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Dispersive liquid–liquid microextraction based on solidification of floating organic drop and fluorescence detection for the determination of nitrated polycyclic aromatic hydrocarbons in aqueous samples

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Abstract

In this study, a dispersive liquid–liquid microextraction method based on the solidification of a floating organic drop (DLLME-SFO) combined with molecular fluorescence detection was developed for the analysis of nitrated polycyclic aromatic hydrocarbons in environmental samples. Parameters affecting the efficiency of the extraction procedure were evaluated and optimized, including the nature of the dispersant agent, extractant and dispersant volumes, salt addition effect, and extraction time. Additionally, various strategies in the emulsion formation process were assayed. After optimization, the values obtained for limits of detection and quantification for 3-nitrofluoranthene were 2.3 ng mL^{-1} and 5 ng mL^{-1} respectively, and for 9-nitroanthracene 1.7 ng mL^{-1} and 2.5 ng mL^{-1}. The recoveries values ranged from 95% to 100% and the enrichment factor attained varied from 380 to 400-fold. The developed method was successfully applied to the analysis of lake water and drinking water samples. The results indicated that the proposed approach is a novel, sensitive, fast and reproducible methodology suitable for the analysis of traces of environmentally important nitrated polycyclic aromatic hydrocarbons in water samples.

Keywords: *Nitrated Polycyclic Aromatic Hydrocarbons; Water samples; DLLME-SFO; Fluorescence detection*

1. Introduction

Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are mutagenic and tumorigenic pollutants that are present in a variety of environment samples [1, 2]. In the atmosphere, PAHs derivatives occur in the gas phase as well as in particulate matter and are chemically transformed by reactive trace gases such as ozone, NO_x or OH radicals or by photolysis reactions. These compounds can be found in airborne particulate matter and migrate across environmental phase boundaries, resulting in exposure from multimedia pathways [1, 3], soil [4-6], sediment [7, 8], water [9-11] and food [12-14].

The importance of the nitro-PAHs depends not only on their relative concentrations in ambient but also on their mutagenicity. While PAHs have proved to be indirect mutagens, nitro-PAHs are direct-acting mutagens [15, 16]. Particularly, 3-NFLUANTH and 9-NANTH have been identified and listed within the IARC Group 3 as "not classifiable as to its carcinogenicity to humans" and no regulation exists for nitro-PAHs. However, these compounds could be important contributors to the high toxicity in air particles at extremely low levels and their persistence in the particulate phase may result in human exposure through inhalation [5, 17, 18].

The nitro-PAHs, 1-nitropyrene and 3-nitrofluoranthene (a four-ring, low vapor pressure molecule) are of great interest because of their presence in diesel exhaust. These compounds have not been observed in any gas-phase reactions [19, 20]; whereas, 9-nitroanthracene (a three-ring structure) was found to be distributed between the particulate and vapor phase and could be directly emitted and/or formed from atmospheric reactions [21, 22]. From seasonal studies, the concentrations of all these compounds were several times higher in winter and autumn than in summer and spring, in particular for 9-NANTH and other volatile nitro-PAHs [21-23]. Therefore, the

concentrations of nitro-PAHs in the atmosphere depend on the season, the type and number of sources (heating used, traffic vehicles and industrial plants). Previous studies have reported nitro-PAHs levels in the low ng m^{-3} in airborne particles from areas with heavy traffic [3, 5, 24].

Although their importance, scarce information about distribution of nitro-PAHs in the different environmental compartments is still available. Certainly, the chemical properties of these compounds, their hydrophobic character and concentration levels in the environment, make their study a challenging analytical task for quantification purposes. Nitro-PAHs are widely distributed in the environment and have been identified in air, soil, sediment, plants and food [1, 9]. PAHs derivatives undergo wet and dry deposition, although wet deposition is more limited due to their hydrophobicity. In addition to the mentioned process, these compounds may also enter into waters through industrial discharges and wastewater treatment plants [25]. Several studies have reported the presence of nitro-PAHs in aquatic biota [13, 21] and marine sediment around the world [7, 8]. Recent studies reported the presence of methyl- and oxygenated-PAHs, but no nitro-PAHs were detected [26]. In other study, nitro-PAHs were found at relatively low levels (ng L^{-1}) in river samples using SPE extraction and GC-MS detection [27]. Consequently, information regarding distribution and accumulation of nitro-PAHs in aqueous samples should be expanded.

The complexity of environmental samples and the expected low concentration range of nitro-PAHs require highly sensitive and selective analytical methods to be optimized.

Existing methods include complex sampling and extraction procedures of nitro-PAHs based on traditional Soxhlet extraction [21], ultrasonic extraction [21], microwave-assisted extraction [24], and accelerated solvent extraction [5, 28]. Because of the complexity of the matrices of environmental samples, the effectiveness of all these

methods depends critically on the "cleanness" of the sample containing the nitro-PAHs. Clean-up steps typically involve gel permeation chromatography, adsorption chromatography, liquid–liquid extraction or solid-phase extraction [28, 29].

Analysis of environmental nitro-PAHs should be highly selective and sufficiently sensitive, for this reason, usually involves the use of high performance liquid chromatography (HPLC) and gas chromatographic (GC) [3, 12, 26, 27]. These chromatographic techniques are usually coupled with several detectors, thus fluorescence, UV, FID and MS detectors are the most frequently used for nitro-PAHs analysis [3, 14].

Current research directions are oriented towards the development of efficient, economical, and miniaturized sample preparation methods and, as result, solid-phase microextraction (SPME) and solvent microextraction (SME) approaches have been developed [30]. In 2006, dispersive liquid–liquid microextraction (DLLME) was introduced for the determination of polycyclic aromatic hydrocarbons in water samples, using gas chromatography-flame ionization detection (GC-FID) [31]. Up to now, DLLME has been successfully applied to the extraction of various families of organic and inorganic compounds in different matrices and several reviews have been written on this issue [31-33]. The advantages of the DLLME methods are simplicity of operation, rapidity, low cost -since it does not require the use of complex equipment-, high recoveries and enrichment factors.

Leong [34] and Xu [35] introduced in 2009 a variant to the dispersive liquid-liquid microextraction method known as solidification of a floating organic drop (SFO). In general, detailed DLLME-SFO stages are: an appropriate mixture of dispersive solvent and extraction solvent with low-density and proper melting point (for example, 1-undecanol or 1-dodecanol) are rapidly injected into an aqueous sample by syringe. The

dispersion of the extractant drops is achieved by using a third liquid phase which is immiscible with both, sample and extractant, and serves as dispersant. Thus an emulsion solution containing the fine drops of the extraction solvent dispersed completely in the aqueous phase is formed. The analytes in the sample are extracted into the fine drops, which are separated by centrifugation and immersed in an ice bath to solidify the extracting agent which had collected on the sample surface. The floated extract is removed with a spatula spoon and then transferred into another vessel where it melts. The extract obtained is dispensed to the appropriate measuring instrument [30, 31].

Hence, the DLLME-SFO has become one of the most interesting sample preparation techniques developed in recent years and has been applied to aqueous samples for determining specific groups of pollutants such as PCBs, OCPs, dinitrobenzenes, polycyclic aromatic hydrocarbons (PAHs), pyrethroid pesticides, and others compounds [36, 37]. However, to the best of our knowledge, the application of this technique for the extraction/enrichment of nitro-PAHs in environmental water samples has not been reported so far.

The foregoing demonstrates that the development of sensitive, selective and green analytical methods for the determination and quantification nitro-PAHs is extremely necessary and important from the environmental and toxicological applications point of view. In this article, a novel extraction methodology based on DLLME-SFO coupled to fluorescence detection for the determination of two nitro-PAHs of environmental concern: 3-nitrofluoranthene (3-NFLUANTH) and 9-nitroanthracene (9-NANTH) in aqueous samples is proposed for first time. Extraction parameters such as ionic strength, pH, mixing assistance (manual-, vortex-, and ultrasonic-assisted modes), time required, and different variants of emulsion formation were studied and optimized. It is important to note that the optimization of the mixing mode and the sample temperature control

variables were truly important since these aspects strongly influenced both analysis time and extraction efficiency. The proposed analytical method was successfully applied to the determination of 3-NFLUANTH and 9-NANTH in lake and drinking water samples from San Luis, Argentina.

2. Experimental

2.1. *Reagents and chemicals*

Two environmentally relevant chemical standards of nitro-PAHs were selected. Thus 3-nitrofluoranthene (3-NFLUANTH) and 9-nitroanthracene (9-NANTH) and the extractant 1-dodecanol (99%) were purchased from Sigma Chemical (St. Louis, MO, USA). Acetonitrile, methanol HPLC® grade were purchased from Fisher Scientific (Fair Lawn, New Jersey). Formic acid was 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

Ethanol-based working standard solutions were prepared by stepwise dilution from 10.0 mg L^{-1} stock standard solutions of each compound. All water samples were kept in darkness at 4°C prior to use. The daily standard working solutions of different concentrations were obtained by diluting the stock solutions with ultrapure water. All solutions prepared were stored in dark containers at 4 ºC.

2.3 Instrumentation

Fluorescence measurements were made using a Shimadzu RF-5301 PC spectrofluorophotometer equipped with a 150W Xenon lamp using semi-micro quartz

cells (300 µL). Excitation (λex) and emission (λem) wavelengths used for the detection of different compounds were 281/463 (slits: 10/5 nm) for 3-NFLUANTH and 258/406 (slits: 3/5 nm) for 9-NANTH, respectively. To accelerate the extraction of the mentioned compounds, an ultrasonic bath with temperature control (Cleanson 1106, Buenos Aires, Argentina) was employed. The pH of the solutions was determined in the field using a portable pH-meter (Orion Research, Inc., Orion 230 A, Beverly, MA, USA) equipped with a 9107 BN Orion glass electrode. For sample centrifugation, a U-320R-BOECO centrifuge was used.

2.4 Sampling and sample preparation

In this assay, lake water samples were collected from the Potrero de Los Funes reservoir (33º 14' 6.2376"S, 66º 13' 59.8908"W, San Luis Province, Argentina) in large 1 L dark-glass bottles. Drinking water samples were collected from our lab (33º 17' 29.5368"S, 66º 20' 24.7194"W, San Luis province, Argentina), between October and November (spring season in the Southern hemisphere). Before sample collection, the bottles were cleaned, the pH of the aqueous samples was adjusted to pH=2 with HNO_3 to suppress all the microbiological activity, the samples were filtrated using 0.45 µm pore filter prior to analysis and were kept in dark bottles at 4°C prior to use.

2.5 DLLME-SFO procedure

The schematic diagram of DLLME-SFO is shown in **Fig. 1**. A mixture of 75 µL of methanol (dispersive solvent) and 25 µL of 1-dodecanol (extraction solvent) was introduced into a screw cap glass test tube. After that, a 10 mL aqueous sample conditioned at 35 °C and spiked with 10 µL (40 mg L^{-1}) nitro-PAHs standards, was slowly added against the tube walls and vortex-mixed for 30 seconds. Consequently, a cloudy suspension (consisting of water, methanol and 1-dodecanol), which resulted

from the dispersion of fine 1-dodecanol droplets in the aqueous solution, was formed in the test tube. This solution was centrifuged for 10 min at 3000 rpm (1106.8 g) and placed then into an ice bath for 5 min. The obtained solidified floating organic solvent lying on top of the solution surface was transferred into a 1.5 mL vial. This extract was diluted with ethanol according to the ratio of 1:10 (v/v), because of the high viscosity of 1-dodecanol and the solution was directly placed to measurement cell the spectrofluorometer.

2.6 Enrichment factor and extraction recovery

There are typically two ways to display and compare data attained during the optimization process: enrichment factor (EF) and relative recovery (RR). Thus, the enrichment factor can be defined as the ratio between the analyte concentration in the floating phase ($C_{floated}$) and the initial concentration of analyte ($C_{initial}$) within the sample:

$$EF = \frac{C_{floated}}{C_{initial}}$$

On the other hand, the relative recovery was obtained from the following equation:

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

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 known amount of standard that was spiked to the real sample [31].

3. Results and discussion

The nitrated polycyclic aromatic hydrocarbons constitute one of the most important classes of environmental pollutants due because of their high toxicity. Thus 3-NFLUANTH is known as a primary pollutant from diesel motor exhaust emissions and it has been found to be one of the most abundant nitro-PAHs in ambient particulate matter [4, 19, 20]. On the other hand, 9-NANTH has also been detected in diesel exhaust, where it is likely to be formed upon high-temperature electrophilic nitration and is also present in the atmosphere due to the presence of HNO_3, N_2O_5, and NO_2 [23].

However, no analytical methods have been reported and evaluated for quantification of this class of compounds in water samples using DLLME-SFO coupled to fluorescence detection. There are different factors that affect the extraction process [36]. For example, some of them are: selection and volumes ratio of mixture of disperser agent to extraction solvent, effect of pH of sample solution and salt addition to the sample, processing time for ice bath and centrifugation steps. As mentioned, it was very important the optimization of these factors in order to obtain a suitable and reliable recovery strategy. In addition, three different strategies of emulsion formation were optimized and evaluated in the present work. Finally, the optimal conditions were used to extract and to detect nitro-PAHs in real aqueous samples.

3.1 *Selection of the nature and volume of extraction solvent*

The extraction solvent used for DLLME-SFO must satisfy several requirements: it must have low volatility, toxicity, cost and low solubility in water, high affinity for analytes and it must be compatible with the instrumentation employed for determination [36].

The extraction solvents most commonly used in DLLME-SFO for persistent organic pollutants (POPs) are for example: 1-octanol, 1-decanol, 1- dodecanol, 2-dodecanol, and 1-undecanol [36]. In a previous reported study 1-dodecanol was chosen as the most

suitable system for extraction of PAHs (including anthracene and fluoranthene [35, 38] and nitro-aromatic compounds in aqueous samples [39]. In addition, 1-dodecanol (melting point of 24°C) has demonstrated to be an extraction solvent having all the above mentioned requirements, for all these reasons 1-dodecanol was selected as the extraction solvent.

The effect of 1-dodecanol volume on the extraction efficiency for nitro-PAHs was also investigated. Different volumes of 1-dodecanol (10, 25, 50, 75 and 100 µL, respectively) as the extraction solvent was studied. At this point, the volumes used for methanol (dispersant reagent) and aqueous sample solution were fixed at 75 µL and 10 mL, respectively.

The extraction efficiency of 3-NFLUANTH and 9-NANTH by 1-dodecanol is illustrated in **Fig. 2**. From the obtained results, it was observed that relative recuperation (RR) decreased as the extraction solvent volume decreased. In all cases, the floating drop was difficult to collect and, as a consequence, the results irreproducible and the results when the volume of 1-dodecanol was lower than 25 µL. Consequently, 25 µL of 1-dodecanol was used as optimal extraction solvent volume, which is lower than the reported by other authors, 100 µL, for PAHs extraction with DLLME-SFO [35, 38].

3.2 *Selection of the dispersive solvent*

The selection of a dispersive solvent should usually be miscible with water and the extraction solvent in order to increase the contact surface area of the selected extraction solvent and to form very fine droplets with the aqueous sample. In addition, it is necessary to consider the toxicity of this agent and its cost.

Then, acetone, acetonitrile and methanol were tested, 1-dodecanol is highly soluble in methanol and, consequently, methanol provided an increased efficiency in the extraction

of the studied compounds in contrast with the other solvents under evaluation (**Fig. 3A**). After that, different volumes of MeOH (25, 75, 125, 175 µL) were tested in presence of a constant volume of 1-dodecanol (25 µL) (**Fig. 3B**). At low volumes of methanol (25 µL), low EF values were obtained, possibly due to the cloudy state was not well formed and the extraction step was disturbed. At the highest volume of methanol tested (175 µL), the solubility of the nitro-compounds in water increased and, probably, other analytes could be extracted, which also resulted in a deficient extraction. From the obtained results, a volume of 75 µL of methanol yielded the highest EF and it was selected for further experiments.

3.3 *Ionic strength and temperature effect*

The salting-out effect can decrease the solubility of analytes on aqueous sample solution and enhance the extraction efficiency. This can be explained due to the salting-out effect reduces the concentration of water available to dissolve the analyte/s and then decrease their solubility into the aqueous phase, facilitating this way their transference to the organic phase. 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 [30, 32, 34, 36]. The effect of salting out on the method was investigated over a sodium chloride concentration range of 0–25% (w/v). As a result, the peak areas of the analytes, and their enrichment factors, decreased slightly with the increase of addition of salt. Consequently, the extraction recovery was constant with the assayed amounts of salt and due to this; no salt was added in subsequent experiments.

Other important factor in DLLME experiments is the temperature. Temperature is the driving force for the complete dispersion of the extractant/dispersant solvents into the aqueous solution and it plays an important role on the whether the achieved sensitivity of the developed method is satisfactory or not. In this work, a temperature increment during the extraction affects both organic solvent solubility in water as well as the emulsification phenomenon and facilitate the transfer of the analytes to the organic phase. A series of experiments were designed for the optimization of temperature. Sample tubes containing the aqueous phase were placed for 10 min in a water bath maintained at 15, 25, 35, 40 and 50°C. The results are exhibited in **Fig. 4**. As can be seen from the figure, the extraction performance was greatest at 35°C and then decreased with the increase of temperature. Degradation of nitro-PAHs at high temperature is most likely the reason. The results were consistent with those reported by Song Xingliang [38, 40] for the extraction of PAHs.

3.4 *Strategies to improve the emulsification phenomenon*

It is known that in DLLME the mixture of extraction and disperser solvents is rapidly injected by syringe into the sample solution (or aqueous phase) and an emulsion is formed (a cloudy solution consisting of water, extraction solvent and dispersive solvent). A large contact surface between the sample and the droplets of extractant/dispersant is created, which improves the mass transfer between the phases, resulting in the analytes's extraction into the fine droplets, which are further separated by centrifugation. It would be also expected that shaking the tube after the addition of the extraction mixture solvent and before centrifugation, could have some influence in the extraction efficiency because it allows a more intimate and prolonged contact between aqueous and organic phases. Various techniques for assisting dispersion have been studied, and these methods can be classified into different groups: manual shaking,

vortex, magnetic stirring or ultrasonic agitation, among others [32, 33, 36]. In view of the importance of this stage on DLLME, different types of emulsification strategies were studied and, to the best of our knowledge, this is the first time a study of this nature was performed to efficiently extract nitro-PAHs by DLLME-SFO.

Initially, to select the optimal technique for emulsion formation, four approaches were tested and they are represented in **Fig. 5A**. The strategies I to III consisted in the classic injection procedure employed in DLLME with different modes of shaking: manual, vortex-based, and ultrasonic-based. Meanwhile, strategy IV is proposed in the present work as a novel methodology for the extraction of the analytes. For this approach, the mixture of MeOH (dispersive solvent) and 1-dodecanol (extraction solvent) was introduced in an empty conical tube and vortexed. After that, the water phase conditioned at 35 °C was slowly added against the tube walls, which was stirred for 30 s by vortex to induce a fine dispersion and subsequent efficient separation of the extractant phase. The results obtained are shown in **Fig. 5B**, as seen strategies I and II showed low extraction efficiencies for both analytes, this may be because the low dispersion of the analytes and incomplete phase separation due to the manual shaking and ultrasound-assisted agitation. By contrast, the strategies III and IV exhibited the best, close to 100%, and comparable extraction efficiencies. Therefore, for the process of emulsion formation, the strategy IV, proposed for first time in this manuscript, was selected for further experiments. The approach IV in contrast to III has the major advantage of the easy operation for the extraction of aqueous samples and a minimum adherence of the organic droplets onto the inner walls of the tube was observed.

3.5 *Effect of extraction, centrifugation and ice bath times*

Extraction time is also an important parameter during any procedure. In the DLLME methods, the extraction time is normally defined as the time elapsed between the injection of the mixture of disperser (methanol) and extractant (1-dodecanol) solvents and their contact with the sample, before the centrifugation step [31, 36]. To investigate the effect of the vortex-assisted extraction time on the extraction efficiency, vortex periods of 0, 0.5, 1, 1.5 and 3 min, under fixed experimental conditions, were assayed. The results revealed that vortex-based mixing has no significant effect on the extraction efficiency at times above 0.5 min. These results are possibly because of the large surface area between the aqueous solution and the extraction solvent. Therefore, the transition of the analytes between these two phases is very fast and an equilibrium state is achieved quickly, which is the obvious advantage of the DLLME technique. Consequently, 0.5 min of extraction time was chosen for the following experiments.

In order to accomplish a clear separation of the formed phases, different centrifugation times from 1 to 15 minutes were assayed. From the results, an adequate separation was obtained after 5 min of centrifugation at 3500 rpm. After phase separation at room temperature, the centrifuge tube was immediately put into an ice bath and the collection of the solid organic drop with a spatula was carried out. The effect of ice bath was examined in the range 3–20 min under constant experimental conditions. While ice bath times lower than 5 min affected negatively the droplet collection, ice bath times above 15 min affected the droplet integrity due to adherence of the organic material to the tube walls. As result, an ice bath time of 5 min was optimum for a rapid and easy collection and of floating organic phase.

As reported, after vortex agitation and centrifugation, the floating organic drop due its melting point is easily collected with a Hamilton syringe or with a spatula. This later is carried out via solidification of the droplet at a low temperature, then is transferred to a

vial using a small spatula and is quickly melted at room temperature and placed (after proper dilution) into the analytical instrument for quantification [30, 32, 33, 36]. This is a tedious, prone to analyte loss and time consuming step (between 3 to 10 min) in DLLME-SFO; therefore, in this work the effect of collecting the organic drop with a Hamilton syringe (at room temperature) or after cooling in an ice bath (at low temperature) during 5 min was studied. This comparison was carried out in order to minimize the time necessary for droplet collection. The results showed that the best extraction efficiency was achieved when the drop was solidified by cooling in a beaker containing crushed ice. In contrast, when a Hamilton syringe was used the efficiency was lower since it was difficult to differentiate the interphase between the organic phase (floating drop) and the aqueous phase. Although the solidification of the extraction phase by cooling in an ice bath is an additional step in DLLME-SFO and this consequently not satisfy the demand of a fast analysis, it was demonstrated in this work that this stage is extremely important when very small volumes of extraction solvent as used for efficient extractions of nitro-PAHs using DLLME-SFO.

3.6 Analytical performance

Under the optimum conditions described above, the analytical performance characteristics of the proposed method was obtained. The DLLME-SFO method-fluorescent detection was validated for linearity, detection and quantification limits, selectivity, accuracy and precision and the performance of this method was summarized in **Table 1**. The calibration curve using was obtained by least-squares linear regression analysis of the relative intensity of fluorescence versus nitro-PAHs concentration, good linearity was observed, with correlation coefficients (r^2) higher than 0.997 for both compounds under study. The F-test demonstrated that linear regression was statistically acceptable in the working range and this model showed goodness of fit. The limits of

detection (LOD) and quantification (LOQ) were calculated based on the signal-to-noise ratios of 3 and 10; respectively. Intra-day and inter-day precisions (5 days) of the spiked 3-NFLUANTH and 9-NANTH at concentration levels from 0.5 to 500 ng mL^{-1} were evaluated. Reproducibility of the proposed method was studied by calculating the relative standard deviations (RSD) of three replicate runs of the proposed procedure. The EFs and RR for the nitro-PAHs ranged from 380 to 400 and from 95.8 to 100%, respectively (**Table 1**). The matrix effect was studied by comparing the slopes of the calibration curves of standards in both pure solvent (ethanol) 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)*. The results show that the matrix under consideration did not significantly affect the DLLME process.

3.7 *Application of the method to real samples*

The real samples collected as described in Section 2.4 were analyzed. To evaluate the recovery of the analytes, the selected samples were spiked with nitro-PAHs standards at concentrations from to 0 to 100 ng mL^{-1}. The sample analysis and recovery studies were performed in triplicate. The relative recoveries for the nitro-PAHs in lake and drinking water samples are summarized in **Table 2**.

The 3-NFLUANTH and 9-NANTH nitro-PAHs were both found in lake water samples while these nitro-PAHs concentrations were below the detection limits in drinking water. These preliminary results indicated that 9-NANTH was most abundant than 3-NFLUANTH in the analyzed samples, possibly due to the fact that the solubility of 9-NANTH ($8.3x10^{-9}$ mg L^{-1}) in water is greater than the solubility of 3-NFLUANTH ($3.71x10^{-16}$ mg L^{-1}) [41]. In addition, due to the surrounding areas of the lake, where the

samples were collected, were recently affected by wildfires could be one of the causes of the presence of nitrated PAHs in lake water samples.

4. Conclusions

A new dispersive liquid–liquid microextraction methodology based on the solidification of a floating organic droplet (DLLME-SFO) combined with fluorescence molecular detection was developed for the first-time for simultaneous extraction of ultratraces of nitrated-PAHs in lake and drinking water samples. The proposed method has demonstrated advantages such as low cost and solvent consumption –low toxicity-, simplicity and rapidity and satisfactory accuracy and precision. Therefore, the proposed methodology can be successfully applied for routine quantitative analysis of trace levels of 3-NFLUANTH and 9-NANTH in natural water samples.

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Table 1. Analytical figures of merit of the DLLME-SFO methodology combined with fluorescence detection.

Compounds	r^2	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	EF	Intra-day precision	Inter-day precision
						(RSD%, n = 5)	
3-NFLUANTH	0.9979	5.0-500	2.3	4.8	385	3.4	4.2
9-NANTH	0.9981	2.5-500	1.7	2.3	399	2.9	3.3

Table 2. Relative Recovery (RR(%)) obtained from the analysis of spiked water samples using the proposed methodology.

Compounds	Spiked Concentration (ng mL^{-1})	Drinking water			Lake water		
		Concentration found (ng mL^{-1})	RR (%)	RSD (%) (n=3)	Concentration found (ng mL^{-1})	RR (%)	RSD (%) (n=3)
3-NFLUANTH	0	N.D.			8.8		
	10	N.D.	97	1.5	9.1	99	4.1
	20	N.D.	99	2.1	9.3	98	3.8
	40	N.D.	95	1.4	8.9	95	2.5
	60	N.D.	96	1.0	9.4	92	1.9
	80	N.D.	94	3.6	9.2	95	3.2
	100	N.D.	95	4.9	9.3	93	4.0
9-NANTHR	0	N.D.			12.5		
	10	N.D.	99	2.1	11.9	96	2.9
	20	N.D.	96	2.7	12.1	98	3.1
	40	N.D.	98	2.5	12.3	99	1.9
	60	N.D.	97	1.9	12.0	97	1.5
	80	N.D.	97	2.0	11.7	96	2.2
	100	N.D.	96	3.8	11.9	94	3.4

*N.D.: not detected

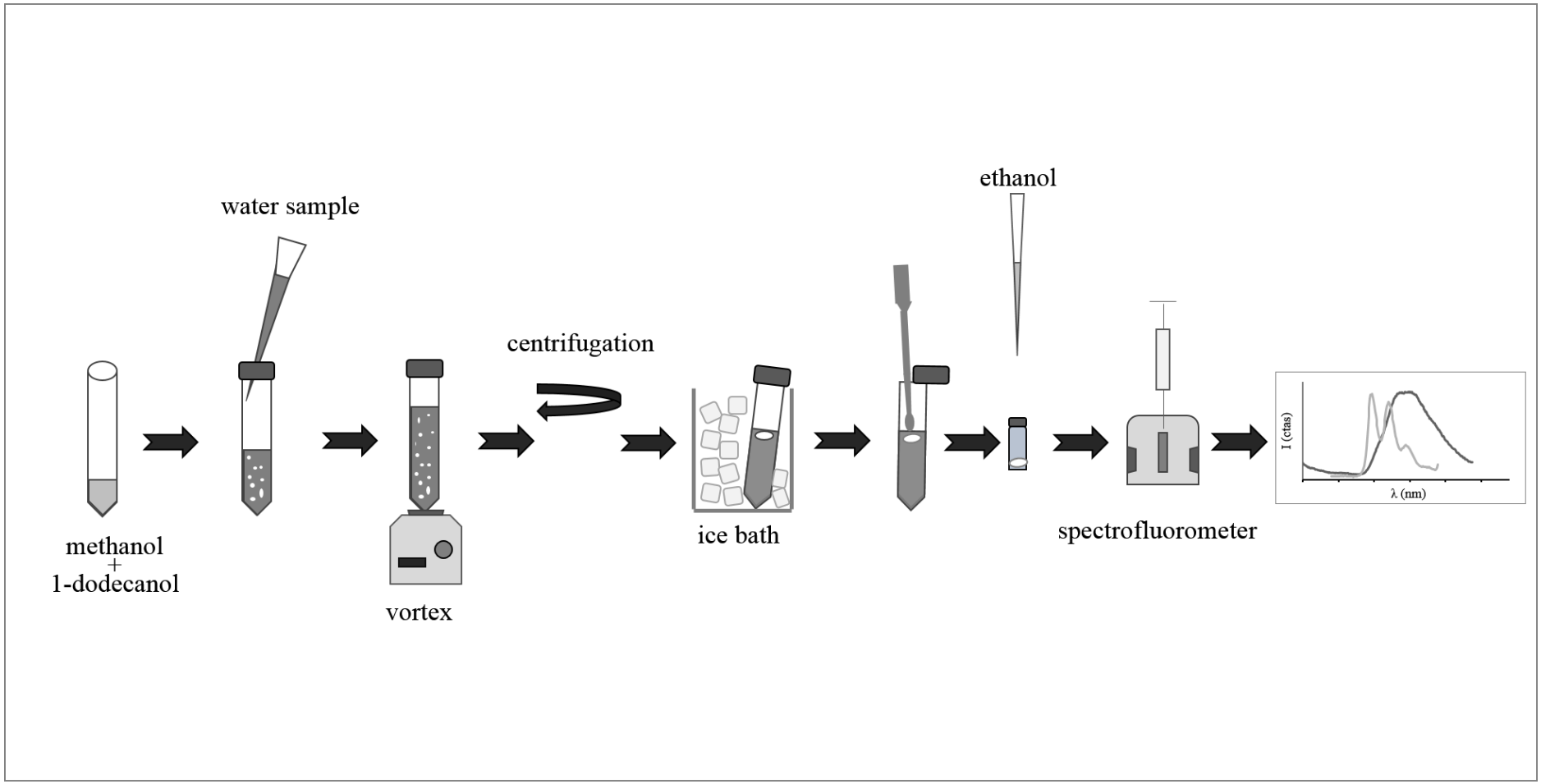

Fig. 1

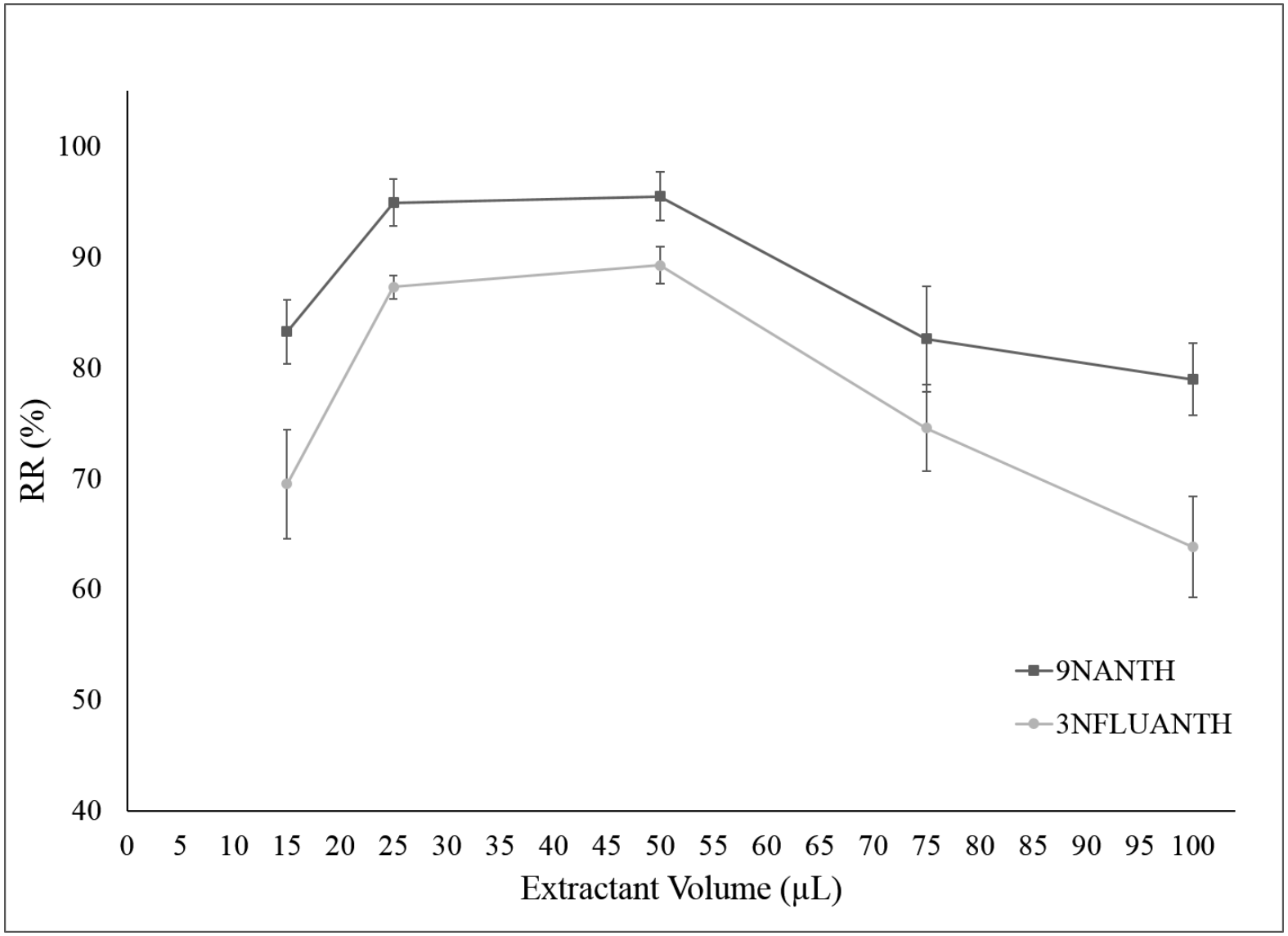

Fig. 2

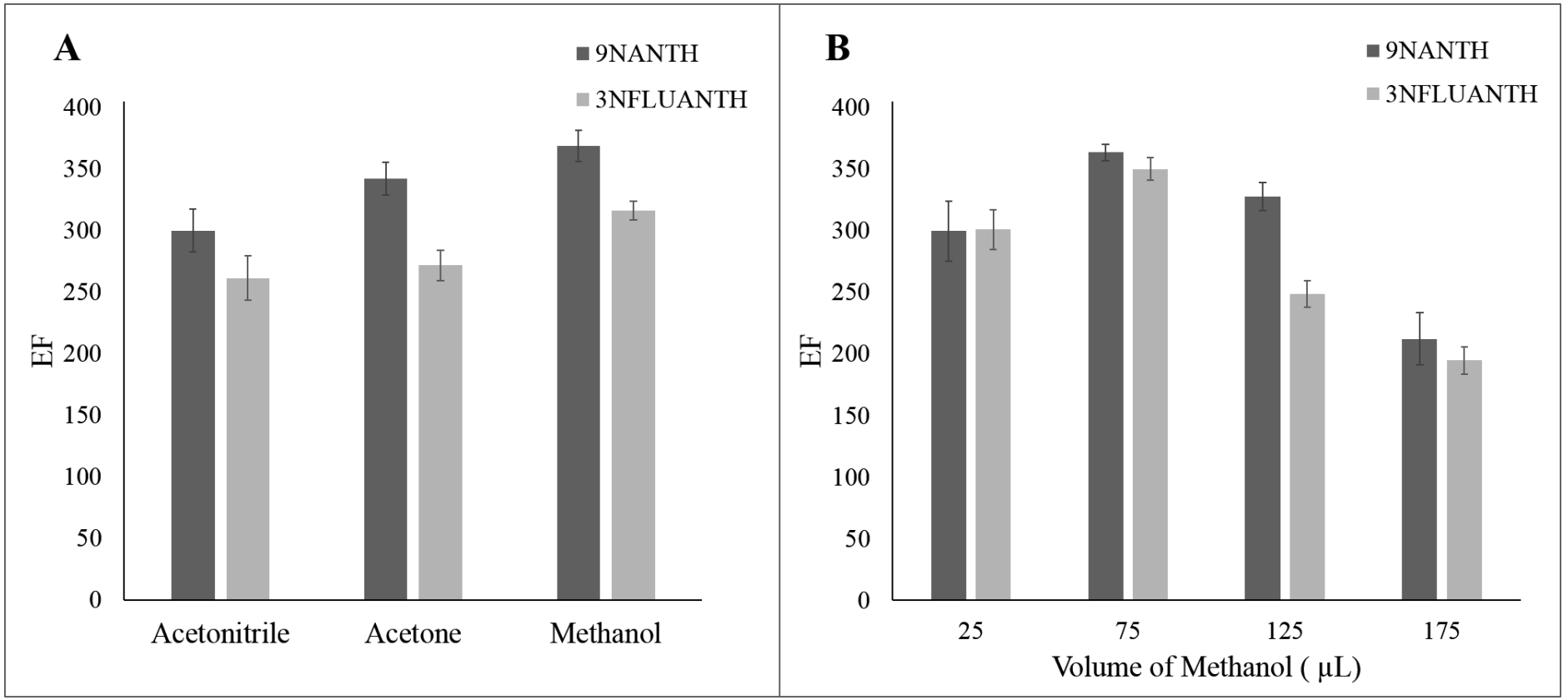

Fig. 3

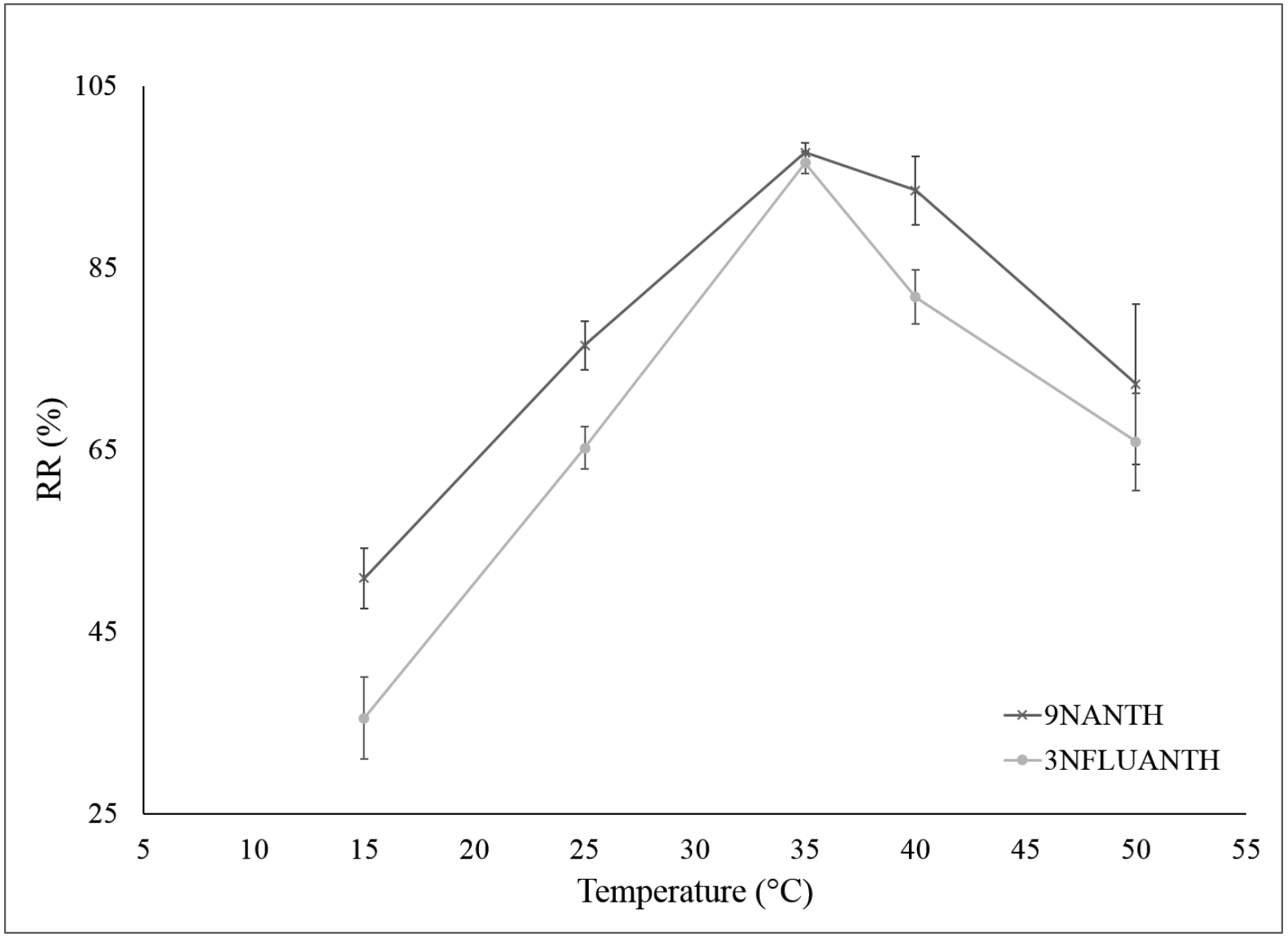

Fig. 4

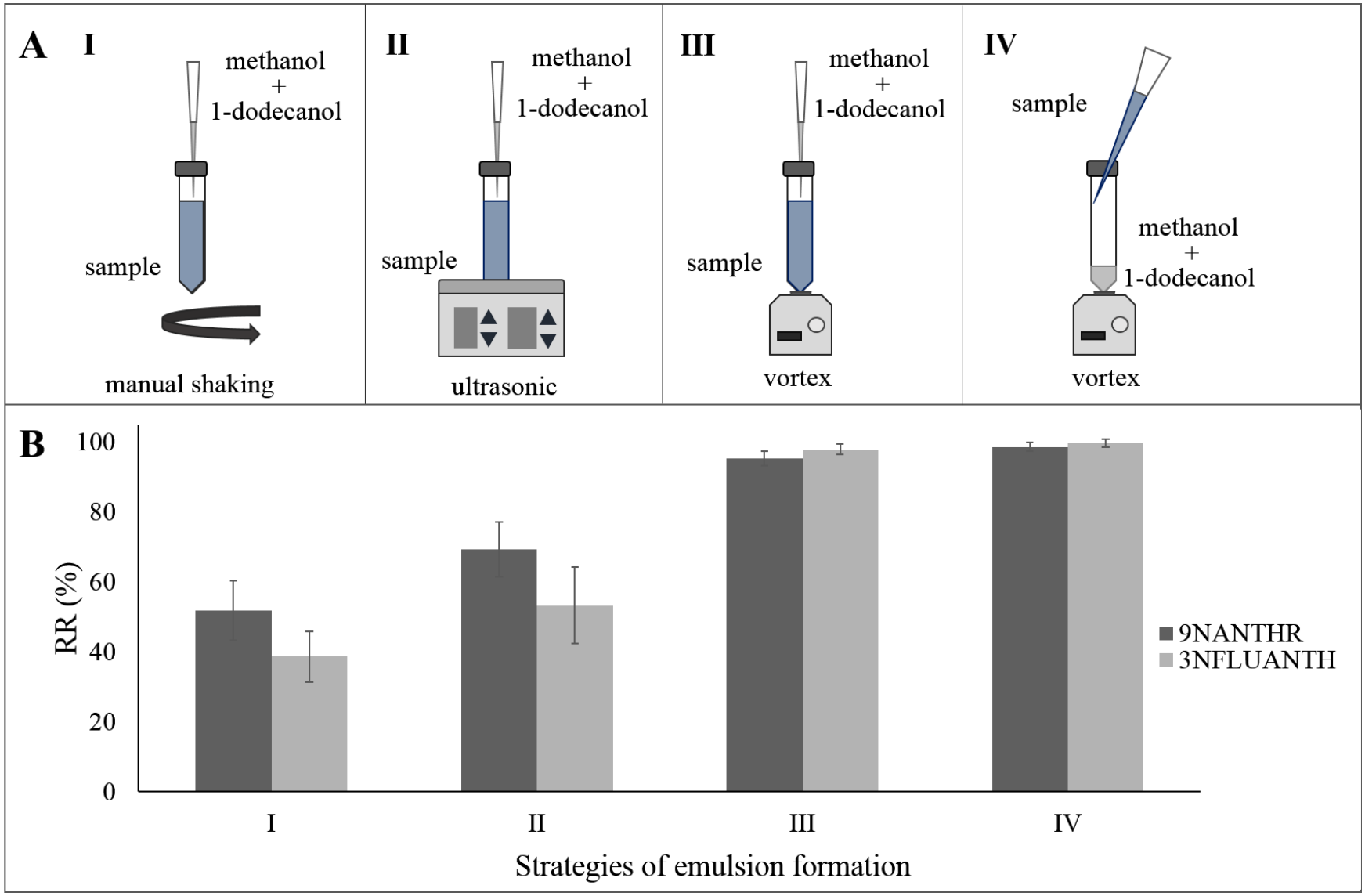
A
I
methanol
+
1-dodecanol
sample
manual shaking
II
methanol
+
1-dodecanol
sample
ultrasonic
III
methanol
+
1-dodecanol
sample
vortex
IV
sample
methanol
+
1-dodecanol
vortex
B
RR (%)
100
80
60
40
20
0
I
II
III
IV
Strategies of emulsion formation
9NANTHR
3NFLUANTH

Fig. 5

Fig. 1. Schematic illustration of the experimental DLLME-SFO procedure applied for nitrated PAHs followed by fluorescence detection.

Fig. 2. Effect of 1-dodecanol volume over the nitro PAHs extraction. Concentration of mixture nitro-PAHs standard solution: 40 ng mL^{-1}; sample volume: 10 mL; methanol (dispersant solvent) volume: 75 µL; no salt addition; vortex time: 0.5 min; centrifugation time and rate: 5 min, 3000 rpm.

Fig. 3. Influence of the type **(A)** and volume **(B)** of the extraction solvent on the EFs of the nitro-PAHs. Concentration of the mixture standard solution: 40 ng L^{-1}; sample volume: 10 mL; volume of 1-dodecanol (extracting solvent) 25 µL; no salt addition; vortex time: 0.5 min; centrifugation time and rate: 5 min, 3000 rpm.

Fig. 4. Effect of temperature on extraction efficiency. Concentration of mixture standard solution: 40 ng L^{-1}; sample volume: 10 mL; 1-dodecanol (extracting solvent) volume: 25 µL; methanol (dispersant solvent) volume: 75 µL; no salt addition; vortex time: 0.5 min; centrifugation time and rate: 5 min, 3000 rpm.

Fig. 5. Strategies of emulsion formation **(A)** and effect on the extraction efficiency (**B)**. Concentration of mixture standard solution: 40 ng L^{-1}; sample volume: 10 mL; 1-dodecanol (extracting solvent) volume: 25 µL; methanol (dispersant solvent) volume: 75 µL; no salt addition; vortex time: 0.5 min; centrifugation time and rate: 5 min, 3000 rpm.

Graphical abstract

Highlights

- *A novel DLLME-SFO strategy was developed for extraction/enrichment of nitro-PAHs.*
- *Nitro-PAHs of environmental concern were studied.*
- *Ultra-trace levels of the PAHs derivatives were detected by fluorescence.*
- *The method was applied to lake and drinking water samples.*