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Micro-focused ultrasonic solid–liquid extraction (μFUSLE) combined with HPLC and fluorescence detection for PAHs determination in sediments: optimization and linking with the analytical minimalism concept

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Abstract

Analytical minimalism is a concept that deals with the optimization of all stages of an analytical procedure so that it becomes less time, cost, sample, reagent and energy consuming. The guide-lines provided in the USEPA extraction method 3550B recommend the use of focused ultrasound (FU), i.e., probe sonication, for the solid–liquid extraction of Polycyclic Aromatic Hydrocarbons, PAHs, but ignore the principle of analytical minimalism. The problems related with the dead sonication zones, often present when high volumes are sonicated with probe, are also not addressed. In this work, we demonstrate that successful extraction and quantification of PAHs from sediments can be done with low sample mass (0.125 g), low reagent volume (4 ml), short sonication time (3 min) and low sonication amplitude (40%). Two variables are here particularly taken into account for total extraction: (i) the design of the extraction vessel and (ii) the solvent used to carry out the extraction. Results showed PAHs recoveries (EPA priority list) ranged between 77 and 101%, accounting for more than 95% for most of the PAHs here studied, as compared with the values obtained after soxhlet extraction. Taking into account the results reported in this work we recommend a revision of the EPA guidelines for PAHs extraction from solid matrices with focused ultrasound, so that these match the analytical minimalism concept.

Keywords: Soxhlet extraction; Focused ultrasound; PAHs; Sediment; Analytical minimalism

1. Introduction

PAHs are common pollutants known to promote the formation of carcinogenic molecules in the living organisms [1]. In addition, PAHs bioaccumulate in the food chain. PAHs form when carbon-containing materials are incompletely burnt, in the production of coal tar derivatives, and also enter the environment as a result of spills of oil from tankers, refineries and offshore oil drilling sites. PAHs are present in all compartments of the environment: atmosphere, soil and water.

The United Estates Environmental Protection Agency (USEPA) recommends the determination and quantification of its 16 priority PAHs pollutants list in its Method 8310 liquid chromatography with fluorescence or absorbance UV detection, while in Method 8100 suggests gas chromatography with mass spectrometry detection [2]. The HPLC methods allow the determination of non-volatile, thermally labile molecules without derivatization. Moreover, samples are not destroyed in the analysis and it is

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Table 1
Selected references: PAH determination in solids with probe sonication

Matrix	Comments: (i) probe device; (ii) sample mass (g)/volume (ml); (iii) extracting agent/sonication time (min); (iv) range PAHs extracted (%)/number of PAHs; (v) other comments; (vi) reference.
Urban dust	(i) 3/4-in probe (240 W); (ii) 0.25/30 (ratio 0.008); (iii) ciclohexane/10; (iv) 100/9; (v) multiple extraction (three times); (vi) [25].
River sediment	(i) Not specified (50% duty cycle, control ouput of 10); (ii) 30/100 (ratio 0.3); (iii) methylene chloride-acetone (1 + 1)/3 min; (iv) 74.4–125/EPA-PAH list for sediments plus others (v) low recoveries for one certified soil. (vi) [26].
Fly ash	(i) 1/2-in probe (350 W, full power setting); (ii) 3/10 (ratio 0.3); (iii) benzene (two extractions)/0.5; (iv) 55–100/4; (v) low recovery for tracers; (vi) [27].
Stack ash	(i) 1/2-in probe (92.5 W) (ii) 0.102/10; (iii) methylene chloride (one extraction)/2.5; (iv) 73%/benzo(a)pyrene; (v) total extraction (91.5% and 93.9%) at 74 W with seven extractions, and 149–230 mg); (vi) [28].
Soils	(i) Not specified (375 W, 50% duty cycle); (ii) 30 g soil + 30 g anhydrous sodium sulphate/75 ml (ratio 0.43); (iii) acetonitrile/75; (iv) not specified (extraction with soxhlet was not different)/18 PAHs; (v) Coal tar contaminated reference materials; (iv) [23].

possible to collect fractions for further analysis by other methods.

For PAHs characterization in solid matrices such as sediments, a sample preparation step is mandatory. This has been made by two classical methods, namely soxhlet and sonication. The USEPA solid–liquid extraction method 3550B recommends for PAHs extraction an ultrasonication with probe. However, ultrasonication with probe, referred to as focused ultrasound (FU), has been rarely reported for PAHs extraction from solid matrices in literature, the ultrasonic bath being the device chosen for most researchers to carry out ultrasonic extractions. The USEPA method 3550B distinguishes two different approaches, depending on the expected concentration of organics in the sample. For low organics concentration a 30 g sample mass/100 ml extraction solvent ratio (three extraction times) is recommended, while 2 g/10 ml (one extraction time) is the recommended mass to volume ratio for high organics concentration. However, different approaches can be found in literature as it can be seen in Table 1. Some drawbacks have been discussed in literature dealing with the implementation of this extraction procedure: (i) it is a time-consuming procedure [3], (ii) it requires large volumes of organic solvents (often toxic, the purchase and disposal being expensive) [3], (iii) there is a progressive decrease in the efficiency of the ultrasonic extraction due to ageing of the sonication probe, frequent replacement being necessary [4], and (iv) low recoveries were reported when comparing ultrasonication with the new extraction procedures that have been developed as alternatives to the classical approaches. The new extraction procedures include supercritical fluid extraction (SFE) [5,6], pressurized liquid extraction (PLE) [7,8], microwave-assisted extraction in closed vessels (MAE) [9,10], focused microwave extraction in open vessels (FMWE) [11] and also sub-critical water extraction [3,12–14]. Most of the drawbacks mentioned above are the result of an incomplete understanding of the ultrasonic probe properties and handling and, since good extraction efficiencies are not achieved, this may lead to inaccurate evaluation of the potential health and environmental risks of the zones from where the sediment has been taken.

This work shows a new perspective in the use of FU for PAHs extraction from solid matrices within the minimalism concept [15]. Moreover, it gives the details of micro solid–liquid extraction with focused ultrasound (μSLEFU) as a technique, as well as of the effect of several experimental parameters on recovery: temperature, extraction solvent/s, shape of extraction vessel, sonication time, sonication power, and probe diameter.

2. Considerations for enhanced extractions with μSLEFU

2.1. Essentials

The solid–liquid extraction with FU is a consequence of the cavitation phenomena: ultrasonic waves crossing a liquid cause the generation, growth, oscillation, splitting and implosion of numerous tiny gas bubbles (cavitation bubbles). As a result of cavitational bubble implosion, extreme temperatures and pressures are generated. In addition, solute thermolysis and formation of hydrogen peroxide and reactive radicals have also been cited in literature [16]. Furthermore, when a cavitating bubble collapses near the surface of a solid sample particle, micro-jets of solvent, propagate toward the surface at velocities greater than 100 m s^{-1}, cause pitting and mechanical erosion of the solid surface, this leading to particle rupture (i.e., disruption), and consequently, to smaller particle size [17]. This characteristic of FU is of great importance since it makes this extraction method unique: the smaller the particle size the higher the total area exposed to the solvent. Hence, zones that otherwise should be not exposed to the liquid phase are subjected toextraction.

2.2. Dead zones and reactor design

The so called “dead zone”, i.e., zones in which cavitation is not achieved, is one of the most important parameters to consider when using probe sonication. This is overlooked by most authors and is also neglected in the USEPA method 3550B guide-lines. On the one hand, the distribution of ultrasonic waves is an important parameter to be considered for the process optimization, since variation of the local cavitational activity and the resulting pressure field with axial

Table 2
Fluorescence detector wavelength program used in this study

Step	Exλ(nm)	Emλ (nm)	PAH determined	Retention time (min)
1	280	330	Naphthalene, acenaphthalene + fluorine	0–70.95
2	246	370	Phenanthrene	7.95–9.03
3	250	375	Anthracene	9.03–10.10
4	280	420	Fluoranthrene	10.10–11.23
5	270	390	Pyrene	11.23–14.45
6	265	380	Benzo(a)antracene, chrysine	14.45–18.00
7	290	430	Benzo(b), benzo (k)fluoranthene; benzo(a)pyrene	18.00–25.00
8	290	410	Dibenzo(a,h)antracene, benzo(g,h,i)perylene	25.00–29.00
9	300	500	Indene(1,2,3) cd pyrene.	29.00–35.00

and radial distance from the horn tip has been demonstrated [18,19]. On the other hand, the high energy density produced from a single probe tip is unlikely to be capable of delivering enough energy density to affect the whole of a large reacting volume [16, p. 80]. Thus, it is of paramount importance to maintain the distance between the horn tip and the wall container as short as possible in order to avoid the "dead zones". As a consequence of these considerations, the design of the reaction vessel in which the sonication is carried out is of major concern. Eppendorf-type vessels should be employed, since the small diameter raises the liquid level of the sample without increasing the volume, thereby allowing the probe to be inserted deep enough into the solution; on the other hand, some energy will be reflected back when the wave sound impinges directly on the conical-shaped bottom. In addition, when the extraction solvent volume is minimised, the number of impacts among particles induced by cavitation increases, causing an enhancement in the analyte extraction as a consequence of the disruption effect.

2.3. *Temperature, pressure and cavitations effects*

The use of high temperatures increases the capacity of solvents to dissolve analytes. Moreover, faster diffusion rates occur with the increase in the temperature of the extraction. Furthermore, the use of high temperatures helps the disruption of the strong solute-matrix interactions which involve Van der Waals forces, hydrogen bonding and dipole attractions between the solute molecules and active sites on the matrix. On the other hand, cavitation is improved as the temperature is lowered when the ultrasonic power setting of the generator is constant [16, p. 68]. Hence, a compromise between temperature and cavitation must be achieved.

3. Experimental

3.1. *Apparatus*

A Branson sonifier 150 ultrasonic cell disruptor/homogeniser (22.5 kHz, Branson Ultrasonics Corporation, USA) equipped with a 3- and a 6-mm titanium microtip was used. Ultrasonic energy irradiation was fixed at any desired level using a power setting in the 40–70% range. The Sonifier 150 has a digital LCD display which provides a continuous information of the watts delivered to the end of the probe (within the 6–7 W range). A 35 W, 35 kHz Transsonic (Elma, Germany) ultrasonic cleaner bath was used for ultrasonic performance comparative purposes. The temperature of the samples was estimated by placing

Table 3
Extraction (ng/g dry weight) with dichloromethane

PAH extracted	Soxhlet ($X \pm$ S.D.) (ng/g dry weight, $n = 5$)	1	2	3	4	ΣPAH	R (%)
Naphthalene	1584 ± 118	550	152	93	43	838	53
Acenaphthene+fluorene	5518 ± 586	1946	204	n.d	n.d.	2150	39
Phenanthrene	1747 ± 128	630	94	17	1	742	42
Anthracene	3229 ± 220	1068	251	n.d	n.d.	1319	41
Fluoranthrene	4253 ± 359	1844	72	61	29	2006	47
Pyrene	3324 ± 290	1254	140	n.d.	n.d.	1394	42
B(a)anthracene	3470 ± 330	1423	113	n.d	n.d.	1536	44
Chrysene	3050 ± 184	1275	27	n.d.	n.d.	1302	43
B(b)fluoranthene	1657 ± 123	747	31	n.d.	n.d.	778	47
B(k)fluoranthene	5158 ± 372	2232	n.d.	n.d.	n.d.	2232	43
B(a)pyrene	1037 ± 80	322	n.d.	n.d.	n.d.	322	31
D(a,h)anthracene	3739 ± 240	1909	262	32	10	2213	60
B(ghi)perylene	2326 ± 220	1029	2	n.d.	n.d.	1029	44
Indene	2326 ± 220	1556	125	n.d.	n.d.	1681	72

Four trials in the same sample.

Table 4
Extraction (ng/g dry weight) with acetonitrile

PAH extracted	Soxhlet ($X \pm$ S.D.) (ng/g dry weight, $n = 5$)	1	2	3	4	ΣPAH	*R* (%)
Naphthalene	1584 ± 118	1035	298	225	169	1727	109
Acenaphthene+fluorine	5518 ± 586	4484	247	n.d.	n.d.	4731	86
Phenanthrene	1747 ± 128	1495	140	35	23	1692	97
Anthracene	3229 ± 220	2580	111	n.d.	n.d.	2692	83
Fluoranthrene	4253 ± 359	3708	410	152	91	4361	103
Pyrene	3324 ± 290	2683	158	n.d.	n.d.	2841	85
B(a)anthracene	3470 ± 330	2836	236	n.d.	n.d.	3071	89
Chrysene	3050 ± 184	2589	206	n.d.	n.d.	2795	92
B(b)fluoranthene	1657 ± 123	1355	64	n.d.	n.d.	1419	86
B(k)fluoranthene	5158 ± 372	4283	161	n.d.	n.d.	4445	86
B(a)pyrene	1037 ± 80	703	n.d.	n.d.	n.d.	703	68
D(a,h)anthracene	3739 ± 240	3249	444	180	115	3988	107
B(ghi)peryilene	2326 ± 220	1819	86	n.d.	n.d.	1905	82
Indene	2326 ± 220	2578	224	n.d.	n.d.	2801	89

Four trials in the same sample.

a glass thermometer immediately after the first ultrasonic extraction was completed.

PAHs analyses were conducted by HPLC with fluorescence detection, using an Agilent model 1100 series, with a Lichrospher PAHs column (Merck, Darmstadt, Germany, cat. num. 1.50149.0001, 250 mm × 4.6 mm, 5 μm particle diameter). The 15 PAHs to be quantified were separated by gradient elution. Details of the program used are given in Table 2. The excitation and emission wavelength pairs were programmed to change during the analytical run to optimise the detection of each component. Data collection and integration were carried out using HPLC D ChemStation software (Hewlett-Packard, Wokingham, Berkshire).

3.2. Reagents

Milli-Q ultrapure water was used throughout. Dichloromethane, acetone, acetonitrile and hexane HPLC grade solvents were purchased from Merck.

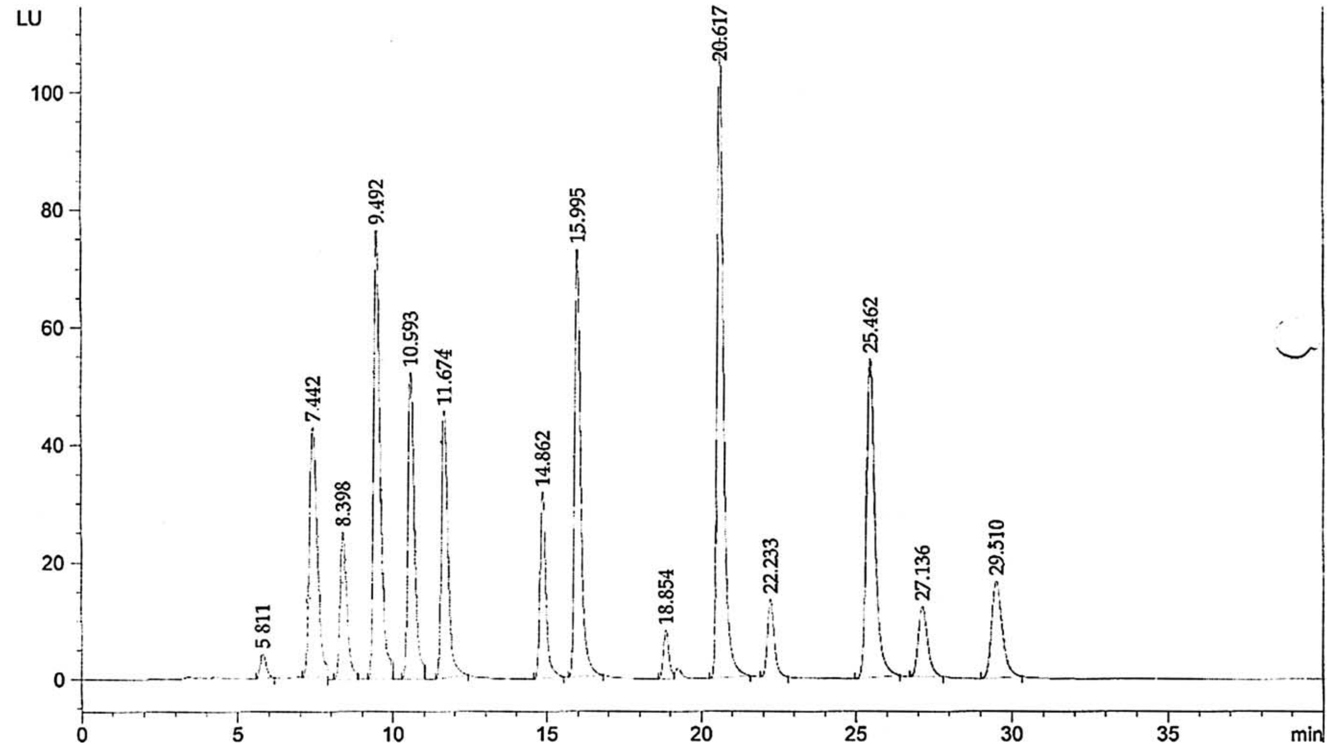

Fig. 1. Chromatogram of surface sediment: flow rate of 0.7 ml min^{-1}; fluorometric detection see Table 2; Peaks: naphthalene, acenaphthalene + fluorine, phenanthrene, anthracene, fluoranthrene, pyrene, benzo(a)antracene, chrysine, benzo(b) fluoranthene, benzo (k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)antracene, benzo(g,h,i) perylene, indene(1,2,3) cd pyrene.

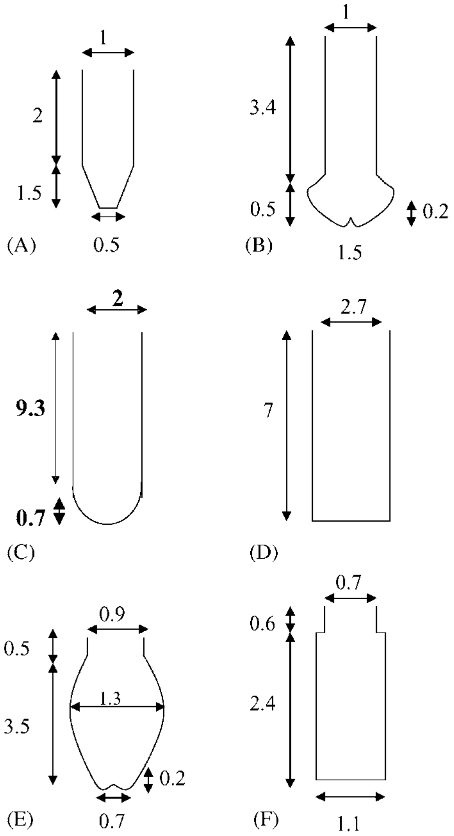

Fig. 2. Vessel designs (cm) used in this work.

3.3. Analytical quality assurance

Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. By running method blanks, under the conditions of the analysis, all of these materials were demonstrated to be free from interferences. Interferences co-extracted from the samples may vary considerably from source to source. The sample selected for parameter optimization was chosen because in preliminary experiments we found no interferences, centrifugation plus filtration being enough to avoid particles. Therefore, although a general cleanup technique is provided as part of this method, other types of samples may require additional cleanup procedures.

The 15 parent PAHs determined were Naphthalene, Acenaphthene + fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)-fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Di-Benzo(a,h)anthracene, Benzo(ghi)perylene, and Indeno(123-cd)pyrene. The 15 PAHs determined were identified on the basis of retention time matches with an external standard mixture run on a weekly basis. All the PAHs here studied (total of 15) are included in the US Environmental Protection Agency (USEPA) priority pollutant list of 16 parent PAHs compounds.

Reference material EC-1 from the National Water Research Institute was used for validation purposes.

Concentrations of PAHs in sediments are expressed on a dry weight basis. Procedural blanks were analysed routinely alongside each batch of daily samples to allow for reagent contamination, if needed.

In addition to the Supelco PAHs standard solution which was analysed each week, a home-made PAHs standard mixture was also analysed at routine intervals to ensure that the quantification standard did not deteriorate with time. Daily, the PAHs standard was added to sample in order to check recoveries. Once the analytes were spiked, extractions were started to prevent the loss of volatile compounds. Recovery calculations of spiked samples were done as described by Lopez-Avila et al. [20] and were always higher than 90%.

3.4. Specimen collection

Contamined samples of surface sediment (0–2 cm deph) were collected at the shoreline of the Tagus estuary (Lisbon, Portugal) in selected sampling points. The samples were

Table 5
Extraction conditions used in this work

Extraction vessel	Sample mass (gr)/volume (ml)	Solvent type	Cooled
A1-polypropylene	0.125/1.3	1 ml acetone/*n*-hexane (1:1, v/v) + acetonitrile	no
A2-polypropilene	0.125/1.3	1 ml acetone/*n*-hexane (1:1, v/v) + acetonitrile	yes
A3-polypropilene	0.125/1.3	Acetonitrile	yes
A4-polypropilene	0.125/1.3	Acetonitrile	no
B1-glass	0.125/1.3	1 ml acetone/*n*-hexane (1:1, v/v) + acetonitrile	no
B2-glass	0.125/1.3	1 ml acetone/*n*-hexane (1:1, v/v) + acetonitrile	yes
B3-glass	0.125/1.3	Acetonitrile	no
B4-glass	0.125/1.3	Dichloromethane	no
C1-glass	0.125/4	Dichloromethane/acetone (1:1, v/v)	no
C2-glass	0.125/4	Dichloromethane/hexane (1:1, v/v)	no
C3-glass	0.125/4	Dichloromethane/acetonitrile (1:1, v/v)	no
C4-glass	0.125/4	Acetonitrile	no
D3-glass	0.478/18	Dichloromethane	no
D4-glass	0.441/5	Dichloromethane	no
E1-glass	0.125/1.3	Acetonitrile	no
F1-glass	0.125/1.3	Acetonitrile	no

transferred to hexane-rinsed glass jars with aluminium foil inserts, and kept refrigerated [21]. One sample was taken from the shoreline at 25 km from Lisbon in a non polluted area called "Costa da Caparica". Once in the laboratory, sediments were dried in a ventilated oven for 48 h at 45 °C and then milled, ground and sieved to get rid of stones and pieces of wood. The samples were bottled and preserved in the dark at 4 °C. Extractions were carried out within 30 days after sampling. For validation purposes one certified certificate material was used.

3.5. Sample treatment

3.5.1. Soxhlet extraction

0.7 g of dried sediment sample was Soxhlet extracted with 180 ml of dichlorometane for 24 h. The extract was concen-

Table 6
Recoveries (on basis in ng g^{-1} dry weight) of PAHs in sediment material as a function of the solvent extraction conditions used

PAH extracted	Extraction procedure							
	Soxhlet ($X \pm$ S.D.) (ng/g dry weight, $n = 5$)	Recovery (focused ultrasound/Soxhlet × 100, $X \pm$ S.D.)						
		A1 ($n = 3$)	A2 ($n = 2$)	A3 ($n = 2$)	A4 ($n = 2$)	B1 ($n = 5$)	B2 ($n = 2$)	B3 ($n = 2$)
Naphthalene	1584 ± 118	71 ± 28	69 ± 11	84 ± 7	72 ± 1	77 ± 8	62 ± 22	89 ± 5
Acenaphthene+fluorene	5518 ± 586	73 ± 18	58 ± 11	81 ± 7	78 ± 3	76 ± 9	51 ± 5	90 ± 4
Phenanthrene	1747 ± 128	78 ± 7	58 ± 13	82 ± 6	83 ± 1	81 ± 6	51 ± 3	91 ± 3
Anthracene	3229 ± 220	77 ± 16	53 ± 11	79 ± 5	81 ± 2	80 ± 7	48 ± 3	91 ± 3
Fluoranthrene	4253 ± 359	96 ± 16	63 ± 11	86 ± 6	94 ± 6	99 ± 6	59 ± 7	99 ± 2
Pyrene	3324 ± 290	100 ± 1	61 ± 13	84 ± 6	90 ± 2	100 ± 6	55 ± 8	97 ± 2
B(a)anthracene	3470 ± 330	96 ± 11	60 ± 13	82 ± 5	92 ± 0	98 ± 8	57 ± 9	98 ± 2
Chrysene	3050 ± 184	97 ± 11	62 ± 13	84 ± 7	93 ± 3	98 ± 6	60 ± 9	100 ± 3
B(b)fluoranthene	1657 ± 123	106 ± 19	65 ± 12	86 ± 8	93 ± 6	103 ± 7	62 ± 8	98 ± 1
B(k)fluoranthene	5158 ± 372	102 ± 18	63 ± 14	84 ± 8	93 ± 6	99 ± 7	60 ± 9	98 ± 1
B(a)pyrene	1037 ± 80	99 ± 14	53 ± 17	74 ± 9	83 ± 6	98 ± 9	49 ± 7	94 ± 5
D(a,h)anthracene	3739 ± 240	104 ± 14	69 ± 13	88 ± 8	96 ± 4	102 ± 7	66 ± 8	101 ± 1
B(ghi)perylene	2326 ± 220	107 ± 5	65 ± 17	82 ± 9	92 ± 4	104 ± 9	60 ± 10	98 ± 5
Indene	2326 ± 220	110 ± 17	72 ± 18	90 ± 9	97 ± 9	105 ± 10	68 ± 10	97 ± 8

		B4 ($n = 1$)	C1 ($n = 2$)	C2 ($n = 2$)	C3 ($n = 2$)	C4 ($n = 2$)	D1 ($n = 1$)	D2 ($n = 2$)
Naphthalene	1584 ± 118	38	39 ± 2	33 ± 6	55 ± 3	60 ± 3	58	66 ± 11
Acenaphthene+fluorene	5518 ± 586	35	45 ± 4	32 ± 8	49 ± 0	63 ± 4	67	69 ± 8
Phenanthrene	1747 ± 128	47	52 ± 6	38 ± 7	53 ± 1	70 ± 5	57	60 ± 4
Anthracene	3229 ± 220	40	50 ± 6	34 ± 6	47 ± 0	65 ± 5	60	61 ± 5
Fluoranthrene	4253 ± 359	52	63 ± 8	46 ± 5	60 ± 0	75 ± 4	65	70 ± 4
Pyrene	3324 ± 290	52	62 ± 8	47 ± 5	60 ± 1	68 ± 3	63	64 ± 7
B(a)anthracene	3470 ± 330	55	62 ± 9	46 ± 5	59 ± 1	73 ± 3	71	68 ± 8
Chrysene	3050 ± 184	57	63 ± 7	48 ± 5	63 ± 0	82 ± 11	70	68 ± 8
B(b)fluoranthene	1657 ± 123	55	68 ± 8	50 ± 3	65 ± 0	74 ± 3	72	71 ± 9
B(k)fluoranthene	5158 ± 372	55	65 ± 8	38 ± 20	63 ± 0	74 ± 4	78	73 ± 9
B(a)pyrene	1037 ± 80	38	55 ± 10	39 ± 3	44 ± 0	55 ± 3	65	63 ± 12
D(a,h)anthracene	3739 ± 240	64	72 ± 9	55 ± 5	68 ± 0	78 ± 4	78	75 ± 18
B(ghi)perylene	2326 ± 220	55	70 ± 9	52 ± 3	63 ± 1	67 ± 4	70	68 ± 10
Indene	2326 ± 220	59	72 ± 9	54 ± 4	66 ± 0	73 ± 3	80	82 ± 13

		E1 ($n = 3$)	F1 ($n = 2$)
Naphthalene	1584 ± 118	77 ± 2	54 ± 3
Acenaphthene+fluorene	5518 ± 586	82 ± 2	59 ± 7
Phenanthrene	1747 ± 128	88 ± 4	62 ± 6
Anthracene	3229 ± 220	81 ± 4	58 ± 7
Fluoranthrene	4253 ± 359	97 ± 2	70 ± 7
Pyrene	3324 ± 290	93 ± 3	69 ± 7
B(a)anthracene	3470 ± 330	97 ± 6	70 ± 7
Chrysene	3050 ± 184	97 ± 4	69 ± 7
B(b)fluoranthene	1657 ± 123	96 ± 5	71 ± 7
B(k)fluoranthene	5158 ± 372	98 ± 5	71 ± 7
B(a)pyrene	1037 ± 80	86 ± 6	57 ± 8
D(a,h)anthracene	3739 ± 240	103 ± 5	74 ± 7
B(ghi)perylene	2326 ± 220	98 ± 6	72 ± 8
Indene	2326 ± 220	101 ± 5	67 ± 16

trated first to about 2 ml in a rotary evaporator and then transferred to a Kuderna-Danish evaporator and concentrated to 1 ml with a gentle stream of nitrogen.

3.5.2. Focused ultrasonic extraction

In previously decontaminated home-made glass cups with a conical-shaped bottom, ca. 0.125 g were weighed and 1.3 ml of acetonitrile was added. Each sample was irradiated during 2 min with ultrasound using the 3-mm microtip. The sample was then centrifuged at 2500 rpm during 2 min. The supernatant was withdrawn and the extraction procedure was repeated twice. All fractions were collected, filtered and concentrated in a rotary evaporator (2 min). The final concentration to a volume of 0.6 ml was achieved with a N_2 flux (15 min). The samples were then ready for analysis by HPLC.

4. Results

Tables 3 and 4 show that the number of ultrasonic extractions per sample needed to achieve maximum PAHs recoveries was 3, either with dichloromethane (recoveries ranging from 31 to 72%), or with acetonitrile (recoveries ranging from 62 to 109%) [22,23,25].

Since HPLC with fluorescence detection is a quite selective technique, to speed up the whole procedure we also tested the possibility of developing the sonic extraction directly in acetonitrile (which is the mobile phase in the HPLC procedure), and then investigate the samples without a clean-up step. A typical chromatogram is shown in Fig. 1.

With dichloromethane the results reported in literature are contradictory. In fact, at concentration levels of $\mu g\,g^{-1}$, extraction efficiencies of 100% have been reported, whereas at $ng\,g^{-1}$ levels less than 30% of several representative PAHs (four-ring and large PAHs) were extracted by sonic treatment [25,27]. This can be explained on the basis of the saturation of the cavity bubbles with the PAHs in their gas phase: induced thermolysis inside the cavity destroy the molecules, so the gas-liquid equilibrium is never reached. Hence, rapid and continuously, cavitation induce PAHs molecules to be vaporized, transported and decomposed inside the bubbles. We suggest that this process is faster at low concentrations, this explaining the low recoveries achieved at low concentrations, mainly due to the degradation of the PAHs and not because of a lack of extraction efficiency in the ultrasonication.

Extracts were filtered after centrifugation. Recoveries obtained with and without filtration were compared and did not show significant differences ($n = 3$, t-test, $P = 0.95$).

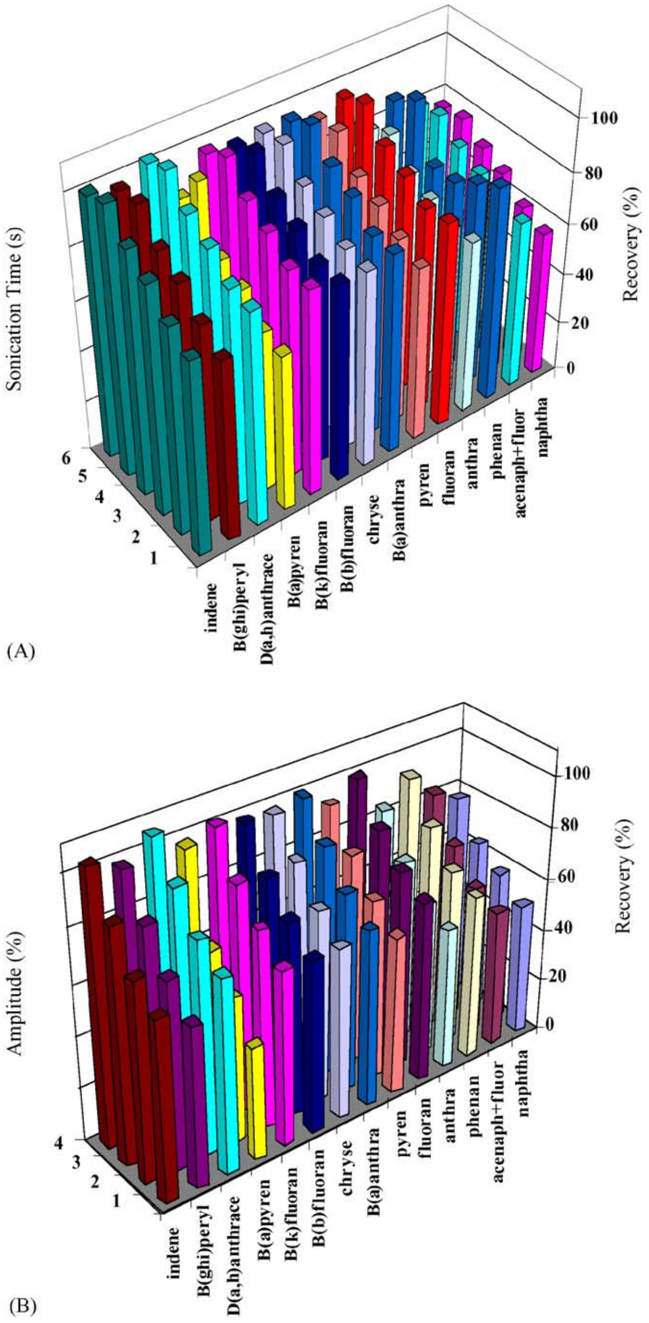

Fig. 3. Optimization of the ultrasonic extraction. (A) Optimization of the ultrasonication time: 1 = 15 s; 2 = 30 s; 3 = 60 s; 4 = 75 s; 4 = 90 s; 5 = 120 s; sonication amplitude 40%. (B) Optimization of the sonication amplitude: 1 = 10%, 2 = 20%; 3 = 30%; 4 = 40%; sonication time 90 S. Sample mass 0.125 g; probe diameter: 3 mm and sample volume: 1.3 ml for conditions (A) and (B).

During the optimization procedure different solvents, solvent mixtures and vessels with different shapes were tested to evaluate the performance of FU micro-extraction. The designs and characteristics are depicted in Fig. 2. Extraction conditions are described in Table 5. Design A corresponds to a classical eppendorf cup made from polypropylene, designs B and E have a bump in its base and were home-made from glass. These latter designs assist sonic mixing by dispersing the sonic waves as they impinge upon the bump and are reflected from the base. Design C has a spherical bottom whilst designs D and F have a flat-bottom. Recipients A, B, E and F were specially designed to work with low volumes, to reduce the dead zones during sonication. Moreover, the distance from the probe sonicator to the wall container was kept as small as possible to maintain the particles inside the area under the effects of cavitation. Cavitation phenomena decreases axially and radially rapidly. The low sonication volume allows to induce more collisions among particles, hence, increasing the total area exposed to solvent extraction (see above). Recipient C was used with a volume of 4 ml, whereas recipient D was used with larger volumes (5 ml and 18 ml). In the latter case, 18 ml, a 6 mm diameter probe was used.

The results are shown in Table 6. From these we may conclude that among the variables that control the total extraction of the PAHs (i) temperature, (ii) vessel shape, and (iii) solvent used are particularly important.

As far as temperature is concerned, recoveries using cooling conditions were below those adequate (A2: 58–73%, B2: 48–70%, F1: 54–74%). When cooling conditions were used in conjunction with acetonitrile, the recoveries were higher, A3: 84–90%. However, when no cooling was employed, A4, the recoveries were up to 6–14% higher.

Concerning vessel shape eppendorf-type containers (A, B, C), these were found appropriate to achieve total extractions, whereas the extraction efficiency using design F was the lowest of all attempts done with containers with a total volume lower than 2 ml. This is important when performing micro-extractions with FU, since we observed that the particles climbed the ultrasonic tip when a flat bottom (case F) was used, leading to poor cavitation contact and, hence, to lower extraction efficiencies. Sonications carried out with containers C and D led to recoveries below 70%. This low extraction may be the result of the “dead cavitation zones” due to the high volume to be sonicated in these cases (4, 5 and 18 ml). As an example, the distance between the probe and the wall container in the case of design C is 8.5 mm, in the case of D it is 12 mm, whereas the distances for containers A, B, E and F are 3.5, 3.5, 3 and 2 mm, respectively.

Concerning the solvent used, extractions with acetonitrile, either pure or associated with other reagents, were found optimal and gave the highest recoveries. For the other combinations used (see Table 5) recoveries were below 80% for all the PAHs. An especial explanation is required for cases F1 and C3, in which acetonitrile was used. The low recoveries reported here can be attributed to the flat bottom (case F1), or to the high vessel volume (case C3).

It was reported elsewhere [24] that cavitation is achieved at lower acoustic intensity as a consequence of the rise in vapour pressure associated with solvent heating. Unfortunately, the effects resulting from cavitational collapse are also reduced. In other words, to get maximum sonochemical benefit any experiment should be conducted at as low temperature as is feasible or with a solvent of low vapour pressure. Since, as previously stated, cooling should be avoided, the low vapour solvent pressure plays an important role to achieve high extraction efficiency. Acetonitrile has a vapour pressure, VP, of 9700 Pa at 293 K, whereas dichloromethane has a VP of 47400 Pa at the same temperature. This difference may explain why under similar conditions for extraction of PAHs with focused ultrasound.

4.1. Optimization of the ultrasonic extraction

The sonication time and the sonication amplitude were optimised for micro-extractions using vessel design E, no cooling conditions and ciclohexane as solvent reagent. As can be seen in Fig. 3A, the sonication time was studied in the range 15–120 s, but 75 s was enough to achieve recoveries of 90–100% for most of the PAHs tested. The time chosen as optimum was 90 s. During the sonication procedure it was observed that the energy delivered by the ultrasonic generator to the ultrasonic probe to achieve the desired amplitude was

Table 7
Validation of the proposed methodology

PAH	Costa de Caparica Sediment		Recovery (%)	Reference material EC-1			Recovery (%)	t_{exp}
	Soxhlet values[a]	Sonication values[a]		Certified values[b]	Soxlet values ($n=2$)[b]	Sonication values ($n=4$)[b]		
Naphthalene	253	160	63	–	–	–	–	–
Acenaphthene+fluorine	444	349	79	–	–	–	–	–
Phenanthrene	178	165	93	15.8 ± 1.2	15012 ± 1051	14111 ± 564	89	5.98
Anthracene	84	74	87	1.2 ± 0.3	1141 ± 80	970 ± 48	81	9.58
Fluoranthrene	34	33	98	23.2 ± 2.0	22575 ± 1582	21898 ± 452	94	2.99
Pyrene	41	41	101	16.7 ± 2.0	17201 ± 1376	16340 ± 557	95	1.29
B(a)anthracene	33	43	129	8.7 ± 0.8	8923 ± 803	8834 ± 516	102	0.52
Chrysene	64	66	103	9.2 ± 0.9[c]	10001 ± 600	9301 ± 363	101	0.56
B(b)fluoranthene	40	36	90	7.9 ± 0.9	7325 ± 514	6895 ± 633	87	3.17
B(k)fluoranthene	7	0	n.d.	4.4 ± 0.5	4325 ± 297	4282 ± 195	97	1.20
B(a)pyrene	18	16	89	5.3 ± 0.7	5538 ± 445	4984 ± 358	94	1.76
D(a,h)anthracene	51	51	99	1.3 ± 0.2[c]	1433 ± 125	1453 ± 101	111	3.03
B(ghi)perylene	48	46	95	4.9 ± 0.7	5193 ± 559	5141 ± 319	105	1.5
Indene	18	14	82	5.7 ± 0.6	5877 ± 631	5701 ± 331	97	0.01

$t_{exp} = 3.18$, $P = 0.05$.

[a] ng g^{-1}.

[b] μg g^{-1}.

[c] Informative values.

decreasing, while the temperature of the solution was increasing as consequence of the sonication itself. Thus, for 120 s, the maximum time use for sonication, the watts being delivered to the end of the probe were automatically adjusted from 9, at the beginning of the sonication procedure, to 4 W at the end. The effect of the sonication amplitude was also assessed performing the extractions at different values, ranging from 10 to 40%. As can be seen (Fig. 3 B) the best results were obtained with the amplitude value of 40%. Higher amplitudes led to several problems such as sputtering of the sample from the vessel and/or to decoupling of the probe from the sonication process, as consequence of the high and rapid increment on the solution temperature.

4.2. Analytical application

The optimised procedure was applied to a sediment taken from the Costa de Caparica, Portugal, and to a reference material kindly provided by Dr. Riekkola, University from Helsinky, Finland. These results are shown in Table 7.

5. Conclusions

In this work, we demonstrate that ultrasonic extraction of PAHs can be achieved matching the principles of the ultrasonic minimalism, that is, keeping the analytical process as simple as possible with a minimum consumption of resources. Extractions are achieved in short times, with low sample masses (0.125 g) and low reagent volumes (4 ml). Minimum waste products are generated, and the procedure is easy to implement and handle. The success of the extraction depends on the extraction vessel design that must be eppendorf-type, and on the reagent used for extraction, in our case acetonitrile was found adequate. Considering the results here reported we recommend a revision of the EPA guidelines for the PAHs extraction from solid matrices with focused ultrasound so that these match the analytical minimalism concept.

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