Determination of Polycyclic Aromatic Hydrocarbons in Coastal Sediments from the Porto Region (Portugal) by Microwave-Assisted Extraction, Followed by SPME and GC–MS

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Abstract

A simple low-cost, analytical method based on microwave-assisted extraction of sediments, followed by solid phase micro-extraction and gas chromatography mass spectrometry, was developed and validated for the quantification of sixteen polycyclic aromatic hydrocarbons (PAHs) in marine and estuarine sediment samples. The PAHs were those included in the United States Environmental Protection Agency (US EPA) priority list. Method detection limits were between 0.07 and 0.76 µg/kg dry weight (dw), which makes the current method suitable for environmental analysis. Sediments screened for PAHs from the Douro River estuary and the Porto seacoast exhibit total concentrations that ranged from 58.98 to 156.45 µg/kg dw, and from 51.98 to 54.79 µg/kg dw, respectively. The presence of almost all human carcinogenic PAHs in the analyzed areas indicate that these sediments can be considered polluted, suggesting that future monitoring programs together with an effective coastal management program must be implemented to guarantee the safe usage of the current areas for fishing and bathing.

Introduction

Environmental pollution by polycyclic aromatic hydrocarbons (PAHs) is an issue of great concern because of their huge dissemination in the environment (1,2) due to lipophilicity and high toxicity to aquatic organisms and humans (3,4). These facts alerted international health organizations and lead to the inclusion of 16 parent PAHs in the United States Environmental Protection Agency priority list (US EPA) (5). Also, restrictive legislation has been implemented by the European Union (Water Framework Directive 2000/60/EC, WFD 2000) (6), and the mobilization of many economic and technical resources were recently considered necessary for the environmental monitorization of these pollutants (7). For this purpose, several methods for the evaluation of PAHs in organisms, water and soils have been developed (7,8). Nonetheless, among all environmental compartments, the evaluation of PAHs in sediments are extremely relevant, because certain members of the PAH class remain well preserved in ancient sediments, indicating that sorption onto them can greatly prolong those pollutants lifetime in the environment (9). Additionally, the evaluation of PAHs in marine/estuarine sediments allows for the establishment of possible risks for aquatic life and human health, especially during coastal activities (bathing waters, aquaculture, etc.) (7). Therefore, the development of analytical methods, highly sensitive and accurate to monitor these compounds in complex environmental matrices which are needed under the legal European directives and regulations that establish maximal concentrations of 0.1 µg/L for the sum of PAHs in drinking water (EU Council Directive 98/83/EC) (10). Recently, the WFD 2000 also established regulatory limits for PAH concentrations for inland, transitional, and coastal waters; unfortunately, no limits have been set yet for coastal (marine and estuarine) sediments, although they are the final receivers of these compounds in the aquatic environment (2,11–13). In estuaries, anthropogenic activities are the main sources of a number of PAHs, some of them being considered potentially carcinogenic for humans; in particular benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene, and benzo[ghi]perylene (14). Because the first step of all PAHs sediment analysis involves its concentration and purification from complex matrices, several methods are reported in literature (7). Among them, Soxhlet extraction has been the most popular (7,15,16). Nonetheless, this technique involves huge volumes of solvents, as well as a time-consuming analysis. Therefore, other techniques such as ultrasonic extraction, microwave dissolution, pressurized liquid extraction, and super-

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critical fluid extraction have been implemented (18,19). However, an extra extraction step is still required after these procedures. In this sense, solid phase microextraction (SPME) techniques started gaining interest for coastal sediment samples, as it requires less extraction solvent volumes than those reported for solid phase extraction procedures (7,20). Furthermore, the analysis and detection of PAHs is mostly based on chromatographic techniques [i.e., high-performance liquid chromatography (HPLC) or gas chromatography combined with mass spectrometry (GC–MS) (7)]. Although similar in sensitivity, GC–MS provides a higher selectivity, as it can be adjusted by the selection of the appropriate molecular and fragmentation ions, therefore avoiding interferences from co-extracted sample materials.

Thus, the present study aimed to develop a microwave-assisted extraction (MAE) protocol to extract 16 PAHs from coastal sediments, followed by a SPME and a GC–MS identification and quantification procedure. To test the applicability of the developed MAE followed by SPME and GC–MS method, 16 US EPA priority PAHs were quantified; naphthalene (N), acenaphthylene (Ace), acenaphthene (AcP), fluorene (F), phenanthrene (P), anthracene (A), fluoranthene (Fluo), pyrene (Py), benzo[a] anthracene (BaA), chrysene (Ch), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno [1,2,3-cd]pyrene (IP), dibenzo[a,h]anthracene (DBA), and benzo [g,h,i]perylene (Bper) in several sediment samples taken from the Douro River estuary and from the nearby coast (Porto), from where no such kind of data has been reported yet.

Experimental

Chemicals and materials

An acetone solvent (chromatographic grade) was acquired from Merk (Darmstadt, Germany). Ultrapure water was supplied by a Milli-Q water system (conductivity = 0.054 µS/cm, at 25°C). The Florisil (pro-analysis grade), used for cleaning the microwave extracts, was from Sigma-Aldrich (Steinheim, Germany) and 1.0 µm glass fiber filters were purchased from Millipore (Dublin, Ireland).

Reference standards

PAHs standards (EPA TCL Polynuclear Aromatic Hydrocarbons mix) were purchased from Supelco (Bellefonte, PA). This stock mixture contained the 16 US EPA prioritary PAHs, each at 2000 µg/mL in dichloromethane–benzene (1:1, v:v). The surrogate standard was a mixture containing naphthalene-d_8 (N-d_8), acenaphthene-d_{10} (Ace-d_{10}), phenanthrene-d_{10} (P-d_{10}), chrysene-d_{12} (Ch-d_{12}), and perylene-d_{12} (Per-d_{12}), which were added to the samples before extraction and used as internal standards (IS) for quantification. This standard mix solution at 2000 µg/mL in dichloromethane was purchased from Supelco (Bellefonte, PA). Both stock solutions were kept in the dark at –20ºC to minimize their potential decay. All standard solutions were stable for one year and evidence of decomposing was never observed. Stock solutions were used to prepare working standard solutions for calibration and spiking experiments. From the stock solutions, six nominal calibration standard mixtures were prepared and spiked in clean coastal sediment samples (i.e., free of all target PAHs) taken from an uncontaminated salt marsh located in an inhabited area of north Portugal (Minho). These fortified matrices were used as calibration standards and to demonstrate the applicability of the method. The range of concentrations added to the sediment matrices, and used to produce the calibration curves, was 20–100 ng/kg. The surrogate IS were added to the spiked sediment samples at 100 ng/kg. The response factors were then calculated using the response obtained from the desorption of a standard solution containing 40 ng/kg of the 16 PAHs of interest and 100 ng/kg of each IS. For precision, accuracy, and recovery assays, three quality control (QC) standard solutions, containing each target PAHs and IS, were prepared and added to clean coastal sediment samples at different concentrations: 30, 70, and 90 ng/kg. The usage of the certified reference material (CRM) supplied by NIST (NIST 1941b, Organics in Marine Sediment) guaranteed the quality of the current protocol.

Sample collection and preparation

Collections took place during March, 2009. The sampling sites were four areas of the Douro River estuary and two marine beaches (Figure 1). Sediment samples were taken into aluminium foil packets from a depth of approximately 50 cm, in low tide. These samples were kept refrigerated (± 4°C) and transported in the dark to the laboratory, where they were divided for physico-chemical assessment and for further PAHs evaluation. The considered physico-chemical parameters included the evaluation of humidity, organic matter, and sediment characterization.

MAE

All target PAHs were extracted from the spiked sediment samples, CRM, and real sediment samples by MAE. This protocol required a common domestic microwave (Panasonic NE1037). Here, all sediment samples were (*i*) dried at room temperature until reaching a constant weight and sieved through a 2 mm metal net to remove large stones; (*ii*) then, approximately 1 g of dry sediment was accurately weight and quantitatively transferred into a teflon vessel (Parr system, model 4782) containing 3 mL of acetone and submitted for 4 min at a potency of 340 W; (*iii*) after 1 h in ice, the last extraction procedure was repeated;

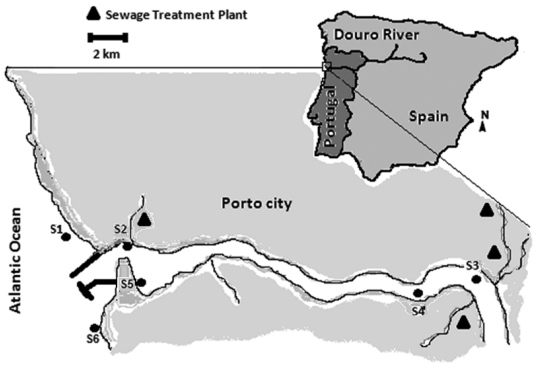

Figure 1. Location of the sampling sites in the Douro River estuary (S2 to S5), and in the Porto seacoast (S1 and S6).

(iv) later on, after another cooler step of 1 h, the extract (ca. 3 mL) was transferred into a 15 mL flask and added with 3 mL of acetone and 300 mg of Florisil; (*v*) finally, the extract was cleaned by filtration through a 25 mm syringe glass fiber filter (1.0 µm, BGB Analytik AG).

SPME

The SPME device and the fibers, coated with polydimethylsiloxane (PDMS) with a 100 µm film thickness, were from Supelco (Bellefonte, PA). Prior to use, the fibers were conditioned, following the instructions provided by manufacturer, in the hot injection port of the GC–MS apparatus at 250°C for 30 min. For cleaning, the fibers were introduced in the hot injection port of the GC–MS at 270°C for 6 min. This protocol was done between different samples to ensure that no contamination occurred. For SPME extractions, the fibers were immersed in a solution in a 15 mL amber glass vials capped with PTFE-coated septa. The final volume of the sample to be extracted was always 15 mL. This way the vial was practically full, ensuring that the fiber was always completely immersed in the solution. To enhance extraction, all samples were continuously agitated with a magnetic stirring bar (1 cm long, PTFE coated) on a stir plate at 600 rpm for a period of 1 h at 60°C.

GC–MS analysis

GC–MS analysis was performed using a gas chromatograph (Varian CP-3800), coupled with an ion trap mass spectrometer (Varian Saturn 2200). A Varian FactorFour (VF-5 ms) fused silica capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) was used for separating the PAHs. The injection port, transfer line, and ion trap temperatures were set at 270°C, 250°C, and 230°C, respectively. Helium carrier gas (99.99 % purity) was maintained at a constant flow rate of 1.3 mL/min. The SPME fiber thermally desorpted in the GC–MS hot injection port. The oven temperatures were programmed from: (*i*) 40°C (initial equilibrium time 4 min) to 150°C at 40°C/min; (*ii*) from 150°C to 280°C at 2.9°C/min (hold time 4 min); and finally (*iii*) from 280ºC to 300°C at 4°C/min. The identification of 16 PAHs was achieved in MS by electron impact ionization on Selected Ion Storage mode (microSIS) and confirmed with the CRM (NIST 1941b, Organics in Marine Sediment) (Figure 2). The target compounds were then quantified by programming seven acquisition groups (Table I). The entire chromatographic analysis took approximately 60 min per run.

Matrix effect

The matrix effect was evaluated by spiking real sediment samples from both marine and estuarine

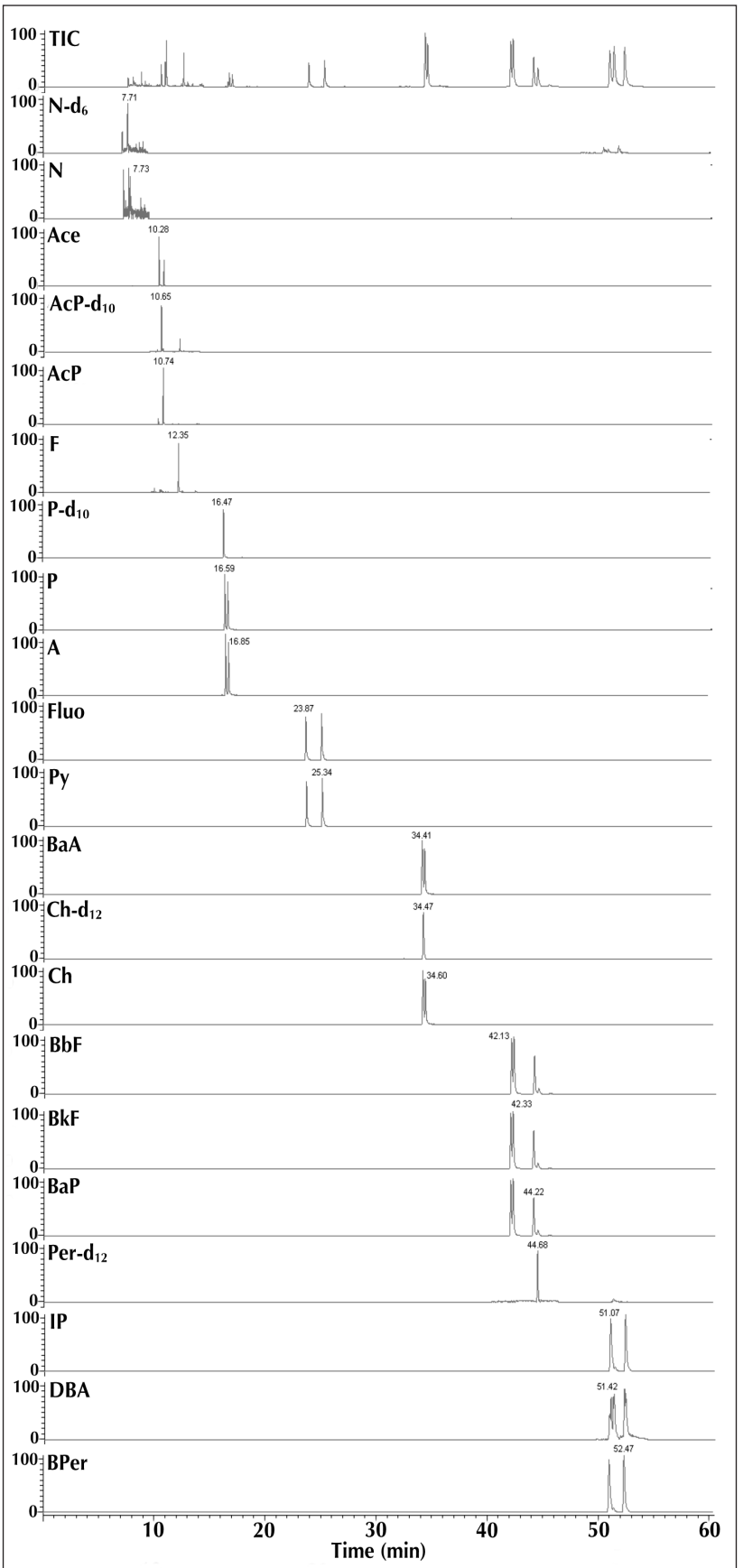

Figure 2. Chromatogram of a standard mixture of the target PAHs at 100 ng/kg and their five deuterated internal standards in selected Ion Storage mode (microSIS). The nomenclature for every 16 PAHs corresponds to that in Table I.

sediments with QC standards, added with both IS at three different levels and injected in triplicate, as referred by the IUPAC validation guidelines (21). The ratio areas and MS spectra of the standards spiked in real samples were compared with those of clean sediment fortified matrices.

Validation studies

The method was validated following the analytical performance parameters established by the International Conference of Harmonisation (CPMP/ICH/281/96) and the IUPAC validation guidelines (21,22). According to that, the validation process included the evaluation of linearity, accuracy, precision, and the limits of detection (LOD) and quantification (LOQ). Clean sediment material, collected from a non-polluted area and submitted overnight to high temperatures (> 100°C), was used as a blank matrix. Accuracy and precision (intra and inter batch) were evaluated analysing three replicates of each QC samples and using the CRM NIST. Precision was expressed in terms of the relative standard deviation (RSD) of the replicate measurements. Accuracy was estimated as the percentage of agreement between the method results and the nominal amount of the added compound. The blank matrices of the sediments, fortified at three QCs concentrations, allowed for the calculation of the recovery and the effectiveness of the extraction step. These values were obtained comparing the QCs concentrations, calculated after the SPME procedure, with those of the equivalent QCs prepared in acetone. The LODs and LOQs were determined evaluating the signal/noise ratio (S/N = 3 for the LODs, and S/N = 10 for the LOQs).

Table I. Quantification and the Diagnostic Ions Used in the GC–MS Analysis. The Inside Brackets Refer to the Relative Abundance of Ions (*m/z*) for Each Target PAH

Compound	t_R (min)	Molar mass	Quantification ions (*m/z*)	Diagnostic ions (*m/z*)	Segment times (min)
N-d_8	7.71	136	136	–	7.20–9.50
N	7.73	128	128	127	7.20–9.50
Ace	10.28	152	152	151	9.50–14.00
AcP-d_{10}	10.65	164	164	–	9.50–14.00
AcP	10.74	154	153	154	9.50–14.00
F	12.35	166	165	166	9.50–14.00
P-d_{10}	16.47	188	188	–	14.00–18.50
P	16.59	178	178	176	14.00–18.50
A	16.85	178	178	176	14.00–18.50
Fluo	23.87	202	202	101	22.00–27.00
Py	25.34	202	202	101	22.00–27.00
BaA	34.41	228	228	226	31.50–36.00
Ch-d_{12}	34.47	240	240	–	31.50–36.00
Ch	34.60	228	228	226	31.50–36.00
BbF	42.13	252	252	126, 250	40.00–46.00
BkF	42.33	252	252	126, 250	40.00–46.00
BaP	44.22	252	252	126, 250	40.00–46.00
Per-d_{12}	44.68	264	264	–	40.00–46.00
IP	51.07	276	276	274	48.50–54.00
DBA	51.42	278	278	248	48.50–54.00
BPer	52.47	276	276	248	48.50–54.00

Results and Discussion

MAE

The sample pretreatment, herein, was optimized for the extraction of sixteen US EPA priority PAHs in sediments collected from coastal areas (marine and estuarine sediments). The analysis of both one blind sample, prepared in triplicate and containing 100 ng/L, and a sample using the CRM, produced accuracies from 80.7 to 111.9%, and precisions (RSD values) from 0.4% to 15.1%; similar values were obtained for all assayed levels within the dynamic range of the calibration curves (Table II). The extraction protocol developed herein produced higher recovery results (70.0% to 109.6%) than others previously published using MAE (20,23,24), and show the advantage of using a conventional domestic microwave system which lowered the overall costs per sample. Moreover, the recoveries (Table III) obtained by this MAE system were higher than those obtained by other extraction techniques, such as ultrasonication (7) (65–100%), or within the same order of magnitude of those using the most recent Soxhlet techniques (14,25). Nonetheless, because the current method used less amounts of organic solvents and lower manipulation-time than the latter techniques, it becomes a particularly advantageous tool when huge amounts of samples are to be processed.

Matrix effects

Humic substances (humic and fulvic acids) constitute the greatest part of the dissolved organic matter in surface waters. They generally impair the efficiency of the sample extraction and the detection of the target compounds in aquatic sediments (26). To confirm that the matrix did not affect the last process, the prepared QC standard solutions, spiked in real sediment samples, were analyzed. Data confirmed that neither the reten-

Table II. Analytical Characteristics of the Optimized GC–MS Method: Calibration Equations, Coefficients of Determination (R^2), LOD and LOQ for all PAHs Spiked in Sediment Samples

Compound	Linearity parameters* Calibration Equations [$y(K_{counts}) = a.x(ng/L) + b$]	R^2	$LOD_{sediments}$ (µg/kg dw)	$LOQ_{sediments}$ (µg/kg dw)
N	$y = 337.0x + 3175$	0.994	0.27	0.91
Ace	$y = 664.3x - 1868$	0.997	0.12	0.40
AcP	$y = 871.2x - 4297$	0.993	0.25	0.83
F	$y = 1141.0x + 1509$	0.994	0.08	0.28
P	$y = 820.9x + 2752$	0.996	0.37	1.23
A	$y = 847.1x - 4454$	0.996	0.07	0.23
Fluo	$y = 3269.0x - 31823$	0.992	0.17	0.56
Py	$y = 1991.1x + 6777$	0.998	0.36	1.18
BaA	$y = 2161.3x + 100526$	0.992	0.76	2.53
Ch	$y = 2038.7x + 6051$	0.995	0.09	0.30
BbF	$y = 1943.2x + 23724$	0.991	0.36	1.20
BkF	$y = 1856.1x + 17457$	0.990	0.07	0.22
BaP	$y = 1638.7x + 6402$	0.990	0.13	0.43
IP	$y = 1377.8x + 8108$	0.991	0.15	0.49
DBA	$y = 835.3x + 478$	0.994	0.13	0.43
BPer	$y = 1745.0x + 8168$	0.991	0.14	0.48

Table III. Intra and Inter-Day Precision, Accuracy, and Recovery Data for PAHs Spiked in Sediment Samples

PAHs* (ng/kg)		1st Day† Accuracy (%)	1st Day† RSD (%)	2nd Day† Accuracy (%)	2nd Day† RSD (%)	3rd Day† Accuracy (%)	3rd Day† RSD (%)	Recovery (%)† (RSD %)
N	30	93.4	15.1	102.5	5.2	105.8	8.3	70.0 (8.6)
	70	88.9	2.9	90.4	5.5	101.6	7.1	86.8 (6.9)
	90	90.0	1.3	103.3	8.6	100.0	2.3	82.6 (6.4)
Ace	30	99.9	9.6	97.7	5.2	98.8	6.1	86.7 (5.1)
	70	95.3	6.1	81.5	2.2	94.9	7.2	99.3 (9.2)
	90	87.4	7.3	86.3	6.6	85.9	3.6	93.4 (2.7)
AcP	30	102.1	10.5	99.1	3.3	99.6	4.3	108.4 (7.7)
	70	96.5	6.5	83.3	1.6	98.6	8.2	84.5 (4.4)
	90	87.4	6.0	82.3	3.8	83.8	4.1	96.8 (1.7)
F	30	93.1	9.0	99.4	1.7	101.0	6.8	100.5 (3.9)
	70	93.0	6.4	83.4	6.5	95.6	2.6	84.1 (6.7)
	90	89.4	9.3	90.0	3.7	86.5	5.8	92.1 (5.3)
P	30	95.3	9.9	95.3	3.0	97.0	13.9	94.6 (2.3)
	70	92.0	5.3	81.4	2.4	96.9	6.5	100.4 (2.8)
	90	87.1	4.8	87.5	2.6	87.7	2.7	98.9 (2.1)
A	30	99.7	9.0	98.3	4.9	98.9	7.1	88.1 (3.5)
	70	97.5	3.4	85.9	4.8	96.2	3.7	94.1 (6.6)
	90	96.7	8.9	95.8	2.6	95.9	3.8	90.3 (4.1)
Fluo	30	98.7	10.8	95.8	9.0	92.9	9.1	94.7 (1.6)
	70	84.5	5.9	81.8	3.4	84.0	3.2	89.4 (3.0)
	90	85.4	10.6	84.9	5.6	83.8	3.7	86.3 (1.5)
Py	30	93.0	6.5	90.6	2.0	88.9	6.0	105.6 (3.0)
	70	94.2	2.9	86.2	5.4	93.4	2.4	89.1 (1.2)
	90	87.3	8.1	81.1	1.3	80.7	3.0	99.7 (1.1)
BaA	30	101.1	9.8	96.1	1.1	84.0	12.3	99.9 (1.3)
	70	111.5	2.1	93.0	8.8	87.0	5.5	89.6 (7.4)
	90	96.7	8.0	86.1	1.9	82.9	6.5	81.9 (4.4)
Ch	30	85.2	0.4	91.5	2.9	93.8	2.3	100.3 (3.2)
	70	97.7	3.6	93.6	7.0	95.4	1.2	94.7 (4.9)
	90	91.8	7.6	90.5	4.8	90.3	5.4	100.7 (8.2)
BbF	30	100.8	3.7	100.1	2.3	96.2	1.7	105.6 (3.4)
	70	105.9	10.5	102.6	1.6	100.8	5.3	83.6 (4.7)
	90	86.7	12.7	85.4	9.0	89.0	13.5	92.2 (6.6)
BkF	30	105.2	4.2	100.8	2.3	94.8	1.3	93.6 (7.9)
	70	99.3	11.3	102.3	1.2	101.8	7.5	90.3 (8.0)
	90	95.9	2.4	95.7	10.5	92.2	9.6	92.2 (6.5)
BaP	30	105.7	9.4	100.4	7.8	101.8	2.3	108.3 (4.7)
	70	104.9	12.3	88.0	3.2	98.2	8.0	102.9 (7.3)
	90	86.6	10.5	84.0	12.0	92.9	1.7	104.0 (6.5)
IP	30	102.6	6.3	106.3	4.6	97.6	1.6	105.5 (4.8)
	70	95.3	4.9	111.9	3.6	101.7	4.7	99.0 (8.4)
	90	87.0	1.9	81.6	3.0	93.0	1.8	107.7 (8.0)
DBA	30	95.2	6.2	100.4	5.2	96.5	3.1	103.8 (0.9)
	70	91.2	4.5	107.1	2.7	100.6	6.5	104.6 (7.2)
	90	101.1	1.4	94.1	1.8	90.6	2.4	103.0 (6.2)
BPer	30	94.3	5.2	96.9	2.6	104.0	3.8	97.8 (1.5)
	70	101.0	3.1	105.1	1.8	98.5	5.9	109.6 (7.8)
	90	93.7	8.6	82.7	1.7	96.0	2.3	104.3 (0.9)

* Spiked in sediments matrix (ng/kg)

† $n = 3$

tion time (tR) nor the ion fragmentation were affected (RSD < 9.2%). Relatively to the last item, all fragments were within the ranges proposed by the 2002/657/EC European Commission Decision (i.e., the tolerances were ± 10% for ions with a relative intensity > 50% of the base peak, ± 15% for ions with a relative intensity of 20–50%, ± 20% for ions with a relative intensity of 10–20%, and ± 50% for ions with a relative intensity of < 10%) (24). Also, peak areas were similar when comparing QC standards spiked in real sediments or in certified sediments. Similar data was obtained when the NIST CRM material was submitted to the current MAE followed by SPME and GC–MS protocol. Thus, it was concluded that external calibration using the 16 PAHs standards mix (EPA TCL Polycyclic Aromatic Hydrocarbons mix) was possible and unaffected by innate matrix components. Consequently, because extractions from real samples were carried out using exactly the same parameters used for the extraction of the 16 PAHs from both CRM and clean sediments, the concentration of each analyte was calculated using relative response factors and the equations in Table II.

Method validation parameters

The linearity and the range of application were established by the calibration curves with coefficients of determination (R^2) values ranging from 0.990 and 0.998 (Table II). It is important to stress that these data are in conformity with all the ICH validation requisites used in this work.

Precision and accuracy

The precision of this method was based on the determination of the repeatability (intra-day assays) and the intermediary precision (inter-day assays) (Table III). In this method, precision ranged from 0.40 to 15.10%, and accuracy from 80.70% to 111.90% for almost all calibration concentrations (Table III). These results are suitable to rank both PAHs and polluted environmental sites, because the values obtained cover the complete sample preparation, and not only a consecutive sequence of injections of the same sample (27).

Limits of detection and quantification

The LODs ranged from 0.07 to 0.76 µg/kg dry weight (dw) and the LOQs from 0.22 to 2.53 µg/kg dw (Table II). These values were considered suitable for environmental analysis, taking into account the usual range of concentrations of PAHs found in sediments (7).

Selectivity

The PAHs showed well resolved peaks when the QCs were spiked in real samples. The identity of each chromatographic peak was confirmed not only by its retention time, but also by its mass spectrum. Similar agreements were found in other environmental validation methods (26).

Application of the present method to real sediment samples

To evaluate the applicability of the validated method to assess the present PAHs, sediment samples were collected from several areas of the Douro River estuary and in the Porto seacoast (Figure 1); which was formerly identified as polluted and screened for other classes of toxicants (28,29). The positive quantification findings in the six sampling sites (S1 to S6) are shown in Table IV. Here, with the exception of BaA, all the other PAHs were detected, and the majority were quantified. The most polluted areas correspond to locals close to a marina (S3) and to a small harbor (S5). There, the total amounts of PAHs were 98.4 µg/kg dw (S5) and 156.5 µg/kg dw (S3), values that are in line with a previous finding of contaminated fish organisms, usually taken in this area for human consumption (30). The physico-chemical data (Table V) of these two sampling stations revealed that, together with S2, they had higher amounts of organic matter (ca. 13%), humidity (ca. 20%), and fine sand than the other sampling zones, in particular those reported for marine sediments (areas S1 and S6). In the sea coast, the sediments were less contaminated than those in the estuary; herein, the total amounts of PAHs were 54.8 µg/kg dw (S1) and 52.0 µg/kg dw (S6). Besides, the presence of potential human carcinogens, such as BbF, BaP, and Bper, in both S1 and S6, which are very popular beaches that attract large crowds, raises questions of toxicity impacts not only for humans, but also for aquatic organisms. Compared with other highly urbanized coastal zones located in China, India, and Porto Rico, the present 16 PAHs ranged from 77 to 305 µg/kg dw in Daya Bay (31) from 132 to 2938 µg/kg dw in Sundarban Mangrove (31), and from 40.4 to 1912 µg/kg dw in Jobos Bay (33). Thus, the finding of considerable amounts of almost all PAHs in the Porto coastal region, with particular emphasis to the four carcinogenic compounds reported in Table IV, has led to the belief that this area is polluted. Consequently, an obvious public health concern rises in the Porto coastal area that deserves attention from local authorities.

Table IV. Environmental Levels of the Measured PAHs Taken from the Sediment Coastal Samples (*n* = 3)

	Environmental levels (µg/kg dw)					
PAHs	S1	S2	S3	S4	S5	S6
N	12.50	13.09	23.62	24.46	23.96	17.59
Ace	< 0.12	< 0.12	3.53	<0.12	2.58	< 0.12
Ac	13.42	13.40	19.21	16.61	18.50	15.52
F	< 0.08	< 0.08	13.37	<0.08	15.13	< 0.08
P	7.01	8.68	10.13	8.07	12.63	7.74
A	< 0.07	< 0.07	2.03	< 0.07	< 0.07	< 0.07
Fluo	1.46	2.64	6.47	1.34	3.64	1.15
Py	0.95	1.88	6.57	0.73	2.72	0.83
BaA	< 0.76	< 0.76	< 0.76	< 0.76	< 0.76	< 0.76
Ch	< 0.09	< 0.09	9.30	< 0.09	1.22	< 0.09
BbF	4.11	3.52	9.82	2.75	3.55	1.85
BkF	2.40	2.46	3.57	2.29	2.34	1.34
BaP	1.34	1.04	7.52	0.84	1.21	0.59
IP	4.79	4.69	12.11	2.87	4.34	2.23
DBA	3.16	3.10	12.92	2.29	3.45	1.52
Bper	3.65	4.48	16.28	2.20	3.14	1.62

Conclusions

A MAE followed by SPME and GC–MS method has been developed and validated for the simultaneous evaluation of 16 US EPA priority PAHs in estuarine and marine sediment samples. International validation guidelines were strictly followed to guarantee the quality of the results. The present methodology proved to be a reproducible and suitable alternative to the conventional methods and other chromatographic techniques, used to the analysis of PAHs in estuarine and marine sediment samples. The feasibility of the developed method was then demonstrated by analysing several sediment samples from the Douro River estuary and the nearby seacoast, proving to be efficient for all selected PAHs. The field data proved that the sampled area is polluted with PAHs, calling for a consistent monitoring and protective program for the evaluation of these compounds.

Acknowledgments

This work was financially supported by FEDER funds through the Competitiveness and Trade Expansion Program (COMPETE), and by National Funds provided by Fundação para a Ciência e a Tecnologia (FCT), via the project PTDC/MAR/70436/2006. Acknowledgements are also due to Francisco Figueiredo for his help in sampling the sediments.

Table V. Physico-Chemical Characteristics of the Sediment Coastal Samples (*n* = 3)

	Sediment dimensions* (%)					
Location	>1400 µm	> 800 µm	> 355 µm	>125 µm	Humidity* (%, w/w)	Organic matter* (%, w/w)
S1	84	5	9	2	2.6	0.9
S2	57	15	21	7	16.5	13.3
S3	69	12	9	10	23.6	14.0
S4	53	25	17	5	2.6	12.9
S5	32	33	27	8	20.0	11.4
S6	77	13	9	1	6.0	1.0

* *n* = 3; Humidity was measured by drying the samples to constant weight at 105ºC; Organic matter was determined by loss-on-ignition at 550ºC in a muffle furnace for 3 h; Sediment characterization was evaluated by sieving.

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Manuscript received November 20, 2010;
revision received May 14, 2011.