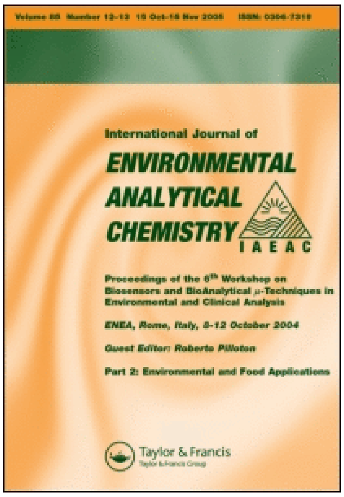

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Micellar Extraction of Polycyclic Aromatic Hydrocarbons from Certified Marine Sediment

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MICELLAR EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM CERTIFIED MARINE SEDIMENT

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In the present study the extraction of polycyclic aromatic hydrocarbons from a certified marine sediment (SRM 1941a) with a micellar medium of polyoxyethylene 10 lauryl ether by microwave- and ultrasound-assisted method has been evaluated. The analysis of extracts has been carried out by HPLC with fluorimetric detection and wavelength programming. Hydrocarbons with more than three rings gave average recoveries of 77.4% and 86.5%, by microwave- and ultrasound-assisted methods respectively, with a mean relative standard deviation better than 6.2%.

Keywords: Microwave- and ultrasound-assisted extraction; Micellar medium; Certified reference marine sediment; Polycyclic aromatic hydrocarbons

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants mostly originating from various emission sources. Due to the mutagenic and carcinogenic potential of some representatives compounds of this group, the content of PAHs has been monitored in a variety of environmental matrices.

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Marine sediments are one of most commonly used matrices to develop new extraction methods since they act as reservoirs of environmental pollutants and are highly variable and complex[1].

The extraction of organic pollutants from marine sediments can be achieved with a number of established methods. Soxhlet extraction[2,3] has a number of drawbacks because sample preparation requires 12–24 h and high consumption of toxic organic solvents. Sonication method[4] also requires large volumes of organic solvents.

In the last few years, new extraction techniques have been developed to reduce the volume of solvents required for extraction, to improve the precision of analyte recoveries, and to reduce extraction time and sample preparation costs. In this sense, microwave-assisted extraction (MAE) is a viable alternative to the conventional techniques[5]. At present, it appears that MAE exhibits many substantial improvements in analytical sample preparations because it requires much lower volumes of organic solvent, reduces extraction time and increases sample throughput through extraction of multiple samples[6–11].

The PAHs extraction and/or preconcentration with micellar systems have been recently proposed[12–16]. The use of this methodology offers some advantages for the extraction of PAHs compared with solvent extraction: safety and cost benefits; easy disposal of the surfactant; ability to concentrate PAHs with high recoveries and very high preconcentration factors and compatibility with micellar and aqueous mobile phases in HPLC.

Recently, we have proposed the use of a micellar medium of polyoxethylene 10 lauryl ether (POLE) to extract PAHs from spiked marine sediments. The use of microwaves[17] or ultrasounds[18] accelerates the extraction process. After the optimization, both methods gave successful results.

The analysis of certified reference materials is a key issue when an analytical method is implemented. When a novel method of analysis or a modified method is proposed, the best way to achieve the target results is by analysing reference materials. This has been the approach of the present paper. This paper follows previous works from our group on the use of micellar media for extraction and preconcentration of PAHs from environmental samples.

In this paper, we present a comparison of the procedures previously developed attending the extraction times and the obtained recoveries of PAHs. The obtained results using a certified reference marine sediment show that PAHs extraction with a micellar medium of POLE, assisted by microwave or ultrasound, allows to diminish the extraction times compared with conventional methods, it does not require the use of potentially hazardous organic solvents and the extracts can be chromatographied without subsequent clean-up steps.

EXPERIMENTAL

Chemical and reagents

Naphthalene (Nph) was supplied by Merck (Darmstadt, Germany); acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (A), fluoranthene (Ft), pyrene (Py), benz(a)anthracene (B(a)A), chrysene (Chy), benzo(b)fluoranthene (B(b)Ft), benzo(a)pyrene (B(a)Py), dibenz(a,h)-anthracene (diB(a,h)A) and benzo(ghi)perylene (B(ghi)Per) were supplied by Aldrich–Chemie (Beerse, Belgium); indeno(1,2,3-cd)pyrene (I(1,2,3-cd)Py) was supplied by Supelco (Bellefonte, PA, USA), and benzo(k)fluoranthene (B(k)Ft) was supplied by Fluka (Buchs, Switzwerland). They were dissolved in Ethanol (Merck).

Polyoxyethylene 10 lauryl ether (POLE) was supplied by Sigma (St. Louis, MO, USA).

For chromatographic analysis, acetonitrile of HPLC grade (Merck) and water purified with a Milli-Q system (Millipore, Bedford, MA, USA) were used throughout.

Marine sediments and spiked procedure

Marine sediments were collected from the coasts of Canary Islands between 8 and 15 m in depth. After sieving, fractions of sediment with particle size under 250 μm were taken. The spike of this sediment sample was performed as follows: An amount of 0.8 g of sediment was mixed with 1 mL of ethanol containing known concentrations of each PAH, which was slowly added to form a dough that was mechanically stirred for some minutes. The sample was then stored in the dark and allowed to dry. The concentration of PAHs spiked oscillate between 0.63 and 22 μg/g on dry-weight basis.

Reference material

The methods were validated using the standard reference material SRM 1941a, provided by the National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA). This reference material is a well-characterized marine sediment which is certified for 23 individual PAHs[19].

Instrument and apparatus

All measurements were made with a Waters (Milford, MA, USA) Model 600 Multisolvent Delivery System equipped with a Waters U6K sample injector and a Waters 474 Scanning fluorescence detector. Autoanalysis 2.4 (Sciware, vcerda@p01.uib.es) software was used for acquisition data. The analytical column was a Vydac 201TP54 reversed-phase C_{18}, 5 µm, 300 Å (25 cm × 4.6 mm I.D.) with a µBondapak C_{18} guard column, 10 µm, 125 Å (20 mm × 3.9 mm I.D.) supplied by Waters.

The microwave extraction system was a CEM (Matthews, NC, USA) MIDS 2000 microwave digestion system. The system delivers approximately to 630 W (100%) of microwave energy at a frequency of 2450 MHz at full power and provides constant feedback control of extraction conditions through continuous monitoring of pressure data on a control vessel. The system is provided with method and data storage capabilities and a printer. The microwave extraction vessels were CEM PFA PTFE-lined advanced composite digestion vessels used for sediments extraction. The vessels are constructed with a PFA PTFE liner and a cap for high-purity analysis that are capable of sustaining temperatures up to 200°C and pressure of 13.9 bar.

Ultrasound-assisted extractions were performed with an ultrasonic bath Selecta-Ultrasons (Barcelona, Spain).

Microwave-assisted extraction method

Once the reference sediment was accurately weighed (100 mg), 10 ml aqueous micellar solution of POLE 0.02 M is added. After ensuring that a new rupture membrane was in place, the extraction vessel was closed and introduced into the microwave cavity. Extraction was performed at a fixed maximum pressure inside the vessels of 2.03 bar and with a 60% of microwave oven power. After fixed extraction time of 5 min was completed, the vessels were allowed to cool at room temperature before they were opened. The supernatant was quantitatively transferred, filtered through 0.45 µm HV Durapore® Membrane Filter (Millipore) and an aliquot of 25 µl was directly injected in the HPLC system.

Sonication extraction method

The reference sediment was accurately weighed (100 mg) and sonicated for 210 min with 10 ml of POLE 0.1 M in an ultrasonic bath with fixed power. The supernatant was quantitatively transferred, filtered through 0.45 µm HV Durapore® Membrane Filter (Millipore) and an aliquot of 25 µl was directly injectecd in the HPLC system.

Chromatographic method

The HPLC method used for the determination of PAHs consisted of a gradient elution procedure with a fluorescence wavelength program. A mixture of acetonitrile and water with a linear gradient from 55 to 100% of acetonitrile over 30 min and 100% acetonitrile for 10 min, at a flow-rate of 1 ml min^{-1}, was as mobile phase. The wavelength program of the detector is shown in Table I.

RESULTS AND DISCUSSION

HPLC Analysis

As a consequence of using the certified sediment instead of the spiked sediments, and so, lower amount of sediment, it is necessary to work with diluted PAHs solutions. For getting the adequate quantification limits we had to substitute the visible–UV detector by a fluorescence detector in the HPLC system.

Figure 1 shows (A) the chromatogram obtained for a standard solution of 15 PAHs under described experimental conditions and (B) the chromatogram obtained after microwave-assisted extraction of the reference sediment. The different PAHs gave satisfactory retention times with RSD between 0.64 and 1.23%.

TABLE I Wavelength program of the detector used for the PAHs determination

Time (min)	*Wavelength* (nm)		*Compounds*
	Excitation	*Emission*	
0	222	329	Naphthalene, Acenaphthene
10.7	226	310	Fluorene, Phenanthrene
13.9	248	370	Anthracene, Fluoranthene
17.7	275	419	Pyrene, Benzo(a)anthracene
22.1	273	384	Chrysene
28.5	254	451	Benzo(b)fluoranthene, Benzo(k)fluoranthene
32.2	288	406	Benzo(a)pyrene, Dibenzo(a,h)anthracene
35.9	289	422	Benzo(g,h,i)perylene
40.0	297	496	Indeno(1,2,3-cd)pyrene

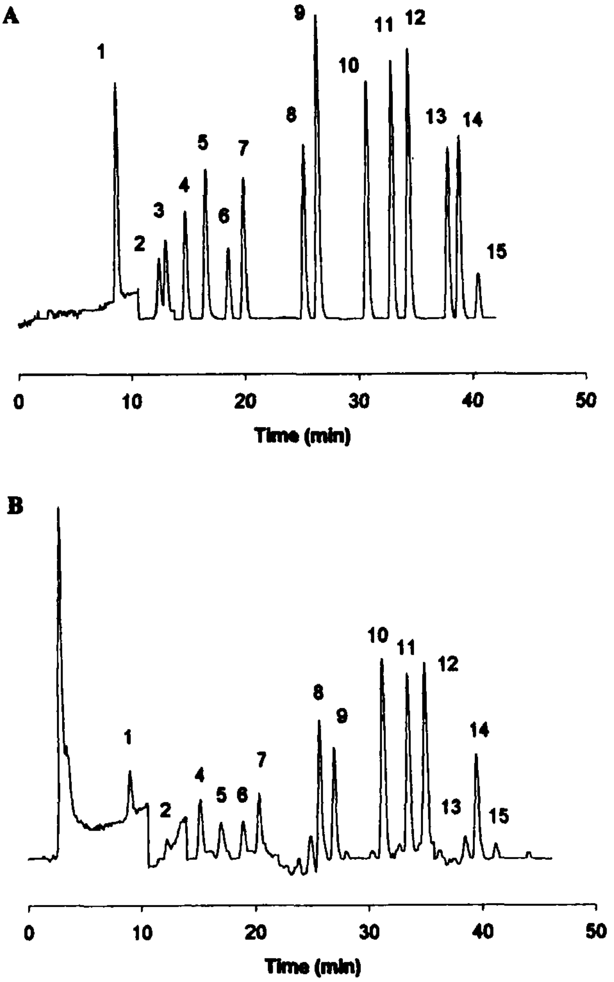

FIGURE 1 (A) Chromatogram of a standard solution of fifteen PAHs. (B) Chromatogram of the reference sediment extracted by microwaves using the optimum conditions given in Table III. The assignment of peaks is as follows: 1, Nph; 2. Ace; 3. Fl; 4. Phe; 5. A; 6. Ft; 7. Py; 8. B(a)A; 9. Chy; 10. B(b)Ft; 11. B(k)Ft; 12. B(a)Py; 13. diB(a,h)A; 14. B(ghi)Per and 15. I(1,2,3-cd)Py. For chromatographic conditions, see text.

Calibration graphs were constructed by plotting the peak-area against the PAH concentration. A linear relationship with $R > 0.990$ was always obtained. Quality parameters for the chromatographic method are reported in Table II.

Detections limits are between 0.03 ng/ml for benzo(k)fluoranthene and 1.09 ng/ml for pyrene. They were calculated as three times the standard

TABLE II Quality parameters for the chromatographic method

PAH	*Lineal Range* (ng/mL)	*Slope* ± *SD*	*Intercept* ± *SD*	*R*	$S_{Y/X}$	*LOD* (ng/mL)	*RSD* (%)
Nph	0.9–15.0	0.861 ± 0.027	0.564 ± 0.224	0.995	0.533	0.36	6.14 (6.5)
Ace	2.0–10.0	0.343 ± 0.010	−0.150 ± 0.060	0.994	0.113	0.83	5.65 (4.5)
Fl	1.3–10.0	0.499 ± 0.009	−0.103 ± 0.054	0.997	0.122	0.62	5.40 (4.5)
Phe	1.0–10.0	0.789 ± 0.021	−0.217 ± 0.119	0.995	0.273	0.49	1.02 (4.0)
A	0.4–5.0	2.058 ± 0.027	−0.059 ± 0.074	0.998	0.189	0.15	1.73 (2.5)
Ft	3.5–35.0	0.197 ± 0.002	−0.098 ± 0.044	0.999	0.108	0.66	2.59 (12.0)
Py	2.0–35.0	0.427 ± 0.019	−0.011 ± 0.228	0.996	0.302	1.09	2.64 (12.0)
B(a)A	0.5–5.0	2.548 ± 0.086	0.609 ± 0.215	0.995	0.458	0.22	0.20 (1.6)
Chy	0.4–15.0	1.015 ± 0.032	0.099 ± 0.213	0.995	0.504	0.42	1.73 (7.0)
B(b)Ft	0.6–10.0	1.743 ± 0.029	0.310 ± 0.155	0.998	0.402	0.16	2.20 (4.0)
B(k)Ft	0.3–5.0	3.383 ± 0.086	0.119 ± 0.222	0.994	0.594	0.03	1.93 (2.5)
B(a)Py	0.3–3.0	3.330 ± 0.115	−0.483 ± 0.204	0.994	0.383	0.30	1.68 (2.0)
DiB(a,h)A	1.4–15.0	0.920 ± 0.025	−0.114 ± 0.156	0.998	0.257	0.80	1.44 (6.5)
B(ghi)Per	1.0–10.0	1.307 ± 0.049	0.108 ± 0.231	0.996	0.358	0.52	4.23 (5.0)
I(1,2,3-cd)Py	4.0–30.0	0.179 ± 0.003	−0.106 ± 0.044	0.998	0.092	0.69	4.29 (10.0)

R: Correlation coefficient ($n = 6$). $S_{Y/X}$: Standard error of the estimate. LOD: Limit of detection.
RSD (%): Relative Standard Deviation for the chromatographic measures ($n = 6$). Values in parentheses are the concentration (ng/mL) used.

deviation of a signal corresponding to a solution with PAHs concentration near to the lowest value of the lineal range of each PAH. The chromatographic system repeatability has been also evaluated, and the obtained relative standard deviations are between 0.20 and 6.14% for the different PAHs.

To study the influence of the micellar medium on the chromatographic determination of the selected PAHs, the different standards have been prepared in POLE 0.02 or 0.1 M, depending on the extraction method used. All the studied compounds appeared to be completely separated from the surfactant in the chromatograms obtained after injections of the standards. There is a signal of low intensity that affect the more volatile PAHs but they can be quantified without problems except fluorene (as it can be seen in Fig. 1B). On the other hand, these PAHs are the ones poorly extracted in both methods. Nevertheless, no influence of the micellar medium was observed on the measured fluorescence intensity for PAHs with the number of rings higher than three. Furthermore, no changes on C_{18} column efficiency was observed after repeated injections of the surfactant.

Extraction from spiked samples

In previous papers[17,18] we proposed the extraction of PAHs from marine sediments by microwave and ultrasound-assisted extraction with a micellar medium of POLE as a prior step to the their determination by HPLC with UV detection. In both cases, the optimal set of operational conditions was found by using the factorial design experiments. This optimization was targeted to obtain the maximum recovery of each PAH from spiked sediment samples prepared in the laboratory.

Advantages and disadvantages of both methods can be established by means of a comparison between the obtained results. The parameters selected for reaching the optimum experimental conditions for the extractions are shown in Table III. It can be seen that, for the same amount of sediment, ultrasound-assisted extraction requires a higher surfactant concentration and higher extraction times.

As can be seen in Table IV, there are significative differences between the extraction efficiency of PAHs of lower molecular weight and PAHs with more than three rings. The comparison of PAHs recoveries when six independent extractions from spiked sediments were carried out, showed that there were important losses of the more volatile PAHs. In this sense, naphthalene and acenaphthene gave recoveries near zero. Fluorene and phenanthrene, using microwave- or ultrasound-assisted extraction, gave maximum recoveries of 49 and 34% respectively.

TABLE III Optimum parameters of the extraction methods

Parameter	*Microwave extraction*		*Ultrasonic extraction*	
	Spiked sediment	*SRM-1941a*	*Spiked sediment*	*SRM-1941a*
Extraction time (min)	5	5	30	210
Extractant volume (ml)	10	10	10	10
Extractant Concentration (M)	0.02	0.02	0.10	0.10
Maximum pressure (bar)	2.03	2.03	–	–
Power (W)	378	378	–	–
Amount of sample (mg)	800	100	800	100

TABLE IV Average recoveries and percent RSDs of PAHs after microwave- and ultrasound-assisted extraction

PAHs	*Microwave Extraction*		*Ultrasonic Extraction*		D_{rel}
	Recovery (%)	*RSD (%)*	*Recovery (%)*	*RSD (%)*	
Fl	11.51	62.13	5.20	42.50	54.8
Phe	49.45	29.37	33.70	10.70	31.8
Ft	87.79	7.00	89.60	7.02	−2.06
Py	87.81	6.58	86.67	6.36	1.29
B(a)A	85.70	3.78	99.20	2.63	−15.8
Chy	95.04	1.78	99.70	3.76	−4.90
B(b)Ft	100.73	3.51	106.60	1.71	−5.83
B(b)Py	84.37	5.77	94.13	4.25	−11.6
DiB(a,h)A	99.43	3.48	98.00	3.05	1.44
B(ghi)Pe	98.45	3.93	97.55	2.07	0.91
I(1,2,3-cd)Py	95.97	4.02	103.13	2.40	−7.46
Recovery (%)*	92.81		97.18		
SD*	6.38		6.25		

*Values of average recovery and Standard Deviation of PAHs with a number of rings higher than three.

Different reasons could contribute to the low recoveries of the more volatile PAHs, the degradation of PAHs, which may occur during microwave or sonication extraction due to the heating, or the loss of these hydrocarbons during the sediment fortification process. Studies carried out with PAHs solubilized in a micellar medium of POLE and subjected to the established extraction conditions, show the stability of PAHs versus microwave and sonication extraction. It is possible that during the fortification process, some hydrocarbons could volatilize. Furthermore, the different interactions that PAHs present with the organic and/or inorganic matter of the sediment, could influence their solubility or diffusivity into the micellar medium.

However, PAHs with the number of rings higher than three gave percentages of recoveries higher than 85%. The average recoveries are slightly higher with ultrasound-assisted extraction. Both extraction methods have similar standard deviations (SD).

On the other hand, the average recoveries have been evaluated for each individual extraction method using as representative parameter the constant systematic error[20]:

$$D_{\mathrm{rel}}(\%) = [(X_1 - X_2)/X_1] \cdot 100$$

being X_1 and X_2 each PAH recovery in microwave- and ultrasound-assisted extraction respectively.

PAHs with the number of rings higher than three like pyrene, dibenz(a,h) anthracene and benzo(ghi)perylene gave values for D_{rel} lower than 2%. PAHs like fluorene and phenanthrene gave D_{rel} values of 54.8 and 31.8% respectively, but the extraction efficiency is not very good. Rest of PAHs gave negative D_{rel} values which oscillate between 2 and 16%, which means that ultrasound-assisted extraction is more efficient than microwave-assisted extraction.

Taking into account average recoveries for hydrocarbons with more than three rings, the Student test shows that there are not significative differences ($p = 0.05$) between the average recoveries found by the ultrasound-assisted extraction and the theoretical value of 100%. However, the F-test at a confidence level of 95% shows that there are not significative differences between the varianes of both methods.

Extraction of PAHs from reference material

The cerfitied marine sediment (SRM 1941a) has been used to validate the two extraction methods above compared. It was not possible to use the same optimized conditions for spiked sediments and for the certified marine sediment because both sediments have different characteristics, specially different organic matter content. In this sense, it was necessary to use a lower amount of certified sediment and, as a consequence, to decrease the PAHs concentration in solution. For this reason a new optimization of the extraction methods was made.

The proposed extraction methods have several important advantages like short extraction times, specially when compared to conventional ones. To compare this parameter, and establish its influence in extraction efficiency, the certified sediment was subjected to microwave- and ultrasound-assisted extraction with POLE.

Figure 2 shows the average recoveries with a 95% confidence intervals as a function of the extraction time. In microwave-assisted extraction there are slight increases in the percentage of recoveries when passing from 5 to 30 min, but it is not statistically significant. In the other hand, in ultrasound-assisted extraction there are significative increases in recoveries when passing from 30 to 210 min. No more significative increases in recoveries took place with higher extraction times. For these reasons, the extraction times were established to 5 and 210 min for microwave- and ultrasound-assisted extraction respectively. These conclusions have been established with experiences using 100 mg of certified sediment. When the sample amount increases until 200 mg the recoveries does not suffer significative differences.

As shown in Table III, the rest of the optimized experimental parameters with spiked sediments are also adequate conditions for the certified sediment. In general, the obtained results show that in microwave-assisted extraction the extraction time is not a critical parameter if this time is equal or higher than 5 min. This extraction time is really short when it is compared with conventional extraction methods. When ultrasound-assisted extraction is used, it is necessary to find the optimum extraction time when the sediments characteristics are different, specially the organic matter content and the size grain distribution.

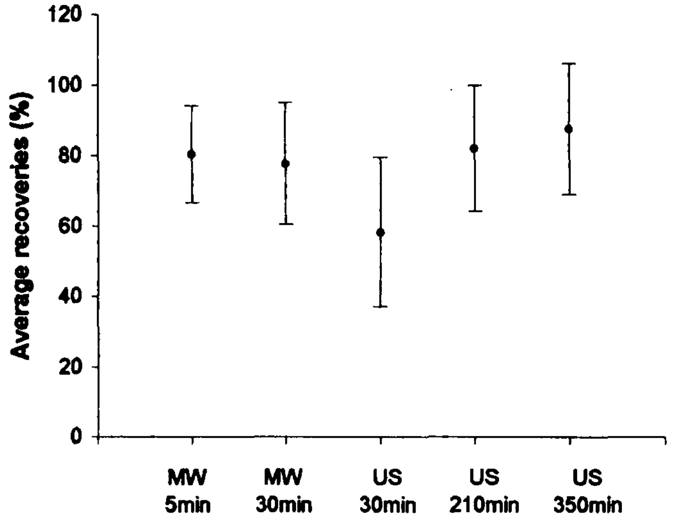

FIGURE 2 Average recoveries with 95% confidence intervals as a function of the extraction time, for microwave- and ultrasound-assisted extraction.

TABLE V Average recoveries and percent RSDs for the SRM 1941a marine sediment

PAH	*Certified values* (ng/g)	*SD*[a] (ng/g)	*RSD*[b] *(%)*	*Microwave Extraction*				*Ultrasonic Extraction*			
				Experimental values (ng/g)	*SD*[c] (ng/g)	*RSD*[d] *(%)*	*Recovery (%)*	*Experimental values* (ng/g)	*SD*[c] (ng/g)	*RSD*[d] *(%)*	*Recovery (%)*
Ft	981	78	7.9	895	48	5.4	91	819	12	1.5	83
Py	811	24	2.9	533	32	6.0	66	683	59	8.6	84
B(a)A	427	25	5.8	244	15	6.4	57	215	18	8.3	50
Chy	380	24	6.3	386	37	9.7	102	381	11	2.8	100
B(b)Ft	740	110	14.9	543	22	4.1	73	591	9.1	1.5	80
B(k)Ft	361	18	4.9	266	19	7.0	74	298	12	4.0	82
B(a)Py	628	52	8.3	304	11	3.8	48	462	29	6.3	74
DiB(a,h)A	73.9	9.7	13.1	73	2.0	3.4	99	85	12	14.8	115
B(ghi)Per	525	67	12.8	374	1.1	0.1	71	511	15	3.0	97
I(1,2,3-cd)Py	501	72	14.4	465	4.2	0.8	93	501	56	11.3	100
Mean						4.7	77.4			6.2	86.5

[a] Standard deviation of certified values.
[b] Relative standard deviation of certified values.
[c] Standard deviation of experimental values.
[d] Relative standard deviation of experimental values.

Table V shows the experimental recovery values for the considered 9 PAHs in the established experimental conditions. To determine accuracy and precision of the methods, six portions of 100 mg of the standard reference sediment was extracted with 10 ml of POLE 0.02 and 0.10 M by microwave- and ultrasound-assisted extraction method respectively, using the optimized extraction times. Volatile PAHs recoveries are practically zero or very low, as in spiked sediments. Hydrocarbons with a number of rings higher than three have significatively higher average recoveries with ultrasound- than with microwave-assisted extraction. The obtained average recovery, 86.5% with ultrasounds, can be considered as high, although it is 10% less than the one obtained with spiked sediments. The precision obtained with both methods have been estimated with the RSD. In general, for each considered hydrocarbon this parameter has the same value, or lower than the certified one.

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