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Optimization of the extraction of polycyclic aromatic hydrocarbons from wood samples by the use of microwave energy

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Abstract

A simple and rapid microwave-assisted extraction (MAE) procedure was developed and optimized for benzo[*a*]anthracene, benzo[*e*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene in wood samples. The spiked wood used was prepared 3 months before analysis to simulate weathering processes and to allow the formation of analyte–matrix interaction. The samples, immersed in acetonitrile were irradiated with microwaves in a closed-vessel system. Optimization of the method was achieved by using a factorial design approach on parameters such as extraction time, temperature and sample amount. The analysis of extracts has been carried out by reversed-phase high-performance liquid chromatography with fluorescence detection for quantification and UV-diode-array detection for confirmation. The MAE procedure yielded extracts that could be analyzed directly without any preliminary clean-up or solvent exchange steps.

Keywords: Wood; Microwave-assisted extraction; Extraction methods; Optimization; Factorial design; Polynuclear aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are an extensive group of compounds distributed in the environment due to natural processes or anthropogenic inputs [1,2]. Today's main sources are anthropogenic: incomplete combustion of both fossil fuels and wood and the application of PAH-containing products in wood preservation, ship hulk protection and aluminium production. PAHs are serious environmental pollutants because of their high carcinogenicity and mutagenicity [3,4].

The usual presence of PAHs in environmental samples mixed with a variety of potentially interfering compounds makes it necessary to use separation techniques, usually involving an extraction step and the final separation of each compound using chromatography or electrophoresis, before the detection and quantification stage. The most common way to analyze polycyclic aromatic hydrocarbons is by gas chromatography with mass spectrometry detection, or liquid chromatography with fluorescence detection and/or photodiode-array detection [5–8]. Fluorescence detection is often used for compounds quantification, and UV-diode array detection is used in order to confirm identity of the analytes, since UV spectra are characteristic and different enough for each PAH.

The extractions of PAHs from solid materials can be achieved with several established methods. The most conventional approach is Soxhlet extraction [9].

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This time-consuming preparation technique requires 12–24 h. Moreover, the high consumption of hazardous and toxic organic solvents is another disadvantage of this technique. Currently, other techniques, such as supercritical-fluid extraction [6,10–14], needing much less time and solvent usage, while providing at the same time high recoveries for PAHs, are gradually replacing the conventional Soxhlet technique. More recently microwave-assisted extraction (MAE) has been reported as an advantageous sample preparation technique for various solid samples [15]. The main advantage of MAE is the rapid heating of solvent, in closed vessels, at a temperature above boiling point. This high temperature and pressure allow sample extraction in a few minutes, with very good efficiency. Besides time saving, another important advantage is the use of small solvent volumes.

The most common solvents used for the MAE of PAHs from solid samples are acetone–hexane, dichloromethane, acetone–light petroleum and methanol–toluene [10,16–18]. However, the selection of solvent to extract analytes must take also into consideration the technique that is to be used in the final determination. Most solvents or solvent mixtures used for PAHs extraction appear perfectly suited for gas chromatography, but if liquid chromatography is to be used a solvent exchange would be necessary.

In this study, we used MAE for the extraction of five PAHs from wood in a closed-vessel system. These five compounds were chosen based on their abundance in construction wood. Acetonitrile was used as solvent, so it is not necessary to make any solvent exchange to analyze the obtained extracts by HPLC (avoiding in this way evaporation steps and the associated errors). The procedure was developed and optimized using factorial designs. Parameters studied included extraction time, temperature, and sample weight. A spiked wood material that was prepared 3 months before analysis was used for method development and optimization.

2. Experimental

2.1. Chemicals and reagents

The acetonitrile was HPLC grade obtained from Scharlau (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q water system. The standards used, viz., benzo[*a*]anthracene (B[*a*]A), benzo[*e*]pyrene (B[*e*]P), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P) and benzo[*ghi*]perylene (B[*ghi*]P), were purchased from Supelco (Bellefonte, PA, USA). They were of 99% purity except for B[*e*]P, which was 98.50%. They were employed to prepare individual stock standard solutions of each compound in toluene. Serial dilutions were then carried out, in acetonitrile, to produce the required standard concentrations. Individual solutions were used to form PAH mixtures by appropriate dilution with acetonitrile. The preparation of the standards for calibration was made by weighting, using a calibrated balance.

2.2. Equipment

MAE were done in a MES-1000 (950 W, 2.45 GHz) (CEM, Matthews, USA) microwave extraction system, equipped with 12 closed vessels with a nominal volume of 100 ml. Maximum values for pressure and temperature inside of extraction vessels are 13.8 bar and 200°C.

Chromatographic equipment was a 600E Multisolvent Delivery System (Waters, Milford, MA, USA), a Waters 700 Wisp autosampler and two detectors connected in series: a PU4027 programmable fluorescence detector (Philips, Cambridge, UK) and a Waters 996 diode array detection (DAD) spectrophotometer. The two detectors used were interfaced to a Millenium 2.10 data station (Waters) in order to monitor both signals simultaneously. The temperature of the column was controlled by means of a Waters TCM-HCM oven. Separations were performed using a 250×2.1-mm I.D. Vydac 201 TP52 column, with a particle size of 5 μm, purchased from Hewlett-Packard. A 30×2-mm guard column Vydac 201 TP was employed to protect the analytical column.

2.3. Chromatographic conditions

Acetonitrile and water were used as eluents at a flow-rate of 0.4 ml min^{-1}. The gradient elution program profile was: 50% acetonitrile initially, then increasing linearly to 100% acetonitrile in 10 min, staying there for 22 min and finally back to initial

conditions in 10 min. The column temperature was maintained at 35°C. The fluorescence intensity was measured at excitation and emission wavelengths of 286 and 400 nm, respectively. The DAD spectrophotometer was used over a wavelength range 200–450 nm.

2.4. Wood material and spiking procedure

Optimization experiments were performed using a spiked pine sawdust. After sieving, fractions between 1 mm and 300 μm were taken. One thousand grams of the sieved material were slurried with 6 l of methanol (enough volume to soak the wood) containing a known concentration of PAHs, which was slowly added to form a dough that was mechanically stirred. The sample was allowed to air-dry with occasional mixing at ambient temperature, protected from draughts, for 2 weeks. The wood was then bottled and stored at 0°C for 3 months in dark, before the first extractions. The dry-mass wood concentration, on the basis of added amounts were: B[*a*]A (8.88 μg/g), B[*e*]P (8.98 μg/g), B[*b*]F (7.76 μg/g), B[*k*]F (5.18 μg/g) and B[*a*]P (6.93 μg/g).

2.5. Sample preparation

Irrespective of the working conditions imposed by the particular factorial design, all samples were prepared using the same procedure. An portion of wood material (the amount depending on the particular experiment to be carried out) was accurately weighted in the extractor PTFE-lined extraction vessel and 30 ml of acetonitrile were added. This acetonitrile volume was weighed in order to check possible solvent losses during the MAE. After ensuring that a new rupture membrane was in place, the extraction vessel was closed. Extractions were performed at fixed temperature (also dependent on the particular experiment as dictated by the factorial design) at 40% microwave oven power. After extraction time was completed (according to the value fixed by the particular experiment), the vessels were allowed to cool down to room temperature before they were opened. The extraction vessel with the wood and the solvent was weighed again. Next, using a pipette, 2 ml of this extract were transferred to a 10-ml calibrated flask, 1 ml of B[*ghi*]P solution was added as internal standard, and the flask volume was completed with acetonitrile. All mixed liquids (extract, internal standard solution and solvent) were accurately weighed using a calibrated balance. The extract was filtered through a 0.22-μm Millex GV (Millipore, Bedford, MA, USA). Ten μl were injected in the HPLC system.

3. Results and discussion

3.1. Optimization of the procedure for PAHs by HPLC

The chromatographic conditions were optimized for the resolution of the five PAHs considered. Fig. 1 shows typical chromatograms of a PAHs standard mixture (Fig. 1c) and an acetonitrile extract from the spiked wood sample (Fig. 1b) obtained by fluorescence detection and using the chromatographic conditions described in Section 2.

Calibration curves were constructed using appropriately diluted standards in acetonitrile. A five-point internal standard calibration in the range 40–800 ng/g of PAHs was performed. The concentration of the B[*ghi*]P used as internal standard was 225 ng/g. Each concentration level was injected in triplicate. Chromatographic peak areas were fitted by linear regression and the results are given in Table 1. For quantification we used the average response factors from the multilevel calibration.

The reproducibility of the chromatographic procedure was assessed by performing six injections in different days. The results (between-injection reproducibility data) are also given in Table 1 along with detection and quantification limits for direct injections of standards at signal-to-noise ratios of 3 and 10, respectively.

In the method described above, identification of compounds is based on retention times of fluorescence signals. To confirm identity of compounds extracted from wood we have used the UV-DAD response. The UV chromatogram corresponding to a MAE extract is given in Fig. 2 with some UV spectra taken at peak apex, showing very good agreement with those referenced in the UV-DAD library. This demonstrates the possibility of analyzing acetonitrile extracts of wood samples without any further clean-up procedure.

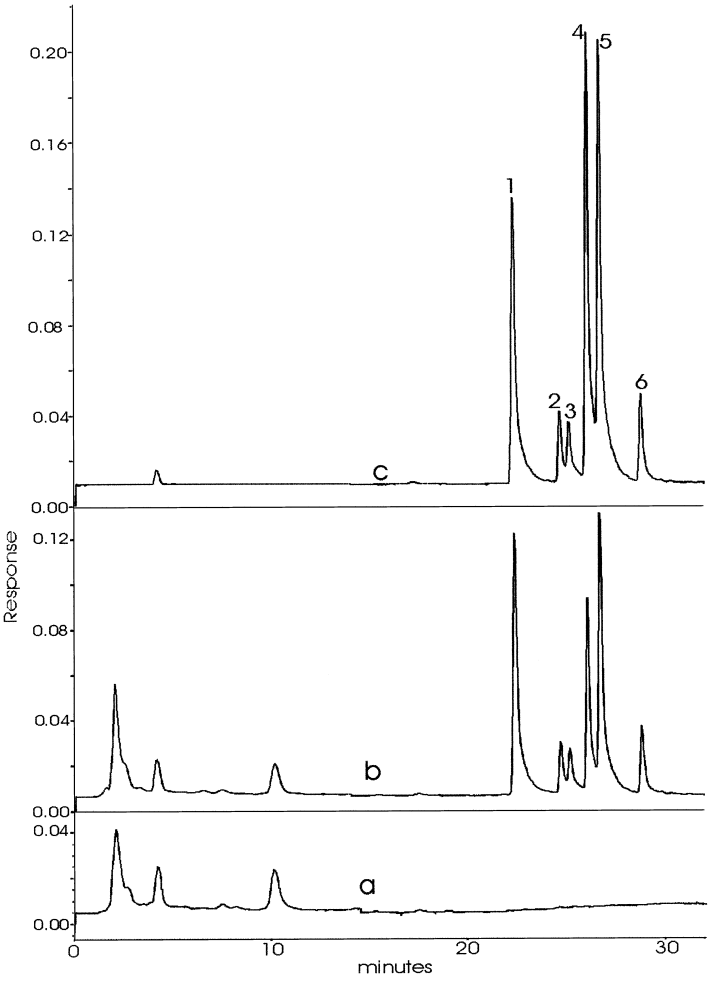

Fig. 1. Chromatograms of a sample blank (a), an acetonitrile extract from a spiked wood sample (b) and, PAHs standard mixture (c) obtained by fluorescence detection. The assignment of peaks is as follows: (1) benzo[*a*]anthracene; (2) benzo[*e*]pyrene; (3) benzo[*b*]fluoranthene; (4) benzo[*k*]fluoranthene; (5) benzo[*a*]pyrene; (6) benzo[*ghi*]perylene.

Table 1
Calibration and statistical validation parameters

Parameter	B[*a*]A	B[*e*]P	B[*b*]F	B[*k*]F	B[*a*]P
Calibration range (ng/g)	37.8–654.1	47.8–827.4	37.5–649.1	45.5–788.4	39.2–679.2
Correlation coefficient	0.9996	0.9998	0.9999	0.9999	0.9999
RSD (%) between injections (n=6)	1.59	0.78	1.31	1.16	1.44
Detection limit (ng/g) (S/N=3)	1.27	7.48	5.55	1.11	0.74
Quantification limit (ng/g) (S/N=10)	4.23	24.93	18.50	3.70	2.46

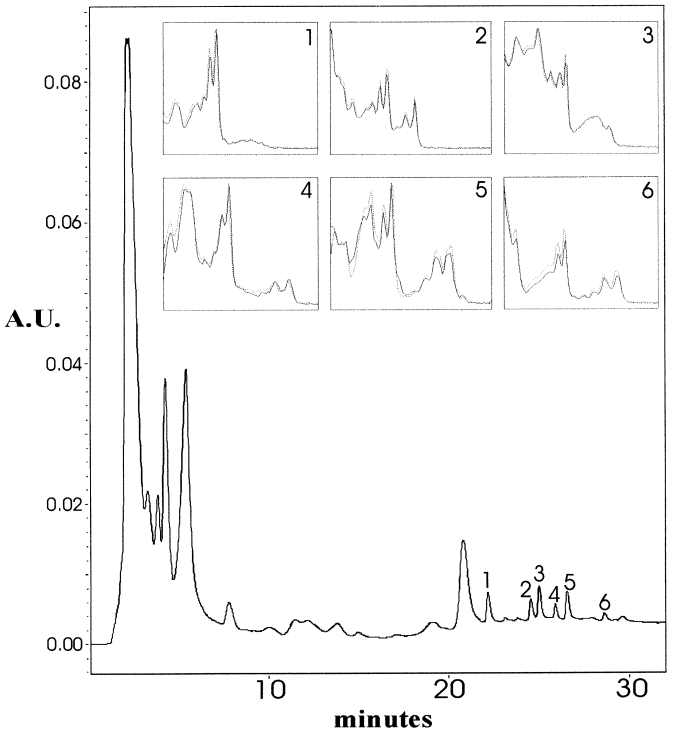

Fig. 2. Chromatogram obtained by UV detection at 254 nm corresponding to an MAE extract of spiked wood sample. Inner graphs show matching between the UV spectrum in the DAD library and the one at the peaks 1, 2, 3, 4, 5 and 6 apex in wood samples (peak numbering as in Fig. 1).

3.2. Evaluation of the homogeneity of the spiked wood

Before starting the optimization study of the extraction process, the homogeneity of the spiked material was assessed. The objective was that between-sample portions variability could not mask the effects of experimental factors to be optimized. A set of extractions were performed under the conditions given in Table 2. The relative standard deviations for each compound in the experiments (Table 2) were compared to that obtained from the injection of calibration standards (Table 1). The variability for 4 g of sample (RSD 0.65–1.5%) was very close to that obtained from the injection of calibration standards alone.

In conclusion, the material was considered to be homogeneous as far as the target analytes are concerned, provided that sample amounts equal to or greater than 4 g were taken in the experiments.

Some blank (starting wood material) samples of 4 g were extracted under the same conditions (Table 2).

Table 2
Evaluation of wood homogeneity used in MAE optimization process[a]

Sample size (g)	Replicates	Amount (μg/g)/RSD (%, $n=3$)				
		B[*a*]A	B[*e*]P	B[*b*]F	B[*k*]F	B[*a*]P
1	3	7.17/3.64	7.92/1.46	6.27/3.02	4.25/2.20	5.39/2.75
2	3	7.22/2.97	7.92/2.53	6.30/2.33	4.30/1.61	5.38/1.84
4	3	8.13/1.43	8.38/0.65	6.90/1.49	5.03/0.92	5.85/0.68

[a] Extraction time, 20 min; temperature, 120°C; solvent volume, 30 ml acetonitrile.

Table 3
Factor levels in the experimental design

Variable	Key	Low (−)	High (+)
Sample mass (g)	A	4	6
Temperature (°C)	B	90	120
Extraction time (min)	C	15	20

The results (Fig. 1a) showed the absence of an analytical signal at the retention times of the compounds studied.

3.3. Optimization of microwave extraction: factorial design

We have considered three variables that could potentially affect the efficiency of the extraction. The variables considered in MAE optimization were temperature, extraction time and sample weight. Solvent volume (30 ml of acetonitrile) was constant in all experiments. A two-level factorial design, 2^3, with two central points was used, involving 10 runs divided in two blocks. This model allows the evaluation of the effects of each variable and also the interaction effects between variables. Table 3 lists the high and low levels given to each factor. Data analysis was performed using the statistical package Statgraphics Plus for Windows V 3.3 [19]. Table 4 gives the experimental design matrix, and the concentrations obtained in each run for the compounds studied. B[*a*]P was chosen as a representative example of all compounds, since the five PAHs studied present the same tendency versus the three variables considered. An analysis of B[*a*]P results, given in Table 4, produced the Pareto Chart of main effects shown in Fig. 3. In this chart, bar lengths are proportional to the absolute value of the estimated effects, helping in comparing the relative importance of effects. Pareto chart tests the significance of each effect compared with the experimental error, and in this design, experimental error is greater than the effect of any factor. Only the interaction between sample weight and extraction time was statistically significant. Effects of main factors in the response are shown in Fig. 4a. Sample mass and extraction time have a negative effect in response (extraction efficiency is inversely proportional to both factors), while extraction temperature has a positive effect. Optimum conditions would be 4 g of sample, extraction temperature of 120°C and extraction time of 15 min as can be seen in Fig. 4a. Fig. 4b shows the estimated response surface considering the factors: sample weight and the extraction temperature.

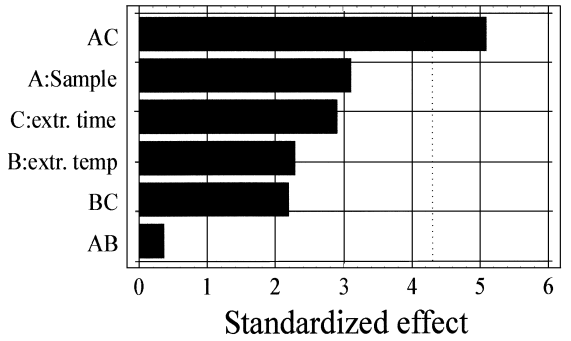

Fig. 3. Standardized Pareto chart for benzo[*a*]pyrene in the factorial design (dotted vertical line indicates the statistical significance boundary for the effects).

Table 4
Design matrix and response values in the factorial design

Run	Block	Sample (g)	Temp. (°C)	Time (min)	B[*a*]A (μg/g)	B[*e*]P (μg/g)	B[*b*]F (μg/g)	B[*k*]F (μg/g)	B[*a*]P (μg/g)
1	1	4	120	15	7.77	8.01	6.69	4.40	5.63
2	1	5	105	17.5	7.55	8.24	6.58	4.53	5.69
3	1	6	120	20	7.19	7.98	6.28	4.29	5.42
4	1	4	90	20	7.38	8.03	6.29	4.43	5.61
5	1	6	90	15	7.61	8.19	6.44	4.52	5.72
6	2	6	90	20	7.36	8.11	6.35	4.40	5.56
7	2	5	105	17.5	7.71	8.57	6.76	4.75	5.87
8	2	4	90	15	8.01	8.49	6.82	4.80	5.94
9	2	6	120	15	8.09	8.94	7.01	4.89	6.04
10	2	4	120	20	8.27	8.89	7.20	4.94	6.18

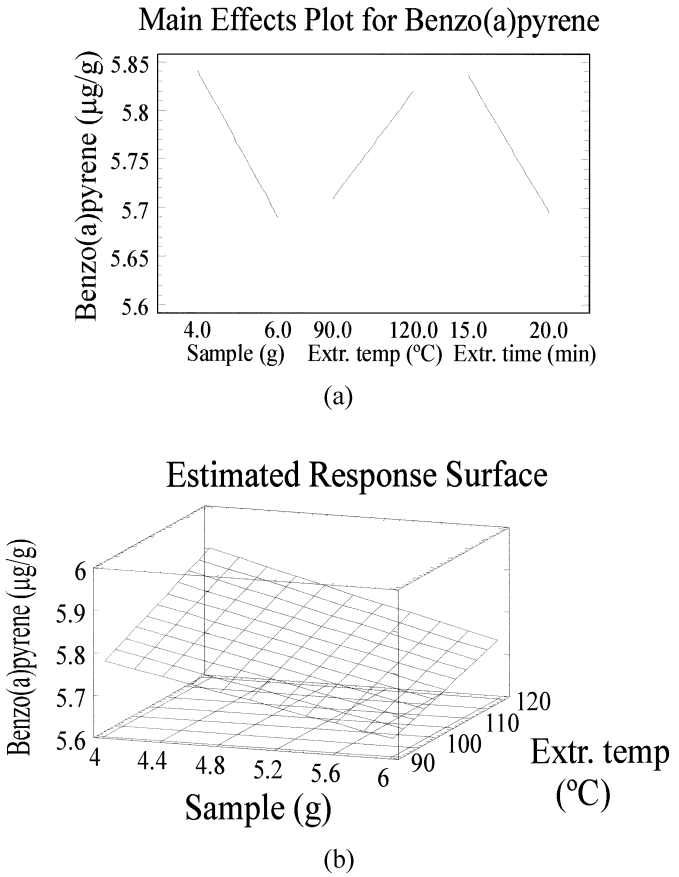

Fig. 4. (a) Main effects plot for benzo[*a*]pyrene in the factorial design; (b) estimated response surface for benzo[*a*]pyrene (sample mass versus extraction temperature factors).

Fig. 5a shows the interaction between factors. As can be seen, there is an interaction between sample weight and extraction time. Fig. 5b shows the response surface function developed by the model considering the sample weight and the extraction time factors. The response reaches the maximum value when the sample amount is at its lowest level and the extraction time at the maximum. This behavior could be attributed to difficulties to appropriately weathering higher sample amounts with the fixed solvent volume.

Given these findings, we decided to work with 4 g of sample, extraction temperature of 120°C and extraction time of 20 min. The selection of this extraction time was based on a consideration of practical attainment of maximum extraction efficiencies working with the smallest amount of wood.

3.4. Reproducibility and recovery of the microwave extraction

Using 30 ml of acetonitrile and the optimum conditions developed above, six individual extraction experiments were carried out in different days to establish the day-to-day precision (Table 5). RSDs from 4.62 to 1.19% were obtained showing a good reproducibility of the extraction method. Recoveries, based on target values are given in Table 5. Recoveries from 87 to 99% were obtained.

The recoveries of the PAHs were also calculated by freshly spiking 4-g portions of the starting wood material, with standard solutions in toluene. Each PAH was spiked at concentrations ca. 0.5, 1 and 1.5 times the actual concentration in the original wood samples. After spiking, samples were equilibrated

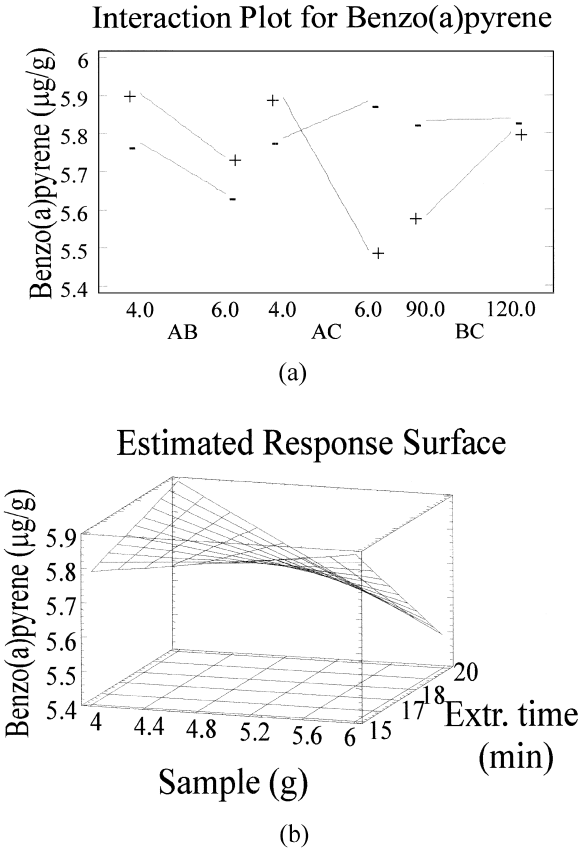

Fig. 5. (a) Interaction plot for benzo[*a*]pyrene in the factorial design; (b) estimated response surface for benzo[*a*]pyrene (sample mass versus extraction time factors).

during 48 h at room temperature before extraction. Calibration graphs were constructed from the representation of the μg recovered/g sample in relation to the μg spiked/g sample. The recovery was calculated as the slope (×100) of the calibration graph for each compound. The results, means and standard deviations, obtained are also given in Table 5. Good recoveries for all the PAHs (between 90.5 and 108.6%) and adequate reproducibility were obtained. At the time of conducting these experiments, to the best of our knowledge no certified reference material for PAHs in wood was available so a true validation could not be carried out. Thus, the comparison between recoveries of aged and freshly spiked material was the only means to asses the obtained figures. Because in many cases analyte behaviors appear to be quite different, good agreement of recoveries in both cases can be considered a proof of procedural convenience, and at the same time indicates that no significant losses of analytes have occurred during sample spiking and storage, except in the case of B[*a*]P.

4. Conclusion

The use of a microwave-assisted extraction procedure has been shown to be a valid alternative for sample preparation in the determination of PAHs in wood samples. Microwave-assisted extraction using acetonitrile as solvent yield extracts that can be directly injected in HPLC without the need of further preliminary clean-up or solvent exchange steps. The optimized procedure allows satisfactory recoveries and good reproducibility for all the studied PAHs.

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Table 5
Reproducibility and recovery study for each compound using the optimum operating conditions

Compound	Average (μg/g) (n=6)	Recovery (%) based on spiked target	RSD (%, n=6)	Recovery (%) (slope×100)	Slope RSD (%)
B[*a*]A	8.36	94.0	4.62	105.8	2.5
B[*e*]P	8.54	95.3	1.46	90.5	2.5
B[*b*]F	7.31	94.2	1.33	102.6	0.8
B[*k*]F	4.82	98.5	1.33	108.6	2.3
B[*a*]P	6.03	87.2	1.19	100.2	1.4

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