Dispersive Liquid–Liquid Microextraction for the Simultaneous Determination of Parent and Nitrated Polycyclic Aromatic Hydrocarbons in Water Samples

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A new method for simultaneous extraction and quantification of 6 nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) and 16 parent polycyclic aromatic hydrocarbons (PAHs) in water matrices was optimized and validated.
The extraction procedure was based on dispersive liquid-liquid microextraction technique, followed by gas chromatography-mass detection. The optimum conditions of extraction (volume of the extraction solvent, dispersive solvents and amount of salt) were selected using central composite design. The best results were found by using 200 μL of acetonitrile as dispersive solvent, 60 μL of chloroform as extraction solvent, and 10% (w/v) NaCl. Excellent linearity was observed in the range of 10–150 ng L^{-1} with correlation coefficients (r^2) ranging between 0.9996 and 0.9999 for nitro-PAHs and in the range of 5–150 ng L^{-1} with r^2 ranging from 0.9998 to 1.000 for PAHs. The limits of detection for the nitro-PAHs studied ranged from 0.82 to 3.37 ng L^{-1}, whereas for PAHs ranged from 0.62 to 3.48 ng L^{-1}. The intra- and inter-day precisions for nitro-PAHs were in the range of 0.45 to 19.54% and 0.43 to 19.62%, respectively, and for PAHs ranged between 0.45 to 17.42% and 0.38 to 18.97%, respectively. The proposed method was successfully applied in analyses of groundwater, sea, rain water and river water, being appropriate for routine analyses.

Keywords: Nitro-polycyclic aromatic hydrocarbons, dispersive liquid–liquid microextraction (DLLME), GC–MS, experimental design, routine analyses

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants in the atmosphere, soil, water, and food. PAHs are known for their potential teratogenicity, carcinogenic, and mutagenic properties. Due to their high toxicity and adverse effects, 16 PAHs were designated as priority pollutants and regulated by the United States Environmental Protection Agency (USEPA) [1, 2]. PAHs are also designated as priority hazardous substances by the European Commission, in Directive on Environmental Quality Standards (Directive 2008/105/EC) [3].

Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) belong to the family of substituted PAHs. These compounds can have 100,000 times more mutagenic and 10 times more carcinogenic characteristics than PAHs [4, 5]. The International Agency for Research of Cancer (IARC) has classified some nitro-PAHs as belonging to 2A group (probably carcinogenic to humans), such as 1-nitropyrene and 6-nitrochrysene, and 2B group (possibly carcinogenic to humans) compounds like 2-nitrofluorene, among others [6–8].

Similarly to PAHs, nitro-PAHs can be emitted from incomplete combustion reactions in vehicles engines, combustion processes to produce energy by burning fossil fuels and aluminum smelters, etc. However, the nitro-PAHs may also be formed by biological and chemical degradation of PAHs. The chemical degradation of PAHs occurs mainly through the oxidation of PAHs by atmospheric radicals (OH, NO_3, and O_3) in gas phase [5, 6, 9–12]. The most abundant nitro-PAH in atmosphere is 1-nitropyrene, originated from the incomplete combustion of diesel fuels. Additionally, the 9-nitroantracene, 3-nitrofluoranthene, and 2-nitrofluorene are other nitro-PAHs easily found in atmosphere resulting from emissions of diesel engines [5, 6, 10].

Actually, parent and nitro-PAHs compounds are considered as a class of organic pollutants widely distributed in the environment; therefore, its analysis in environmental samples becomes mandatory, including water samples. Most of the studies concerning the analysis of parent and nitro-PAHs are in air and soil samples due to the very low concentration levels in environmental waters. In this context, appropriate extraction methods for determination of these compounds in water samples are necessary, in order to achieve good sensitivity and selectivity [7, 8]. Solid-phase extraction (SPE) is the most usual technique used as pretreatment/preconcentration method for parent and nitro-PAHs analyses in water samples [8, 10, 13, 14]. In addition, experimental studies using liquid–liquid extraction (LLE) and solid-phase microextraction (SPME) were also reported for determination of trace levels of nitro-PAHs [7, 13, 15]. However, these procedures are highly costly and time-consuming.

The dispersive liquid–liquid microextraction (DLLME) technique is an alternative extraction procedure already used for PAHs [16], but not described for nitro-PAHs and PAHs simultaneously. DLLME methods are based on a ternary component solvent system. The extraction occurs in an appropriate mixture of high density organic solvent (extraction solvent) and water miscible polar solvent (dispersive solvent) that is rapidly injected into an aqueous sample containing the analytes. After injection, the extraction solvent is dispersed by a dispersant solvent into sample and a cloudy solution is formed. Thereby, the extraction equilibrium is achieved

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more quickly. After this step, the cloudy solution is centrifuged and a sediment phase containing the analytes is formed. Finally, the determination of the analytes in the sedimented phase can be performed by instrumental analysis [17–21].

In this work, an innovative DLLME method was optimized for the simultaneous extraction of six nitro-PAHs and 16 PAHs from environmental water samples, by controlling several experimental parameters using an experimental design approach. The performance of the optimized method was properly validated, allowing its applicability in routine analyses.

Materials and Methods

Chemicals and Materials. PAH calibration mix (naphthalene, Nap; acenaphthylene, Acy; acenaphthene, Ace; fluorene, Flu; phenanthrene, Phe; anthracene, Ant; fluoranthene, Flt; pyrene, Pyr; benz[a]anthracene, BaA; chrysene, Chr; benzo[b]fluoranthene, BbF; benzo[k]fluoranthene (BkF); benzo[a]pyrene, BaP; dibenzo[a,h]anthracene, DahA; benzo[ghi]perylene, BghiP; and indeno[1,2,3-cd]pyrene, Ind) was purchased from Sigma-Aldrich (Steinheim, Germany). 2-Nitrofluorene (≥98%), 9-nitroanthracene (≥93%), 3-nitrofluoranthene (≥90%), and 1-nitropyrene (≥99%) were from Sigma-Aldrich (Steinheim, Germany) whereas 6-nitrochrysene 50 μg mL^{-1} in toluene and 7-nitrobenz(a)anthracene (≥99.5%) were from LGC Standards. As internal standard, PAH-Mix31 from Dr. Ehrenstorfer (Augsburg, Germany) constituted by the deuterated standards (naphthalene-d8, acenaphthene-d10, pyrene-d12, chrysene-d12, and phenanthrene-d10) was used. Methanol, acetonitrile, and acetone high-performance liquid chromatography (HPLC)-grade LiChrosolv were supplied by Merck (Darmstadt, Germany). Ultrapure water was purified by a Milli-Q gradient system (18.2 mΩ cm^{-1}) from Millipore (Milford, MA, USA). Trichloromethane, chlorobenzene, dichloromethane, tetrachloromethane, and tetrachloroethylene were high-purity solvents from Fluka for HPLC analysis. Sodium chloride (NaCl) was analytical grade from Riedel-de-Haën (Buchs, Switzerland).

Stock standards of each compound were prepared in acetonitrile by exact weighing of the high purity substances and accurate dilution and stored in amber glass flasks at 4 °C. A mixture containing the six nitro-PAHs and 16 PAHs was then prepared, in acetonitrile, to obtain working solutions of appropriate concentrations that were kept at low temperature in the dark. Daily calibration solutions at concentration levels ranging from 5 ng L^{-1} to 150 ng L^{-1} were prepared by spiking 10 mL of water with different volumes of the working solutions.

Apparatus and GC–MS Conditions. Chromatographic analyses were carried out in a Shimadzu GCMS-QP2010 Gas Chromatograph Mass Spectrometer equipped with an autoinjector AOC-5000. Injections of 1 μL were made in the splitless mode with a 1.0 min purge-off time, and the injector temperature was set at 280 °C. Helium (99.9999%), at a constant flow rate of 1.5, mL min^{-1} was used as the carrier gas. Samples were analyzed using a fused-silica capillary column Zebron ZB-5MS W/Guardian (Phenomenex) coated with 5% polysilarylene and 95% of polydimethylsiloxane (30 m × 0.25 mm ID, 0.25 μm film thickness) with 10 m of Guardian capillary column but not with stationary phase. The analysis was done following the oven program temperature: initial temperature 70 °C (held for 2 min), increased by 25 °C min^{-1} to 180 °C (held for 2 min), next increased by 15 °C min^{-1} to 280 °C and held at this temperature for 2 min, and increased again by 10 °C min^{-1} to 300 °C, and held at this temperature for 5 min. The transfer line was set at 270 °C and ion source at 200 °C with an electron impact ionization of 70 eV. Positive fragment ions (*m*/*z*, ions mass-to-charge ratio) were analyzed over the 50–500 *m*/*z* mass range in full scan mode and in selected-ion monitoring (SIM) mode.

The identification of PAHs and nitro-PAHs was done according the following conditions: (1) the selected diagnostic ions were present at the substance specific retention time; (2) the relative retention time of the sample component matched that of the authentic compound within a limit deviation of ±0.04 min in the chromatogram of the latest calibration standard, measured under identical conditions; and (3) the relative intensities of two of the chosen diagnostic ions measured in the sample do not deviate by more than 20% from the relative intensities determined in the reference standard working solution.

Positive fragment ions (*m*/*z*, ions mass-to-charge ratio) selected for quantification and identification purposes are shown in Table 1 and the respective chromatogram in Figure 1A.

Table 1. The target nitro-PAHs and PAHs, CAS number and their MS conditions

	Compounds	Class	CAS number	$t_{R\ (min)}$	Identification ions (*m*/*z*)	TWs
1	Naphthalene-d8	IS	1146-65-2	5.748	68; **136**; 137	1
2	Naphthalene	PAH	91-20-3	5.771	127; **128**; 129	1
3	Acenaphthylene	PAH	208-96-8	7.571	151; **152**; 153; 154	2
4	Acenaphthene-d10	IS	15067-26-2	7.783	162; **164**; 165	2
5	Acenaphthene	PAH	83-32-9	7.828	151; 152; 153; **154**	2
6	Fluorene	PAH	86-73-7	8.766	165; **166**; 167	2
7	Phenanthrene-d10	IS	1517-22-2	10.617	94; **188**; 189	3
8	Phenanthrene	PAH	85-01-8	10.663	176; **178**; 179	3
9	Anthracene	PAH	120-12-7	10.76	176; **178**; 179	3
10	Fluoranthene	PAH	206-44-0	12.892	101; **202**; 203	4
11	2-Nitrofluorene	nitro-PAH	607-57-8	13.161	**168**; 194; 211	4
12	Pyrene	PAH	129-00-0	13.281	101; **202**; 203	4
13	9-Nitroanthracene	nitro-PAH	602-60-8	13.332	176; 193; **223**	4
14	Benzo(a)anthracene	PAH	56-55-3	15.367	226; **228**; 229	5
15	Chrysene-d12	IS	1719-03-5	15.378	120; **240**; 241	5
16	Chrysene	PAH	218-01-9	15.43	226; **228**; 229	5
17	3-Nitrofluoranthene	nitro-PAH	892-21-7	15.921	200; 217; **247**	5
18	1-Nitropyrene	nitro-PAH	5522-43-0	16.321	201; 217; **247**	5
19	Benzo(b)fluoranthene	PAH	205-99-2	17.619	126; **252**; 253	6
20	Benzo(k)fluoranthene	PAH	207-08-9	17.687	126; **252**; 253	6
21	7-Nitrobenzo(a)anthracene	nitro-PAH	20268-51-3	17.882	**226**; 243	6
22	Benzo(a)pyrene	PAH	50-32-8	18.350	126; **252**; 253	6
23	Perylene-d12	IS	1520-96-3	18.470	260; **264**; 265	7
24	6-Nitrochrysene	nitro-PAH	7496-02-8	18.807	226; 244; **273**	7
25	Indeno(1.2.3-cd)pyrene	PAH	193-39-5	20.945	138; 139; **276**; 277; 278	7
26	Dibenzo(a,h)anthracene	PAH	53-70-3	21.048	138; 139; 276; 277; **278**	7
27	Benzo(ghi)perylene	PAH	191-24-2	21.642	138; 139; **276**; 277; 278	7

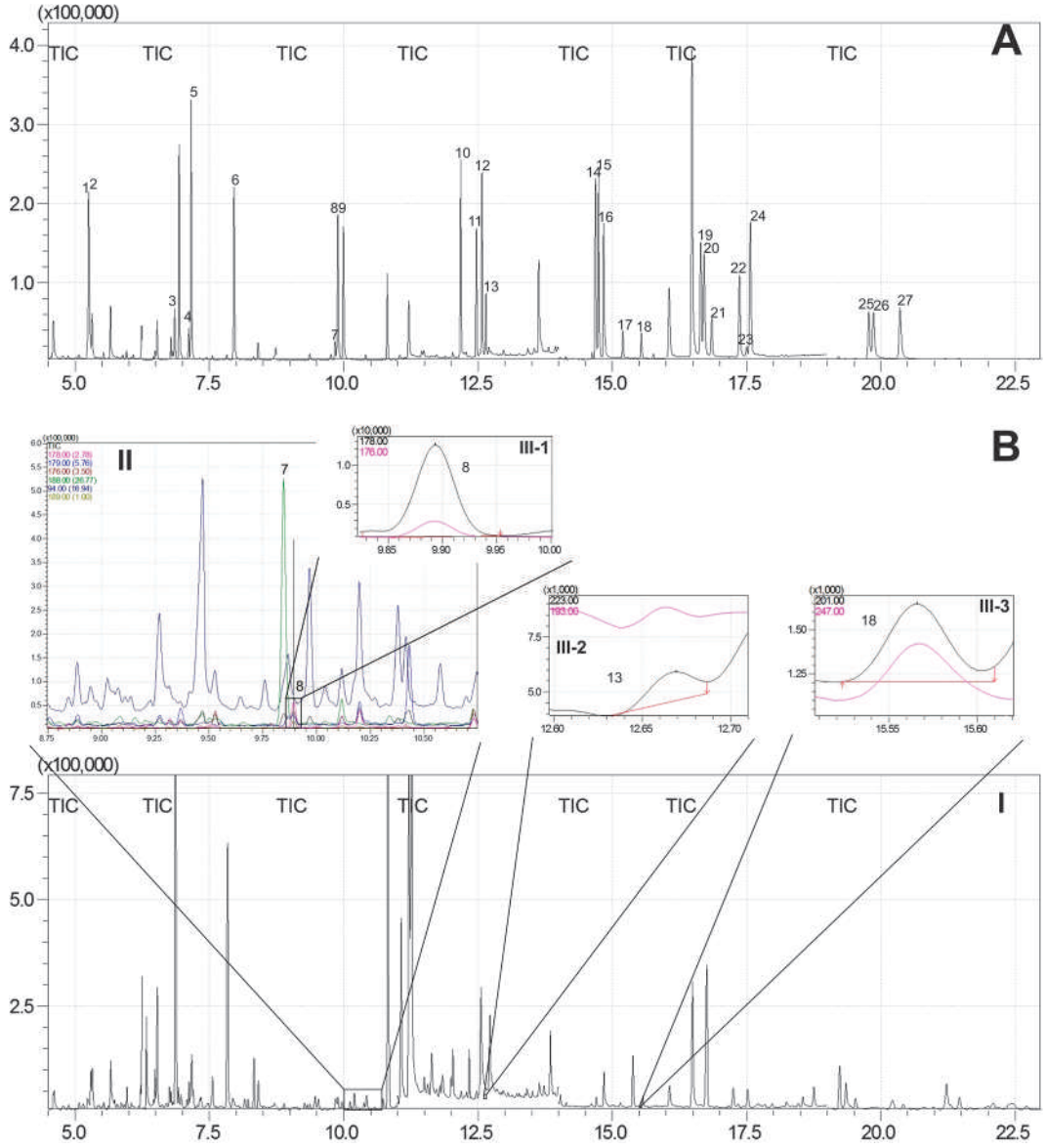

Figure 1. (A) GC–MS separation of nitro-PAHs and PAHs added to ultrapure water analyzed in selected monitoring ion mode and represented as total ion chromatogram (each compound was fortified at the concentrations 150 ng L^{-1}; the legend of peaks and used ions are in Table 1). (B) GC–MS chromatogram of groundwater sample with representation of total ion chromatogram (B-I), selected monitoring ion of time window between 8.75 min and 10.75 min (B-II), and extracted ion chromatograms in SIM mode identification of phenanthrene (B-III-1), 9-nitroanthracene (B-III-2), and 1-nitropyrene (B-III-3)

Instrument control and mass spectrometry data were managed by a personal computer with the LabSolutions GCMS software (2.50 SU3 version).

Extraction Procedure. Extraction was carried out using a volume of 10 mL of water sample placed into a 15 mL glass conical test tube and spiked with internal standard (0.025 μg L^{-1} final concentration). The analytes were extracted by the DLLME method, after addition of 200 μL of acetonitrile and 1 g of sodium chloride, followed by the quick injection of 60 μL of trichloromethane (extraction solvent), resulting in a cloudy solution. This solution was vigorously shacked by hand until the total dissolution of sodium chloride. Finally, the conical tubes were centrifuged at 2000 rpm and 4 °C for 7 min. Dispersive particles were collected at the bottom of the centrifuge tube by a glass Pasteur pipette with latex bulb. The resulting organic phase was transferred to a microinsert of 100 μL and injected in gas chromatography–mass spectrometry (GC–MS) for analysis.

Experimental Design. A central composite design (CCD) was built for optimization of variables affecting the extraction procedure of nitro-PAHs and PAHs under analysis. Table 2 presents the three coded variables at ranged levels of low (−1) and high (+1) concentrations in a total of 19 runs with five center points. CCD consisting of a complete 2^3-factorial design as cubic points, with six axial points at a distance of α = 1.682 from the design center and five center points. The response used in experimental designs was the sum of peak area for PAHs and for nitro-PAHs. The statistical analyses were performed by using the software Design Expert Trial Version 7 (Stat-Ease Inc., Minneapolis, MN).

Table 2. Central composite design values for extraction condition, with independent variable levels

Independent variable	Unit	Symbol	Coded levels				
			−1.682	−1	0	+1	+1.682
Solvent extraction volume	μL	X_1	49.8	60	75	90	100.2
Solvent dispersive volume	μL	X_2	12.5	200	475	750	937.5
NaCl amount	mg	X_3	659.1	1000	1500	2000	2340.9

Validation and Quality Control. The method was validated mainly in accordance with the guidelines established by International Conference on Harmonization recommendations

[22], as well as some European and American validation guidelines [23, 24] with specifications for environmental pollutants analysis and/or GC–MS methodology.

In order to develop and optimize the DLLME procedure followed by GC–MS, for an effective and reproducible detection and quantification of low concentrations of PAHs and nitro-PAHs, several parameters such as specificity and selectivity, linearity and linear range, limits of detection and quantification, precision, accuracy, and trueness (recovery) were determined. The calibration curves were constructed with matrix-matched standards, that is, the analysis was carried out by spiking water matrix samples with different amounts of standards using deuterated PAH-Mix31 as internal standard. Calibration curves were constructed using the least squares linear regression model, plotting the peak area ratios of the different compounds and respective internal standard versus the concentration of each analyte under study. Standard calibration curves were prepared using seven calibration points for PAHs (0.005, 0.010, 0.025, 0.050, 0.075, 0.100, and 0.150 μg L^{-1} of each analyte) and six points for nitro-PAHs (without 0.005 μg L^{-1} point) prepared by adding the correct amount under the conditions of point 2.3. Each test was performed at least in five independent experiments. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the calibration curve parameters [25, 26], where the LOD was equal to the calculated intercept of the linear regression (a) plus three times the $S_{y/x}$ and for LOQ ten times this value.

In order to transfer the validated method into laboratory working routine, its performance was also evaluated through the participation in an interlaboratory study for PAHs provided by LGC Standards Proficiency Testing, Aquacheck Sample 7C. Collaborative trials allow to estimate accuracy and to evaluate the possible bias of an analytical method. To assess the performance of the method, *z*-scores were provided for each analyte. *Z*-scores are indicators that compare the difference between the reported result of the laboratory and the assigned value (bias), with a standard error.

To evaluate the accuracy and applicability of the proposed method, analyses were carried out in different natural water sample matrices, including groundwater, rainwater, river, and sea water collected in Portugal in January of 2015.

Several samples were collected in Zone A (Figure 2), corresponding to Oporto city, namely, groundwater samples from dug wells in public supplies, river water samples from Douro River, and sea water samples from Atlantic Ocean. Rain water was also collected in this Zone. There were also collected five samples of groundwater in mountain range of Estrela and four samples of groundwater in Lisbon region, marked as B and C, respectively (Figure 2). Groundwater samples were collected in these 3 regions since the chemical composition of water matrices is very different, which could interfere in PAHs and nitro-PAHs recovery tests. Five hundred milliliters of each sample was filtered through glass fibber filters (Whatman, GF/F 47 mm, Maidstone, England) and maintained in amber glass containers at 4 °C until analysis.

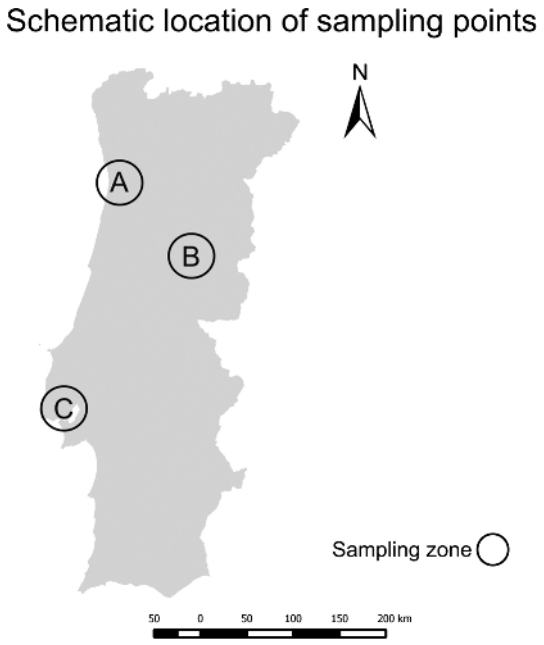

Figure 2. Schematic location of sampling zones

Results and Discussion

Preliminary experiments were conducted in order to select the extracting and the dispersive solvents of DLLME method. These preliminary experiments were performed using 10 mL of ultrapure water spiked with the mix containing six nitro-PAHs and 16 PAHs to obtain a final concentration of 0.25 μg L^{-1} each. Then, 0.5 mL of acetonitrile and 800 mg of NaCl were added. Different solvents of extraction were tested by adding an experimental volume of 100 μL to form the cloudy suspension. The other procedures followed the conditions described in Extraction Procedure section. The organic solvents tested were trichloromethane, chlorobenzene, tetrachloroethylene, and trichloroethylene. Trichloromethane was selected as the extraction solvent. This solvent presented the highest peak response for heavy PAHs compounds with a higher sum of total peak area in relation of the other extraction solvents (data not shown). For nitro-PAHs, trichloromethane and tetrachloroethylene were able to extract all nitro compounds; however, in general, the first had a higher sum of peak area. Chlorobenzene was not able to extract 3-nitrofluoranthene, and trichloroethylene only was able of extract two compounds, 2-nitrofluorene and 9-nitroantracene (Figure 3A).

For the selection of the dispersive solvent, the previous conditions were used: 100 μL of trichloromethane and 0.5 mL of the different organic solvents under study, i.e., acetonitrile, methanol, and acetone (Figure 3B). Acetonitrile was chosen as dispersive solvent and showed the highest peak response for heavy PAHs compounds, like B[a]P and B[g,h,i]P, but light PAHs, like Na and Acy, presented higher peak response with methanol. The sum of total peak area for dispersive solvents had similar response for acetonitrile and methanol and lower for acetone. Thus, acetonitrile was preferred as dispersive solvent.

Optimization of Extraction Conditions. Optimization of the extraction conditions for PAHs and nitro-PAHs was carried out using a statistical design, by CCD. Afterwards, the chosen variables were optimized and the range parameters for extraction solvent (X_1), dispersive solvent (X_2), and NaCl amount (X_3) are presented in Table 2. The experiments were performed in a random manner at different combinations of these parameters using statistically designed experiments. The sum of nitro-PAHs peak area and the sum of PAHs peak area were used as response factor for CCD. The complete design had 19 combinations, including five replicates of the center point.

Regression analyses were performed to fit the response functions, and the final model was obtained to the nitro-PAHs and parent compounds. Adequacy and significance of the quadratic model were evaluated by analysis of the variance (ANOVA) by means of Fisher's *F*-test. The models presented a good fitness to quadratic interaction with an *F*-test value of 24.10 and 55.69 for PAHs and nitro-PAHs, respectively, which implies that the models were significant for compounds. A model *F* value was obtained, indicating the high significance of the model to the four different responses with only 0.01% possibility that a large model

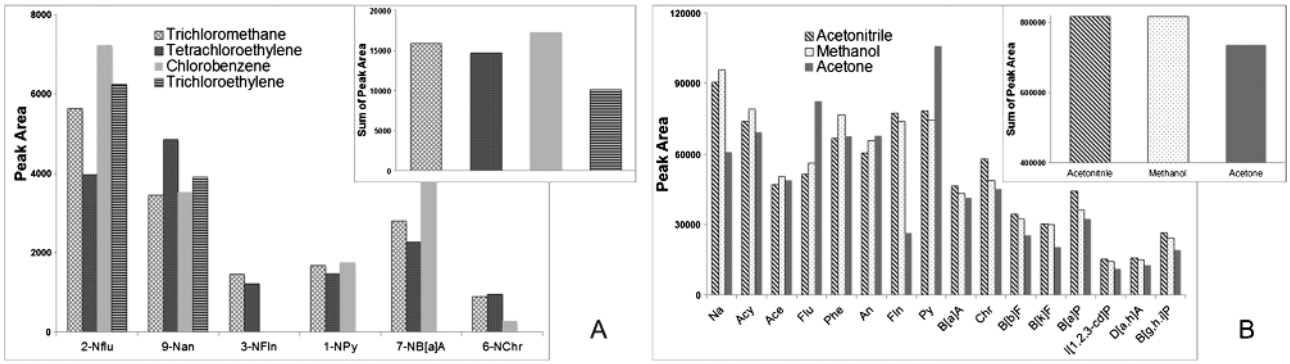

Figure 3. Bar chart for selection of extraction (A) and dispersive (B) solvent

F value could occur due to noise. Models presented a good fitness for quadratic interaction, with R^2 pred values of 0.8608 and 0.9266 for PAHs and nitro-PAHs, respectively, which are in agreement with the R^2 adj value of 0.9203 for PAHs and 0.9647 for nitro-PAHs. The achieved signal to noise ratios were 15.19 and 22.79 for PAHs and nitro-PAHs, respectively, which indicates adequate signals of the designs.

The significant terms for each response were those that presented a value of Prob > *F* lower than 0.05. In the case of PAHs, the significant terms were X_1, X_2, X_3, X_1X_3, X_1^2 and X_2^2, and that of nitro-PAHs, it was X_1, X_2, X_3, X_1X_2, X_1X_3, X_1^2, X_2^2, and X_3^2. The "lack of fit value" for all responses was also evaluated, and a Prob > *F* of 0.8876 and 0.7437 to PAHs and nitro-PAHs, respectively, was obtained, indicating non-significant lack of fit ($p > 0.05$), which means that the model is valid for the present study.

Curve analysis of response surfaces for all experimental designs allowed prediction of response function (sum of peak area for PAHs and nitro-PAHs) due to the effects of the three variables under study. Surface and contour plot demonstrating the effects of dispersive solvent amount and extraction solvent amount in PAHs data are shown in Figure 4A. Figure 4B displays the effects of NaCl amount and extraction solvent amount; the third variable (NaCl amount and dispersive solvent amount, respectively) was kept constant at the higher level on the response function. According to this optimization study, higher responses were obtained with lower extraction solvent and lower NaCl amount, but for dispersive solvent amount, the higher responses were achieved for both lower and higher amount of dispersive solvent in PAHs response. Surface and contour plot for nitro-PAHs response are shown in Figure 4C and D. nitro-PAHs response presented a behavior same as that of PAHs. The effects of dispersive solvent amount and extraction solvent amount are shown in Figure 4C, and the effects of NaCl amount and dispersive solvent amount in Figure 4D; third variable of design was maintained at higher level on response function. The higher response was achieved for lower extraction solvent and lower NaCl amount; for dispersive solvent amount, higher responses were achieved for both lower and higher amount of dispersive solvent.

Therefore, the following was established as optimized conditions: 60 μL of extraction solvent, 200 μL of dispersive

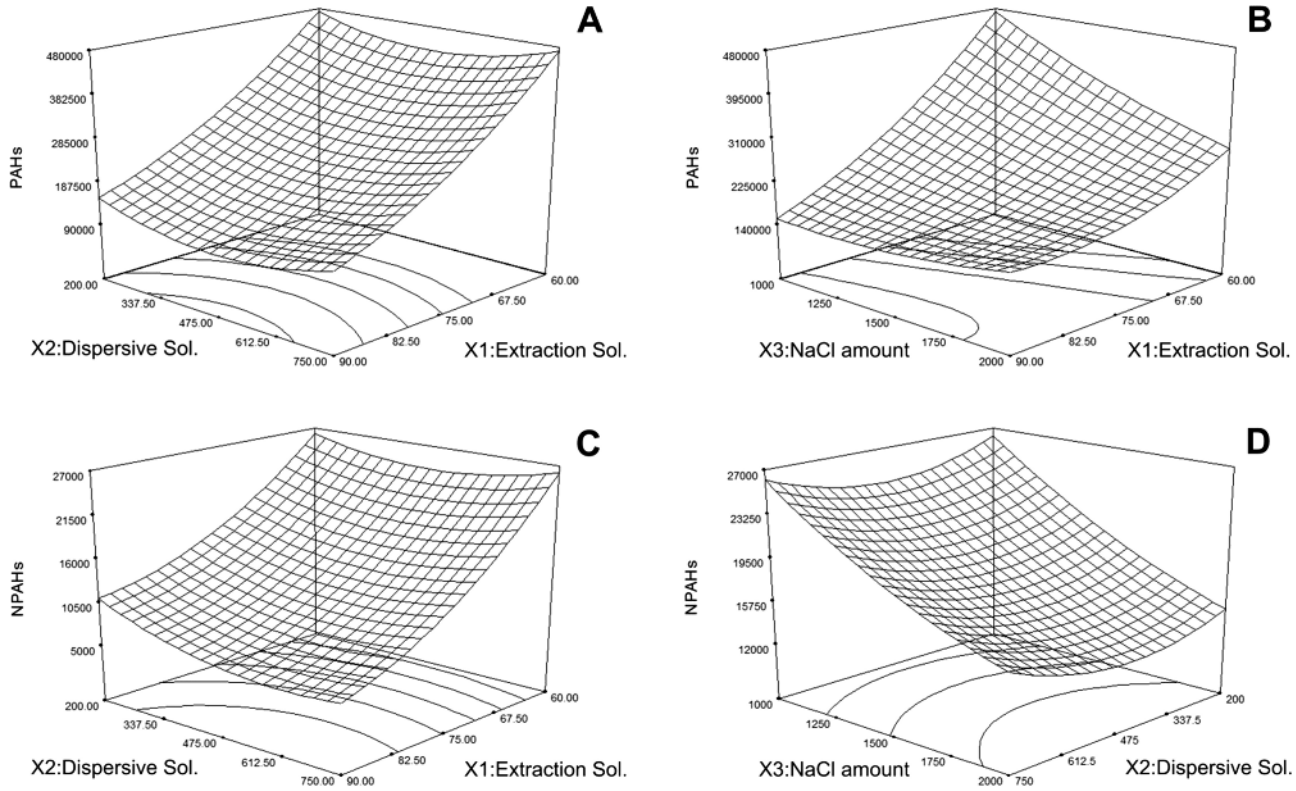

Figure 4. Response surfaces estimated for the central composite design of extraction procedure and the combined effect of variables on the sum of peak area, A and B for PAHs and C and D for nitro-PAHs

Table 3. Calibration parameters of the purposed method for nitro-PAHs and PAHs analysis

Compounds	$b \pm S_b$	$a \pm S_a$	$S_{y/x}$	r^2	LOD (ng L^{-1})	LOQ (ng L^{-1})
PAHs						
Naphthalene	0.0578 ± 0.0006	0.2071 ± 0.05	0.0317	0.9999	1.64	5.48
Acenaphthylene	0.0843 ± 0.0008	0.1852 ± 0.06	0.0392	0.9999	1.40	4.65
Acenaphthene	0.0539 ± 0.0010	0.5695 ± 0.07	0.0491	0.9998	2.73	9.10
Fluorene	0.0484 ± 0.0004	0.0262 ± 0.03	0.0191	0.9999	1.18	3.94
Phenanthrene	0.0518 ± 0.0006	0.5005 ± 0.05	0.0321	0.9999	1.86	6.20
Anthracene	0.0498 ± 0.0002	0.0428 ± 0.02	0.0103	0.9999	0.62	2.08
Fluoranthene	0.0574 ± 0.0020	0.1595 ± 0.07	0.0466	0.9998	2.44	8.13
Pyrene	0.0628 ± 0.0005	0.1822 ± 0.04	0.0243	0.9999	1.16	3.87
Benzo(a)anthracene	0.0420 ± 0.0005	−0.0368 ± 0.04	0.0269	0.9999	1.92	6.39
Chrysene	0.0356 ± 0.0008	0.0726 ± 0.06	0.0393	0.9996	3.32	11.1
Benzo(b)fluoranthene	0.0519 ± 0.0006	−0.0588 ± 0.04	0.0285	0.9999	1.65	5.50
Benzo(k)fluoranthene	0.0306 ± 0.0004	0.0225 ± 0.03	0.0208	0.9999	2.04	6.80
Benzo(a)pyrene	0.0463 ± 0.0008	−0.0289 ± 0.06	0.0386	0.9998	2.51	8.35
Indeno(1,2,3-cd)pyrene	0.0285 ± 0.0004	0.0398 ± 0.03	0.0183	0.9999	1.93	6.42
Dibenzo(a,h)anthracene	0.0299 ± 0.0004	0.0610 ± 0.03	0.0216	0.9998	2.17	7.22
Benzo(ghi)perylene	0.0360 ± 0.0008	0.1742 ± 0.06	0.0418	0.9996	3.48	11.6
nitro-PAHs						
2-Nitrofluorene	0,0072 ± 0.00005	0.0543 ± 0.004	0.0020	0.9999	0.82	2.75
9-Nitroanthracene	0,0036 ± 0.00009	0.0329 ± 0.008	0.0038	0.9997	3.21	10.7
3-Nitrofluoranthene	0,0021 ± 0.00002	−0.0029 ± 0.002	0.0009	0.9999	1.35	4.50
1-Nitropyrene	0,0045 ± 0.0001	−0.0053 ± 0.01	0.0051	0.9996	3.37	11.3
7-Nitrobenzo(a)anthracene	0,0102 ± 0.0002	0.0162 ± 0.01	0.0066	0.9999	1.94	6.48
6-Nitrochrysene	0,0229 ± 0.0003	−0.0531 ± 0.03	0.0132	0.9999	1.73	5.77

b indicates slope; *a*, intercept; r^2, correlation coefficient; S_b and S_a, standard deviations of slope and intercept; $S_{y/x}$, standard deviation of *y*-residuals of regression line; LOD, limit of detection; LOQ, limit of quantification.

solvent amount, and 1 g of NaCl amount, which leads to the achievement of the better global response for both groups or target compounds.

Method Performance. The applicability of the optimized method was verified; for this purpose, the analytical performance parameters such as specificity and selectivity, linearity and linear range, limits of detection and quantification, precision, accuracy, and trueness (recovery) were determined and assessed.

Specificity and selectivity were evaluated by comparing the chromatograms of matrix-blank samples (different samples of different water matrices) with an aqueous solution of the analytes at concentrations near the limits of quantification. No significant interferences have been detected at the retention time of different compounds.

Regression parameters obtained after application of the least squares linear regression were calculated and presented in Table 3. An excellent linear response was achieved for all analytes, where the lower correlation factor determined was equal to 0.9996 for Chr, B[ghi]P, and 1-nitropyrene. Experimental LOD based on the calibration curve parameters ranged between 0.62 and 3.48 ng L^{-1}, and LOQ ranged between 2.08 and 11.6 μg L^{-1}, for PAHs. The LOD and LOQ of the proposed method were below those found in the scientific literature regarding miniaturized sample pre-treatment techniques coupled

Table 4. Intra-day and inter-day results on repeatability (RSD) and accuracy (% Bias) of the proposed method

Compounds	Intra-day						Inter-day					
	Precision (RSD)			Accuracy (% Bias)			Precision (RSD)			Accuracy (% Bias)		
Nominal conc. (ng L^{-1})	5	50	150	5	50	150	5	50	150	5	50	150
PAHs												
Naphthalene	2.28	4.31	3.49	2.12	0.01	−4.34	17.22	6.90	5.07	−9.36	−3.67	−0.81
Acenaphthylene	0.45	17.42	3.46	−6.28	7.31	8.17	18.97	5.59	9.85	−16.14	14.51	14.58
Acenaphthene	19.29	7.45	1.47	−16.38	4.76	19.91	17.40	13.13	10.33	5.35	10.33	6.59
Fluorene	9.22	1.84	3.05	1.10	−1.68	2.61	18.40	5.36	1.05	−8.88	0.54	−0.15
Phenanthrene	3.32	2.87	0.97	5.53	5.98	3.51	17.48	0.38	3.61	19.20	2.89	0.23
Anthracene	6.32	12.16	1.25	18.25	9.61	3.39	12.07	13.40	16.50	−7.02	2.67	0.92
Fluoranthene	6.91	5.18	2.19	19.15	−4.78	0.88	12.11	5.00	1.30	−1.62	−4.66	−0.63
Pyrene	14.68	14.07	3.01	9.02	−0.02	13.97	17.23	7.41	5.88	−0.02	−9.86	1.43
Benzo(a)anthracene	10.28	9.32	3.86	5.20	2.03	6.83	11.53	2.40	1.39	10.15	−7.12	3.42
Chrysene	1.43	16.48	2.45	−3.83	−0.62	−10.16	7.02	8.65	10.88	6.47	4.03	1.41
Benzo(b) fluoranthene	1.50	11.12	1.72	−15.43	13.95	14.05	2.74	11.25	9.10	−13.53	−5.63	2.14
Benzo(k)fluoranthene	7.01	0.98	3.81	−11.08	2.10	2.39	5.98	7.55	8.78	−12.96	−2.47	−4.13
Benzo(a)pyrene	7.25	8.51	1.98	−4.01	−1.71	10.04	12.64	6.24	8.36	5.22	−0.45	−10.47
Indeno(1,2,3-cd)pyrene	9.36	5.30	2.98	16.17	−11.48	3.99	18.76	7.25	4.42	3.09	0.51	0.63
Dibenzo(a,h)anthracene	5.97	3.22	4.92	19.29	11.83	−6.86	11.87	8.45	6.14	0.66	0.53	−1.11
Benzo(ghi)perylene	6.35	8.61	3.61	−13.95	−8.53	−5.74	2.71	9.74	3.70	−2.01	10.92	1.04
Compounds	Intra-day						Inter-day					
	Precision (RSD)			Accuracy (% Bias)			Precision (RSD)			Accuracy (% Bias)		
Nominal Conc. (ng L^{-1})	10	50	150	10	50	150	10	50	150	10	50	150
nitro-PAHs												
2-Nitrofluorene	14.03	8.91	2.33	−14.24	−3.51	−2.27	9.22	2.33	0.43	−9.09	2.85	6.83
9-Nitroanthracene	19.54	5.61	13.29	6.097	1.60	5.52	19.62	13.29	10.68	6.10	−6.55	−12.03
3-Nitrofluoranthene	10.39	9.36	9.96	−16.27	0.20	1.54	19.44	9.96	11.62	−12.44	0.76	−5.19
1-Nitropyrene	3.72	1.24	4.12	15.69	11.04	−9.82	9.67	4.12	7.25	7.43	8.88	10.23
7-Nitrobenzo(a)anthracene	3.21	7.84	1.57	−11.24	−11.64	5.08	4.16	1.57	5.55	−3.60	−0.50	−6.98
6-Nitrochrysene	19.79	6.33	5.31	2.09	2.08	8.47	13.90	5.31	9.75	−15.03	−13.12	2.79

Table 5. Results on concentration and recoveries of the purposed method in different water matrix

Compounds	Samples (n = 13)													
	Concentration range		River water			Groundwater			Seawater			Rainwater		
			Conc.	Exp. conc.	Recovery	Conc.	Exp. conc.	Recovery	Conc.	Exp. conc.	Recovery	Conc.	Exp. conc.	Recovery
	min	max	(ng L^{-1})	50 (ng L^{-1})		(ng L^{-1})	50 (ng L^{-1})		(ng L^{-1})	50 (ng L^{-1})		(ng L^{-1})	50 (ng L^{-1})	
PAHs														
Naphthalene	<LOD	<LOQ	<LOD	42.22	81.62	<LOQ	41.38	77.65	<LOQ	40.32	71.25	<LOQ	44.78	79.51
Acenaphthylene	<LOD	<LOD	<LOD	40.50	81.00	<LOD	47.47	89.27	<LOD	35.78	71.56	<LOQ	38.61	70.32
Acenaphthene	<LOD	<LOD	<LOD	59.61	119.23	<LOD	57.11	114.22	<LOD	40.21	80.41	<LOD	51.23	102.47
Fluorene	<LOD	11.90	<LOD	60.11	120.23	9.25	65.14	111.77	11.86	50.78	77.84	11.90	57.02	90.24
Phenanthrene	<LOD	18.67	<LOD	28.04	73.55	11.75	49.58	75.66	18.67	54.15	70.96	13.70	52.53	77.66
Anthracene	<LOD	6.67	6.67	59.60	105.87	<LOD	40.85	81.70	<LOQ	39.58	77.03	<LOD	39.13	78.25
Fluoranthene	<LOD	<LOD	<LOD	42.00	86.13	<LOD	43.24	82.76	<LOQ	42.97	75.71	<LOD	40.73	81.47
Pyrene	<LOD	<LOD	<LOD	37.12	74.25	<LOD	38.59	77.20	<LOD	38.24	76.48	<LOD	35.13	70.27
Benzo(a)anthracene	<LOD	<LOQ	<LOD	43.54	87.08	<LOQ	49.62	93.69	<LOQ	47.01	88.40	<LOQ	50.20	94.64
Chrysene	<LOD	<LOD	<LOD	32.97	65.94	<LOD	36.75	73.76	<LOD	38.86	72.03	<LOD	35.09	70.78
Benzo(b)fluoranthene	<LOD	<LOQ	<LOQ	44.82	85.62	<LOD	51.13	102.27	<LOD	49.63	99.26	<LOD	54.83	109.66
Benzo(k)fluoranthene	<LOD	<LOD	<LOD	42.36	84.72	<LOD	50.50	101.07	<LOD	45.41	90.83	<LOD	44.92	90.91
Benzo(a)pyrene	<LOD	<LOQ	<LOD	46.16	92.31	<LOD	49.15	98.30	<LOD	49.34	98.68	<LOQ	48.45	89.16
Indeno(1,2,3-cd)pyrene	<LOD	<LOQ	<LOD	49.29	96.05	<LOD	44.49	88.99	<LOQ	52.04	99.68	<LOD	37.43	74.85
Dibenzo(a,h)anthracene	<LOD	<LOD	<LOD	51.53	103.06	<LOD	41.53	83.06	<LOD	55.96	109.23	<LOD	36.29	72.57
Benzo(ghi)perylene	<LOD	<LOD	<LOD	54.88	109.75	<LOD	43.79	94.45	<LOD	54.61	109.37	<LOD	39.19	78.38
nitro-PAHs														
2-Nitrofluorene	<LOD	<LOD	<LOD	52.11	104.23	<LOD	44.03	88.06	<LOD	53.52	105.44	<LOD	50.00	99.99
9-Nitroanthracene	<LOD	37.85	29.08	79.80	101.45	16.46	73.00	113.07	12,48	69.21	113.45	37.85	81.66	87.62
3-Nitrofluoranthene	<LOD	<LOD	<LOD	58.44	116.88	<LOD	53.97	107.95	<LOD	58.47	116.94	<LOD	86.20	119.36
1-Nitropyrene	<LOD	23.06	23.06	64.58	83.05	15.62	72.18	113.10	<LOD	52.38	104.77	<LOQ	65.40	105.45
7-Nitrobenzo(a)anthracene	<LOD	<LOD	<LOD	47.81	95.61	<LOD	59.01	118.01	<LOD	55.52	108.01	<LOD	54.21	108.42
6-Nitrochrysene	<LOD	<LOQ	<LOD	56.39	112.78	<LOD	50.92	101.83	<LOQ	60.48	116.02	<LOD	45.15	90.29

Conc. indicates sample concentration; conc. exp., experimental concentration; recovery, recovery percentage.

to GC–flame ionization detector (FID) [27, 28] and GC–MS [18, 29] determination, and in a same range than a similar method of DLLME–HPLC–fluorescence (FL) [16]. Concerning the applicability of DLLME, from our knowledge, it is the first time that this method is used in nitro-PAHs determination. The proposed methodology for nitro-PAHs presents good limits for LODs and LOQs, ranged from 0.82 to 3.37 ng L^{-1} and 2.75 to 11.3, respectively. When comparing these data values with results obtained by SPE–GC–MS [15] (SPE is the most applied extraction methodology for these compounds) or HPLC–chemiluminescence [8] detection, they are lower, and only slightly worse than the values obtained by headspace (HS)–SPME [7]. In comparison with a SPE–GC–MS methodology for simultaneous determination of PAHs and nitro-PAHs, the detection limits are in the same range [10]. However, it should be noted that SPE has a high cost, is time-consuming, and is high sample handling.

Evaluation of repeatability and accuracy was performed (Table 4). Results expressed as relative standard deviation (RSD) ranged between 0.45% and 19.79% for intra-day precision and between 0.38% and 19.62% for inter-day precision. Concerning accuracy, bias values varied between −16.38% and 19.29%. These values were below the 20% recommended by regulatory authorities and major international bodies [22, 23] in all three concentration levels.

Performance was also evaluated through the participation in an interlaboratory study for PAHs; the overall performance of the method was seen to be satisfactory, and all z-scores were between −3.42 and 0.97. For the majority of the analytes (70%), z-scores indicated satisfactory ($|z| \leq 2$) performance of the method. However, three determinations were classified as questionable ($|z|$-score from 2 to 3) and two, unsatisfactory ($|z| \geq 3$). The unsatisfactory results were from benzo(ghi)perylene and benzo(k)fluoranthene, interlaboratory sample concentrations of which were beyond the lower limit of validated linear range. The questionable results were due to reporting concentrations at approximately the limits of detection or close to the limits of quantitation.

The applicability of the proposed method was tested in four different types of natural water samples (river, groundwater, sea, and rainwater). Extraction by DLLME was performed as described in Extraction Procedure section. Samples were screened for the different compounds under study, and concentrations were taken in consideration to recovery analysis. Recovery studies were performed at 0.050 μg L^{-1} level of concentration. The mean recoveries ranged from 70.25% in pyrene for rainwater to 120.27% for fluorene in river water (Table 5). These results confirm the accuracy of the method in these different types of matrices. The thirteen samples of groundwater were the samples that presented the lowest concentration of the compounds under study. Detected PAHs were fluorene, phenanthrene, and anthracene. The heavy PAHs were not detected in those samples. For the nitro-PAHs compounds, 9-nitroanthracene and 1-nitropyrene were the ones that were found more often (an example chromatogram of groundwater is presented in Figure 1B). Groundwater samples from zones B and C presented a very low concentration of PAHs and nitro-PAHs only with trace levels of different compounds.

Conclusions

A quick, cost-effective, and eco-friendly method was developed for simultaneous analysis of nitro-PAHs and PAHs in water samples by applying a DLLME extraction methodology and GC–MS analyses. Multivariate chemometric techniques were successfully used to establish the optimum DLLME extraction conditions of the different variables, namely, NaCl amount of 1000 mg, extraction solvent (60 μL), and buffer (200 μL) amount for DLLME extraction. Establishment of optimal conditions was obtained with minimal number of assays to obtain maximum compound response.

The proposed method was successfully validated in terms of limit of detection and quantification, precision, and recovery. The simultaneous quantification of nitro-PAHs and parent compounds

is advantageous when applied to routine analyses of different types of water samples, namely, groundwater, river, sea, and rainwater. The different samples analyzed were not found to have problematic values. The overall performance of the method was seen to be also satisfactory, through the participation in an interlaboratory study for PAHs.

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