

Optimization, validation and comparison of various extraction techniques for the trace determination of polycyclic aromatic hydrocarbons in sewage sludges by liquid chromatography coupled to diode-array and fluorescence detection

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Received 13 May 2002; received in revised form 3 March 2003; accepted 13 March 2003

Abstract

There is a need for a better characterization of sludges from wastewater treatment plants which are destined to be spread on agricultural lands. Inorganic pollutants are regularly controlled but organic pollutants have received few attention up to now. On this paper, we have been interested on the analysis of the 16 polycyclic aromatic hydrocarbons (PAHs) listed in the US Environmental Protection Agency (US EPA) priority list and more particularly of the six PAHs listed in the European community list (fluoranthene, benzo[*b*] and *k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene). The analysis step consists on liquid chromatography with both fluorescence and UV detections as described in the EPA Method 8310. As for the extraction step, several techniques such as supercritical fluid extraction, pressurized liquid extraction, focused microwave extraction in open vessels, Soxhlet and ultrasonic extractions are compared after optimization of the experimental conditions (solvent nature and quantity, temperature, pressure, duration, . . .) and validation with certified sludges. When optimized, these five extraction techniques are as much efficient with similar relative standard deviation. Whatever the extraction techniques used, the whole analysis protocol permits to quantify PAHs in the range of 0.09 to 0.9 mg/kg of dried sludges.

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Keywords: Sewage sludge; Extraction methods; Optimization; Validation; Polynuclear aromatic hydrocarbons

1. Introduction

Because of their low solubility in water and their

high hydrophobicity, polycyclic aromatic hydrocarbons (PAHs) are quantitatively removed from sewage and adsorbed on solid particles during sedimentation. This results in the formation of sewage sludges. Thus, sludges which are spread on agricultural lands as fertilizers are likely to contain such PAHs that are persistent and develop high carcinogenic and muta-

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genic toxicity. Therefore, there is a risk of soil contamination that must be reduced by developing analytical methods to allow routine monitoring of sludge matrices.

As for the analysis step, the best available methods for PAHs are liquid chromatography (LC) with both fluorescence (FL) and absorbance UV detections or gas chromatography (GC) with mass spectrometric detection (MS). These two separation–detection steps are described by US Environmental Protection Agency (EPA) Methods 8310 and 8100, using LC with fluorescence or absorbance UV detection and GC–MS, respectively. To characterize PAHs in solid matrices such as sludges, an extraction step is required prior to the analysis. Supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), microwave-assisted extraction in closed vessels (MAE), focused microwave extraction in open vessels (FMWE) and also subcritical water extraction have been developed as alternative techniques to replace classical Soxhlet and ultrasonic extractions. SFE has already been tested to extract PAHs from petroleum waste sludges [1], urban sludges [2–4], or contaminated soils [5]. PLE has been tested for PAHs in contaminated soils [5–9], in sediments [10–12] and other environmental matrices such as urban dust [10,11], diesel particulate matter and mussel tissues [11]. MAE has been tested for PAHs in soils [6,13,14] and sediments [14,15]. FMWE, which is no more commercialized since 1999, has been tested for PAHs in soils [4,16–19], sediments [17–20], air particulates [17] and biological tissues [20]. Subcritical water extraction of PAHs were achieved in certified soils and compost [5,21,22].

The aim of this paper is to present the optimization and the validation of various extraction techniques which are SFE, FMWE, PLE, ultrasonic and Soxhlet extractions and to compare their performances when applied for the routine analysis of the 16 PAHs included in the US EPA priority pollutants list, and contained in sludges destined to be used as soil amendment. The sludge extracts were analysed by LC using simultaneously fluorescence and UV diode array detection (DAD). Extractions were investigated without any clean-up step because of the high selectivity that can be obtained with PAHs when analysing by LC–FL.

2. Experimental

2.1. Apparatus

The HPLC system consisted of a Varian 9012 pump with a Rheodyne 20- μ l loop injector coupled with a Varian 9070 fluorescence detector and a Varian 9065 photodiode-array detector (Varian, Les Ulis, France). A 250 \times 3 mm Bakerbond PAH-16 Plus column including guard column was used (J.T. Baker–Mallinckrodt, Noisy le Sec, France). A Croco-Cil oven (Touzart et Matignon, Les Ulis, France) was used for setting up the column temperature. Supercritical-fluid extractions were performed with a Star SFE Prepmaster apparatus in conjunction with a star SFE pump modifier and equipped with a Duraflo variable restrictor (Varian, Les Ulis, France). Focused microwave extractions were performed with a Soxwave 100 apparatus (Prolabo, Fontenay-sous-Bois, France). Pressurized liquid extractions were performed with an ASE 200 apparatus (Dionex, Jouy en Josas, France). Ultrasonic extractions were performed with a Transsonic 257/H apparatus (Prolabo). Soxhlet extractions were performed with a 60-ml siphon system (Prolabo).

2.2. Chemicals

Acetonitrile of “HPLC ultra” grade was obtained from J.T. Baker–Mallinckrodt. Dichloromethane, cyclohexane and toluene of “spectroscopic” grade and acetone of “chromatographic” grade were obtained from Merck (Nogent sur Marne, France). Ultrapure water was obtained from a Milli-Q water system (Millipore, St. Quentin en Yvelines, France). Glassbeads for SFE were obtained from Phase Sep (St. Quentin en Yvelines, France). CO₂ of “SFC” grade was obtained from Air Liquide (Paris, France). The standard mixture of the 16 priority PAHs in dichloromethane was obtained from J.T. Baker–Mallinckrodt at concentrations of 100 or 200 ppm depending on the PAH except for the naphthalene at 1000 ppm.

2.3. Sludge samples

Certified sludges (CRM No. 088) were prepared

by the Community Bureau of Reference from Brussels and were obtained from Promochem (Molsheim, France). Certified concentrations of PAHs in these sludges are as follows (mass fractions of dry matter expressed as mg/kg): pyrene, 2.16 ± 0.09 ; benz[*a*]anthracene, 0.93 ± 0.09 ; benzo[*a*]pyrene, 0.91 ± 0.09 ; benzo[*b*]fluoranthene, 1.17 ± 0.08 ; benzo[*k*]fluoranthene, 0.57 ± 0.05 ; indeno[1,2,3-*cd*]pyrene, 0.81 ± 0.06 . Standard deviations are mentioned for $n=9-12$.

Sewage sludge samples were obtained from an urban sludge treatment plant and were collected in great quantity at the end of the treatment, dehydrated to 52% of dry matter. Before extraction, sludges were dried in a ventilated oven for 24 h at 80 °C and then milled, ground and sieved to get rid of stones and pieces of wood. These urban sludges naturally contaminated with PAHs were used as a non spiked matrix to optimize the extraction step included in the analysis protocol. Concentrations of PAHs in these sludges were determined in a precedent paper [3] by validated Soxhlet extraction and are as follows (mass fractions of dry matter expressed as mg/kg): fluoranthene, 2.21 ± 0.26 ; pyrene, 2.31 ± 0.27 ; benz[*a*]anthracene, 0.63 ± 0.09 ; chrysene, 1.17 ± 0.15 ; benzo[*a*]pyrene, 0.64 ± 0.09 ; benzo[*b*]fluoranthene, 0.66 ± 0.08 ; benzo[*k*]fluoranthene, 0.33 ± 0.05 ; benzo[*ghi*]perylene, 0.28 ± 0.04 ; indeno[1,2,3-*cd*]pyrene, 0.75 ± 0.12 . Standard deviations are mentioned for $n=3$.

2.4. Liquid chromatography procedure

Acetonitrile and water were used as eluent solvents at a flow-rate of 0.5 ml/min. The gradient elution program was 0–5 min: 40% acetonitrile and 60% water; then a linear gradient elution from 40% acetonitrile at 5 min to 100% acetonitrile at 30 min, followed by isocratic elution with 100% acetonitrile for 5 min. The column temperature was maintained at 35 °C. The UV wavelength was set at 254 nm. The fluorescence excitation and emission wavelengths were changed during the chromatographic separation in order to obtain better sensitivity. The excitation/emission wavelengths were set as follows: 280/340 nm for the naphthalene to the fluorene elution, 295/380 nm for the phenanthrene elution, 280/430 nm

for the anthracene to the chrysene elution and 285/460 nm for the benzo[*b*]fluoranthene to the indeno[1,2,3-*cd*]pyrene elution.

2.5. Spiking procedure

The PAHs mixture was deposited with a micropipet directly onto the sludge sample. Once the analytes were spiked, extractions were starting up to prevent the loss of volatile compounds.

2.6. Extraction procedures

Soxhlet extraction procedure: 1 g of dried sludge sample was Soxhlet extracted with 80 ml of toluene for 8 h.

Supercritical-fluid extraction procedure [1–5]: The extraction cell having an internal volume of 3 ml was packed with 1 g sample of dried sludge and glassbeads were added to fill the rest of the volume of the extraction cell. Pure or modified CO₂ (with 5% of toluene during dynamic extraction step) was used as supercritical fluid. All extractions were performed at 500 atm and 150 °C during 10 min in static mode and then 30 min in dynamic mode (1 atm=101 325 Pa). SFE flow-rate was controlled at 1 ml/min. All extracts were collected by inserting the outlet end of the restrictor into a vial containing 10 ml of solvent toluene. The collecting solvent corresponds to the solvent used as modifier in order to avoid incomplete solubilization.

Ultrasonic extraction procedure: 1 g of dried sludge sample was extracted with 80 ml of toluene for 30 min.

Pressurized liquid extraction procedure [5–12]: 1 g of dried sludge sample was extracted with 16 ml of solvent (toluene or heptane–acetone (1:1, v/v) or heptane–dichloromethane (1:1, v/v)) for 20 min. Pressures of 100, 140, 160 and 200 bar and temperatures of 80, 100 and 120 °C were tested with a heat-up time of 5 min. Two cycles of extractions were performed during 5 min in static mode and then in dynamic mode with 8 ml of extraction solvent.

Focused microwave extraction procedure [4,16–20]: The cellulose extraction cartridge was packed with 1 g of dried sludge. The extractions were carried out at 30, 60, 90 and 120 W with 40 or 60 ml

of a mixture of toluene–acetone or toluene–acetonitrile or dichloromethane, and lasted 10, 15, 20 or 30 min. During the extraction process, the cartridge was in a low position at about 1 cm from the bottom of the 250-ml tube. A rinsing step ended the extraction process. It consisted in positioning the cartridge in a higher position (near the top of the 250 ml tube) while continuing to heat the tube for 5 min. Next, a concentration step was carried out by opening the tap and thereby allowing evaporation for 10 min until almost 40 or 25 ml of solvent remain, depending on the initial quantity which can be 60 or 40 ml, respectively.

After the extraction step, the extract was evaporated with a rotary evaporator until 5 ml and under nitrogen flow to dryness. After that, the extract was redissolved in 3 ml of acetonitrile and 0.75 ml of water [acetonitrile–water (8:2, v/v)] for HPLC injection.

3. Results and discussion

3.1. Analytical separation of PAHs

The separation of the 16 PAHs was carried out in 40 min. Only acenaphthylene does not fluoresce, this is the reason why it is not reported in Tables 1 and 2. HPLC with fluorescence and absorbance UV detections provided a linear response from amount injected in the range of 0.1–50 ng. Detection limits, obtained by direct injection of the 16 PAHs standard mixture and calculated with a signal-to-noise ratio of 3, are between 2- and 100-fold lower using fluorescence detection compared with UV detection, except for anthracene and chrysene, with similar UV and fluorescence limits of detection. Fluorescence limits of detection are in the range of 0.04 ng injected for benzo[*b*]fluoranthene and benzo[*k*]fluoranthene to 1.3 ng for pyrene and chrysene. Whereas, detection limits with UV (254 nm) range from 0.3 ng for anthracene to 12 ng for naphthalene, acenaphthylene and acenaphthene.

3.2. Performances of the evaporation step of the extracts

The whole protocol includes an evaporation to

dryness step of the sludge extract before its analysis. The losses of the PAHs during this evaporation step is evaluated in this section. A solution consisted on 60 ml of toluene with the 16 PAHs at quantity of 100 or 200 µg according to the PAH has been evaporated to dryness with successively rotavapor and nitrogen flow. The recoveries of non lost compounds are reported on Table 1. For the less volatile PAHs (from No. 7 to No. 16), losses are under 13%. But losses are more important for the more volatile PAHs (from No. 1 to No. 6), since they are higher than 43% and they even attain 72% for the naphthalene. The relative standard deviations are also higher (from 16 to 25%) for the more volatile PAHs than for the other ones (around 8%).

3.3. Optimization and validation of the various extraction techniques

It is well-known that recoveries obtained with spiked compounds may not be representative of those obtained with native compounds. Actually, spiked analytes on real-world samples are neither situated on the same binding sites as those of the native analytes nor adsorbed in the same manner. Spiked analytes are generally lightly coated on the surface of the matrix whereas native ones can be strongly adsorbed inside the porous matrix. This can be explained by the diffusional and the kinetic

Table 1
Recoveries and RSDs ($n=3$) obtained from a standard solution evaporated to dryness: analysis by LC–FL

	Recovery (%)	RSD (%)
(1) Naphthalene	28	25
(3) Acenaphthene	36	22
(4) Fluorene	42	18
(5) Phenanthrene	57	16
(6) Anthracene	51	17
(7) Fluoranthene	87	8
(8) Pyrene	91	9
(9) Benz[<i>a</i>]anthracene	89	8
(10) Chrysene	90	10
(11) Benzo[<i>b</i>]fluoranthene	93	7
(12) Benzo[<i>k</i>]fluoranthene	92	7
(13) Benzo[<i>a</i>]pyrene	95	9
(14) Dibenz[<i>ah</i>]anthracene	89	8
(15) Benzo[<i>ghi</i>]perylene	91	7
(16) Indeno[1,2,3- <i>cd</i>]pyrene	92	9

limitations of the sorption process, and the several interactions which can have been simultaneously established between a native analyte and a complex matrix [1,10,16]. Therefore, spiked analytes are always less retained in the environmental matrices than the native ones and the use of spike recovery studies may overestimate the efficiencies of extraction methods with real sample. That is the reason why it is necessary to validate the extraction procedures with certified reference matrices when available or at least with non spiked matrices. However, due to the high price of the certified reference materials, the optimization of the extraction techniques was performed with urban sludges that have been collected in great quantity at the end of their stabilization in a wastewater and sludge treatment plant. These sludges, naturally contaminated with PAHs (see Section 2.3) have been homogenized and treated as described in the experimental section.

SFE and Soxhlet extractions have been already optimized and validated in a previous paper [3]. In the present paper, we just reexamine which parameters was tested and selected and how accurate is the analysis protocol when Soxhlet or SFE is used. Soxhlet extraction was chosen as a reference technique to achieve the optimization of PLE, FMWE and ultrasonic extraction. In fact, recoveries obtained by Soxhlet for the PAHs from the non spiked urban sludges are compared with recoveries obtained by

PLE, FMWE and ultrasonic method; by this way the optimal experimental conditions can be fitted.

3.3.1. Soxhlet extraction: the reference technique

3.3.1.1. Optimization of the Soxhlet extraction conditions

The optimization of the Soxhlet extraction, which essentially consisted on the choice of the solvent, were achieved with naturally contaminated sludges in another paper [3]. The selected solvent were toluene. We proceeded as described in Section 2.6.

3.3.1.2. Performances of the Soxhlet extraction and validation with certified sludges

In this section, the urban sludges are spiked with the 16 PAHs at 1–2 mg/kg. Since the urban sludges are contaminated with original PAHs, non-spiked samples were systematically compared with spiked samples, and extraction recoveries have been calculated from the increase in peak areas between chromatograms corresponding to the same non-spiked and spiked samples.

After the extraction step, the sludge extract is evaporated to dryness, dissolved in the appropriate solvent and analysed by LC–FL. Extraction recoveries and relative standard deviations are reported in Table 2. Phenanthrene is not reported because it cannot be quantified at the spiked concentrations

Table 2
Recoveries and RSDs ($n=3$) obtained from spiked urban sludges: analysis by LC–FL

	Soxhlet		Ultrasonic		SFE		FMWE		PLE	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
(1) Naphthalene	61	23	56	24	35	26	58	22	55	23
(3) Acenaphthene	64	19	62	22	49	25	64	20	65	17
(4) Fluorene	79	19	72	19	74	20	76	16	76	18
(6) Anthracene	88	17	74	17	83	17	85	18	85	17
(7) Fluoranthene	88	10	81	9	84	9	82	11	83	9
(8) Pyrene	87	11	79	13	84	11	83	10	85	12
(9) Benz[<i>a</i>]anthracene	89	8	77	9	87	8	80	10	87	8
(10) Chrysene	94	16	80	16	95	16	86	15	92	16
(11) Benzo[<i>b</i>]fluoranthene	90	9	82	9	89	9	89	12	91	11
(12) Benzo[<i>k</i>]fluoranthene	89	9	84	10	87	8	88	10	88	10
(13) Benzo[<i>a</i>]pyrene	85	15	78	16	84	16	85	14	88	14
(14) Dibenz[<i>ah</i>]anthracene	89	10	81	11	85	10	84	10	87	10
(15) Benzo[<i>ghi</i>]perylene	83	12	78	10	82	11	82	11	81	11
(16) Indeno[1,2,3- <i>cd</i>]pyrene	86	13	80	12	84	11	87	11	89	13

because of interferent compounds. Except for the two more volatile PAHs (Nos. 1 and 2), the extraction recoveries are satisfying since they range from 79% for the fluorene to 94% for the chrysene. When comparing with Table 1, we notice that losses of volatile PAHs are lower in Table 2. This can be explained by a matrix effect: contrary to what happens in pure solvent, PAHs in the extract are retained during the evaporation step by sorption onto the matter in suspension. When evaporation is achieved to dryness, PAHs can also be sorbed onto the solid residue of the extract and then, losses by volatilization are avoided and recoveries are higher.

As for the relative standard deviations, they varies from 17 to 23% for the PAHs from Nos. 1 to 6 and from 8 to 16% for the other PAHs. When comparing with Table 1, we notice that RSD are higher in Table 2, especially with the non volatile PAHs (from Nos. 7 to 16). This can be explained by the multi-step analytical protocol and by the combination of the successive RSD (spiking, extraction, evaporation).

The protocol has been applied to certified sludges (CRM No. 088). The quantification has been realized taking into account the recoveries from Table 2. Certified and calculated concentrations have been reported on Table 3 and their concordance permits to validate the whole protocol included the Soxhlet extraction.

3.3.2. Optimization of the other extraction methods

All the extraction methods were optimized with non spiked urban sludges (see Section 2.3).

3.3.2.1. The supercritical fluid extraction conditions

The optimization of the supercritical fluid extraction, which consisted on the choice of solvent modifier, pressure and temperature, were achieved in another paper [3]. We proceeded as described in Section 2.6.

3.3.2.2. The ultrasonic extraction conditions

The optimization essentially consists on the choice of an extraction solvent. Toluene is selected because it has been proved to be efficient for Soxhlet and supercritical fluid extractions. The 150-W power of the extraction is imposed by the ultrasonic apparatus. The solvent volume is set at 80 ml as for the Soxhlet extraction. According to the literature, a 30-min

duration to achieve extraction of PAHs from solid matrices is necessary [23].

In these conditions, the extraction recoveries for the nine native PAHs in the urban sludges range from 86 to 98% with relative standard deviation around 12%, which is quite satisfying.

3.3.2.3. The pressurized liquid extraction conditions

3.3.2.3.1. *Choice of the nature of the extraction solvent* We tested toluene, a mixture of heptane–acetone and a mixture of heptane–dichloromethane. For these experiments, temperature and pressure were fixed to 100 °C and 140 bar, respectively. Relative extraction recoveries (for Soxhlet recoveries considered as equal to 100%) and residual standard deviations are represented in Fig. 1a. We finally selected toluene since it allows to obtain the best recoveries.

3.3.2.3.2. *Choice of the extraction temperature* Extraction were performed to 80, 100 and 120 °C with a pressure of 140 bar and toluene as solvent. Relative extraction recoveries (for Soxhlet recoveries considered as equal to 100%) and residual standard deviations are represented in Fig. 1b. Recoveries are slightly higher to 100 instead of 80 °C. But there is not significant increase of recoveries between 100 and 120 °C. Therefore, we chosen to proceed to 100 °C.

3.3.2.3.3. *Choice of the extraction pressure* Extraction were performed to 100, 140, 160 and 200 bar with temperature of 100 °C and toluene as solvent. Relative extraction recoveries (for Soxhlet recoveries considered as equal to 100%) and residual standard deviations are represented in Fig. 1c. Recoveries are slightly higher to 140 instead of 100 bar. There is not significant increase of recoveries between 140 and 160 bar. But we noticed a great decrease of recoveries when proceeding to 200 bar, due probably to a crushing of the matrix and so a trapping of the analytes inside the closed pores. We chosen to proceed to 140 bar.

3.3.2.4. The focused microwave extraction conditions

3.3.2.4.1. *Choice of the nature and the volume of the extraction solvent* As for the nature of the extraction solvent, we tested mixtures of toluene–acetone or toluene–acetonitrile or the dichlorome-

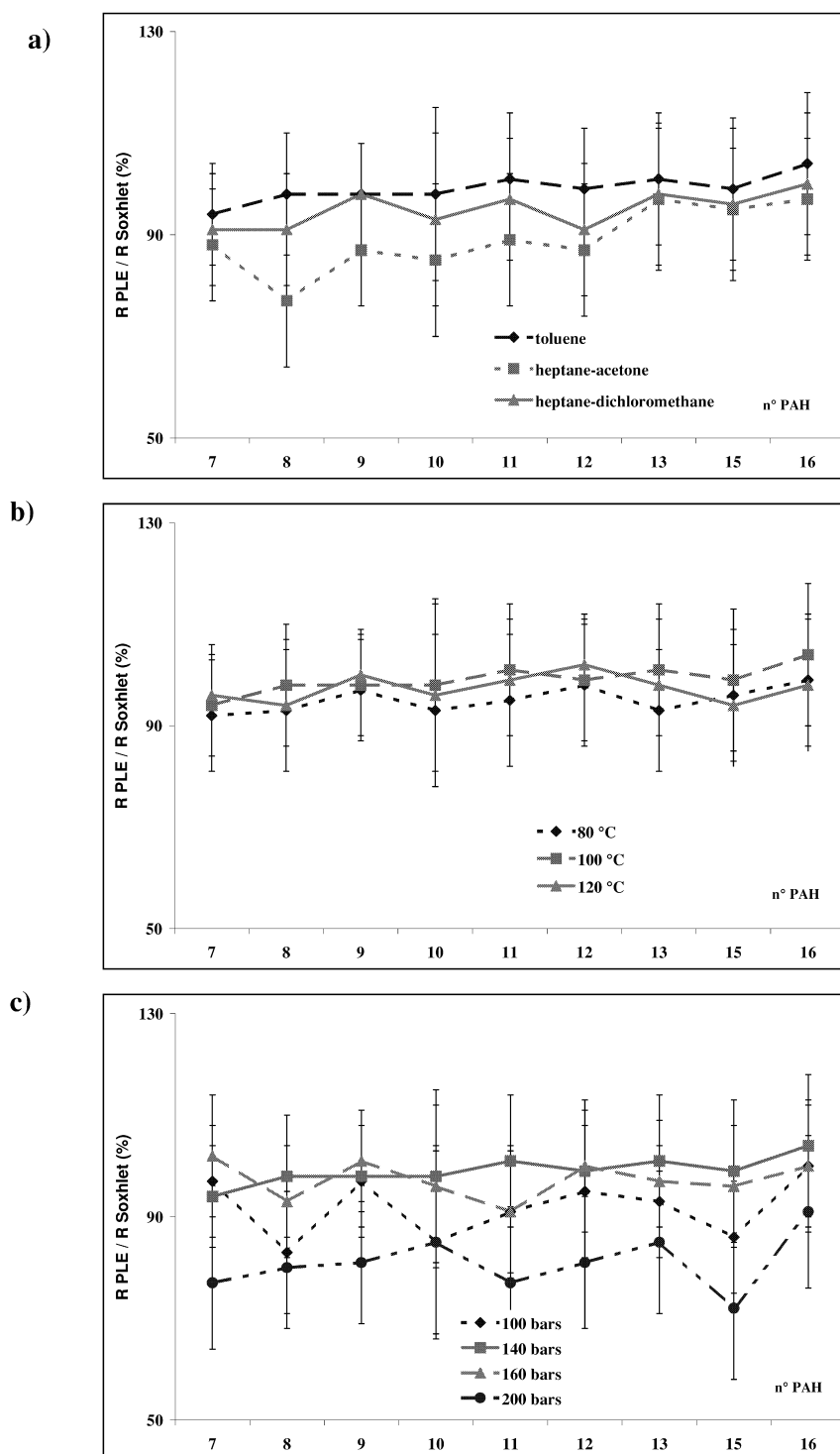


Fig. 1. Influence of solvent nature (a), temperature (b) and pressure (c) on PLE efficiency.

thane alone. In fact, toluene is often used with PAHs since its apolarity is well adapted with apolarity of PAHs, and complexations based upon specific interactions of PAHs with chemically bonded electron acceptors compounds like toluene may occur. Nevertheless, apolar solvent such as toluene unfortunately cannot absorb electromagnetic microwave radiations. It must be mixed with a solvent which is sensible to the electric field of the microwave radiations just like polar or polarizable solvents with a dielectric constant (ϵ). Moreover, the solvent must be able to convert electromagnetic energy into thermic energy, and this phenomenon depends in part on its calorific capacity, its dielectric losses ability and its thermic conductivity. Acetone and acetonitrile have been tested because they both are relatively polar with a consequent dielectric constant (ϵ). Moreover, acetonitrile and acetone are completely miscible with toluene. Incomplete miscibility of the solvents would induce heterogeneity that may cause existence of cool points areas where absorption of energy is slight and hot points areas where absorption and dissipation of energy is intense; and this phenomenon could lead to non reproducible extraction recoveries. Dichloromethane has also been tested since it is an efficient solvent of PAHs and it can absorb electromagnetic microwave radiations.

The recoveries of the extractions have been represented on Fig. 2a. The higher recoveries are obtained with the mixture of acetone–toluene (1:1, v/v), and the lower recoveries are obtained with dichloromethane. Acetone in combination with toluene seems to be more adapted to the extraction of sludge matrices than acetonitrile. This cannot be explained by the dielectric constant (ϵ') but the dielectric loss factor (ϵ'') allows to explain this phenomenon since it is higher for acetone and that induces a higher dissipation factor ($\tan \delta$) and then a more efficient heating with the mixture toluene–acetone instead of toluene–acetonitrile.

As for the volume of the extraction solvent(s), the fact that the sludge samples were packed in a cartridge and this cartridge was placed at about 1 cm from the bottom of the 250-ml tube, constrain us to use larger quantities of solvent than without cartridge. Anyway, the use of a cartridge allows to avoid a centrifugation step to separate the extract from the sludge sample. In order to completely

immerse the sludge samples, we used in a first time 40 ml of solvent. But we rapidly observed that a non-negligible proportion of sludge was thrown against the upper surface of the cartridge and then was not anymore in contact with the solvent. Therefore, we decided to use a volume of 60 ml of solvent.

We tested mixtures of acetone–toluene with different proportions (30:30, 20:40, 10:50 ml). Since acetone is more volatile than toluene, it is firstly evaporated and it fills in a great part the 10-ml dead volume located below the cooling system. Therefore, the mixture with only 10 ml of acetone cannot absorb the microwave radiations as soon as the 10-ml of acetone is imprisoned below the cooling system. With only 20 ml of acetone, the system is not optimal, light explosions occur and the mixture does not correctly absorb the microwave radiations. We finally chose to proceed with a 30:30-ml mixture of acetone–toluene.

3.3.2.4.2. Choice of the extraction power
Extractions were investigated at 30, 60, 90 and 120 W. The recoveries of the extractions have been represented on Fig. 2b. With 30 and 60 W, we do not observe any ebullition of the solvent mixture, this can explain the lower recoveries. Ebullition occurs as early as 90 W is attained. According to Fig. 2b, 120 W extractions do not bring more quantitative recoveries than 90 W extractions. We finally chose to work at 90 W.

3.3.2.4.3. Choice of the irradiation time
Extractions were investigated during 10, 15, 20, 30 min, and the recoveries of the extractions are represented Fig. 2c. Quantitative recoveries are obtained after 20 min even for the higher molecular mass PAHs which are supposed to be the more strongly adsorbed on the sludge matrix, and which are not quantitatively removed from sludges after 15 min of extraction. There is not significant increase of recoveries between 20 and 30 min. We finally chose to extract during 20 min.

3.3.2.4.4. Humidification of the matrix
Some papers pointed out the fact that higher extraction recoveries (10–20% higher) are obtained when the matrix (soils, sediments, sludges) is humidified before extraction (20–30% of the weight) [19]. With the extraction conditions defined above [acetone–toluene (1:1, v/v), 90 W, 20 min], we have tested the extraction of PAHs from wet sludges but we did not

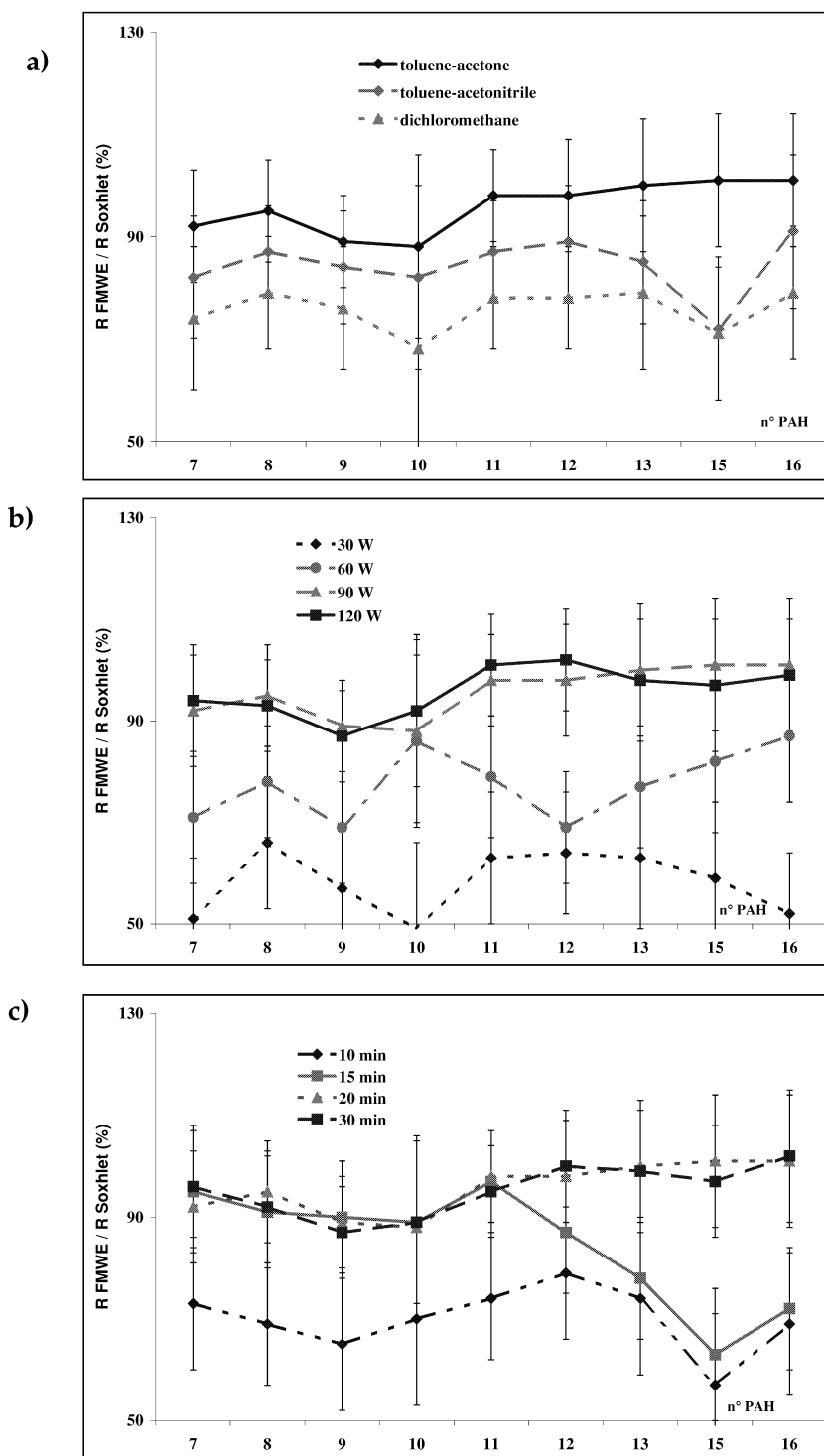


Fig. 2. Influence of solvent nature (a), power (b) and extraction duration (c) on FMWE efficiency.

Table 3
Certified and calculated PAH concentrations (mg/kg) in CRM No. 088: analysis by HPLC–FL

	Certified concentrations ^a	Soxhlet ^b	Ultrasonic ^b	SFE ^b	FMWE ^b	PLE ^b
(8) Pyrene	2.16±0.09	2.21±0.25	2.13±0.26	2.11±0.22	2.19±0.22	2.10±0.23
(9) Benz[<i>a</i>]anthracene	0.93±0.09	0.91±0.14	0.99±0.13	0.98±0.13	0.92±0.15	1.10±0.12
(11) Benzo[<i>b</i>]fluoranthene	1.17±0.08	1.22±0.15	1.19±0.14	1.13±0.17	1.21±0.16	1.14±0.15
(12) Benzo[<i>k</i>]fluoranthene	0.57±0.05	0.61±0.08	0.55±0.09	0.51±0.08	0.55±0.10	0.62±0.09
(13) Benzo[<i>a</i>]pyrene	0.91±0.09	0.85±0.11	0.90±0.12	0.98±0.13	0.89±0.11	0.99±0.12
(16) Indeno[1,2,3, <i>cd</i>]pyrene	0.81±0.06	0.86±0.10	0.77±0.13	0.87±0.09	0.84±0.12	0.85±0.14

^a $n = 9-12$.

^b $n = 3$.

notice any significant increase of recoveries. Therefore we chose not to humidify the matrix.

3.3.3. Comparison of the different extraction methods performances and validation with certified sludges

We used spiked urban sludges as for the Soxhlet extraction (see Section 3.3.1.2). Extraction recoveries and relative standard deviations are reported on Table 2. Phenanthrene is not reported because it cannot be quantified at the spiked concentrations because of interferent compounds.

With regard to Table 2, except for the two more volatile PAHs (Nos. 1 and 3), the extraction recoveries for the five extraction techniques are totally satisfying since they are over 72%. Relative standard deviations varies from 16 to 26% for the PAHs from Nos. 1 to 6 and from 8 to 16% for the other PAHs.

As for SFE, the very low recoveries for naphthalene (35%) and acenaphthene (49%) can be explained either by the fact that SFE extracts are known to contain less matrix material [5], and then more evaporative losses are expected if the extracts are taken to dryness (just as it happened for standard solutions in Section 3.2); or it can also be explained by possible losses during the collection of the extract consisting of inserting the end of the restrictor directly into a vial containing 10 ml of toluene, and then if one wants to quantify these two volatile PAHs, it is necessary to add an adsorbant above the collecting vial.

These different extraction methods have been applied to certified sludges (CRM No. 088). The quantification has been realized taking into account the recoveries from Table 2. Certified and calculated

concentrations have been reported on Table 3 and their concordance permits to validate all of these four extraction protocols.

4. Conclusion

Some papers pointed out the fact that PLE allows to obtain higher recoveries than others methods because of the combination of high temperature and pressure conditions [24], but it is not very significant for us (Table 3). Since the relative standard deviations are equivalent and because the concentration of PAH is corrected taking into account the recoveries of the analytical procedure, we conclude, with regards to the results from certified sludges (Table 3), that the five extraction techniques, when optimized, are as much efficient concerning the quantification of PAHs.

Acknowledgements

Agence de l'Environnement et de la Maîtrise de l'Energie (ADEME, Angers, France) is thanked for the grant to CM. We also thank Dionex for the loan of the ASE 200 extractor, Prolabo France for the gift of the FMW extractor and J.T. Baker, France for the gift of PAH columns and various chemicals.

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