitrary unit, range = 1−500). For MS/MS the parent ion was selected at $m/z \pm 0.5$, with CID voltage set from 1.5 to 2.0 V and the q value set at 0.30 or 0.45. Selection of the parent ion was programmed as follows: 5 min, m/z 128; 6.2 min, m/z 152; 7.2 min, m/z 154; 8 min, m/z 166; 10 min, m/z 178; 12 min, m/z 202; 14.2 min, m/z 228; 17 min, m/z 252; 21 min, m/z 276; 25.88 min, m/z 278; 26.7 min, m/z 276. Data analysis was performed using the Xcalibur 1.2 software.

RESULTS AND DISCUSSION

Acenaphthene and fluorene are not discussed as, due to interferent compounds, their quantification may be biased.

Optimization of Cleanup Methods after PLE. Different cleanup procedures were tested after PLE as indicated in **Table 2**. The addition of in situ alumina (experiment 1) was left out as some lipidic phase remained in the PLE extract, leading to nonquantitative and non-reproducible results. For the same

Table 3. Description of the Different Experiments Carried out To Test the Extraction Step with Spiked Ground Coffee Samples

expt no.	extraction technique	extraction solvent	saponification	cleanup	final treatment	recovery (%)	comments[a]
5	PLE (100 °C)	different solvents tested: (a) hexane; (b) hexane/acetone 94:6; (c) hexane/acetone 50:50; (d) acetone; (e) methanol	same as expt 7	same as expt 7	same as expt 7	(a) 39.3–69.4 (b) 36.1–55.7 (c) 57.5–83.3 (d) 36.9–91.2 (e) 47.2–100.4	losses due to insufficient PLE extraction
6	PLE (120 °C)	hexane/acetone 50:50	same as expt 7	same as expt 7	same as expt 7	57.2–102.2	
7	PLE (150 °C)	hexane/acetone 50:50	KOH/EtOH cyclohexane extraction	water washing Na_2SO_4 drying concentration silica (SPE cartridge) cyclohexane elution	evaporation to dryness THF redissolution	63.9–106.1	final retained analytical procedure
8	Soxhlet (6 h)	acetone	same as expt 7	same as expt 7	same as expt 7	66.2–86.0 except Phen (218.5)	overestimation for Phen due to interferent compounds
9	none	none	same as expt 7	same as expt 7	same as expt 7	48.7–84.1	

[a] Some additional experiments were performed to explain some observed losses, in which spiking was performed just before the saponification step, the SPE cleanup step, or the evaporation to dryness.

Table 4. Extraction of PAHs from Spiked Samples under Different Conditions Followed by Alkaline Saponification, Cyclohexane Extraction, and Silica SPE: Recovery (Percent) (RSD, %)

	PLE conditions							Soxhlet	no extraction
PAH	expt 5(a) 100 °C *n*-hexane $n_{exp} = 2$	expt 5(b) 100 °C hexane/acetone 94:6 $n_{exp} = 1$	expt 5(c) 100 °C hexane/acetone 50:50 $n_{exp} = 2$	expt 5(d) 100 °C acetone $n_{exp} = 2$	expt 5(e) 100 °C MeOH $n_{exp} = 2$	expt 6 120 °C hexane/acetone 50:50 $n_{exp} = 2$	expt 7 150 °C hexane/acetone 50:50 $n_{exp} = 2$	expt 8 $n_{exp} = 2$	expt 9 $n_{exp} = 3$
Phen	39.3 (96.6)	49.4	57.9 (15.6)	36.9 (111.1)	48.7 (93.3)	57.2 (2.2)	101.9 (9.2)	218.5 (16.2)	63.8 (11.8)
Anthr	45.5 (22.3)	36.1	57.5 (6.7)	60.5 (3.9)	52.0 (28.2)	72.3 (1.1)	63.9 (12.4)	78.9 (7.1)	69.8 (3.4)
F	57.6 (17.7)	42.8	82.0 (19.1)	91.2 (32.4)	66.1 (127.8)	102.2 (2.7)	100.6 (7.7)	66.2 (139.9)	48.7 (7.2)
Pyr	69.4 (35.8)	47.9	83.3 (5.7)	48.1 (25.5)	100.4 (38.2)	90.1 (9.0)	81.8 (11.4)	77.7 (48.3)	69.3 (6.3)
B[a]A	51.1 (9.5)	55.7	64.2 (5.5)	81.3 (7.7)	71.0 (22.7)	96.3 (3.2)	99.4 (4.4)	80.3 (4.6)	80.6 (1.4)
Chrys	43.1 (9.7)	50.5	60.7 (5.1)	68.5 (9.4)	57.8 (28.1)	86.1 (2.8)	106.1 (6.8)	69.9 (4.7)	84.1 (2.4)
B[b]F	53.2 (12.9)	51.2	67.7 (5.3)	80.0 (2.4)	63.5 (21.6)	65.1 (1.2)	81.0 (3.3)	80.7 (1.3)	75.2 (2.5)
B[k]F	54.4 (10.7)	49.7	68.9 (4.4)	81.7 (1.9)	47.2 (28.4)	65.5 (1.7)	76.5 (1.4)	86.0 (2.5)	74.6 (1.7)
B[a]P	52.8 (12.9)	47.3	66.6 (5.2)	81.1 (2.1)	70.1 (18.9)	65.0 (2.8)	78.6 (7.8)	78.3 (2.4)	71.2 (1.9)
DB[ah]A	49.7 (3.6)	46.6	66.4 (3.3)	82.7 (6.9)	68.0 (16.1)	57.4 (2.7)	70.3 (1.5)	76.4 (0.1)	73.7 (1.7)
B[ghi]P	50.5 (4.0)	47.2	70.9 (4.1)	88.3 (6.2)	72.7 (15.5)	65.1 (4.4)	74.7 (3.2)	76.2 (1.5)	73.8 (2.9)

reason, acid digestion was inadequate under the conditions tested (experiment 3), even though it was effective for the elimination of fats after PLE extraction of PAHs from smoked foods (*22*). Cleanup on C_{18}-silica (experiments 4 and 4bis) required the complete evaporation of the hydrophobic extraction solvent for sufficient PAHs retention on the sorbent; besides, numerous interferent compounds remained that hindered the determination of some PAHs (data not shown). Silica cleanup was preferred (experiments 2 and 2bis), as it is compatible with the PLE extraction solvent, even though reduction of the volume to near 2 mL is necessary to avoid PAH entrainment through the sorbent. Yet, as numerous interferent compounds remained in the extract despite this cleanup, an alkaline saponification of the PLE extract was performed with subsequent cyclohexane extraction. As illustrated in **Figure 1**, numerous interferent compounds (possibly triglycerides) could be removed upon saponification.

Choice of PLE Conditions. All previous experiments revealed insufficient benzo[*a*]pyrene extraction during PLE under the conditions used. To achieve quantitative recoveries, both the nature of the extraction solvent and temperature were tested as indicated in **Table 3** (experiments 5−7). Exhaustive values of obtained recoveries under each conditions are given in **Table 4**, and results of the statistical tests are presented in **Table 5**. With regard to the different solvents tested under 100 °C, hexane gave significantly lower recoveries than either methanol, acetone, or hexane/acetone 50:50 (v/v). The single experiment with hexane/acetone 94:6 (v/v) gave also low recoveries. No statistical difference could be observed between hexane/acetone 50:50 (v/v), acetone, and methanol for recoveries, but hexane/acetone 50:50 (v/v) was preferred due to its better precision. Yet, this solvent under 100 °C led to nonquantitative recoveries, with mean values in the range of 57.5−83.3% for the 11 PAHs quantified. Under 120 °C results were not statistically better, but improvement in recoveries is significant at 150 °C as indicated in **Table 5**, with recoveries ranging from 63.9 to 106.1% and acceptable RSDs for most of the PAHs (<13%). Consequently, we retained a PLE with hexane/acetone 50:50 (v/v) under 150 °C followed by alkaline saponification, cyclohexane extraction, and subsequent SPE silica cleanup (experiment 7) for the determination of PAHs in ground coffee samples.

Benefit of Using PLE. Direct saponification of ground coffee (experiment 9) and Soxhlet extraction followed by saponification (experiment 8) were also tested, keeping all of the cleanup conditions the same as with PLE. Recoveries are presented in **Table 4** and results of the statistical tests in **Table 5**. PLE improved recoveries as compared to direct saponification. Despite similar recoveries with Soxhlet extraction, PLE was advantageous due to a reduced extraction time, less solvent used, and cleaner extracts.

Application to the Determination of PAHs in Ground Coffee Samples. As the behaviors of spiked and nonspiked PAHs could be different, it is also important to compare results obtained using different treatment procedures for native PAHs. Due to the absence of certified reference material, this was done

Table 5. Results (Differences Observed between Mean Recoveries) of Statistical Analyses (Using the Paired *t* Test) for the Different Extraction Procedures Investigated (Experiments 5–9)[a,b]

extraction procedure comparison	different solvents for PLE under 100 °C						different temperatures for PLE with hexane/acetone 50:50			different extraction techniques		
2 by 2 comparison:	MeOH–acetone	hexane–acetone	hexane/acetone 50:50–acetone	hexane–MeOH	hexane/acetone 50:50–MeOH	hexane/acetone 50:50–hexane	120 °C–100 °C	150 °C– 120 °C	150 °C – 100 °C	Soxhlet–none	PLE–Soxhlet	PLE–none
experiments compared: PAH	5(e)–5(d)	5(a)–5(d)	5(c)–5(d)	5(a)–5(e)	5(c)–5(e)	5(c)–5(a)	6–5(c)	7–6	7–5(c)	8–9	7–8	7–9
Phen	11.8	2.4	21	–9.4	9.2	18.6	–0.74	44.7	44.0	excluded	excluded	38.1
Anthr	–8.5	–15	–3	–6.5	5.5	12	14.8	–8.5	6.3	9.1	–15.0	–5.9
F	–25.1	–33.6	–9.2	–8.5	15.9	24.4	20.2	–1.6	18.7	17.5	34.4	51.9
Pyr	52.3	21.3	35.2	–31	–17.1	13.9	6.8	–8.3	–1.6	8.4	4.1	12.5
B[a]A	–10.3	–30.2	–17.1	–19.9	–6.8	13.1	32.2	3.0	35.2	–0.3	19.1	18.8
Chrys	–10.7	–25.4	–7.8	–14.7	2.9	17.6	25.4	20.0	45.4	–14.2	36.2	22.0
B[b]F	–16.5	–26.8	–12.3	–10.3	4.2	14.5	–2.7	15.9	13.2	5.5	0.25	5.7
B[k]F	–34.5	–27.3	–12.8	7.2	21.7	14.5	–3.3	11.0	7.6	11.4	–9.5	1.9
B[a]P	–11	–28.3	–14.5	–17.3	–3.5	13.8	–1.6	13.6	12.0	7.1	0.31	7.4
DB[ah]A	–14.7	–33	–16.3	–18.3	–1.6	16.7	–9.0	12.9	3.9	2.7	–6.1	–3.4
B[ghi]P	–15.6	–37.8	–17.4	–22.2	–1.8	20.4	–5.7	9.5	3.8	2.4	–1.5	0.9
mean difference	– 7.5	– 21.2	– 4.9	– 13.7	2.6	16.3	6.9	10.2	17.1	4.9	6.2	13.6
standard deviation	22.8	17.8	17.2	9.9	10.7	3.7	14.0	14.9	16.7	8.4	17.8	18.0
$\|t\|_{calcd}$ value	1.09	3.95	0.95	4.57	0.81	14.63	1.64	2.27	3.40	1.86	1.11	2.51
n value	11	11	11	11	11	11	11	11	11	10	10	11
$\|t\|_{crit}$ value ($\alpha' = 0.01$)				3.17				3.17		3.25	3.25	3.17
$\|t\|_{crit}$ value ($\alpha' = 0.02$)				2.76				2.76		2.82	2.82	2.76
$\|t\|_{crit}$ value ($\alpha' = 0.05$)				2.23				2.23		2.26	2.26	2.23
$\|t\|_{crit}$ value ($\alpha' = 0.10$)				1.81				1.81		1.83	1.83	1.81

[a] Experiment 5(b) is not discussed as only one experiment was performed. [b] α' is the significance level for individual comparisons. In case of 2 by 2 comparisons, α is the overall significance level and k the number of comparisons ($k = 3$ or 6 here), where $\alpha' = 1 - (1 - \alpha)^{1/k}$ (Bonferroni's adjustment). The following correspondence is given: $\alpha' = 0.01/\alpha = 0.058$ (for $k = 6$) or $\alpha = 0.029$ (for $k = 3$); $\alpha' = 0.02/\alpha = 0.11$ (for $k = 6$) or $\alpha = 0.059$ (for $k = 3$); $\alpha' = 0.05/\alpha = 0.026$ (for $k = 6$) or $\alpha = 0.014$ (for $k = 3$); $\alpha' = 0.10/\alpha = 0.47$ (for $k = 6$) or $\alpha = 0.27$ (for $k = 3$).

Table 6. Determination of PAHs in Ground Arabica Coffee Lots from Colombia Using Different Extraction Techniques [Mean Concentration (Micrograms per Kilogram) (RSD %)][a]

	no extraction	Soxhlet	PLE conditions				
	expt 9	expt 8	expt 5(b) 100 °C hexane/ acetone 94:6	expt 5(b) 100 °C hexane/ acetone 94:6	expt 5(c) 100 °C hexane/ acetone 50:50	expt 6 120 °C hexane/ acetone 50:50	expt 7 150 °C hexane/ acetone 50:50
	lot A	lot A	lot A	lot B	lot C	lot C	lot C
PAH[b,c]	$n_{exp} = 3$	$n_{exp} = 2$	$n_{exp} = 1$	$n_{exp} = 2$	$n_{exp} = 4$	$n_{exp} = 1$	$n_{exp} = 1$
Phenb[b,c]	2.4 (42.4)	9.3 (7.7)	6.23	12.4 (13.8)	20.2 (25.8)	17.3	20.2
Anthr[b,c]	0.90 (13.4)	1.2 (3.1)	0.62	1.3 (0.8)	3.5 (38.4)	1.3	2.2
F[b,c]	7.7 (12.1)	34.0 (4.0)	9.98	11.8 (2.4)	21.5 (12.4)	20.3	24.6
Pyr[b,c]	3.4 (12.1)	12.0 (3.7)	4.03	8.0 (0.2)	12.3 (27.5)	9.5	13.6
B[a]A	ND[d]	0.24 (5.9)	ND	ND	0.91 (15.7)	0.54	1.3
Chrys[c]	ND	0.59 (17.5)	0.81	ND	2.0 (35.4)	0.86	1.6
B[b]F	0.13 (34.0)	0.056 (33.3)	0.052	0.32 (12.2)	0.48 (28.0)	0.63	0.60
B[k]F	0.037 (28.6)	0.042 (6.0)	0.064	0.16 (8.8)	0.21 (23.3)	0.22	0.31
B[a]P	0.063 (47.2)	0.039 (13.0)	0.024	0.15 (2.8)	0.31 (29.1)	0.24	0.33
DB[ah]A	ND	ND	ND	ND	ND	ND	ND
B[ghi]P	ND	ND	ND	ND	ND	ND	ND
sum of PAHs	14.7 (13.0)	57.5 (4.6)	21.8	34.0 (4.0)	61.4 (19.5)	50.9	64.7
TEQ	0.17 (22.4)	0.45 (2.2)	0.16	0.35 (3.1)	0.77 (17.2)	0.63	0.87

[a] Concentrations were estimated without correcting the values to take into account uncomplete recoveries. Toxicity equivalent factors (TEF) are used to estimate the toxicity equivalence (TEQ) (μg kg^{-1}) of the PAH mix. [b,c] Identification was confirmed using HPLC-UV-DAD and/or GC-MS/MS. [d] Not detected.

Table 7. Relative Toxicity of PAHs (from Reference *23*) and Performance of the Proposed Method (Fluorescence Detection) for the Determination of PAHs

PAH	IARC class[a]	TEF	instrument linearity range[b] (μg L^{-1})	regression curve[c]	correlation coefficient (r^2)	analytical LOD[d] (μg L^{-1})	method LOD[e] (μg kg^{-1})
Phen	3	0.001	5–500	$y = 413.67x + 773.57$	0.9977	1.65	0.13
Anthr	3	0.01	5–600	$y = 8595.8x + 25633$	0.9983	1.65	0.13
F	3	0.01	5–800	$y = 1239.7x + 430.43$	0.9983	1.65	0.13
Pyr	3	0.001	5–800	$y = 2455.4x + 3328.2$	0.9976	1.95	0.16
B[a]A	2A	0.1	5–100	$y = 2946.2x + 779.28$	0.9973	1.65	0.13
Chrys	3	0.01	5–500	$y = 877.18x - 1345.6$	0.9983	1.35	0.11
B[b]F	2B	0.1	5–800	$y = 5777.8x + 9652.2$	0.9993	1.65	0.13
B[k]F	2B	0.1	5–150	$y = 38885x + 28956$	0.9976	1.95	0.16
B[a]P	2A	1	5–600	$y = 9681.6x + 25566$	0.9992	1.80	0.14
DB[ah]A	2A	1	5–150	$y = 3462.4x - 3769.3$	0.9982	1.95	0.16
B[ghi]P	3	0.01	5–600	$y = 1477.8x + 918.71$	0.9980	2.25	0.18

[a] IARC (International Agency for Research on Cancer) class: 2A, probably carcinogenic to humans; 2B, possibly carcinogenic to humans; 3, not classifiable as to carcinogenicity to humans. [b] Based on ($n = 11$) determinations in the range 5–800 μg L^{-1} (5, 10, 25, 50, 100, 150, 200, 250, 500, 600, 800 μg L^{-1}). [c] y = peak area; x = concentration (μg L^{-1}). [d] S/N = 3 for LOD, based on the analysis of blanks ($n = 10$). [e] Estimated LODs for the whole method under our conditions (i.e., 5 g of ground coffee sample and final extract of 0.4 mL).

on the basis of estimated PAH concentrations after their extraction from different Arabica coffee lots. Results are presented in **Table 6**. Confirmation of the presence of phenanthrene, anthracene, fluoranthene, pyrene, and chrysene could be done using GC-MS/MS analysis, as well as HPLC-DAD for most of them as indicated in the table.

Comparisons can be made between the different extraction procedures for the same coffee lot analyzed. With regard to lot A, results using direct saponification (experiment 9) and Soxhlet (experiment 8) differ from those with PLE [experiment 5(b)] for the early-eluted compounds, due to interferent compounds as already discussed. This results in an overestimation of the PAH content and the toxicity equivalence (TEQ) for Soxhlet extraction. For lot C, overall concentrations are slightly higher with PLE under 150 °C (experiment 7) than with PLE under lower temperatures [experiments 5(c) and 6], which is consistent with our previous observation of slightly better extraction under such conditions.

Phenanthrene appears as one of the most abundant PAHs along with pyrene, whatever the coffee lot considered, as already reported for ground coffee samples (*8*). As these compounds are considered to be nontoxic [toxicity equivalent factor (TEF) = 0.001 as indicated in **Table 7**], their contribution to the TEQ is minor (around 4% for their sum in lot C). In contrast, the presence of similar concentrations of fluoranthene (i.e., range = 10−25 μg kg^{-1} using PLE) is of great concern as this compound presents some toxicity; consequently, it contributes around 30% of the TEQ in the case of lot C, whereas 40% was attributed to benzo[*a*]pyrene. It is also interesting to note that the overall 11 PAH content varied from one coffee lot to another, with values in the range from 21.8 to 64.7 μg kg^{-1} (for PLE), leading to an overall TEQ of 0.16−0.87 μg kg^{-1}. The estimated benzo[*a*]pyrene contents of the coffee samples (from below the detection limit to 0.33 μg kg^{-1}) are consistent with previously reported values of 0.1−0.7 μg kg^{-1} for coffee samples (*15*). Of great interest is also the absence of the highly toxic dibenzo[*a*,*h*]anthracene in the coffee lots considered. In fact, the contributions of toxic and nontoxic PAHs to the overall concentration are rather equivalent whatever the lot analyzed as illustrated in **Figure 2**.

Performances of the Proposed Method. The performances of the overall analysis method are summarized in **Table 7** for each PAH. Limits of detection (LODs) were estimated on the basis of the determination of the noise for blank samples. In

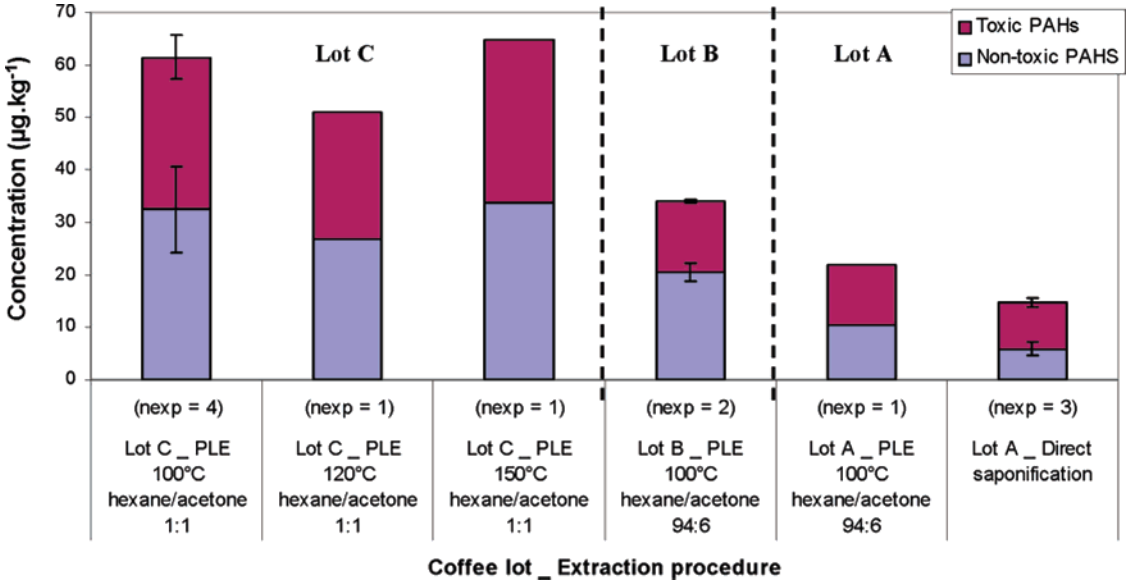

Figure 2. Concentrations of toxic and nontoxic PAHs estimated in three distinct lots of Arabica coffee from Colombia using different extraction procedures. Numbers of experiments performed (n_{exp}) are indicated; in the case of multiple experiments, mean concentrations are given (± standard deviations). Toxic PAHs (i.e., TEF ≥ 0.01): Anthr, F, B[a]A, Chrys, B[b]F, B[k]F, B[a]P, DB[ah]A, B[ghi]P. Nontoxic PAHs (i.e., TEF < 0.01): Phen, Pyr. Soxhlet extraction (for lot A) is not presented due to overestimation of some PAHs because of interferent compounds.

the final analyzed extract, LODs ranged from 1.35 to 2.25 μg L^{-1}, giving for the whole procedure LODs ranging from 0.11 to 0.18 μg kg^{-1}, which is acceptable.

Conclusions. Different sample treatments have been tested for the determination of PAHs in ground coffee. The final analytical procedure retained is PLE with hexane/acetone 50:50 (v/v) under 150 °C, followed by alkaline saponification, cyclohexane extraction, and silica SPE. This procedure enabled the determination of 11 PAHs in ground coffee with LODs in the range of 0.11−0.18 μg kg^{-1}. Its application to ground Arabica coffee lots from Colombia revealed the presence of several PAHs, giving an overall TEQ in the range of 0.16−0.87 μg kg^{-1}. In agreement with previous studies, low levels were observed for benzo[*a*]pyrene, ranging from below the detection limit to 0.33 μg kg^{-1}.

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; Anthr, anthracene; B[a]A, benzo[*a*]anthracene; B[a]P, benzo[*a*]pyrene; B[b]F, benzo[*b*]fluoranthene; B[e]P, benzo[*e*]pyrene; B[ghi]P, benzo[*g,h,i*]perylene; B[k]F, benzo[*k*]fluoranthene; Chrys, chrysene; DB[ah]A, dibenzo[*a,h*]-anthracene; DMF, dimethylformamide; EtOH, ethanol; F, fluoranthene; FD, fluorometric detection; GC, gas chromatography; HPLC, high-performance liquid chromatography; IARC, International Agency for Research on Cancer; I[cd]P, indeno[1,2,3-*c,d*]pyrene; LLE, liquid−liquid extraction; LOD, limit of detection; MeOH, methanol; PAH, polycyclic aromatic hydrocarbon; Phen, phenanthrene; PLE, pressurized liquid extraction; PTV, programmed temperature vaporizer; Pyr, pyrene; RSD, relative standard deviation; SIM, selected ion monitoring; SPE, solid-phase extraction; TEF, toxicity equivalent factor; TEQ, toxicity equivalence; THF, tetrahydrofuran.

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