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Simplified procedures for the analysis of polycyclic aromatic hydrocarbons in water, sediments and mussels☆

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Abstract

We describe in this paper simple and robust analytical protocols to determine the 16 polycyclic aromatic hydrocarbons (PAHs) of the US Environmental Protection Agency priority list in water, sediment and mussels. For water samples, eight different solid-phase extraction (SPE) sorbents have been compared and among them, C_{18} provided highest recoveries and limits of detection of 0.3–15 ng/L. For lyophilized sediments, Soxhlet and ultrasonic extraction were compared, and the last one permitted to recover all analytes with highest repetitivity and was validated by analysing a certified reference material. Finally, the analysis of mussels was undertaken using Soxhlet, ultrasonic and pressurized liquid extraction (PLE) and the performance of several clean-up steps are compared. Whereas for the former two, incomplete recovery or losses of some analytes were evidenced, PLE permitted a more efficient extraction and although alkaline digestion was necessary to remove coextracted compounds, the method gave acceptable recoveries and limits of detection of 0.5–7.7 μg/kg dry mass, as for sediments. In all cases, analysis was performed by gas chromatography coupled to mass spectrometry and internal standard quantification was performed using five deuterated PAHs. Each method performance is discussed for the three matrices analysed and the paper reports advantages and disadvantages of each for their routine application in monitoring programs.

Keywords: Water analysis; Sediments; Mussels; Extraction methods; Polycyclic aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are known for their carinogenetic and mutagenic properties and for being responsible of background level contamination in environmental matrices. PAHs are formed from anthropogenic (e.g. emissions in the environment as a result of vehicle exhausts, asphalt pavements, unvented radiant and convective kerosene space heaters, heating appliances) and natural sources (all incomplete combustion at high temperature and pyrolytic processes involving fossil fuels such as peat, coal and petroleum). Diffuse contamination of these compounds is generalized due basically to atmospheric transport, deposition and dispersion in the environment and residues levels have been found in domestic, industrial and rain water [1]. Levels up to 800 mg/kg were found in surface sediments in Finland and from 10 to 144 mg/kg in deeper layers [2], and mussels were capable to bioaccumulate such compounds, being the levels up to 32 mg/kg in finish lakes [3]. Owing to their semivolatility, these contaminants have also been detected in air [4] and due to long distance transport, in high mountain fish [5]. Their physico chemical properties define their environmental distribution and Tabak et al. [6] studied their bioavailability and degradation in interstitial and sediment samples.

As a result of their widespread presence and inclusion in European Union (EU) Directives, PAHs are generally introduced in monitoring programs. In this sense, in 1976, PAHs were already included in Directive 76/464/CEE [7]. In the year 2000, PAHs remain legislated in the New Framework Water Directive (2000/60/CE) [8] which includes all those

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compounds with demonstrated carcinogenic, mutagenic, esteroidogenic or affecting endocrine functions of the organisms. Directives 75/440/CE [9] and 80/778/CEE [10] indicate maximum residue levels of 0.2 μg/L in surface water directed to produce drinking water for human consumption. In addition, fluoranthene has been included in decision 2455/2001/CE [11] of the European Parliament due to the high production and use of this compound worldwide. At the moment, maximum admissible levels have not been fixed for sediment and biota although throughout yearly monitoring campaigns, the objective is to reduce their concentration in relation to previous years. In Europe, integrated monitoring programs are being established and include the analysis of water, sediment and biota all the way through a river basin, to check background concentrations in the different matrices, identify point source pollution and indicate the overall quality of the aquatic environment.

Due to the low water solubility and high hydrophobicity (Table 1), PAHs have a high affinity for the organic fraction of the sample and in water, they are adsorbed on particulate matter, which can be deposited as sediments [12]. In addition, PAHs are accumulated in the fat tissue of filtrating organisms such as mussels, oysters, clams, etc., which for long time have been used as bioindicators [13]. Fish, have the capacity of metabolizing such compounds through the P-450 cytochrome oxidase, and hence, they are not generally found. Under such circumstances, it is necessary to develop analytical methodologies capable to monitor water, sediment and mussels to allow a routine monitoring of a large number of samples.

Several reference methods have been proposed for the analysis of PAHs, the most common being HPLC–UV–fluorescence (FL) detection [14], or GC–MS [15]. Specific analytical protocols are described in detail by Manoli and Samara [16] and in the US Environmental Protection Agency (EPA) methods 550, 610 and 525, respectively [17]. For the extraction of water samples, most EPA methods use liquid–liquid extraction, whereas solid-phase extraction [18,19] and solid-phase microextraction [20] have been developed more recently. In the case of solid samples, Soxhlet extraction [21] is the reference method which is used to compare the results of more innovative techniques, such as microwave [22], ultrasounds [23] and pressurized liquid extraction (PLE) [24]. All these methods are applied and compared for the analysis of PAHs in sludges [14]. The analytical protocols described for mussels are even more complex due to the need of releasing PAHs from the matrix and removing the big amount of lipids of these organisms. Richardson et al. [25] used liquid extraction of 10 g of homogenized material with anhydrous sodium sulphate and methylene chroride whereas Mooibroek et al. [26] compared liquid extraction with microwave-assisted solvent extraction for the determination of PAHs in worms and concluded that less volatile PAHs were insufficiently recovered and at the same time, provided a significant higher amount of co-extracted material. The performance of PLE for PAH extraction PAHs in mussels has not been evaluated.

The objective of this work is to present simplified and robust extraction and clean-up methods for the analysis of 16 PAHs included in the EPA prioritary pollutants list in water, sediment and mussels which can be thereafter used in routine monitoring programs of PAHs in environmental matrices.

2. Materials and methods

2.1. Chemicals and reagents

Sixteen PAHs considered of primary environmental concern according to the EPA, were analysed: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chry-

Table 1
Physico chemical properties of the 16 EPA PAHs considered in this study

Compound	M_r	Molecular formula	CAS number	Solubility (mmol/L)	log K_{ow}	Vapor pressure 25 °C (Pa)	K_H (amt m^3/mol)
Naphthalene	128	$C_{10}H_8$	91-20-3	2.4×10^{-1}	3.37	10.9	4.5×10^{-3}
Acenaphtylene	152	$C_{12}H_8$	208-96-8	n.f.	3.98	n.f.	n.f.
Acenaphthene	154	$C_{12}H_{10}$	83-82-9	2.9×10^{-2}	4.07	5.96×10^{-1}	2.4×10^{-4}
Fluorene	166	$C_{13}H_{10}$	86-73-7	1.2×10^{-2}	4.18	8.81×10^{-2}	7.4×10^{-5}
Phenantrene	178	$C_{14}H_{10}$	85-01-8	7.2×10^{-3}	4.45	$(1.8 + 0) \times 10^{-2}$	2.7×10^{-4}
Anthracene	178	$C_{14}H_{10}$	120-12-7	3.7×10^{-4}	4.45	$(7.5 + 0) \times 10^{-4}$	1.8×10^{-6}
Fluoranthene	202	$C_{16}H_{10}$	206-44-00	1.3×10^{-3}	4.90	2.54×10^{-1}	1.95×10^{-3}
Pyrene	202	$C_{16}H_{10}$	129-00-0	7.2×10^{-4}	4.88	8.86×10^{-4}	1.3×10^{-5}
Benzo[*a*]anthracene	228	$C_{18}H_{12}$	56-55-3	n.f.	5.61	$(7.3 + 1.3) \times 10^{-6}$	1.2×10^{-6}
Crysene	228	$C_{18}H_{12}$	218-01-9	5.7×10^{-7}	5.16	1.3×10^{-5}	n.f.
Benzo[*b*]fluoranthene	252	$C_{20}H_{12}$	205-99-2	n.f.	6.04	1.2×10^{-7}	n.f.
Benzo[*k*]fluoranthene	252	$C_{20}H_{12}$	207-08-9	n.f.	6.06	5.5×10^{-8}	2.7×10^{-7}
Benzo[*a*]pyrene	252	$C_{20}H_{12}$	50-32-8	8.4×10^{-7}	6.06	1.5×10^{-5}	7.4×10^{-5}
Indeno[1,2,3-*cd*]pyrene	276	$C_{22}H_{12}$	193-39-5	n.f.	6.58	n.f.	n.f.
Dibenzo[*a*, *h*]anthracene	278	$C_{22}H_{12}$	53-70-3	$(3.7 + 1.8) \times 10^{-10}$	6.50	0.8×10^{-6}	2.0×10^{-9}
Benzo[*ghi*]perylene	276	$C_{22}H_{12}$	191-24-2	6.0×10^{-8}	6.84	2×10^{-5}	2.0×10^{-7}

n.f., not found; K_{ow}, Octanol–water partition coefficient; K_H, Henry's Law Constant.

sene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*] pyrene, indeno[1,2,3-*cd*]pyrene dibenzo[*a,h*]anthracene and benzo[*ghi*]perylene. They were purchased from Supelco as mix solution of 1000 μg/L in methanol. The surrogate standard was a mixture containing [$^2H_8$] naphthalene (naphthalene-d_8), acenaphthene [$^2H_{10}$], (acenaphthene-d_{10}), [$^2H_{10}$] phenanthrene (phenanthrene-d_{10}), [$^2H_{12}$] chrysene (chrysene-d_{12}) and [$^2H_{12}$] (perylene-d_{12}) from Supelco as a mix solution of 2000 μg/L in methanol. Solvents used were from Merck (Germany). Different solid-phase extraction (SPE) cartridges were used: Envi-18 (C_{18}, endapped, 500 mg from Supelco), tC18 (C_{18}, 500 mg from Waters), C_{18} were from IST (500 mg, International Sorbent Technology, UK), Envicrom (SDB 500 mg from Supelco), Porapak (DB + VP, 500 mg from Waters), Oasis 30 and 60 (PDB + VP, 30 mg or 60 mg, from Waters) and Envicarb (graphitised carbon, 250 mg, Supelco).

Alumina SPE cartridges of 5 g were from IST (International Sorbent Technology, UK). Hidromatrix was from Varian (USA). Nitrogen for drying with 99.995% of purity was from Air Liquide (Spain).

2.2. Extraction of PAHs in water

The method was optimised using groundwater. To avoid adsorption of PAHs upon glassware, 10% (v/v) methanol was added to 200 mL of water and the solution was mixed thoroughly. This solution was spiked with target analytes at a concentration of 2 μg/L. The surrogate standard was added at this stage at a concentration of 0.5 μg/L. In all cases, water was filtered through 0.45 μm filters. For the preconcentration step, a Baker vacuum system (J.T. Baker, The Netherlands) was used. SPE cartridges were conditioned with 5 mL of ethyl acetate followed by 5 mL of methanol and 5 mL distilled water containing 2% (v/v) methanol, all at a flow rate of 5 mL/min. The 200 mL of sample was percolated through the cartridges at a flow rate of flow 10 mL/min. Finally, the cartridge was rinsed with 5 mL of HPLC-grade water. The cartridge was dried under vacuum and elution was performed with 5 × 5 mL ethyl acetate. The extract was evaporated at low temperature under nitrogen and reconstituted in 250 μL of hexane.

2.3. Extraction and purification of PAHs in sediments

Sediment samples of low total organic carbon content were used to optimise the protocol. Sediments were placed in glass pots, were frozen at −18 °C and were lyophilized during 48 h (10^{-2} mbar vacuum) in a liophylizator (Lioalfa, Telstar, Spain). Then, samples were sieved through 500 and 120 μm mesh to obtain an homogeneous sediment material. This last fraction was spiked with the target solution at a concentration of 12.5 μg/kg and with the surrogate standard at a concentration of 20 μg/kg and extracted by Soxhlet and sonication.

2.3.1. Soxhlet extraction

One gram of sediment was inserted in a cellulose cartridge and extraction was performed using 100 mL of hexane–dichloromethane (1:1) for 24 h. The extract was rotaevaporated to almost dryness and afterwards it was purified using SPE cartridges as depicted below.

2.3.2. Ultrasonic extraction

One gram of sample was inserted in a glass tube with 30 mL hexane–dichloromethane (1:1) and placed in the ultrasonic bath for 10 min. Afterwards, the solution was centrifuged during 5 min at 2500 rpm. The two last steps were repeated three times. The sonicated extracts were evaporated in a rotary evaporator to almost dryness (0.5 mL approximately) for further clean up.

In both cases the extracts were purified following a clean up procedure using solid-phase extraction cartridges of neutral alumina of 5 g. The alumina was solvated and conditioned prior to sample loading with 20 mL hexane–dichloromethane (2:1) and 20 mL hexane–dichloromethane (10:1). The sediment extract was added to the top of the column and analyte elution was performed with 100 mL hexane–dichloromethane (10:1) and afterwards with 100 mL hexane–dichloromethane (2:1). The two fractions were collected into the same heart balloon recipient. Then, the fractions were preconcentrated in a rotary evaporator to 0.5 mL and transferred into vials. Extracts were evaporated at room temperature under nitrogen and reconstituted in 250 μL of hexane.

2.4. Extraction and purification of PAHs in mussels

Mussels from the fish shop which had undergone a purification treatment using ozone were used to optimise the method. Thirty individuals were cleaned, the flesh removed with a spoon, placed on a filter paper to remove the exceeding water, wrapped in aluminium foil and frozen at −18 °C. Samples were lyophilized during 7 days (10^{-2} mbar vacuum) and afterwards were smashed in a mortar until obtaining a fine sand. Mussels were spiked with the 16 target analytes and surrogate at a concentration of 50 μg/kg each. In all cases, 0.5 g were extracted using three different methods: Soxhlet, ultrasonic and pressurized liquid extraction.

2.4.1. Sohxlet extraction

Extraction was performed as depicted for sediments.

2.4.2. Ultrasonic extraction

Extraction was performed as depicted above for sediment samples.

2.4.3. Pressurized liquid extraction

Extraction was carried using the ASE 2000 System (Dionex, USA), using either hexane–acetone (1:1 (v/v)) or hexane–dichloromethane (1:1 (v/v)) for 10 min. Pressures of 1500 and 2000 psi and temperatures of 100 (60% flow) and

150 °C (100% flow) were tested with a heat-up time of 5 or 7 min (1 psi = 6894.76 Pa). Two cycles of extraction were performed during 10 min in static mode. The purge time was of 90 s.

The extracts were evaporated in a rotary evaporator to 0.5 mL. Due to the great amount of coextracted compounds noticed by the colour of the extract, and due to the fact that alumina SPE did not succeed in removing matrix components, those extracts were purified using alkaline digestion either by adding 20 mL of 6 M KOH and letting the solution in contact for 18 h at ambient temperature or 20 mL of 0.5 M KOH and letting the solution in contact for 4 h at 80 °C. Extraction was then carried out with 20 mL of hexane (×3) and finally the extract was rotaevaporated to approximately 0.5 mL, transferred into a 1.7 mL amber vial, evaporated at room temperature under nitrogen and reconstituted in 250 μL of hexane. If this extract was still viscous and colour, it was necessary to follow a clean-up step following the alumina SPE clean-up as depicted for sediments.

2.5. Instrumental analysis

Samples were analysed by a GC System (Carlo Erba GC 8000) coupled to a quadrupole mass spectrometer (Fisons MD 800). The system was operated in electron impact mode (EI, 70 eV). The separation was achieved with a 30 m × 0.25 mm i.d. DB-5 column (J&W Scientific, Folsom, CA, USA) coated with 5% diphenyl–polydimethylsiloxane (film thickness 0.25 μm). The oven temperature was programmed from 60 °C (holding time 1 min) to 175 °C at 6 °C/min (holding time 4 min) to 235 °C at 3 °C/min and finally to 300 °C at 8 °C/min, keeping the final temperature for 5 min. Injection was performed in the splitless mode, keeping the split valve closed for 48 s. Helium was the carrier gas (50 cm/s). Injector, transfer line and ion source temperatures were 280, 250 and 200 °C, respectively.

Peak detection and integration were carried out using Masslab software. For increased sensitivity and specificity, quantification was performed in time scheduled selected Ion monitoring (SIM) using three ions for each compound. Internal standard quantification was performed using the deuterated compound present in each elution window. The ion mass program used for quantification is detailed in Table 2.

3. Results and discussion

3.1. GC–MS separation and quality parameters

Table 2 shows the experimental mass conditions used in the GC–MS analysis. Although PAHs undergo very little fragmentation in EI mode, two and when possible three ions were used to identify each compound and the base peak, which in all cases corresponded to the molecular mass, was used for quantification. For the analysis of PAHs in water, sediment and biota, which have a very variable matrix, it is compulsory to use suitable internal/surrogate standards to correct retention time shifts, and rectify losses produced in the sample preparation step. In the described protocol, we have used five deuterated PAHs, one in each chromatographic window to quantify all target compounds within the window. The response factors of each PAH in relation to the deuterated

Table 2
GC–MS conditions under time scheduled selected ion monitoring indicating retention time windows, retention time of each analyte including native and labelled compound, molecular mass, specific ions of each compound indicating in italics the base peak and overall monitored ions in each retention window

Time window (min)	t_R compound (min)	Compound	M_r	Ions	*m*/*z* Window
6.00–14.50	10.57	Naphthalene	128	*128*, 127, 51	51, 127, 128, 136
	10.57	*Naphthalene-d_8*	136	*136*	
14.50–20.50	16.39	Acenaphthylene	152	*152*, 76, 151	76, 82, 151, 152, 154, 164, 165, 166
	17.01	*Acenaphthene-d_{10}*	*164*	*164*	
	17.12	Acenaphthene	154	154, 152, 76	
	19.07	Fluorene	166	*166*, 165, 82	
20.50-34.00	23.17	*Phenanthrene-d_{10}*	188	*188*	89, 101, 152, 178, 188, 200, 202
	23.30	Phenanthrene	178	*178*, 152, 89	
	23.56	Anthracene	178	*178*, 152, 89	
	31.64	Fluoranthrene	202	*202*, 200, 101	
	33.16	Pyrene	202	*202*, 200, 101	
34.00-48.00	42.53	Benzo[*a*]anthracene	228	*228*, 226, 114	114, 226, 228, 240
	42.63	*Crysene-d_{12}*	240	*240*	
	42.81	Crysene	228	*228*, 226, 114	
48.00-65.00	51.25	Benzo[*b*]fluoranthene	252	*252*, 250, 126	126, 138, 139, 250, 252, 264, 274, 276, 278
	51.49	Benzo[*k*]fluoranthene	252	*252*, 126	
	53.64	Benzo[*a*]pyrene	252	*252*, 250, 126	
	54.06	*Perylene-d_{12}*	264	*264*	
	59.06	Indeno[1,2,3-*cd*]pyrene	276	*276*, 138	
	59.27	Dibenzo[*a*,*h*]anthracene	278	*278*, 276, 139	
	59.87	Benzo[*ghi*]perylene	276	*276*, 274, 138	

Table 3
PAHs studied, identification number and recoveries (%) in water using different types of SPE sorbents

Compound	Id. no.	Envicrom-P	Porapak	Oasis 30	Oasis 60	Envicarb	Envi C_{18}	t-C_{18}
Naphtalene	1	27	32	41	36	48	45	48
Acenaphtylene	2	38	35	68	60	n.d.	68	72
Acenaphthene	3	112	129	93	98	94	95	125
Fluorene	4	73	130	65	56	n.d.	27	101
Phenantrene	5	72	85	89	75	n.d.	85	93
Anthracene	6	128	91	122	88	38	75	115
Fluoranthene	7	108	93	90	86	n.d.	116	104
Pyrene	8	121	97	96	93	n.d.	124	123
Benzo[*a*]anthracene	9	98	89	73	86	n.d.	90	81
Crysene	10	101	83	70	68	n.d.	85	79
Benzo[*b*]fluoranthene	11	87	58	66	57	n.d.	72	72
Benzo[*k*]fluoranthene	12	86	54	66	54	n.d.	77	67
Benzo[*a*]pyrene	13	108	65	74	64	n.d.	91	84
Indeno(1,2,3-*cd*)pyrene	14	76	19	60	15	n.d.	27	23
Dibenzo[*a*,*h*]anthracene	15	66	43	50	38	n.d.	52	42
Benzo[*ghi*]perylene	16	84	41	57	35	n.d.	44	40

congener were between 0.5 and 3, and over a concentration range from 0.05 to 2 μg/mL, the system behaved linear with a coefficient of correlation above 0.99 and the relative standard deviation of five consecutive injections at a concentration of 0.5 μg/mL was below 5%. With the program used, complete separation of all compounds was achieved in 65 min. Injection T_a was increased to 280 °C to enhance the recovery of high-molecular-mass PAHs.

3.2. Extraction of water samples

The recoveries of PAHs in groundwater spiked at a concentration of 2 μg/L are shown in Table 3. Among polymeric based sorbents, carbon based and C_{18}, best performance was obtained with C_{18} cartridges, which were chosen as preferred option for the analysis of PAHs in water. In general, polymeric sorbents produced low recoveries of the more volatile compounds and excessive retention of the most apolar ones. Envicarb could recover only 3 compounds out of the 16 under study. Out of the C_{18} cartridges used, all of them were suitable to extract PAHs and Table 4 shows the quality parameters using C_{18} cartridges of 500 mg of IST. The recoveries of PAHs in water varied from 35 to 113%. Naphthalene and acenaphthene were the least recovered compounds due to the fact that they are the most volatile whereas the more apolar ones (benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a*,*h*]anthracene and benzo[*ghi*]perylene) showed up to 42% losses. To overcome the problem of irreversible adsorption, it is important to add 10% MeOH in solution prior to extraction and to use a surrogate standard to recovery the losses during the extraction and evaporation steps. The overall repeatability of the method is very good (relative standard deviations between 0.5 and 6% for most of the compounds and up top 13% for fluoranthene). The limits of detection

Table 4
Recoveries, R.S.D.s (n = 3) and LOD (ng/L for water and μg/kg dry mass for sediment and biota) obtained from spiked waters (using SPE with C_{18} of IST), sediments (using ultrasounds and alumina SPE clean-up) and mussels (PLE, alkaline digestion and alumina SPE clean-up)

Compound	Water (C_{18})			Sediment			Mussel		
	Recovery (%)	R.S.D. (%)	LOD	Recovery (%)	R.S.D. (%)	LOD	Recovery (%)	R.S.D. (%)	LOD
Naphthalene	35	5	6	20	16	2	116	30	5
Acenaphtylene	46	2	6	83	11	1	90	12	1
Acenaphthene	105	5	12	97	7	2	107	12	8
Fluorene	97	3	15	114	3	2	121	11	4
Phenantrene	102	6	0.6	85	2	0.6	96	9	4
Anthracene	86	2	3	83	3	0.6	81	9	3
Fluoranthene	113	13	0.1	96	5	0.3	66	7	0.8
Pyrene	112	10	2	86	7	0.3	69	10	0.8
Benzo[*a*]anthracene	68	5	0.3	74	7	0.1	104	4	1
Crysene	67	6	2	79	8	0.1	106	4	0.6
Benzo[*b*]fluoranthene	86	12	0.3	60	7	0.2	93	9	1
Benzo[*k*]fluoranthene	73	6	0.3	60	19	0.5	64	14	1
Benzo[*a*]pyrene	61	12	12	63	10	0.4	94	5	0.5
Indeno[1,2,3-*cd*]pyrene	63	6	0.9	51	11	0.5	82	3	1
Dibenzo[*a*,*h*]anthracene	58	0.5	3	57	11	0.4	100	8	3
Benzo[*ghi*]perylene	67	5	0.3	47	12	0.5	95	4	1

Analysis by GC–MS.

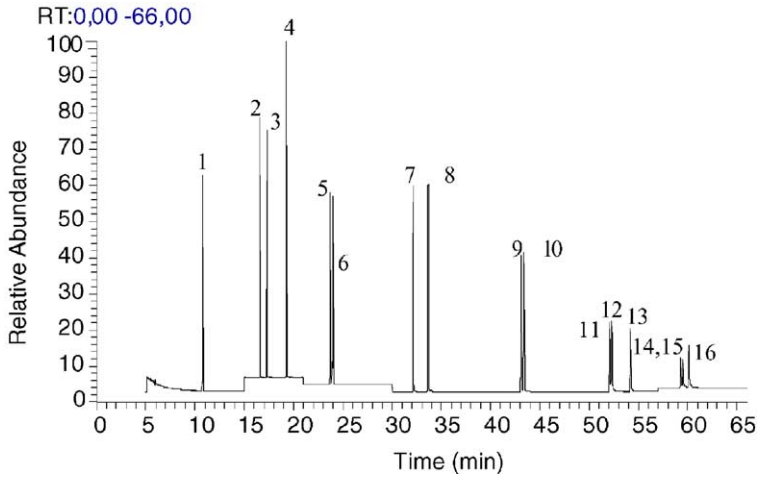

Fig. 1. GC–MS total ion chromatogram of a spiked sediment extract. Identification number as in Table 3.

(LODs) calculated at a signal-to-noise ratio of 3 are also indicated in Table 4 and are between 0.1 and 15 ng/L.

3.3. Extraction of sediment samples

Fig. 1 shows the total ion chromatogram of a spiked sediment, where it is possible to detect all 16 PAHs in a neat baseline chromatogram. Such chromatograms can only be achieved after a clean up step, despite the type of extraction that is used. In this study, for both Soxhlet and ultrasonic extraction, we used hexane–dichloromethane (1:1 (v/v)) as reported in previous methods [27,28]. Fig. 2 compares the recoveries of PAHs using Soxhlet and using ultrasonic extraction. PLE was not tested for sediment samples since many studies report on the optimisation, recoveries and overall performance [14,28–30] for the 16 EPA PAHs, indicating the suitability of the method although the amount of coextracted compounds increased. For Soxhlet and ultrasounds, similar recoveries were obtained with both methods, with relative standard deviation below 20%. With Soxhlet extraction, we obtained recoveries from 22 to 112% except for naphthalene and acenaphthene which were lost during the process. Due to its easier use and faster operation, ultrasonic extraction was chosen as the preferred option, even if compared with PLE from bibliographic data where a more exhaustive clean-up is needed. Table 4 shows the quality parameters obtained after

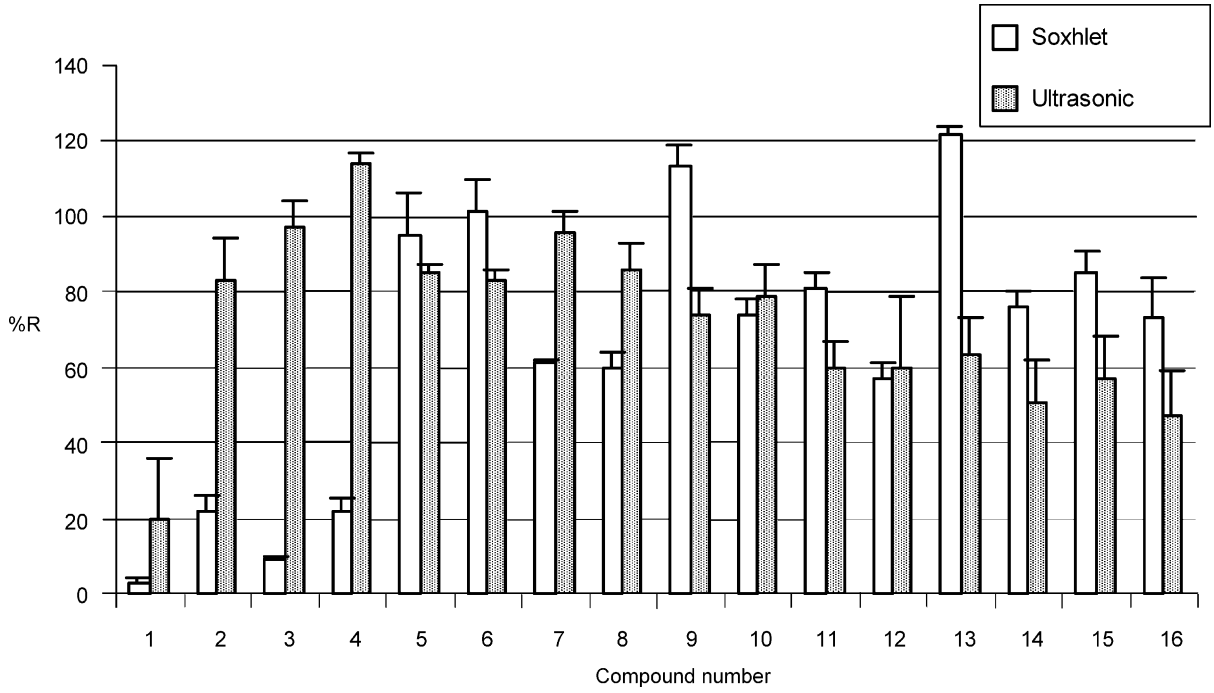

Fig. 2. Recoveries and coefficient of variation (n = 3) of target compounds in sediment spiked at 20 μg/kg dry mass after Soxhlet and ultrasonic extraction.

Table 5
Compounds included in the reference material Equate 96049, measured concentration (n = 3) and %R.S.D., certified concentration and R.S.D. and percentage of error made after ultrasonic extraction, alumina SPE and GC–MS analysis

Compound	Measured concentration (μg/kg) and R.S.D. (%)	Certified equate concentration (μg/kg)	Error (%)
Phenanthrene	220 ± 11	201 ± 7	9
Anthracene	46 ± 11	56 ± 14	18
Fluoranthene	361 ± 5	326 ± 7	11
Crysene	174 ± 4	175 ± 1	0
Benzo[*k*]fluoranthene	88 ± 12	138 ± 31	36
Indeno[1,2,3-*cd*]pyrene	118 ± 6	133 ± 8	11
Dibenzo[*a*,*h*]anthracene	17 ± 7	34 ± 47	50
Benzo[*ghi*]perylene	94 ± 13	107 ± 8	12

extraction of 1 g of sediment spiked at 12.5 μg/kg using ultrasonic extraction. This spiking level was chosen due to the fact that this is in the lower detected concentrations found in environmental samples. All compounds were recovered at 47–114%. Naphthalene was the least recovered compound due basically to losses during lyophilization. The R.S.D.s were highest for naphthalene and acenaphthene and also for the least volatile compounds, which presented values up to 19%. For the rest of the compounds, the R.S.D.s varied from 2 to 8%.

Ultrasonic extraction followed by alumina SPE clean-up was applied to analyse certified sediments (Equate 96049). Quantification was been corrected by the recoveries obtained in Table 4. Certified and calculated concentrations are reported in Table 5 and there is a good agreement among results being the error below 18% for most compounds.

3.4. Extraction of mussels

The analysis of PAHs in mussels has some additional difficulties related to the fact that mussel is a complex matrix which contains large amounts of lipids and proteins which have to be removed to eliminate chromatographic interferences and retention time shifts and secondly, PAHs have to be released from the lipidic tissue to enhance recoveries. Good method performance can only be obtained by optimising both the extraction step so as to recover all target analytes and the clean-up step, to remove co-extracted compounds. For such matrix, we have optimised PLE and we have compared this method with Soxhlet and ultrasonic extraction. Several parameters were optimised in PLE extraction of mussels. Among the different extraction solvents used, hexane–dichloromethane (1:1 (v/v)) yielded better recoveries than using hexane–acetone (1:1 (v/v)) and among the other parameters tested, system pressure of 1500 psi, extraction temperature of 150 °C and heat time of 7 min permitted to extract all compounds. Table 4 indicates the recoveries, R.S.D.s and LODs obtained using PLE. Fig. 3 compares the recoveries obtained with Soxhlet, ultrasonic and PLE extraction. Highest recoveries were obtained with PLE, which permitted to recover all PAHs. Ultrasonic extraction proved also efficient, but in general we observed higher R.S.D.s and benzo[*k*]fluoranthene and indene[1,2,3-*cd*]pyrene could not be recovered. Soxhlet extraction provided the poorest recoveries and highest R.S.D.s. According to the results obtained in this work, Soxhlet was the least efficient method whereas ultrasonic extraction and PLE are both reproducible methods showing the last one better recoveries. This is due to the fact the PLE is a more aggressive extraction technique capable to break the lipidic cells and release the encapsulated PAHs. However, PLE extracts are much "dirtier", by the appearance of the colour and consistency of the extract, than Soxhlet and ultrasonic extracts, and a simple SPE clean-up is not sufficient to remove co-extracted compounds. Therefore, PLE extracts were submitted to alkaline digestion using either 25 mL 0.5 M KOH at 80 °C during 4 h or 25 mL 6 M KOH at ambient T_a for 24 h. This latter option permitted to recover all PAHs at values between 80 and 120%. Alkaline digestion is in general enough to eliminate co-extracted impurities and provide clean baseline chromatograms. However, we have experienced that mussels vary in composition according to the sampling area and physiological condition and that alkaline digestion in some cases is not enough to remove coextracted compounds. In such cases, we suggest to follow a SPE clean-up step using 5 g alumina to ob-

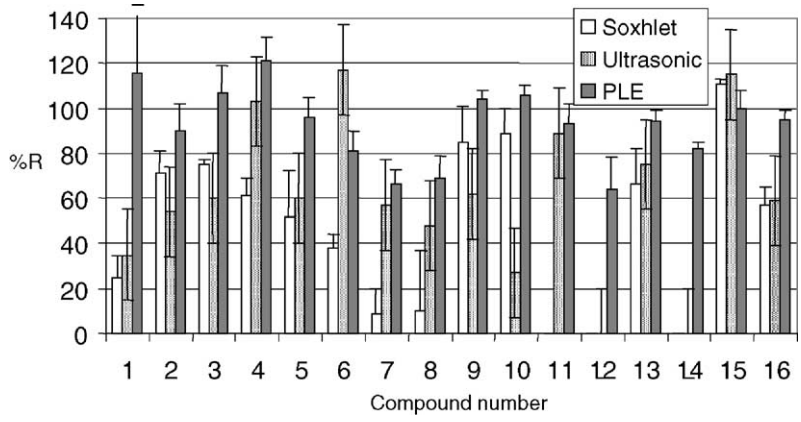

Fig. 3. Recoveries and coefficient of variation (n = 3) of target compounds in mussels spiked at 50 μg/kg dry mass after Soxhlet, ultrasonic and PLE extraction.

tain a neat chromatogram, as can be observed in Fig. 1 for sediments.

4. Conclusions

Several methods have been optimised to extract 16 priority EPA PAHs from water, sediment and mussels. The methods developed involved the use of a surrogate standard containing five deuterated PAHs, GC–EI–MS analysis and internal standard quantification. For water samples, SPE was tested using eight different sorbents including polymeric, Envicarb and C_{18} of different brands. C_{18} of 500 mg was the most efficient and repetitive extraction method and provided good recoveries and LODs at the ppt level, although 10% methanol had to added to the water sample to enhance extraction efficiency.

For sediment samples, both Soxhlet and ultrasonic extraction provided recoveries from 47 to 122% except for naphthalene, being ultrasonic extraction the method of choice for its faster performance. The analysis of PAHs in sediments needs a clean-up step to remove sample interferences and retention time shifts, which can be easily performed using SPE alumina cartridges.

In the case of the analysis of mussels, we have optimised a PLE extraction method and recoveries obtained were between 65 and 150%, with LODs between 0.51 and 7.77 μg/kg. PLE recoveries were similar than with Soxhlet and ultrasonic extraction, although these last two methods did not recover all analytes. The advantage of using PLE is that target compounds are released from the matrix rendering higher recoveries, despite the fact that PLE extraction provided "dirtier" extracts and the clean-up step had to be optimised using alkaline digestion and alumina SPE.

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