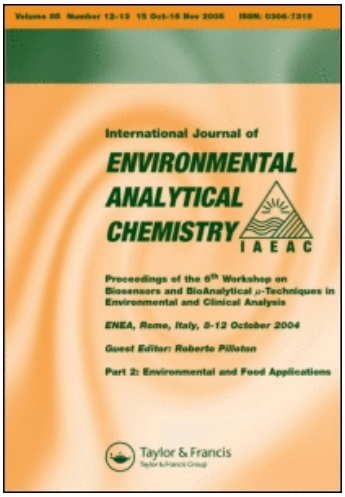

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Statistical tools for the optimization of a highly reproducible method for the analysis of polycyclic aromatic hydrocarbons in sludge samples

Eric Trably[a]; Nadine Delgènes[a]; Dominique Patureau[a]; Jean Philippe Delgènes[a]
[a] Institut National de la Recherche Agronomique (INRA), Laboratoire de Biotechnologie de l'Environnement, F-11100 Narbonne, France

To cite this Article Trably, Eric , Delgènes, Nadine , Patureau, Dominique and Delgènes, Jean Philippe(2004) 'Statistical tools for the optimization of a highly reproducible method for the analysis of polycyclic aromatic hydrocarbons in sludge samples', International Journal of Environmental Analytical Chemistry, 84: 13, 995 — 1008
To link to this Article: DOI: 10.1080/03067310412331298412
URL: http://dx.doi.org/10.1080/03067310412331298412

Intern. J. Environ. Anal. Chem.
Vol. 84, No. 13, pp. 995–1008, 15 November 2004

STATISTICAL TOOLS FOR THE OPTIMIZATION OF A HIGHLY REPRODUCIBLE METHOD FOR THE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN SLUDGE SAMPLES

ERIC TRABLY, NADINE DELGÈNES,
DOMINIQUE PATUREAU* and JEAN PHILIPPE DELGÈNES

Institut National de la Recherche Agronomique (INRA), Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, F-11100 Narbonne, France

(Received 26 January 2004; In final form 15 July 2004)

The aim of this study is to develop and optimize an analytical method for the determination of 14 priority PAHs in sludge samples based on Accelerated Solvent Extraction (ASE) coupled to RP-HPLC/fluorescence detection. Statistical tools were used to demonstrate the influence of the parameters during the optimization steps. The final parameters were selected to provide analytical errors statistically as low as possible. First, couples of excitation/emission detection wavelengths were tested, and some were finally selected to provide errors lower than 2%. It was then demonstrated that PAH extraction efficiencies are not statistically influenced by the ASE parameters. It was also found that the ASE extraction from sludge samples provides statistically similar results to those obtained with traditional Soxhlet extraction, but with a lower reproducibility error. After optimization, the accuracy of the method was validated with a certified sludge. In conclusion, an optimized analytical procedure has been proposed to monitor PAHs during lab-scale experiments requiring highly repeatable and accurate results from a low sample volume contaminated by PAHs at trace levels.

Keywords: Accelerated solvent extraction; RP-HPLC; Statistical tools; PAHs; Sewage sludge

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment, especially in atmospheric particles, soils, sediments, and sewage sludges. Their widespread distribution is due to numerous anthropogenic and natural sources. Mainly, the PAHs are formed by incomplete combustion of organic matter, petroleum, coke or fossil fuels [1–2]. More than 74 PAHs have been identified, but only 16 are currently monitored by the US Environmental Protection Agency (EPA) and the Environmental Commission of the European Community [3]. Most of these 16 priority PAHs are

*Corresponding author. Fax: +33-468425160. E-mail: patureau@ensam.inra.fr

ISSN 0306-7319 print: ISSN 1029-0397 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/03067310412331298412

suspected to show toxic, carcinogenic, and/or mutagenic properties at low concentrations. Since the PAHs are highly hydrophobic, they are readily adsorbed onto the suspended particles of primary and secondary sludge in wastewater treatment plants (WWTPs) [4]. Such contaminated sludges cannot therefore be recycled by spreading on agricultural soils because of the potential toxic effects and the high persistence of PAHs in the environment. Consequently, the fate of PAHs during sludge treatment has become a significant subject of study over the last ten years for the WWTP managers. However, the lack of a standardized procedure for PAH analysis in sewage sludge is highly prejudicial for inter-laboratory studies.

Several PAH analytical methods have already been described in the literature by registered laboratories and governmental agencies [5–10]. Since 1986, the US Environmental Protection Agency (EPA) has proposed standard methodologies for the extraction and the analysis of PAHs in sewage sludge [6]. However, the proposed methods require high sludge quantities, from 10 to 100 g dry weight, and the validation of a method requiring lower amounts of samples (down to 1 g dry weight) is desirable. In addition, practical the reproducibility errors of these methods are high – from 21 to 44% – and the PAH concentrations provided by registered laboratories may vary up to 300% [11]. The high variability of the results is likely due to the large variety of PAH extraction/analysis procedures involved [7]. In this study, two steps are considered for the optimization of the PAH analytical method: the first is the PAH extraction from dried sample of sludge, and the second is the PAH quantification from the extracts.

Many PAH extraction methods have already been described in the literature: Soxhlet, methanolic saponification, ultrasonication, microwaves, and, more recently, accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE). All these methods provide similar extraction yields but not the same analytical errors [9–10,12]. In the present work, two of these methods are studied: (1) Soxhlet extraction, which is considered as the reference but requires long extraction time (6–24 h) and high solvent volumes (150–250 mL); (2) ASE, an automated method providing a high reproducibility and enhanced security. In addition, ASE requires a short extraction time (20 min) and a low amount of solvent (20 mL). Despite a relatively important investment, the extraction of PAHs by ASE is especially recommended for intensive use in lab-scale assays [13,14]. Among the parameters, only few experimental values have been reported for the temperature, static time, pressure, sample amount and solvent mixture composition (Table I). Recent results showed that the ASE extraction yields are very similar to the other PAH extraction method efficiencies in the case of contaminated soils and sediments [9–10,12–15]. Such results need to be statistically demonstrated in the case of the sludge because of the highly specific interactions between the organic matrix, the solvent, and the PAHs.

After extraction, PAH concentrations of the extracts are determined by gas chromatography coupled with a flame ionization detector, or mass spectrometry (GC/FID or GC/MS), or by reverse phase high-performance liquid chromatography coupled with a photodiode array or a fluorescence detector (RP-HPLC/PDA or RP-HPLC/FLU). Since 1970, the last method has provided good results considering the highly specific detection of the PAHs within complex samples. Indeed, the excitation and emission wavelengths are highly specific for each molecular formula [17]. However, many different PAH detection wavelengths have been reported in the literature (Table II). The main disadvantage of the RP-HPLC/FLU method lies in the resolution of the peaks, and the highest PAH separation efficiencies are generally found with a C_{18}

TABLE I Summary of the accelerated solvent extraction parameters found in the literature[a]

Matrix	*Sample amount* (g)	*Solvent mixture*	*T* (°C)	*Pressure* (bar)	*Static time* (min)	*Reference*
Soil, sludge	20	Hexane : acetone (50 : 50)	100	100–140	5	[6]
Sludge	1	Hexane : acetone (50 : 50)	100	100	8	[8]
Sediment	0.3	Hexane : acetone (50 : 50)	100	140	5	[9]
Soil	7	DCM : acetone (50 : 50)	100	140	5	[10]
Soil, sludge	20	Hexane : acetone : toluene (10 : 5 : 1)	100	138	10	[12]
Soil	–	Hexane : acetone (50 : 50)	100	140	5	[14]
Sediment, soil, sludge	–	DCM : acetone (50 : 50)	100	140	5	[15]
Soil	7	Hexane : acetone or DCM : acetone (50 : 50)	70–200	90–140	5–16	[16]

[a]DCM : dichloromethane.
–: not reported.

column presenting a selective polymeric phase (Bakerbond C18 Widepore, Supelcosil LC-PAH) [17]. Two other parameters also influence the PAH separation by RP-HPLC: the elution temperature and the length of the solvent gradient. The separation efficiency increases significantly with the lowest temperature and the longest solvent gradient [17,19]. Several experimental values are reported in Table II. Since numerous analytical conditions are available in the literature, the detection wavelengths, the elution temperature, and the length of the gradient need to be tested to optimize the accuracy and reproducibility of the analytical method.

The aim of this study is the optimization and the validation of an analytical method of 14 of the 16 priority PAHs, as described below (the acenaphtene and the acenaphthylene compounds were removed because of their low fluorescent properties). This method was developed to monitor PAHs in lab-scale experiments using low-contaminated sludge samples. Thus, the purpose of this study is to obtain the highest reproducibility of the analysis in spite of low PAH concentrations and low sample volumes (200–300 mL). Statistical tools were used to demonstrate the effect of each parameter. The optimization of the analytical method was carried out in three steps. First, the PAHs analysis by RP-HPLC and the fluorescence detection were optimized to obtain repeatability and reproducibility errors lower than 2%. Then, the influence of the ASE extraction parameters was evaluated. The PAH extraction efficiencies by ASE were compared with those obtained by the classical Soxhlet method. Finally, the optimized method was validated by the determination of the PAH recoveries in spiked sludge and in certified material.

The 14 studied PAHs are as follows: Na, naphthalene; Fl, fluorene; Ph, phenanthrene; An, anthracene; Flu, fluoranthene; Py, pyrene; BaA, benzo(a)anthracene; Ch, chrysene; BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene; BaP, benzo(a)pyrene; DB, dibenzo(ah)anthracene; BP, benzo(ghi)perylene; Ind, indeno(123-cd)pyrene.

EXPERIMENTAL

Chemicals

All chemicals were of analytical grade. The solvents were provided by J.T. Baker-Mallinkrodt (Noisy le Sec, France) with a purity higher than 98% for acetone,

TABLE II Summary of the elution parameters and the fluorescence wavelengths found in the literature for the PAH analysis by RP-HPLC–fluorescence detection[a]

References	*[6]*	*[7]*	*[12]*	*[17]*	*[18]*	*[19]*	*[20]*	*[21]*	*[22]*	*[23]*	*[24]*	*[25]*
Gradient time (min)	*25*	–	*30*	*30*	*5*	*25*	*5*	*25*	–	*25*	*5*	*16.5*
Flow rate (mL/min)	*0.5*	–	*1*	*2*	–	*0.5*	*2*	*1*	*1*	–	*0.5*	*1*
Excitation/emission wavelengths												
Na		–	220/330	280/340	–	280/330	–	280/340	–		280/340	–
Fl		–	225/315	249/362	320/404		–		–	280/340		–
Ph		–	244/370	250/400	–	250/370	–	250/376	259/370		295 /380	265/350
An		–		285/450	–	250/405			252/405			
Flu		268/462	237/460	333/390	–	280/450	260/430	286/460	284/460			265/430
Py		–	237/385	285/385	320/404	270/390			336/398		280/430	
BaA	280/389	–	277/376	260/360	257/407	265/380	285/385	–	–			–
Ch		–		295/425	269/361			–	368/384			–
BbF		234/420	255/420		290/409	290/430	305/405	305/403	–	280/410	285 /460	–
BkF		298/424		296/405	284/427				–			–
BaP		268/398							378/406			–
DB		–	300/415		–	290/410	–	–				–
BP		234/420			–		300/455	305/425	–			295/460
Ind		302/500	250/495	300/500	303/500	300/500			–	305/500		–

[a]The gradient of elution was performed from acetonitrile/water (60%–40% v/v) to acetonitrile (100%).
–: not reported.

acetonitrile, hexane, methanol, and toluene. The borosilicate glassware and the experimental apparatus were previously rinsed with a mixture of acetonitrile : acetone (50 : 50).

The 10 mg/L standard solution of the 16 priority PAHs was prepared by Dr-Ehrenstorfer-Schäfers laboratory (Augsburg, Germany, PAH Mix-9, purity over 98%). Ten- to 1000-fold dilutions of the standard solution were prepared in acetonitrile, and the diluted solutions were stored at −20°C.

The certified sludge (CRM n° 088 – PAH in dried sewage sludge) was provided by Promochem (Molsheim, France) with the following certified PAH concentrations (mg/kg of dry weight): pyrene, 2.16 ± 0.09; benzo(a)anthracene, 0.93 ± 0.09; benzo(a)pyrene, 0.94 ± 0.09; benzo(b)fluoranthene, 1.17 ± 0.08; benzo(k)fluoranthene, 0.57 ± 0.05; indeno(123-cd)pyrene, 0.81 ± 0.06.

Sludge Sample Preparation

A long-term PAH-contaminated sludge was used as a stock mixture during the optimization steps. The sludge corresponded to a mixture of primary and secondary sludge (50 : 50, v : v). Prior to PAH extraction, 300 mL of the sludge mixture was centrifuged (20 000 g, 25 min). The supernatant was stored at −20°C for further solid-phase extraction. The pellet was ground with 4 mm glass beads, dried in a ventilated oven (60 h at 40°C), sieved on a 2-mm mesh size, and stored at −20°C for further ASE or Soxhlet extraction.

Liquid Chromatography Procedure

The analytical system was composed of a sampler injector (Waters 717plus Autosampler), a solvent degasser (Waters Inline Degasser), a peristaltic pump system (Waters 600 Controller) and a programmable fluorimetric detector (JASCO FP-1520). The excitation and emission wavelengths were changed according to the elution time of each PAH. The C_{18} column was provided by Bakerbond (PAH 16-Plus Bakerbond^{TM}: 5 µm, 3 × 250 mm, 120 Å). The column temperature was maintained at 25°C by immersion in a regulated water bath. The elution sequence was as follows (flow rate of 0.3 mL/min): 5 min of isocratic elution (acetonitrile : water, 60 : 40), 30 min of linear gradient from 60 to 100% acetonitrile, 30 min of isocratic elution (acetonitrile-100%) and 30 min of isocratic rinsing of the column by a mixture of acetonitrile : water (60 : 40).

Extraction Procedures

Solid-Phase Extraction (SPE)

The PAHs were extracted from the liquid phase (supernatant) by solid-phase extraction (SPE). The affinity column was provided by Supelco^{TM} (Supelclean ENVI-18). The extraction was performed according to the Supelco^{TM} procedure. The sample was passed three times through the column. The PAHs were eluted with 6 mL of a mixture of toluene : methanol (10 : 1). The sample was then evaporated under nitrogen flow to dryness, and the residue was dissolved in 2 mL of acetonitrile.

Accelerated Solvent Extraction (ASE)

The extraction from dried sludge samples was performed with an ASE-200 system (DIONEX^{TM}). The extraction solvent consisted of a mixture of hexane : acetone (50 : 50). The ASE cells were filled as follows (from bottom to the top): a filter of glass fiber (Diameter 19 mm, Whatmann^{TM}), 1 g of Alumina (Sigma^{TM}), 1 g or 0.5 g of dried sludge sample and 1.5 g of Hydromatrix (Varian^{TM}). After extraction, the sample was evaporated under nitrogen flow to dryness. The residue was then dissolved in 5 mL of acetonitrile and was immediately analysed (no storage).

Soxhlet Extraction

The Soxhlet extraction procedure was based on EPA method 8310 [6]. The method was previously optimized and validated internally on certified material. The Soxhlet was filled with 0.5 g of sludge sample and 120 mL of hexane : acetone (50 : 50). The PAH extraction was performed at 50°C during 16 h. The extract was first evaporated under vacuum in a Rotavapor (Buchi^{TM}) at 40°C and then evaporated to dryness under a gentle nitrogen flow. The residue was dissolved in 5 mL of acetonitrile and immediately analysed (no storage).

Experimental Plans and Statistical Analysis

Three independent half-experimental plans were performed to optimize the ASE extraction parameters and to reduce the number of extractions by grouping by two variables [26]. If one group of variable statistically influenced the extraction efficiency, each variable was then tested separately. In the first half-plan experiment, four parameters possessing a low or a high level were studied: the temperature, 100°C or 120°C; the number of cycle, 2 or 3; the static time of extraction, 5 or 8 min; the composition of the solvent mixture (hexane : acetone), 50 : 50 or 25 : 75. The second half-plan was performed to reduce the sludge amount (lab-scale requirement): 1 g or 0.5 g. The third half-plan was performed on two parameters: the solvent volume, 60 or 90% of the cell and the gas purging time, 60 or 100 s.

The results were compared with a statistical test of multiple variances (ANOVA). Each extraction assay was repeated three times, and the averages were compared by a one-factor ANOVA test [26]. The efficiencies of the Soxhlet and ASE extractions were compared by a t-test under a Student law at 5%. The hypothesis of normality and independence between the assays was formulated to apply the ANOVA and the statistical t-tests. The acceptance of a null hypothesis at 5% indicated that the tested averages were statistically similar at 95% (no significant difference between the two statistical populations).

RESULTS AND DISCUSSION

Optimization of the RP-HPLC – Fluorescence Detection

Amongst the parameters influencing the resolution of the detected peaks, the temperature of the RP-HPLC column significantly influenced the PAH elution time and the peak separation efficiency (data not shown). According to previous studies, the elu-

tion temperature was fixed at its lowest level for the best peak resolution [17,19]. Since a water bath was used as a regulator of the column temperature, the temperature was regulated at 25°C because of ambient air limitation. The other RP-HPLC elution parameters were optimized according to the values reported in Table I. It was found that an increase in the elution gradient from 5 to 30 min helped to enhance the peak resolution. Similar results were observed with a decrease in the solvent flow rate from 0.5 to 0.3 mL/min (data not shown). A longer elution gradient and a slower flow rate resulted in an extension of the analysis time from 35 to 90 min. Figure 1 shows the chromatograms obtained under these conditions. The PAH peaks can be readily identified either in the standard solution or in the sludge extract. The chromatograms exhibit only a few interfering peaks due to the high specificity

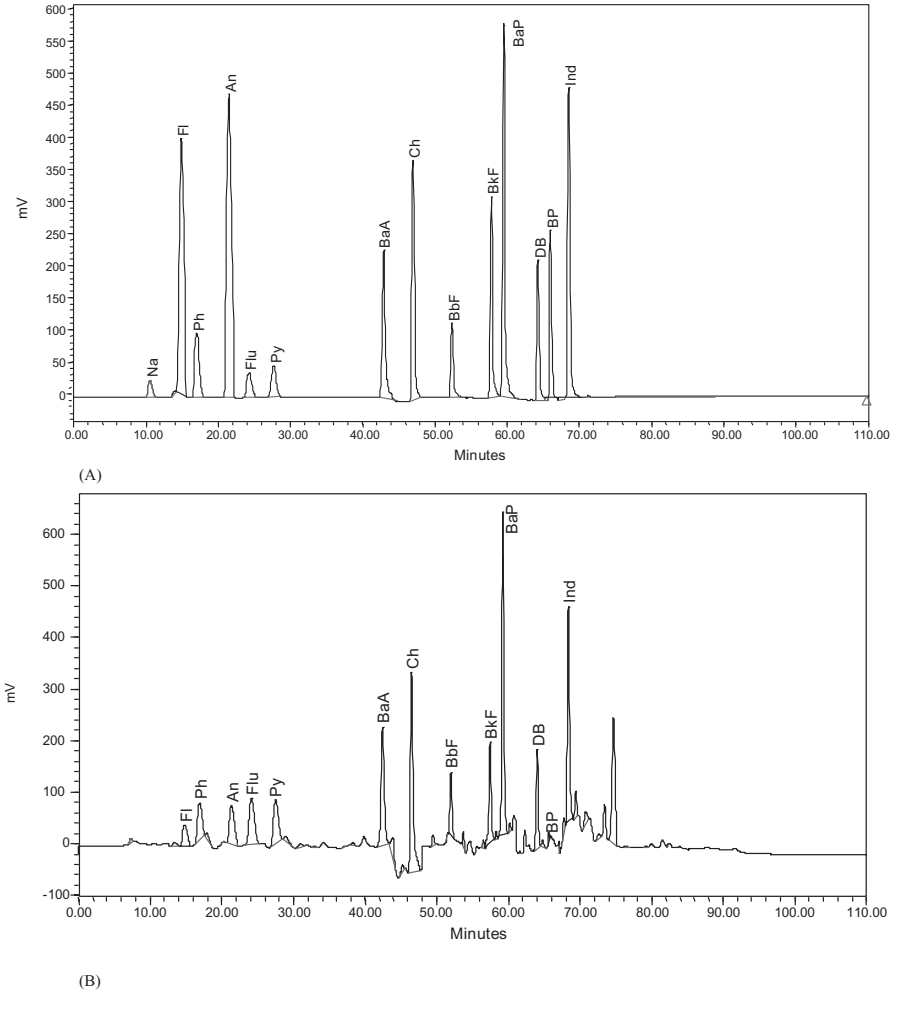

FIGURE 1 Elution chromatogram of the 16 priority PAHs standard solution (A) and the contaminated-sludge extract obtained after accelerated solvent extraction (B). Injection: 20 μL, gradient (30 min) of acetonitrile : water (60 : 40 to 100 : 0), flow rate 0.3 mL/min, temperature 25°C, fluorescence program: 0 min, 280/330, 13 min, 266/312, 17 min, 250/370, 20 min, 250/400; 24 min, 280/430; 27.5 min, 260/410; 32 min, 280/430; 40.2 min, 268/384; 46 min, 234/420; 50.5 min, 270/400; 56 min, 300/407; 60 min, 300/500.

of the fluorescence detection, in contrast with mass-spectrometry detection chromatograms [24]. The identification of the peaks was additionally confirmed with a photodiode array detector (PDA) by comparison of the experimental peak spectrum and the spectra reported in the literature [27] (data not shown).

In contrast with methods based on a high response of the detection system to reach the lowest detection limits [12,21,22,25], well-separated peaks were necessary here to reduce the errors of peak integration. Indeed, the optimization of the fluorescence detection was based on the reduction in the repeatability errors, which corresponded to the relative standard deviation of three analysis of the same sample and was highly dependent on the peak sharpness. Since the test of all excitation/emission wavelengths found in the literature would have been highly complex and unrealizable, only the most common wavelengths were tested and were definitely selected when the repeatability error reached a value lower than 2% (see Table III). Thus, a pair of excitation/emission wavelengths were found to provide highly repeatable results for the analysis of each PAH either in the standard solution (>25 repetitions) or in the sludge sample extracts (four replicates). Moreover, it was observed that the highest areas of the

TABLE III Summary of the repeatability and reproducibility errors according to the excitation/emission wavelengths (PAH fluorescence detection)[a]

PAH	*Excitation/ emission wavelengths*	*Repeatability error* (%) (*maximum*)	*Calibration error* (%) *Average* (*10 repetitions*)	*Repeatibility error on sludge sample* (%) *Average* (*four repetitions*)
Na	272/334	4.7	–	–
	280/330[b]	0.7 (1.4)	1.3	0.4
Fl	266/312[b]	0.4 (1.5)	1.5	0.4
Ph	295/380	4.1	–	–
	250/370[b]	0.7 (1.6)	1.2	0.4
An	250/400[b]	0.4 (1.3)	1.1	0.8
Flu	260/430	n.d.	–	–
	365/460	n.d.	–	–
	280/430[b]	0.6 (1.5)	0.8	0.6
Py	236/394	4.7	–	–
	320/404	2.8	–	–
	270/394	n.d.	–	–
	280/430	n.d.	–	–
	260/410[b]	1.1 (1.9)	0.95	0.7
BaA	268/384	4.4	–	–
	280/430[b]	0.5 (1.6)	0.8	0.5
Ch	268/384[b]	0.6 (1.3)	1.1	0.1
BbF	292/460	7.5	–	–
	234/420[b]	0.4 (1.9)	0.6	0.2
BkF + BaP	292/460	11.3	–	–
	292/430	5.3	–	–
	300/430	4.2	–	–
	270/400[b]	0.6 (1.6)	0.8	0.8
DB + BP	285/460	2.8	–	–
	300/500	n.d.	–	–
	285/400	n.d.	–	–
	300/407[b]	0.5 (0.9)	1	0.8
Ind	285/460	2.3	–	–
	300/500[b]	0.8 (2)	0.6	0.4

[a]The study was performed with injections (20 µL) of a standard solution (100 µg/L of each PAH) according to the optimized HPLC conditions. [b]Finally selected excitation/emission wavelengths (25 repetitions); n.d.: not detected.

peaks did not systematically correspond to the most repeatable results. The analytical errors varied more according to the signal stability than the intensity of the response. The selected excitation/emission wavelengths did not correspond to the lowest detection limit, as normally defined [12,21,22,25].

In the same way, the minimum and maximum PAH concentrations of the calibration curves were determined for repeatability errors exceeding 2%. High errors were encountered for the lowest concentrations or in the case of saturation of the fluorescence detector. The upper and lower limits of the calibration curves were determined as follows: 250–10 000 μg/L (naphthalene); 10–1000 μg/L (fluorene, anthracene, benzo(a)anthracene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, indeno(123-cd)pyrene), 25–1000 μg/L (phenanthrene, fluoranthene, chrysene, benzo(b)-fluoranthene, dibenzo(ah)anthracene). The minimum values were ten times higher than the detection limits (1–1.5 μg/L) but provided higher repeatable results than those found in the literature [8,10,12,21,22].

In addition, the calibration error corresponding to the comparison of a standard solution at 100 μg/L with the theoretical calibration curve was also tested (Table III). It was found that the calibration error was always lower than 2%, and the calibration curve was valid for more than 100 sludge sample analyses.

In conclusion, this study demonstrated for the first time that the analysis of PAHs from sludge extracts by RP-HPLC and fluorimetric detection provide highly accurate and repeatable values and are reliable over time. Therefore, only a limited number of injections (2,3) are needed to estimate the PAH concentration from sludge extracts.

Optimization of the PAH Extraction from Sewage Sludge Samples

PAH Extraction from the Liquid Phase (Solid-phase Extraction)

PAH extraction from the liquid phase was performed by solid-phase extraction. Using a 100 μg/L standard solution, the PAH recoveries in spiked aqueous samples were mostly satisfactory with about 90–100% of PAH recovery except for the fluorene (75%), chrysene (33%), dibenzo(ah)anthracene (32%), benzo(ghi)perylene (28%), benzo(a)pyrene (65%) and indeno(123-cd)pyrene (63%). Moreover, the PAH extraction by SPE yielded high repeatability errors (>20%). In addition, the PAH concentrations in the liquid phase always remain negligible whatever the sludge sample. The soluble fraction of PAHs represents less than 1% of the total amount found in contaminated sludge. The low PAH levels in the aqueous phase result from their low solubility in water and their very strong adsorption onto the sludge organic matrix, as previously reported [4]. Consequently, the PAH concentration from the liquid phase can always be considered as negligible.

PAH Extraction from the Solid Phase (Accelerated Solvent Extraction)

The PAH extraction from the solid phase was performed by ASE. Some parameters were thought to influence the PAH extraction efficiencies, such as cell pressure, temperature, number of cycles, amount of sample, purging time, flush rate of the extraction cell, and solvent mixture composition (hexane : acetone). Thus, three independent experimental plans were implemented to optimize the PAH extraction yields. The extraction pressure was first fixed at 100 bars. This value corresponds to the

TABLE IV Experimental optimization half-plans applied in the case of the ASE PAH extraction[a]

	Experimental plan n° 1		*Experimental plan n° 2*		*Experimental plan n° 3*	
	Average (μg/L)	*F factor* ($H_0 < 2.665$)	*Average* (μg/L)	*F factor* ($H_0 < 9.55$)	*Average* (μg/L)	*F factor* ($H_0 < 4.07$)
Naphthalene	[b]	[b]	[b]	[b]	[b]	[b]
Fluorene	166 ± 12	1.035	165 ± 10	10.77	321 ± 12	0.257
Phenanthrene	497 ± 45	1.662	504 ± 90	4.8	123 ± 3	0.229
Anthracene	97 ± 7	0.707	70 ± 6	2.5	130 ± 4	0.158
Fluoranthene	658 ± 48	0.526	394 ± 33	2.2	643 ± 14	0.064
Pyrene	734 ± 56	0.702	506 ± 53	3.1	883 ± 12	0.033
Chrysene	[c]	[c]	[c]	[c]	[c]	[c]
Benzo(a)anthracene	195 ± 15	0.696	123 ± 9	1.3	203 ± 8	0.148
Benzo(b)fluoranthene	301 ± 25	0.729	198 ± 19	4.0	318 ± 9	0.078
Benzo(k)fluoranthene	130 ± 10	0.776	80 ± 6	2.0	116 ± 3	0.047
Benzo(a)pyrene	250 ± 20	0.656	160 ± 15	2.2	219 ± 5	0.046
Dibenzo(ah)anthracene	44 ± 3.5	0.756	32 ± 4	5.1	55 ± 2	0.073
Benzo(ghi)perylene	154 ± 14	0.997	117 ± 18	9.1	190 ± 8	0.145
Indeno(123-cd)pyrene	77 ± 10	0.242	24 ± 6	0.4	44 ± 11	0.282

[a]The low and high levels were defined for 4 parameters (plan 1: temperature 100–120°C, static time 5–8 min, cycles 2–3, hexane : acetone 25 : 75–50 : 50), one parameter (plan 2: sample amount 0.5–1 g) and two parameters (plan 3: purge time 60–100 s, flush rate 60–90%). Multiple analysis of variance (ANOVA—one factor) were performed between the assays and H_0 (no statistical difference) was confirmed at 95% for F factor lower than 2.665 (first plan), lower than 9.55 (second plan) and 4.07 (third plan). [b]Below detection limit. [c]Not measured.

upper limit for the PAH extraction from the sludge sample, according to DIONEX™ recommendations.

In the first experimental plan, four ASE parameters were tested. A low and high value were defined for each parameter as 100°C and 120°C for the temperature, 2 and 3 for the number of cycle of extraction, 5 min and 8 min for the static extraction time, 25 : 75 and 50 : 50 of hexane : acetone, respectively, for the solvent mixture composition. Each low and high level was tested, and the results were statistically compared by a multiple ANOVA (see Table IV). In the first plan, no significant difference was observed between the assays. Consequently, these ASE parameters have no influence on the PAH extraction efficiencies. Similar results were previously observed in contaminated soils or sediments [9–10,12,14–15]. Therefore, the ASE extraction yields do not seem to be influenced by the sample matrix because of the strong operating conditions (high temperature and pressure), and these results should be valid for any kind of sample. Moreover, this method is suitable for the analysis of the PAHs except the naphthalene, which was not recovered after the sample evaporation because of highly volatile properties (Table IV).

The next two experimental plans were performed to test the influence of secondary parameters. The results are reported in Table IV. The differences in absolute values measured between the experimental plans are explained by the actual low homogeneity of the fresh sludge stock mixture. However, statistical conclusions are independent and remain valid for each half-experimental plan. The main objectives of the second and third plans were to reduce the sample amount, the extraction time, and the solvent consumption. Thus, in the second experimental half-plan, no significant difference was observed between 0.5 and 1 g of sludge sample, except for the fluorene. The volume of sludge sample can therefore be reduced to a minimal level of 0.5 g. Since two repetitions (2×0.5 g) of the PAH extraction require approximately 300 mL of fresh sludge, this amount of sludge is compatible with lab-scale experiments. The third experimental

half-plan was performed to reduce the solvent consumption by decreasing the flush rate of the extraction cell (from 90 to 60%). The extraction time was also reduced by decreasing the final purge time from 100 to 60 s. As for the other factors, the PAH extraction efficiencies were not influenced by these parameters (Table IV).

In conclusion, the ASE parameters can be chosen with a high degree of freedom according to the experiment requirements, such as a low consumption of solvent, a reduced amount of sample, or a short time of analysis. In this study, the ASE parameters were selected to monitor further the PAHs during lab-scale experiments, and the final parameters are as follows: temperature of 120°C, two cycles of extraction, 5 min of static time, hexane : acetone (50 : 50), flush rate of 60%, purging time of 60 s and 0.5 g of sample in the extraction cell. The time of extraction did not exceed 20 min.

Comparison of the Optimized PAH Extraction Method (ASE) and the Soxhlet Reference Method

Considered as the reference method, the PAH extraction by Soxhlet was compared with the previously optimized ASE method. The statistical results are reported in Table V. It appeared that the PAH recoveries by ASE are 94–115% compared with the Soxhlet concentrations. According to the statistical t-test, no differences were observed between the two extraction methods. This result confirms the accuracy of the optimized ASE method. Moreover, similar results between ASE and Soxhlet extractions have already been reported with contaminated soils and sediments [9,14–16]. The ASE and Soxhlet extraction methods are therefore highly comparable whatever the sample matrix.

The reproducibility errors of the ASE and the Soxhlet methods were also calculated by three analyses of the same sludge sample. The Soxhlet method presented the highest reproducibility errors from 5 to 9%, with an average of 7.5%. In comparison, the ASE method provided reproducibility errors lower than 2% for the same sludge sample. This result was confirmed for more than 80 extractions of sludge sample (Table VI). Therefore, the ASE extraction is statistically more reproducible than the Soxhlet extraction.

Validation of the Analytical Method with a Certified Contaminated Sewage Sludge

The PAH losses during the extraction step were first determined to confirm the accuracy of the values. A standard solution of the 14 studied PAHs (from naphthalene to indeno(123cd)pyrene) was added in a fresh sludge sample. The spiked sludge was then analysed by the optimized method, and the spiked values were determined and compared with the non-spiked sludge. The total recoveries are presented in Table VII. It appeared that all added PAHs were recovered, and only the naphthalene was totally

TABLE V PAH recoveries of the ASE extraction compared with the Soxhlet extraction used as reference method[a]

PAH	*Fl*	*Ph*	*An*	*Flu*	*Py*	*BaA*	*Ch*	*BbF*	*BkF*	*BaP*	*DB*	*BP*	*Ind*
Recovery (%)	98.3	100.4	111.1	94.4	98.1	110.5	105.6	108.2	106.6	108.9	106.4	115.8	97.1
t value (< 3.182)	1.280	0.948	0.779	1.507	0.772	0.690	0.905	0.753	0.623	1.724	1.239	1.528	2.304

[a]A t-test (Student law) was performed for the statistical comparison. Hypothesis H_0 of no difference was confirmed at 95% for t values lower than 3.182.

TABLE VI Errors of reproducibility calculated during PAH monitoring of lab-scale experiments (ASE extraction and RP-HPLC/fluorescence detection)[a]

PAH	*Fl*	*Ph*	*An*	*Flu*	*Py*	*BaA*	*Ch*	*BbF*	*BkF*	*BaP*	*DB*	*BP*	*Ind*
Average of errors of reproducibility (%)	1.83	1.75	1.53	1.45	1.68	1.32	1.72	1.47	1.24	1.31	1.86	1.65	1.61
Concentration (mg/kg dw)	0.49	3.52	0.92	10.74	10.69	3.79	4.47	4.94	2.53	4.23	0.75	2.81	4.10

[a]The presented values are the average of more than 80 measurements of the reproducibility errors. The reproducibility error was calculated after three extraction analyses of the same sludge sample.

TABLE VII Measured and expected concentrations of contaminated sludge spiked with 50 µg/L of the 14 PAHs standard mixture

PAH	*Fl*	*Ph*	*An*	*Flu*	*Py*	*BaA*	*Ch*	*BbF*	*BkF*	*BaP*	*DB*	*BP*	*Ind*
Measured concentration (µg/L)	60.4	163	84.7	332	360	171	164	197	139	189	87.8	148	208
Expected concentration (µg/L)	66.6	160	82.6	329	355	165	171	196.9	140.1	190.4	83.1	144	208.1
Recovery (%)	90.8	102.1	101.3	101	101.5	103.5	95.6	99.9	99.8	99.3	105.6	102	99.9

TABLE VIII Measured and certified concentrations of the material CRM088

	Certified concentrations				*Measured concentrations*		*Difference* (%)
	Min	*Average* (mg/kg dw)	*Max*	*RSD* (%)	*Average* (mg/kg dw)	*RSD* (%)	
Pyrene	1.76	2.16	2.70	4.2	2.60	4.9	+20.5%
Benzo(a)anthracene	0.65	0.93	1.14	9.7	0.93	4.2	+0.1%
Benzo(b)fluoranthene	0.99	1.17	1.39	7.7	1.17	4.5	−0.1%
Benzo(k)fluoranthene	0.41	0.57	0.71	8.8	0.52	4.8	−8.8%
Benzo(a)pyrene	0.62	0.91	1.22	9.9	0.80	4.2	−11.8%
Indeno(123-cd)pyrene	0.57	0.81	0.98	7.4	0.72	4.7	−11.2%

lost during the evaporation step. The highly volatile fluorene was also partially lost during the PAH extraction with about 10% of losses. Nevertheless, the proposed method presents no significant losses for the other PAHs.

The accuracy of the analytical method was finally validated by the determination of the PAH concentrations in certified sludge material (CRM088 – Bureau of Reference EUR n° 15039) [8]. The certified concentrations resulted from the sludge analysis by 11 international laboratories using major analytical techniques (GCFID-GCMS-LCFLU). The measured values were in the range of the referenced concentrations (Table VIII). The method described in the present paper seems to extract the lowest PAHs more than the highest (+20.5% *vs.* −11.2%), but measured concentrations are included in the standard deviation of the certified values.

CONCLUSION

The polycyclic aromatic hydrocarbons present high hydrophobic properties, and their monitoring in long-term contaminated environment is particularly complex because

of their strong interactions with the organic compounds. In this study, an analytical method was optimized to monitor 14 priority PAHs during lab-scale experiments. Low PAH concentrations, low sample volume, and a high reproducibility of the analysis were the main analytical constraints. In a first step, it was shown that the separation efficiency of the PAH peaks was strongly dependent of the elution temperature, as low as possible, and of the length of the solvent mixture gradient, as long as possible. After optimization, the fluorescence PAH detection and quantification provided highly accurate and repeatable results (errors lower than 2%). In a second step, the PAH extraction from sewage sludge by ASE was optimized. None of the ASE parameters has a significant effect on the PAH extraction efficiencies. Thus, the operating conditions can be fixed according to the own practical constraints, such as a low consumption of solvent and a short extraction time. Although the PAH extraction by ASE presented similar results to the reference method of Soxhlet, the ASE method presents statistically the highest reproducibility. In addition, the accuracy of the optimized method was validated on certified reference sludge with results statistically similar to the certified concentrations. However, the naphthalene was not recovered after the evaporation step and cannot be analysed by this method. In conclusion, the optimized method was successful according to the high accuracy, the high reproducibility, and the high reliability over time, and consequently is suitable for intensive use during lab-scale experiments.

Acknowledgements

The Agence de l'Environnement et de la Maîtrise de l'Energie (ADEME, Angers, France) is especially thanked for the grant to Eric Trably.

References

[1] M. Feix, J. Jauzein and J. Wiart, *Les micropolluants organiques dans les boues résiduaires de stations d'épuration urbaines* (ADEME Press, Angers, France, 1995), n°. 1800.
[2] E. Wikstrom, M. Tysklind and S. Marklund, *Environ. Sci. Technol.*, **33**, 4263–4269 (1999).
[3] P.K. Lagoy and T. Quirk, *Environ. Health Persp.*, **102**, 348–352 (1994).
[4] M.D. Ferrari, *Rev. Arg. Microbiol.*, **28**, 83–98 (1996).
[5] S. Perez, M. Guillamon and D. Barcelo, *J. Chromatogr. A*, **938**, 57–65 (2001).
[6] US Environmental Protection Agency. *Test Methods for Evaluating Solid Wastes* (EPA Methods, SW846, Method 8310 1986).
[7] Association Française de Normalisation. Project n° X33-0.12. AFNOR, France (2000).
[8] Commission of the European Communities. Report of the Community Bureau (Reference EUR 15039 EN, Bruxelles, Bel, CRM 088, 1994).
[9] O.P. Heemken, N. Theobald and B.W. Wenclawiak, *Anal. Chem.*, **69**, 2171–2180 (1997).
[10] N. Saim, J.R. Dean, M.P. Abdullah and Z. Zakaria, *J. Chromatogr. A*, **791**, 361–366 (1997).
[11] V. Mathevon, In: *Proceedings of the Technical day of the Wastewater Treatment Plant: Wastes Management*. (INSA, Toulouse, France, 1999).
[12] J.D. Berset, M. Ejem, R. Holzer and P. Lischer, *Anal. Chim. Acta.*, **383**, 263–275 (1999).
[13] J. Huau and A.M. Compiano, *Spectra Analyse*, **190**, 34–36 (1996).
[14] B.E. Richter, B.A. Jones, J.L. Ezzell and N.L. Porter, *Anal. Chem.*, **68**, 1033–1039 (1996).
[15] M.M. Schantz, J.J. Nichols and S.A. Wise, *Anal. Chem.*, **69**, 4210–4219 (1997).
[16] N. Saim, J.R. Dean, M.P. Abdullah and Z. Zakaria, *Anal. Chem.*, **70**, 420–424 (1998).
[17] S.A. Wise, L.C. Sander and W.E. May, *J. Chromatogr. A*, **642**, 329–349 (1993).
[18] P. Garrigues and C. Raoux, *Analusis*, **25**, 243–246 (1997).
[19] W. Hesselink, R.H.N.A. Schiffer and P.R. Kootstra, *J. Chromatogr. A*, **697**, 165–174 (1995).
[20] P.W. Kirk and J.N. Lester, *Environ. Technol.*, **12**, 13–20 (1990).

[21] E. Mangas, M.T. Vaquero, L. Comellas and F. Broto-Puig, *Chemosphere*, **36**, 61–72 (1998).
[22] M.D. Aitken, W.T. Stringfellow, R.D. Nagel, C. Kazunga and S.H. Chen, *Can. J. Microbiol.*, **44**, 743–752 (1998).
[23] G. Castello and T.C. Gerbino, *J. Chromatogr. A*, **642**, 351–357 (1993).
[24] C. Miège, J. Dugay and M.C. Hennion, *J. Chromatogr. A*, **823**, 219–230 (1998).
[25] G. Gramss, K.D. Voigt and B. Kirsche, *Biodegradation*, **10**, 51–62 (1999).
[26] R. Tomassone, C. Dervin and J.P. Masson, *Biométrie: modélisation des phénomènes biologiques.* (Ed Masson, Issy les Moulineaux, France, 1993).
[27] E. Touraud, M. Crone and O. Thomas, *Field Anal. Chem. Technol.*, **2**, 221–229 (1998).