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Solvent-based de-emulsification dispersive liquid–liquid microextraction coupled with UPLC-MS/MS for the fast determination of ultratrace levels of nitrated and oxygenated polycyclic aromatic hydrocarbons in environmental samples

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In this study, a new solvent-based de-emulsification dispersive liquid–liquid microextraction strategy followed by liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry has been developed for the determination of the ultratrace levels of nitrated and oxygenated polycyclic aromatic hydrocarbons present in environmental samples. Various parameters affecting the extraction efficiency such as type and volume of the extraction, dispersive and de-emulsifier solvents, temperature, salt addition, extraction time and sample volume were evaluated. Under optimal conditions, calibration plots were linear in the ranges between 0.005 ng mL^{-1} and 100 ng mL^{-1}, with correlation coefficients (r^2) better than 0.995. In addition, satisfactory extraction recoveries ranging from 95.1% to 98.5% were obtained. The proposed method has been found to have excellent detection sensitivity with limits of detection (LODs, S/N = 3) of 8.9–89.0 ng L^{-1} and precisions of 1.4–8.7% (RSDs, n = 5). Enrichment factors of the four target compounds were from 191-folds to 200-folds. The proposed method may be advised as an economical, fast easy, sensitive, accurate approach, even better than conventional DLLME and similar techniques. The results indicated that this methodology was suitable for the analysis of ultratraces of nitrated and oxygenated PAHs in water samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) derivatives belong to a category of organic pollutants ubiquitous in different environmental compartments. A wide variety of different PAHs compounds, including alkyl-PAHs, nitro-PAHs, oxy-PAHs, thio-PAHs; among others, have been identified as having a direct mutagenic potency. Consequently, they have been included in the International Agency for Research on Cancer (IARC) lists (2B and 3) of carcinogens.[1,2]

Multiple transport processes (*e.g.*, dry and wet deposition, wind resuspension, and volatilization) govern distribution of nitro-PAHs and oxy-PAHs in the atmosphere and, concentration levels depend on several factors, such as temperature, seasons, atmospheric particulate size, and anthropogenic factors; among others.[3,4] Once released into the atmosphere, nitro-PAHs and oxy-PAHs are highly persistent in the environment and can be transported long distances from their original source, resulting in exposure from multimedia pathways, air, soil, sediment, water[5–7] and food.[8]

PAHs derivatives undergo wet and dry deposition, although wet deposition is more limited due to their hydrophobicity. In addition, these compounds may also enter into waters through industrial discharges and wastewater treatment plants.[9] Information regarding of nitrated and oxygenated PAHs contents in aquatic samples is limited, probably due to their low concentration levels and sample complexity. A few studies have reported pg L^{-1} to ng L^{-1} levels of nitrated, oxygenated and methylated PAHs derivatives in lake and river water samples.[10–12] Additionally, extraction and clean-up techniques coupled to selective and sensitive analyzers are required in order to determine nitro-PAHs and oxy-PAHs in different environmental matrices. Consequently, the development of new analytical methods is crucial for the above mentioned purposes.

Gas chromatography-mass spectrometry (GC/MS) and liquid chromatography (LC) coupled to various detectors (*e.g.* UV, fluorescence; *etc.*) are widely used in the analysis of PAHs and their derivatives. In addition, a major improvement in sensitivity can be achieved with ultra-high performance liquid chromatography combined with tandem mass spectrometry

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(UHPLC-MS/MS) configured with atmospheric pressure ionization sources, such us electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI); the above mentioned techniques has been applied successfully to analyze several oxy-PAHs and nitro-PAHs.[6,13]

Before instrumental analysis, water sample preparation, including extraction, preconcentration, and clean-up of the PAHs derivatives, need to be performed. A recent study has reported the applicability of solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) for the detection of nitro-PAHs.[14,15] However, this method presents some drawbacks such as high cost, sample carry-over and time. As an alternative to this popular technique is dispersive liquid–liquid microextraction (DLLME).[15] Current objectives of DLLME techniques are to overcome the limitations and to simplify and miniaturize the analytical procedure. Thus, the reduction of organic solvents and the prevention of waste generation make this microextraction technique an environmentally friendly procedure.[15–17] As part of the procedure, the appropriate mixture of extraction solvent and dispersive solvent is injected into the aqueous sample by syringe, rapidly. Thereby, the emulsion is achieved, resulting in a large increase in contact area between the two phases and the analytes are easily transferred into the extraction phase. Subsequently, the solvents are separated from the aqueous phase by centrifugation. The described strategy has been extensively explored during the past few years because of its simplicity, rapidity, convenience, and low cost and high enrichment factors.[15,17–19]

Typically, most DLLME methods have a centrifugation step, which represents the time-consuming stage. Consequently, an alternative, centrifugation-free approach, which was able to simplify the operation and speed up the extraction procedure, was developed. This microextraction technique can be found in literature as solvent terminated dispersive liquid–liquid microextraction (ST-DLLME)[20] or as solvent de-emulsification dispersive liquid–liquid microextraction (SD-DLLME)[21] and has been applied to the simultaneous extraction of pesticides,[20–22] fungicides[23] and PAHs[24,25] from aqueous samples. In these procedures, a de-emulsification step is conducted after the extraction by adding the additional portion of a dispersive solvent, which plays a role of quickly separate the emulsion into two phases.

However, to the best of our knowledge, the combination of SD-DLLME and ultra-high performance liquid chromatography coupled tandem mass spectrometry (UHPLC-MS/MS) with atmospheric pressure chemical ionization (APCI) for the sensitive analysis of nitrated and oxygenated PAHs in waters has not been reported.

In the present study, SD-DLLME coupled to UHPLC-APCI-MS/MS was proposed for the ultratrace determination of the following nitro- and oxy-PAHs of environmental importance: 1-nitropyrene, 2-nitrofluorene, 3-nitrofluoranthene, 9-nitroanthracene, 5,12-naphthacenedione, 9,10-anthracenedione, and 2-fluorenecarboxaldehyde. The effect of the various experimental parameters affecting the SD-DLLME of these compounds was studied and the applicability of the proposed method was tested for the determination of the mentioned nitro- and oxy-PAHs in aqueous environmental samples.

2. Experimental

2.1 Reagents and chemicals

Methanol, acetonitrile, dichloromethane, toluene, acetone, chloroform, cyclohexane, *n*-hexane and water Optima® LC-MS grade were purchased from Fisher Scientific (Fair Lawn, New Jersey). All nitrated and oxygenated PAHs standards were purchased from Sigma Chemical (St. Louis, MO, USA). The following environmentally relevant chemical standards of nitro-PAHs and oxy-PAHs were selected: 1-nitropyrene (1-NPYR), 2-nitrofluorene (2-NFLU), 3-nitrofluoranthene (3-NFLUANTH), 9-nitroanthracene (9-NANTH), 5,12-naphthacenedione (5,12-NAPHTONE), 9,10-anthracenedione (9,10-ANTHRONE), and 2-fluorenecarboxaldehyde (2-FLUCHO). Formic acid and nitric acid were obtained from Fisher Scientific (Loughborough, UK). Ultrapure water (18 mΩ cm) was obtained from a Milli-Q water purification system from EASY pure (RF Barnstead, IA, USA).

2.2 Preparation of standard solutions

Standard working solutions at different concentrations were prepared daily in acetonitrile by appropriate dilution of a 10 mg L^{-1} stock standard solutions of each compound. All water samples and stock standard solutions were protected from light and stored at 4 °C to prevent degradation.

2.3 Sampling and sample preparation

The optimized methodology was applied to river and drinking water samples. Accordingly, river samples were collected from the "Río Chorrillo" located in San Luis city ((33°19′01.7″S, 66°20′19.1″W), San Luis Province, Argentina) and drinking water samples were collected from our lab (33° 17′ 29.5368″S, 66° 20′ 24.7194″W, San Luis province, Argentina). All the water samples were obtained between October and November (spring season in the Southern hemisphere) in large 1 L dark-glass bottles and were all filtered through a 0.45 μm filter and stocked in amber glass at 4 °C. The pH of samples was adjusted to 2 with nitric acid to suppress all the microbiological activity.

2.4 SD-DLLME procedure

The schematic diagram of SD-DLLME is shown in Fig. 1. An aliquot of 20 mL water sample was placed in a glass test tube and was conditioned at 35 °C and spiked, when necessary, with 4 ng mL^{-1} of a mixture of nitro-PAHs and oxy-PAHs standards. After that, a mixture of 750 μL of acetone (dispersive solvent) and 500 μL of dichloromethane (extraction solvent) was introduced rapidly into the sample and vortex-mixed for 1 min. Consequently, a cloudy suspension consisting of water, acetone and dichloromethane, which resulted from the dispersion of fine dichloromethane droplets in the aqueous solution was formed. After the extraction was carried out, 1.0 mL of methanol (de-emulsifier solvent used for phase separation) was injected to break down the emulsion. Thus, the formed mixture cleared and separated into two phases. Accordingly, the dispersed fine

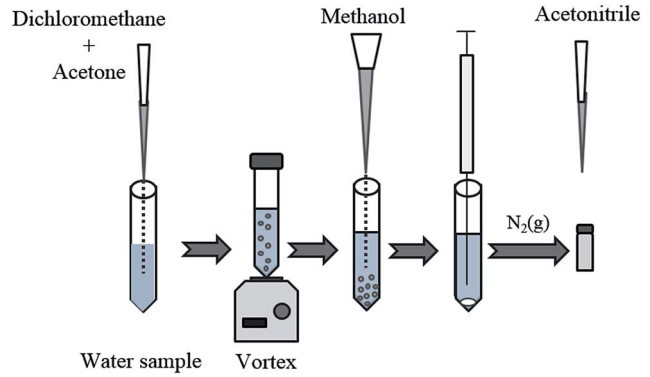

Fig. 1 Scheme of the experimental SD-DLLME procedure applied for extraction and enrichment of nitrated and oxygenated PAHs.

particles of the extracted phase (dichloromethane) settled to the bottom of the test tube. This sedimented phase was removed using a syringe and was transferred into a 1.5 mL amber glass vial to be dried under a N_2 stream. Then 100 μL of acetonitrile was added and the tubes were vortexed for 30 s. Finally, the reconstituted sample was placed for subsequent UHPLC-MS/MS analysis.

2.5 UHPLC-(+)APCI-MS/MS analysis

Analytical determination was performed on an Acquity™ Ultra-High Performance LC system (Waters, Milford) coupled to a Quattro PremierTM XE Micromass MS Technologies, triple quadrupole mass spectrometer with a ZSprayTM equipped with an APCI interface (Waters, Milford, USA), configured in positive ion mode. The source was operated in a positive mode at 400 °C with N_2 as the nebulizer and the source temperature was kept at 120 °C. The corona discharge current was maintained at 3.0 μA and the extractor voltage was set at 4.0 kV. Ultrapure nitrogen was used as desolvation gas with a flow of 200 L h^{-1}. Argon was used as collision gas at a flow of 0.18 mL min^{-1}. The separation was performed by injecting 10 μL sample onto an ACQUITY UPLC® BEH Phenyl (Waters, Milford, USA) analytical column with 2.1 mm internal diameter, 100 mm length, and 1.7 μm particle size. The binary mobile phases consisted of water with 0.1% (v/v) of formic acid (A) and acetonitrile with 0.1% (v/v) of formic acid (B). Variations of the flow rate (flow gradients) combined with solvent gradients were used for the compounds separation. The solvent gradient was started at an initial composition of 60% A and 40% B, then 3.0 min linear gradient to 10% A and 3.7 min linear gradient to 0% A. A return to the initial conditions was accomplished by a 1.3 min gradient to 60% A, where it was held for 0.5 min. The starting flow rate was 0.25 mL min^{-1}, then 3.7 min linear gradient to 0.20 mL min^{-1} and 4.0 min linear gradient to 0.15 mL min^{-1}. Then, flow rate returned to the initial conditions of 0.25 mL min^{-1}, where it was held for 0.5 min. Under the mentioned conditions, the total chromatographic run time was 5.5 min and no sample contamination or sample-to-sample carryover was observed. This total run cycle, was considerably shorter than the reported in recent works[13,26–28] and the herein optimized cycle was similar to the one reported by Fujiwara *et al.*[6] Thus, retention time and MS/MS settings for each compound are summarized in Table 1. As shown in this table, 1-NPYR and 3-NFLUANTH coeluted and

Table 1 Optimized chromatographic retention times and MRM experimental conditions for (+)APCI-MS/MS determination

Compounds	t_r (min)	Cone (V)	Precursor ion (m/z)	Collision (V)	Production (m/z)
Nitro-PAHs					
1-NPYR	4.10	30	248	16	218
				25	202[a]
				30	190
2-NFLU	3.17	30	212	12	195
				17	165[a]
3-NFLUANTH	4.17	19	248	17	231[a]
				16	218
				20	190
9-NANTH	4.49	19	224	8	207
				30	178[a]
Oxy-PAHs					
5,12-NAPHTONA	3.20	10	259	35	242
				21	231
				23	203[a]
9,10-ANTHRONA	2.72	35	209	20	181
				20	153[a]
2-FLUCHO	2.83	32	195	16	167[a]

[a] Transition for quantification.

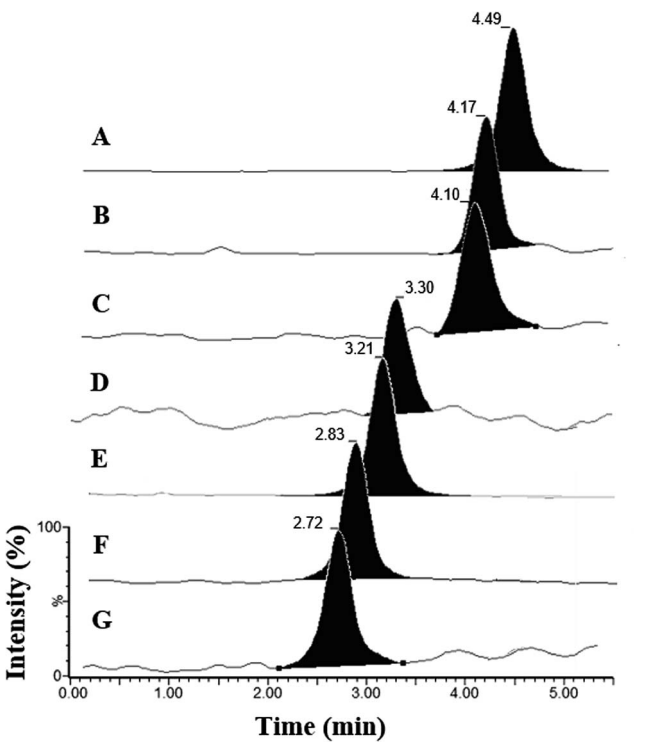

Fig. 2 Chromatograms of the seven compounds determined by UHPLC-(+)APCI-MS/MS: (A) 9-NANTHR (t_r: 4.49 min); (B) 3-NFLUANTH (t_r: 4.17 min); (C) 1-NPYR (t_r: 4.10 min); (D) 5,12-NAPHTONE (t_r: 3.30 min); (E) 2-NFLU (t_r: 3.17 min); (F) 2-FLUCHO (t_r: 2.83 min); (G) 9,10-ANTHRONE (t_r: 2.72 min).

they have the same parent ion (m/z 248). Bearing in mind that retention time and precursor ion were similar for both analytes, the product ions were selected taking into account that they must be different in order to avoid interferences. In this sense, both nitro-PAHs generated an intensive product ion at m/z 218, which could not be used for quantification purposes because both compounds can interfere between them during determination, so specific transitions for each nitro-PAHs were selected to monitor these two compounds. Representative MRM chromatograms of the nitro- and oxy-PAHs are shown in Fig. 2.

3. Results and discussion

The nitrated and oxygenated polycyclic aromatic hydrocarbons group of compounds constitute one of the most important classes of environmental pollutants due to their high toxicity and large distribution. There are different factors that affect this type of extraction process.[21] For example, some of them are selection and volumes of extraction, dispersive and de-emulsifier solvents, addition of a salt, application of ultrasound and/or vortex agitation; and thus all these factors were evaluated to obtain a suitable and reliable extraction strategy. It is important to note that the optimization and control of the sample temperature was also important since it influenced strongly the extraction efficiency.[12] All the mentioned variables were optimized by using the enrichment factor (EF) and/or the recovery (R) as the method's performance indicator. Thus, the enrichment factor can be defined as the ratio between the analyte concentration in the organic phase (C_{org}) and the initial concentration of analyte ($C_{initial}$) within the sample (eqn (1)). The recovery was calculated using the eqn (2).

$$EF = \frac{C_{org}}{C_{initial}} \quad (1)$$

$$R(\%) = \frac{(C_{found} - C_{real})}{(C_{added})} \times 100 \quad (2)$$

where: C_{found} represents the concentration of the analyte after adding a known amount of standard to the real sample, C_{real} is the concentration of the analyte in the real sample, and C_{added} is the concentration of a known amount of standard spiked to the real sample.

3.1 Selection of the nature and volume of extraction solvent

The properties of an extraction solvent are very important for achieving well-performed SD-DLLME. Principally, it should satisfy three properties: (i) high affinity to analytes, (ii) high solubility in the dispersive solvent and (iii) low solubility in water.[21] Therefore, because of all these considerations, *n*-hexane (density: 0.66 g mL^{-1}), cyclohexane (density: 0.78 g mL^{-1}) and toluene (density: 0.87 g mL^{-1}) with lower density than water, and dichloromethane (density: 1.46 g mL^{-1}) and chloroform (density: 1.48 g mL^{-1}) with higher density than water, were tested. Initially, the volume of these solvents was kept constant at 500 μL. The obtained results are shown in Fig. 3A, the best recoveries were obtained with dichloromethane. Consequently, dichloromethane was selected as extraction solvent in the following experiments.

After that, the effect of dichloromethane volume on the extraction efficiency of nitro-PAHs and oxy-PAHs was also investigated. Different volumes of dichloromethane: 50, 100, 250, 500, 750 and 1000 μL were studied. From the obtained results (Fig. 3B), it was observed that the recovery (R) and the precision were poor when the solvent volume was lower than 500 μL, probably due to some practical difficulties during the collection of the organic phase. Consequently, a volume of 500 μL of dichloromethane was selected for subsequent experiments as a compromise between the minimum solvent volume and the required robustness of the extraction procedure.

3.2 Selection of the nature and volume of dispersive solvent

Generally, the dispersive solvent in DLLME must be miscible with both water and extraction solvents. Thus, for the sake of acquiring the most suitable dispersive solvent three typical dispersive solvents, methanol, acetone and acetonitrile were assayed. The result of this study showed that analytes signals with acetone and methanol as dispersive solvents were higher when compared those with acetonitrile (Fig. 4A). Thus, acetone

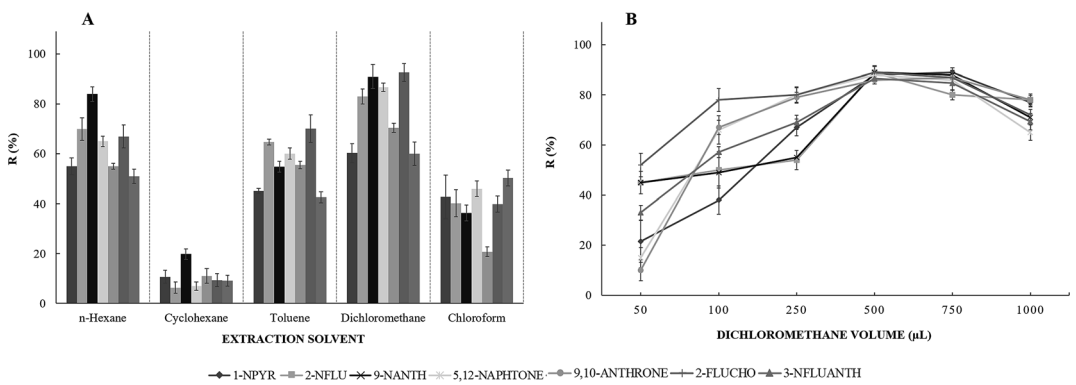

Fig. 3 Influence of the type (A) and volume (B) of the extraction solvent on the nitro and oxy-PAHs extraction. Concentration of mixture PAHs derivatives standard solution: 4 ng mL^{-1}; sample volume: 20 mL; acetone (dispersive solvent) volume: 750 μL; methanol (de-emulsifier solvent) volume: 1 mL; vortex time: 1 min.

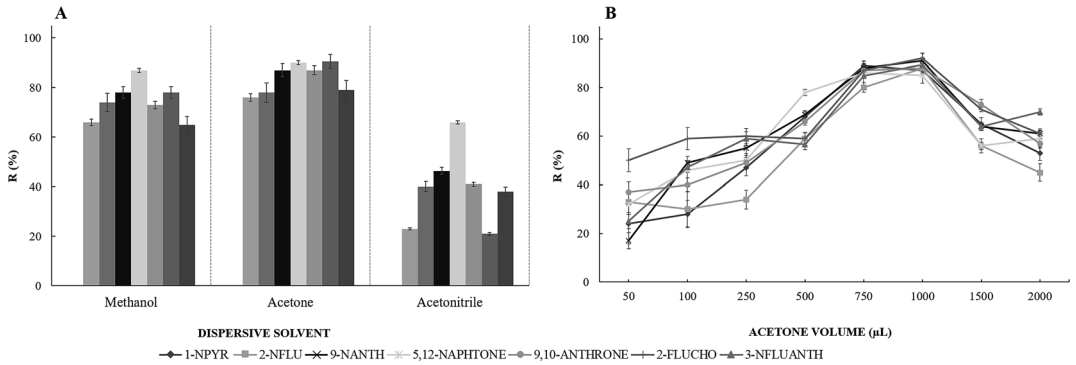

Fig. 4 Influence of the type (A) and volume (B) of the dispersive solvent on the R_s of the nitrated and oxygenated PAHs. Concentration of the mixture standard solution: 4 ng mL^{-1}; sample volume: 20 mL; volume of dichloromethane (extracting solvent) 500 μL; methanol (de-emulsifier solvent) volume: 1 mL; vortex time: 1 min.

was selected as the most suitable dispersive solvent due to its low toxicity and low cost.

The influence of the volume of acetone in the range of 50–2000 μL on the extraction efficiency of nitrated and oxygenated-PAHs was examined. From the obtained results (Fig. 4B), it was observed that the recovery was poor when the dispersive solvent volume was lower than 500 μL, possibly due to the cloudy state was not well formed and the extraction step was disturbed. In contrast, at higher volumes of acetone (1500–2000 μL), the solubility of the PAHs derivatives compounds in water increased and, probably, other analytes could be extracted, which also resulted in a deficient extraction behavior. Consequently, a volume of 750 μL of acetone yielded the highest recoveries for all the compounds and it was selected for further experiments.

3.3 Selection of the nature and volume of de-emulsifier solvent

Demulsification in SD-DLLME is normally conducted after the extraction by adding the additional portion of a dispersive solvent. This solvent quickly separates the emulsion into two phases. As mentioned, generally the dispersive and de-emulsifier solvents are always the same. However, considering that the effectiveness of the de-emulsifier step could also depend on the type of solvent used during the SD-DLLME procedure, in this work, several de-emulsifier solvents were studied. Thus, methanol, acetone (also used as dispersant solvent) and acetonitrile were evaluated. The results (Fig. 5A) revealed that both acetone and methanol provided similar and optimum recoveries when compared with acetonitrile. Thus based on these results, different volumes of acetone and methanol were evaluated: 500, 1000, 1500 and 2000 μL. The results demonstrated that at low volumes of acetone and methanol the extraction efficiency was poor due to a not completed de-emulsifier process (Fig. 5B). On contrary, the extraction efficiency slightly decreased when the de-emulsifier solvents volume increased above 1500 μL probably due to the increase of solubility of the nitrated and oxygenated PAHs in the original aqueous solution. Therefore, 1000 μL of the methanol was used as the optimal volume of de-emulsifier in subsequent experiments (Fig. 5B).

3.4 Temperature and salt addition effect

As described by other authors[29,30] and in our previous study,[12,19] temperature can affect the solubility of analytes in the extraction solvent and the mass-transfer process efficiency. In

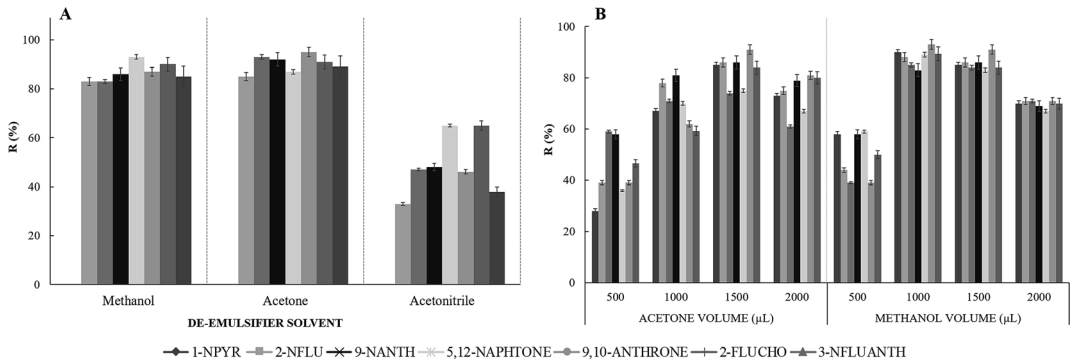

Fig. 5 Influence of the type (A) and volume (B) of the de-emulsifier solvent on the R_s of nitrated and oxygenated PAHs. Concentration of the mixture standard solution: 4 ng mL^{-1}; sample volume: 20 mL; volume of dichloromethane (extracting solvent) 500 μL; acetone (dispersive solvent) volume: 750 μL; vortex time: 1 min.

addition, control of sample temperature has been necessary for the efficient extraction of PAHs and their derivatives in water samples.[31] The effect of sample-solution temperature on the extraction efficiency was studied in the range 15–50 °C. The results are exhibited in Fig. 6A. As can be seen, extraction efficiencies increased and were optimal at temperatures of 35 °C and then decreased at higher temperatures. The high temperature could cause the loss of the settled phase and/or degradation of the PAHs derivatives, which resulted in the reduction of the extraction efficiencies. According to these results, water samples were initially thermostated at 35 °C.

On the other hand, another important factor to take into account in this procedure was the salting-out effect, which may adjust the ionic strength and improve the partition of analytes between aqueous and organic phases. This effect can be explained due that salts addition displaces the extraction equilibrium towards the organic phase, while simultaneously facilitating phase's separation. However, an excess of salt can increase the solubility of the analytes in the aqueous sample solution and, as a consequence, a reduction of the extraction efficiency due to a viscosity increment, which is detrimental to the diffusional transference of the target analytes into the extraction solvent.[29,31–33] The effect of salting out on the extraction procedure was investigated over a sodium chloride concentration ranging from 0 to 25% (w/v). The results are shown in Fig. 6B. As observed, the extraction recoveries of the analytes decreased with the increase of salt addition. Consequently, no salt was added in subsequent experiments.

3.5 Ultrasound application, manual and vortex agitation effect

Application of ultrasound and vortex agitation are efficient treatments to enhance liquid–liquid microextraction.[30] In this study, the influence of different shaking modes: manual, vortex and ultrasonic-based ones, was tested. The experimental results showed that the surface mass transfer process was more rapid and efficient when vortex agitation was used (Fig. 7A). In SD-DLLME, the extraction time is normally defined as the time elapsed between the injection of the mixture of dispersive and extractant solvents, and their contact with the sample, before the de-emulsification solvent is injected.[32] An optimum extraction time is the minimum time necessary to achieve equilibrium between the aqueous and the organic phase so that the extraction of the analytes, the sensitivity, and the speed of extraction is maximized. Accordingly, different vortex agitation times (0.25–5 min) were examined and the results are showed in Fig. 7B. An increase of extraction efficiency was observed when the extraction time was from 0.25 (15 s) to 1 min, meanwhile the extraction efficiency was constant above 1 min. Therefore, 1 min was the selected vortexing time for all the compounds.

The results indicated that the extraction equilibrium in the DLLME was achieved very fast probably due to the large surface area between the extraction solvent and the aqueous phase. Additionally, due to the above mentioned fact, the centrifugation step commonly reported in DLLME extractions can be avoided, reducing drastically the sample preparation time. Consequently, this constitutes a major advantage of the herein proposed method.

3.6 Sample volume

In general, in the DLLME decreasing the ratio of organic solvent volume to sample volume increases the enrichment factor. However, such volumes can lead to practical difficulties related to phase separation due to the solubility of the organic solvent in the aqueous sample. Thus, the sample volume can influence the extraction efficiency and the extraction times.

In this study due to the expected very low nitrated and oxygenated PAHs concentration levels in waters, different sample volumes (5–50 mL) were studied in order to improve their detection capability, while the remaining optimized conditions (extracting solvent (500 μL of dichloromethane), dispersive solvent (750 μL of acetone) and de-emulsifier solvent (1 mL of methanol)) were kept constant.

The obtained results (Fig. 8) showed that there were no significant differences in recovery values by increasing the sample volume from 5 to 20 mL. However, for sample volumes ranging from 30 to 50 mL, the sedimented organic phase diminished due to the previously evaluated optimal ratios among sample, extraction solvent, dispersive solvent, and de-

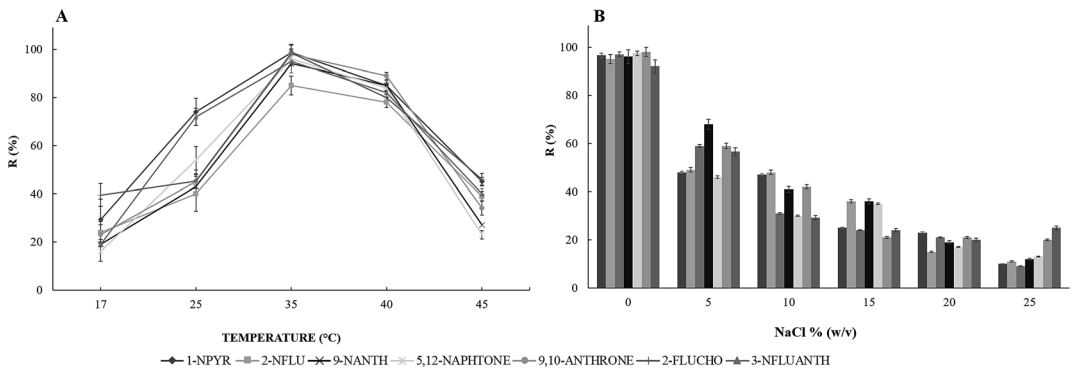

Fig. 6 Effect of (A) temperature and (B) salt addition on extraction efficiency. Concentration of the mixture standard solution: 4 ng mL^{-1}; sample volume: 20 mL; volume of dichloromethane (extracting solvent) 500 μL; acetone (dispersive solvent) volume: 750 μL; methanol (de-emulsifier solvent) volume: 1 mL; vortex time: 1 min.

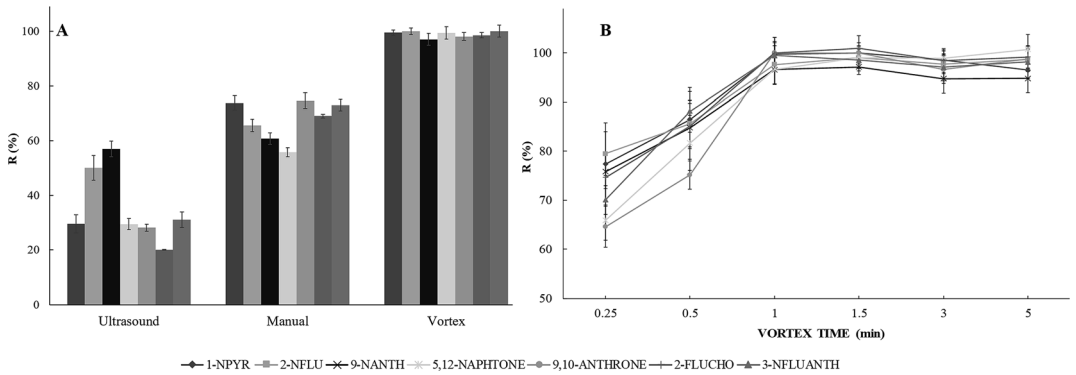

Fig. 7 Effect of (A) type and (B) time agitation on extraction efficiency. Concentration of the mixture standard solution: 4 ng mL^{-1}; sample volume: 20 mL; volume of dichloromethane (extracting solvent) 500 μL; acetone (dispersive solvent) volume: 750 μL; methanol (de-emulsifier solvent) volume: 1 mL.

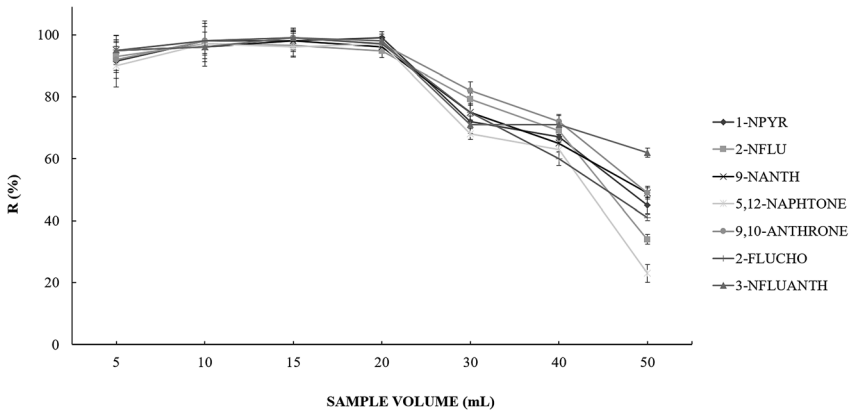

Fig. 8 Effect of sample volume on the R_s of the nitrated and oxygenated PAHs. Concentration of the mixture standard solution: 4 ng mL^{-1}; volume of dichloromethane (extracting solvent) 500 μL; acetone (dispersive solvent) volume: 750 μL; methanol (de-emulsifier solvent) volume: 1 mL; vortex time: 1 min.

emulsifier solvent volumes were not accomplished. Thus, a sample volume of 20 mL was used for further experiments.

3.7 Analytical performance

Under the above-mentioned optimized conditions, linearity, limits of detection, limits of quantification, extraction recovery, accuracy and precision were achieved for the validation of the SD-DLLME-UHPLC-MS/MS method proposed. Experiments were designed for the investigation of such factors; results are listed in Table 2. The analytical method developed showed good linearity, with correlation coefficients (r^2) higher than 0.995, for all nitrated and oxygenated PAHs.

Table 2 Analytical figures of merit of the SD-DLLME methodology combined with UHPLC-MS/MS

							(RSD%, $n = 3$)	
Compounds	r^2	Linear range (ng mL^{-1})	LOD (ng L^{-1})	LOQ (ng L^{-1})	R (%)	EF	Intra-day precision	Inter-day precision
Nitro-PAHs								
1-NPYR	0.9999	0.005–100	13.3	32.61	98.5	200	1.48	2.12
2-NFLU	0.9969	0.020–100	89.0	107.80	96.1	191	4.05	8.71
3-NFLUANTH	0.9983	0.005–100	26.9	45.67	97.3	192	3.43	5.30
9-NANTH	0.9952	0.010–100	11.2	27.21	95.1	193	3.21	6.25
Oxy-PAHs								
5,12-NAPHTONA	0.9984	0.020–100	81.9	120.39	95.7	192	3.22	5.39
9,10-ANTHRONA	0.9978	0.005–100	13.4	19.50	97.8	196	2.35	4.03
2-FLUCHO	0.9999	0.005–100	8.9	14.01	98.3	199	1.39	2.18

The *F*-test demonstrated for all cases that linear regression was statistically acceptable in the working range and this model showed goodness of fit.

The limits of detection (LOD) and quantification for each compound were calculated as the three and ten times of the ratio of the standard deviation of blank measurements to the slope of the calibration curve after extraction; respectively. Repeatability (intra-day precision) and reproducibility (inter-day precision, 5 days) of three replicate runs of the proposed procedure were evaluated by calculating the relative standard deviations (RSD). For this purpose, spiked nitrated and oxygenated PAHs solutions, at concentration levels from 0.001 to 100 ng mL^{-1}, were used.

Standard addition method was applied and performed in triplicate by spiking the samples with each standard at concentration levels from 1 to 20 ng mL^{-1}. The EFs and R_s for the nitro-PAHs and oxy-PAHs ranged from 191-fold to 200-fold and from 96 to 100%, respectively (Tables 2 and 3).

The proposed methodology presented a highly satisfactory analytical performance, the obtained LODs, LOQs and R_s values for the targeted nitro and oxy-PAHs were higher than those reported by other authors.[14,34] In addition, the EFs achieved were considerably higher than those obtained in the DLLME extraction of dinitro-aromatic compounds (EF ~ 85), nitrophenols (EF ~ 90), and PAHs (EF ~ 88–118) reported in the literature.[33,35,36]

The matrix effect was studied by comparing the slopes of the calibration curves of standards in both pure solvent (acetonitrile) and spiked samples. The percentage of the quotient of the slopes (*b*) in the spiked and solvent samples was used as an indicator of the extent of the ion suppression or signal enhancement, which was calculated as 100 − (*b* spiked/*b* solvent × 100). From the obtained results, no statistically significant matrix effect was observed.

3.8 Application of the SD-DLLME-UHPLC-MS/MS method to real samples

To evaluate the efficiency of the proposed SD-DLLME strategy coupled to UHPLC-(+)APCI-MS/MS, several samples including drinking and river waters collected as described in Section 2.3 were analyzed to quantify the targeted nitrated and oxygenated PAHs. In addition, to evaluate the extraction recovery of the analytes, the standard addition method was applied and performed in triplicate by spiking the samples with each standard at concentration levels from 1 to 20 ng mL^{-1}. The extraction recoveries for the nitro-PAHs in river and drinking water samples are summarized in Table 3.

The results in the Table 3 showed that only 1-NPYR (nitro-PAH) and 2-FLUCHO (oxy-PAH) were detected in drinking water samples of at concentrations equivalent to 1 ng mL^{-1}. On the other hand, diverse nitrated and oxygenated PAHs concentrations were found in river waters. It is important to note that these samples were collected from small water river strongly influenced by emissions from vehicular traffic and residential sources. These preliminary results indicated that nitrated and oxygenated PAHs were most abundant than the levels of PAHs reported in water samples,[11,34,37,38] possibly due to the fact that

Table 3 Analysis of water samples using the proposed methodology

	Drinking water samples					River water samples				
Compounds	Sample concentration (ng mL^{-1})[a]	Added concentration (ng mL^{-1})	Found concentration (ng mL^{-1})	*R* (%)[b]	RSD (%) ($n = 3$)	Sample concentration (ng mL^{-1})	Added concentration (ng mL^{-1})	Found concentration (ng mL^{-1})	*R* (%)	RSD (%) ($n = 3$)
1-NPYR	0.96 ± 0.03	1	1.94	98.0	3.4	3.70 ± 0.17	1	4.67	97.0	3.5
		10	10.97	100.1	1.9		10	13.46	97.6	3.4
		20	20.76	99.0	1.6		20	23.08	96.9	1.9
2-NFLU	[c](0.089)	1	0.98	98.0	2.3	2.20 ± 0.13	1	3.14	94.0	4.7
		10	9.56	95.6	2.6		10	11.78	95.8	4.8
		20	18.99	95.0	3.6		20	21.12	94.6	5.4
3-NFLUANTH	[c](0.026)	1	0.96	96.0	3.4	1.33 ± 0.21	1	2.28	95.0	4.6
		10	9.72	97.2	2.1		10	10.90	95.7	5.2
		20	19.38	96.9	3.7		20	20.32	94.9	6.1
9-NANTHR	[c](0.011)	1	0.97	97.0	2.5	6.90 ± 0.46	1	7.86	95.8	5.2
		10	9.55	95.5	4.5		10	16.70	98.0	4.8
		20	19.21	96.0	3.0		20	26.52	98.1	3.9
5,12-NAPHTONA	[c](0.082)	1	0.95	95.0	5.0	1.27 ± 0.72	1	2.22	95.0	3.5
		10	9.67	96.7	4.3		10	10.88	96.1	4.8
		20	19.00	95.0	5.1		20	20.19	94.6	5.0
9,10-ANTHRONA	[c](0.013)	1	0.99	99.0	3.5	2.57 ± 0.31	1	3.52	95.0	4.6
		10	9.76	97.6	2.8		10	12.30	97.3	3.9
		20	19.83	99.2	3.5		20	21.73	95.8	3.2
2-FLUCHO	0.83 ± 0.03	1	1.83	100.0	1.1	9.17 ± 0.20	1	10.13	96.0	5.8
		10	10.62	97.9	2.5		10	18.94	97.7	2.6
		20	20.41	97.9	1.5		20	29.14	99.9	3.9

[a] Mean value ± standard deviation. [b] Recovery, $n = 3$ replicates. [c] n.d., not detected (detection limit).

Table 4 Comparison of the proposed SD-DLLME with other methods for the determination of nitrated and oxygenated PAHs

Method	Separation-detection technique	Compounds	Sample volume (mL)	Extraction solvent (volume, mL)	LOD	R (%)	Total procedure time (min)	Sample	References
SPE	HPLC-chemiluminescence	Nitro-PAHs	8000	Dichloromethane (20)	0.044–0.049[b]	90–98	n.m.[a]	Rain, river and seawater	37
SPE	GC-MS	Nitro-PAHs and oxy-PAHs	4000	Dichloromethane (60)	0.02–7.40[b]	45–158	[a]	River and wastewater	9 and 11
C18-disk	HPLC-fluorescence	Nitro-PAHs	2000	Dichloromethane[a]	0.18–6.24[b]	87–104	[a]	River water	41
SPE	HPLC-chemiluminescence	Nitro-PAHs	1500	Dichloromethane (20)	0.009–0.041[c]	71–103	40	River water	42
SPE	GC-MS	Oxy-PAHs	500	Dichloromethane (20)	0.2–4.8[b]	78–149	[a]	Seawater	43
SPE	μLC-UV	Nitro-PAHs	100	Dichloromethane (10)	0.008–0.058[c]	80–97	[a]	River water	34
SPME	GC-MS	Nitro-PAHs	10	[a]	0.004–0.059[b]	91–102	45	Tap and well water	14
PA/HS-SPME	GC-MS	Nitro-PAHs	10	[a]	0.01–0.11[b]	[a]	45	River water	10
SDME	GC-MS	Nitro-PAHs and oxy-PAHs	10	Toluene (1)	0.6–468[c]	23–134	30	River, sea and groundwater	44
DLLME-SFO	Fluorescence detection	Nitro-PAHs	10	1-Dodecanol (0.025)	2.3–5.0[c]	95–100	∼15	Tap and lake water	12
SD-DLLME	**UHPLC-MS/MS**	**Nitro-PAHs and oxy-PAHs**	**20**	**Dichloromethane (0.5)**	**8.9–89.0[b]**	**95–98**	**2.5**	**Tap and river water**	**This method**

[a] n.m: not mentioned. [b] LOD (ng L^{-1}). [c] LOD (ng mL^{-1}).

the water solubility of PAHs derivatives is greater than the PAHs solubility.[39] Thus, the obtained results demonstrated the presence of nitro-PAHs at concentrations significantly higher than levels reported in previous studies for river, sea, and wastewater samples samples.[10,11,34,37] On the other hand, although literature regarding determination of oxy-PAHs in water samples is still scarce, this work's findings are in agreement with the results reported by other authors.[11,40] These results showed that the proposed method is very practical and useful for the analysis of real samples. As a summary, Table 4 shows a comparison between the analytical performance obtained by the proposed SD-DLLME-UHPLC-MS/MS method and the main characteristics of other works related to the determination of nitrated and oxygenated PAHs in water samples. SPE is an efficient approach that have been widely applied to the trace determination of these compounds for many years, but the strategies related require large amount of toxic organic solvent (10–200 mL), high sample volumes (100–8000 mL) and extraction times (15–45 min), increasing the overall sample treatment time. On the other hand, SPME and Purge-assisted headspace (PA/HS)-SPME are solvent less techniques that offer high sensitivity, but also require exhaustive extraction times (∼45 min). Thus, the optimized SD-DLLME procedure appears to be an advantageous alternative when compared to the others, with the advantages of being faster, simpler and environmentally friendly.

4. Conclusions

In this work, the usefulness and suitability of a novel SD-DLLME strategy combined with UHPLC-MS/MS for the extraction and quantification of seven nitrated and oxygenated PAHs derivatives in drinking and river water samples was demonstrated. The developed procedure provided many advantages such as excellent performance, simplicity, stability, operability, low cost, speed, and minimum consumption of organic solvents; being in agreement with the current demands of Green Chemistry. The coupling of the extraction technique to the characteristics of selectivity of the detection system provided unequivocal identification and sensitive quantification of the individual PAHs derivatives, which could be successfully applied for routine quantitative analysis and environmental monitoring studies.

Conflicts of interest

There are no conflicts to declare.

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