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Determining heterocyclic aromatic amines in aqueous samples: A novel dispersive liquid-liquid micro-extraction method based on solidification of floating organic drop and ultrasound assisted back extraction followed by UPLC-MS/MS

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ABSTRACT

A novel dispersive liquid-liquid microextraction based on solidification of floating organic droplet combined with ultrasound assisted back extraction for the determination of four heterocyclic aromatic amines in natural water samples prior ultra high-performance liquid chromatography-tandem mass spectrometry was developed. The analytes were extracted from the water samples by a dispersive liquid-liquid microextraction procedure based on solidification of floating organic drop, which was performed by a mixture composed by a less dense than water extraction solvent, 1-undecanol, and a dispersive solvent, methanol. After that, a novel ultrasound assisted back extraction step was performed in order to make the clean-up/enrichment procedure compatible with the detection requirements. Under optimum conditions, linearity ranged from 2.2 to 50 ng mL^{-1}, with enrichment factors from 130 to 136-folds. Thus limits of detection between 0.7 and 2.9 ng mL^{-1} were obtained. Precision of the method was evaluated in terms of repeatability, relative standard deviations varied from 4.3% to 6.7%. Relative recoveries ranged from 92% to 106% for all analytes. The satisfactory performance demonstrated that the proposed methodology has a strong potential for application in the multi-residue analysis of heterocyclic aromatic amines present in complex environmental matrices.

1. Introduction

Heterocyclic aromatic amines (HAAs) are a group of compounds known to be mutagenic and carcinogenic. The International Agency for Research on Cancer (IARC) has classified the HAAs as probable or possible human carcinogens (Groups 2A and 2B) and recommends to reduce the exposure to them [1]. The US National Toxicology Program (NTP) has also classified some HAAs as reasonably anticipated human carcinogens [2]. Human contact with these compounds can occur by ingestion of foods and environmental exposure [3]. The HAAs are molecules with multi-ring aromatic structures containing one or more nitrogen atoms in their ring system and exocyclic amino group. These compounds have been classified into two major groups, amino carbolines and aminoimidazoazaarenes (AIAs). Amino carbolines are formed by the pyrolysis of amino acids at temperatures about 300 °C, while AIAs are produced at temperatures about 150 °C through the aldol condensation of pyridine and pyrazines (Maillard reaction) [4,5]. An increasing number of studies also report a link with the combustion of various materials such as wood, biomass, and petroleum; among others [6]. Consequently, HAAs can be detected in cooking fumes, cigarette smoke, diesel exhaust, and airborne particulate matter [7,8].

Heterocyclic aromatic amines are able to migrate into the atmosphere causing pollution in remote areas. It is a well-known fact that water is essential for healthy living, as a consequence, pollution of this resource might be considered as serious health and environmental problems. In this context, HAAs have been identified in water from several areas around the world lately. Ono et al. detected traces of HAAs in river waters near urban areas, where sewage treatment plants discharge their effluents [9]. Therefore, a proper control based on sensitive determination methodologies to ensure HAAs levels in compliance with the safety and water quality requirements are required.

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Many analytical techniques have been reported to detect traces of HAAs in food, biological and environmental samples: gas chromatography-mass spectrometry (GC-MS) [3], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [8,10], high-performance liquid chromatography with photo-diode array detection (HPLC-UV/DAD) [11], ultra-fast liquid chromatography (UFLC) [12], and ultra high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) [13,14].

Owing to the presence of trace amounts of HAAs (ng g^{-1} level) and the complexity of most real samples, both sample pretreatment procedures and preconcentration steps are crucial to improving sensitivity and selectivity of the current analytical methods. Recently, new microextraction techniques have been reported and extensively studied because of the very small amounts of solvent and sample required, which provide more environmentally-friendly analytical alternatives. In this context, as a relatively new sample preparation technique dispersive liquid-liquid microextraction (DLLME) consists in the fast injection of an appropriate binary mixture formed by the extractive and the dispersive solvents into an aqueous sample containing the analytes [15].

Multiple DLLME variants have been developed. Thus a dispersive liquid-liquid microextraction based on solidification of floating organic drop (DLLME-SFO) was introduced by Leong and co-workers in 2008 [16]. This alternative gained popularity since then due to its suitability for organic and inorganic compounds [17–19]. Extraction solvents with lower density than water and melting points near or below room temperature are used. As a consequence, these solvents can be solidified by decreasing the operating temperature. After centrifugation, the solidified floating organic drop (SFO) can be transferred into a glass vial and, as soon as the solidified organic solvent melts and/or is diluted with a proper solvent, the analytical determination can be carried out. As mentioned, the performance of DLLME-SFO has been demonstrated by extraction of different organic and inorganic compounds from different matrices [17,20]. Thus DLLME-SFO promises important applications for trace analysis.

Despite its successful combination with many chromatographic techniques, there are not reports about the application of DLLME-SFO followed by ultrasound assisted back extraction (UABE) prior to UPLC-(+)-ESI-MS/MS. The incompatibility of the final organic phase containing mostly the extractive reagent with the detection system is a drawback when mass spectrometric-based analyses are required. In order to overcome this issue, a novel and simple UABE step based on the use of MS compatible organic solvents to facilitate the injection of the DLLME-SFO extracted HAAs into the chromatographic-spectrometric systems is proposed. In this sense, the coupling of both extraction and back extraction procedures creates novel and important possibilities for ultra-trace analysis.

In summary, the purpose of this study is to propose a novel alternative based on DLLME-SFO-UABE for the isolation and preconcentration of four non-polar HAAs followed by UPLC-(+)-ESI-MS/MS determination of these compounds in natural water samples. To the best of our knowledge, the DLLME-SFO-UABE method has not yet been applied to HAAs and/or efficiently coupled to UPLC-MS/MS. Therefore, the association of DLLME-SFO to mass spectrometry is clearly demonstrated. Also, this work constitutes the first report about the monitoring of non-polar HAAs in both drinking and natural waters from Argentina. The influence of several variables affecting the performance of both microextraction and detection techniques was assessed and optimized. The analytical performance of the DLLME-SFO-UABE approach coupled to UPLC-(+)-ESI-MS/MS was validated and applied.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards of 3-Amino-1,4-dimethyl-5H-pirido-[4,3-b]-indole (Trp-*P*-1), 3-amino-1-methyl-5H-pirido-[4,3-b]-indole (Trp-P-2), 2-amino-9H-pyrido-[2,3-b]-indole (AαC), 2-amino-3-methyl-9H-pyrido-[2,3-b]-indole (MeAαC) were obtained from Toronto Research Chemicals (North York, ON, Canada). The structure of the HAAs studied are shown in Fig. 1.

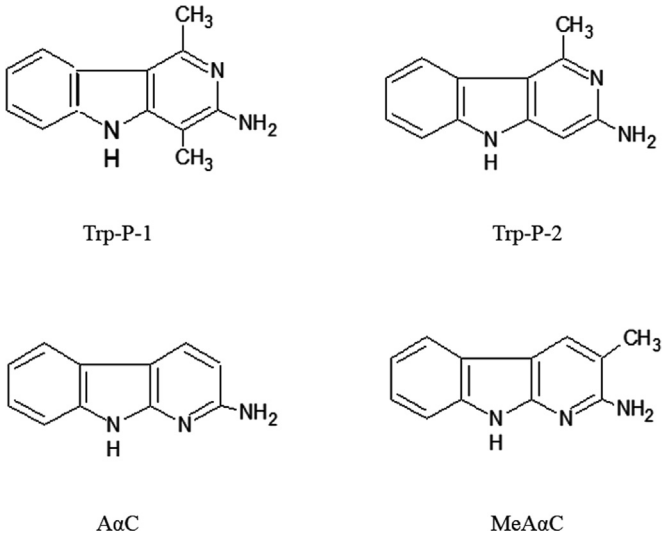

Fig. 1. Structures of the HAAs under study.

Acetonitrile (ACN), methanol (MeOH), acetone and water Optima® LC–MS grade were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). Formic acid was obtained from Fisher Scientific (Loughborough, UK). Working standard solutions prepared in ACN/H_2O (1:3) were prepared daily by appropriate dilution of a 1 mg L^{-1} aqueous stock solution. Quantification was achieved by preparing spiked water samples with proper amounts of the analytes. The solutions were maintained at 4 °C, protected from light and kept in amber flasks. 1-dodecanol for the microextraction step was obtained from Sigma-Aldrich (St. Louis, USA). An ultrasonic cleaner (Testlab, TB-04 TA, Buenos Aires, Argentina), a vortex (Arcano, Buenos Aires, Argentina) and a centrifuge (U-320R-BOECO, Germany) were employed for the sample treatment step.

2.2. Instrumentation

2.2.1. Mass spectrometric instrumentation and MS/MS conditions

Mass spectrometry analyses were performed on a Quattro Premier™ XE Micromass MS Technologies triple quadrupole mass spectrometer with a Z-Spray™ electrospray ionization source (Waters, Milford, USA). For all compounds, the source was operated in a positive mode and the data was acquired in multiple reaction monitoring modes (MRM) of selected ions at the first (Q1) and third quadrupole (Q3). The source working conditions were as follows: capillary voltage, 2.7 kV; extractor voltage, 1.0 kV; source temperature, 150 °C; desolvation temperature, 350 °C; cone gas flow rate, 50 L h^{-1}; desolvation gas flow rate, 400 L h^{-1}. Ultrapure nitrogen and argon were used as cone and collision gases; respectively. To choose the analytes fragmentation patterns in MRM mode, direct infusions (via syringe pump) into the MS of HAAs (0.5 mg L^{-1}) standards solution prepared in ACN/H_2O (1:3) were performed and the product ion scan mass spectra were recorded. The compound-dependent parameters were optimized and listed in Table 1. Data acquisition software was MassLynx Mass Spectrometry (Waters, Milford, USA).

2.2.2. Chromatographic conditions

An Acquity™ Ultra High Performance LC system (Waters, Milford) equipped with autosampler injection and pump systems (Waters, Milford) was used. The autosampler vial tray was maintained at 22 °C. The needle was washed with appropriate mixtures of acetonitrile

Table 1
Parameters and diagnostic fragment ions used for the quantification and confirmation of the four HAAs under study.

Analyte (MW)	Precursor ion (*m/z*)	Cone voltage (V)	Confirmation		Quantification	
			Product ion (*m/z*)	Collision voltage (V)	Product ion (*m/z*)	Collision voltage (V)
Trp-P-1 (211)	212	35	168	19	195	17
Trp-P-2 (197)	198	27	154	30	181	20
AαC (183)	184	25	140	25	167	24
MeαAC (197)	198	25	154/129	25	181	23

and water. The separation was performed by injecting 10 µL sample into an ACQUITY UPLC®BEH C18 (Waters, Milford, USA) analytical column (50 × 2.1 mm i.d., 1.7 µm). The binary mobile phases consisted of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B) delivered at 0.25 mL min^{-1}. The C_{18} gradient was started at an initial composition of 90% A and 10% B, then 2.0 min linear gradient to 35%A, held for 2.0 min. A return to the initial conditions was accomplished by a 0.2 min gradient to 90%A, where it was held for 0.8 min.

Thus, the four HAAs were temporarily resolved within 4 min, being 5.0 min the total chromatographic run time (Fig. 2). The column was held at a temperature of 30 °C. Under these conditions, no sample contamination or sample to sample carryover was observed.

2.3. Enrichment factor, extraction recovery, and relative recovery

The enrichment factor (EF) was defined as the ratio between the analyte concentration in the floating phase ($C_{floated}$) and the initial concentration of the analyte ($C_{initial}$) in the aqueous sample [21], according to Eq. (1).

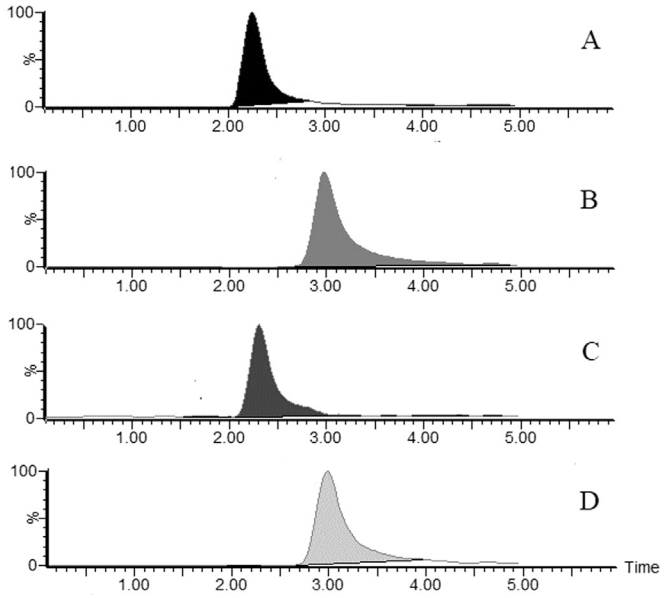

Fig. 2. Chromatograms of the four HAAs determined by UPLC-(+)-ESI-MS/MS: A) AαC (tr: 2, 24 min); B) MeAαC (tr: 2,99 min); C) Trp-P-1 (tr: 2,30 min); D) Trp-P-2 (tr: 2,98 min). As shown in Table 1, MeAαC and Trp-P-2 have the same precursor, but not all the same product ions. It was also observed that the fragment ions did not interfere between them due to possible coelution and/or ionization effects.

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

In this work, the EFs for the compounds were calculated considering the analytes' concentrations in the floating phase since this extraction could be coupled to different separation/detection systems and not only to liquid chromatography-mass spectrometry for which, as mentioned, a back extraction step is required due to compatibility issues.

The extraction recovery (ER) was defined as the percentage of the total analytes (N_0) extracted into the floated phase ($N_{floated}$) as can be seen in Eq. (2). Where $V_{floated}$ and V_{aq} are the volumes of the floating phase and the sample solution; respectively.

$$ER = \frac{N_{floated}}{N_0} \times 100 = \frac{C_{floated} \times V_{floated}}{C_{initial} \times V_{aq}} \times 100 \quad (2)$$

Relative recovery (RR (%)) was calculated as in Eq. (3). Where C_{found} represents the concentration of analyte after adding a known amount of analytical standard to the real sample, C_{real} is the analyte concentration in the real sample and C_{added} refers to the concentration of a known amount of analytical standard that was spiked to the real sample [21].

$$RR(\%) = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (3)$$

2.4. Sample preparation

Natural water samples were collected from the "Potrero de Los Funes" lake located in San Luis (33°21´667S, 66°21´667W), Argentina. Tap water was sampled from our laboratory. All the water samples were filtered through 0.22 µm membrane filters and stored at 4 °C in dark.

2.5. DLLME-SFO-UABE procedure

For the extraction procedure, a mixed solution containing 75 µL of 1-dodecanol (extraction solvent) and 100 µL of MeOH (dispersive solvent) was rapidly injected into a glass tube containing 10 mL of aqueous solution of ultra-pure water spiked with 40 ng mL^{-1} HAAs standards. Once the organic solvents were injected into the water solution, dispersed fine droplets of 1-dodecanol emulsion was formed and, then, this mixture was shaken by vortex for 0.5 min. Consequently, HAAs were quantitatively extracted into the fine droplets of 1-dodecanol. This emulsion was centrifuged for 5 min at 3000 rpm and then, the SFO was observed on the surface of the aqueous solution because of its lower density. After that, the glass tube was placed into an ice bath for 5 min, which allowed SFO solidification because of the low 1-dodecanol melting point (24 °C). After that, the SFO was transferred into a glass tube with a small spatula and the compounds were ultrasound assisted solvent back-extracted whit 600 µL of ACN containing 0.1% formic acid, the system was vortexed for 0.5 min and placed in an ultrasonic bath for 30 min at 45 °C to improve the BE. The obtained extract was centrifuged at 3000 rpm for 5 min. To separate this extract from the remaining 1-dodecanol, the system was placed again in an ice bath for 5 min, thus the solidification of 1-dodecanol was observed at the bottom of the tube. A micropipette was used to withdraw the supernatant HAAs-enriched ACN solution, which was transferred into a glass vial for direct analysis by UPLC-(+)-ESI-MS/MS.

3. Results and discussion

Extraction efficiency of the DLLME-SFO-UABE technique is conditioned by complex mass transfer processes in both the extraction and BE steps, as well as, by other factors including compounds availability,

different type of extraction and BE solvents, the effect of the organic solvent modifiers; among others. Therefore, a careful study to identify variables that affect each step, as well as, to optimize the experimental working conditions to achieve a maximum recovery/enrichment of the compounds in a short time was developed. Thus, extraction and BE related variables were studied as follows.

3.1. DLLME-SFO conditions

3.1.1. Selection of the type and volume of extraction solvent

For the DLLME-SFO procedure, the selection of an appropriate solvent is crucial for maximizing the compounds' extraction efficiency. The extraction solvent should accomplish several requirements such as high extraction efficiency for target compounds, lower density than water, low solubility in water, melting point (MP) close to room temperature, and, preferably, low toxicity, volatility, and cost. Based on the aforementioned requirements, a few solvents are good candidates, among them: 1-undecanol (MP ranges: 13–15 °C), 1-dodecanol (MP ranges: 22–24 °C) and 2-dodecanol (MP ranges: 17–18 °C) [21,22]. As can be noted, 1-undecanol and 2-dodecanol have melting points lower than 1-dodecanol. Accordingly, the main disadvantage of 1-undecanol and 2-dodecanol is the much longer solidification time (> 5 min). Moreover, these solid solvents melt quickly, which makes difficult to draw them out from the sample tube. In this work, due to practical convenience, easy availability and lowest cost, 1-dodecanol was chosen as the extraction solvent for further experiments.

According to some reports, the volume of the extraction solvent has great influence on the extraction efficiency for HAAs [23]. Thus, the effect of the 1-dodecanol volume on the extraction efficiency was investigated. Thus different volumes: 50, 75, 100, and 150 µL; at constants dispersive solvent (MeOH, 100 µL) and aqueous sample (10 mL) volumes were evaluated. From the obtained results (Fig. 3), it can be concluded that an extraction solvent volume of 75 µL was optimal for all compounds. At higher volumes, the recovery decreased in all cases. Also, the increase of volume caused an increase of the floating phase volume and, as a consequence, both enrichment factors and concentration of the compounds in this phase slightly decreased. Thus, a 75 µL of 1-dodecanol volume was chosen as optimal for the next experiments.

3.1.2. Selection of the type and volume of dispersive solvent

The dispersive solvent used for DLLME-SFO should be miscible in both aqueous sample and extraction solvent. Thus the dispersive reagent type and its volume affect the efficiency of the extraction procedure. Hence, acetone, ACN, and MeOH were evaluated as dispersive solvents. As can be seen in Fig. 4**A**, MeOH demonstrated the best efficiency, yielding extraction values between 90% and 100% for all of the compounds. This is probably because 1-dodecanol is highly soluble in MeOH allowing to initially obtain smaller droplets of the extractive solvent, increasing this way the extraction efficiency. Therefore, MeOH was chosen as the dispersive solvent for further experiments.

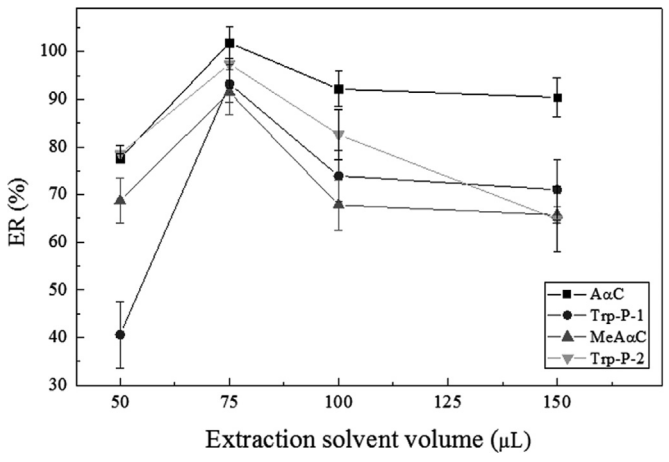

Fig. 3. Effect of the volume of 1-dodecanol (extraction solvent) on the recovery of the HAAs from DLLME-SFO-UA-BE. Extraction conditions: 10 mL sample volume; 100 µL methanol (dispersive solvent); 45 °C; 5 min centrifugation time, 3000 rpm. Back extraction conditions: 600 µL of BE solvent (0.1% (v/v) formic acid in acetonitrile); 30 min ultrasonic bath time; 45 °C; 5 min centrifugation time, 3000 rpm.

To study the effect of the dispersive solvent volume, various experiments were performed by using different volumes of MeOH, which varied from 75 to 500 µL. Efficient extractions were achieved when the MeOH volume ranged from 100 µL to 200 µL as illustrated in Fig. 4**B.** In this context, a MeOH volume of 100 µL was selected since highest extraction efficiencies and, thus, better enrichment factors were attained for all the studied compounds. Therefore, this optimal volume was used for subsequent experiments.

3.1.3. Extraction efficiency vs. shaking time

Extraction time is defined as the interval time between injection of the extracting mixture (disperser plus extraction solvents), and starting the centrifugation process [24]. The sample tube solution after the dispersion of the extraction mixture was immediately vortexed during 0–2 min. In this step, it would be expected that shaking the tube after the addition of the extractive mixture could influence the extraction procedure because this shaking can generate a prolonged contact with the aqueous sample [25]. However, no significant changes in extraction efficiency were observed. These results can be attributed to the quick mass transfer of the analytes from the sample solution to the extraction solvent, suggesting that the extraction equilibrium can be reached within a short time [26]. Nevertheless, a short agitation time of 0.5 min was selected to ensure the formation of a homogeneous emulsion.

3.1.4. Centrifugation time and rate

Centrifugation is needed in any DLLME-SFO to obtain a complete separation of phases. Thus, different centrifugation times (3–10 min) and rates (2500–5000 rpm) were assayed. Centrifugation during a reasonably short time of 5 min at 3000 rpm gave satisfactory results. As a consequence, a well-formed SFO at the surface of a totally transparent aqueous phase, with no traces of organic residues adhered to the tube walls, was observed.

3.1.5. Sample temperature effect on DLLME-SFO efficiency

On the other hand, extraction efficiency might be affected by the temperature of the sample. Thus before injecting the extraction mixture, the sample tube was placed during 3 min in a water bath kept at 25, 35, 45 and 55 °C; respectively. It was observed that no satisfactory emulsion was formed at temperatures below 35 °C, but an acceptable emulsion was obtained at 45 °C and, as a consequence, this temperature was chosen as optimal. These results are in agreement with a previous work in which the shape of the obtained SFO was improved when warming was used during the extraction procedure [27].

3.2. UABE conditions

With the aim of direct transfer of the HAAs from the enriched floating organic drop to a solvent compatible with the UPLC-(+)-ESI-MS/MS detection methodology, different strategies were evaluated. In this sense, a novel BE approach was proposed. It is important to notice that this step made feasible the coupling of DLLME-SFO with the chromatographic separation/spectrometric detection requirements.

The optimized experimental parameters were type and volume of BE solvent, use of organic solvents modifiers, the effect of temperature, and time of ultrasonic bath on the BE; among others.

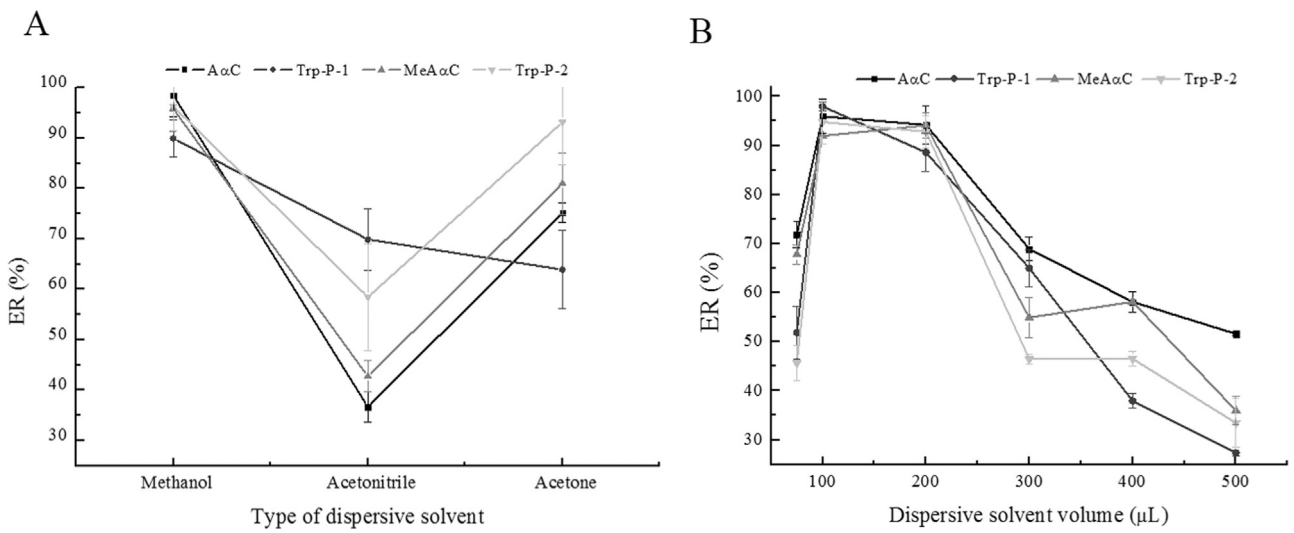

Fig. 4. Effect of type **(A)** and volume **(B)** of dispersive solvent on the recovery of the HAAs from DLLME-SFO-UA-BE. Extraction conditions: 10 mL sample volume; 75 μL of 1-dodecanol (extraction solvent); 45 °C; 5 min centrifugation time, 3000 rpm. Back-extraction conditions: 600 μL of BE solvent (0.1% (v/v) formic acid in acetonitrile); 30 min ultrasonic bath time; 45 °C; 5 min centrifugation, 3000 rpm.

3.2.1. Selection of the type and volume of BE solvent

The most important property of the BE solvent is having a good extraction capability for the compound/s of interest. BE efficiency of solvents such as ACN and H_2O, and mixtures of them, were studied. It was observed that with pure ACN, recoveries up to 60% for all of the compounds were obtained. On the other hand, when only H_2O was used, lower recoveries were achieved. As a consequence, the BE capability of mixtures of these solvents, at several volume ratios, was investigated. In this context, the use of the ACN/H_2O 1:2 (v/v) mixture increased the relative recoveries as compared to the other mixtures, but they were lower in comparison with pure ACN. Thus, ACN was chosen as the BE solvent.

The use of an organic solvent modifier could affect the mass transference from the SFO phase to the BE solvent, improving the relative recoveries considerably [28]. Thus, formic acid and sodium hydroxide as solvent modifiers of ACN were assayed. Good recoveries just for AαC and Trp-P-1 were obtained when NaOH (0.014 mol L^{-1}) was added to ACN. However, as shown in Fig. 5**A**, the addition of formic acid (0.1% (v/v)) to ACN resulted in a higher extraction efficiency for all of the compounds.

In addition to solvent type and solvent modifier, the volume of the selected BE solvent was studied in the range from 400 to 800 μL. The BE efficiency decreased gradually when the volume of the ACN/formic acid mixture was lower than 600 μL. On the other hand, volumes higher than 800 μL could not be used due to a poor phase separation, probably because of the 1-dodecanol miscibility at a high ACN content in the mixture. As indicated in Fig. 5**B**, 600 μL of the ACN/formic acid mixture was selected for the UABE procedure.

3.2.2. Temperature effect on BE

It is well known that temperature affects the analytes partition constants. For this reason, the effect of this variable on the BE procedure was studied. The sample tube was placed in a water bath maintained at 25, 35, 45 and 55 °C; respectively. It was observed that at temperatures below or equal than the 1-dodecanol solidification point (22 °C), no efficient recoveries were obtained.

However, when the temperature reached 45 °C, the extraction recoveries for all of the compounds increased (Fig. 5**C**). However, at temperatures above this value, a marked decrease in the extraction efficiency was observed. As reported in recent literature, The HAAs are stable at ambient temperature, but they are disposed to degradation at higher temperatures. As a consequence, a 45 °C temperature was used as optimal during the BE procedure [29].

3.2.3. Effect of ultrasonic time on BE

In order to achieve a quantitative contact mixing and transference of the analytes between the SFO and the BE solvent. UABE at different times was studied. To analyze the influence of the ultrasonic time on the recovery, 10, 20, 30 and 40 min were evaluated. The temperature of the ultrasonic bath was maintained at 45 °C. As shown in Fig. 5**D**, ultrasonic time is of great importance in this new proposed BE procedure. As the ultrasonic time decreased, the extraction efficiency diminished. The optimal UABE was achieved when a 30 min cycle was applied.

3.3. Method validation

The optimized DLLME-SFO-UABE approach coupled to UPLC-(+)-ESI-MS/MS determination was validated for linearity, detection and quantification limits, selectivity, and precision as summarized in Table 2. An approach based on spiked samples was developed. Thus two types of natural waters were evaluated: tap and lake water samples. These samples composed by five blank samples from each one, 3 replicates at 5, 10, 20, and 50 ng mL^{-1} were used.

The linearity of the calibration curves for spiked natural water was satisfactory with determination coefficients (r^2) in the range between 0.994 and 0.999. The F-test demonstrated that linear regressions were statistically acceptable in the working ranges (LR) and this model showed goodness of fit. The limit of detection (LOD) and limit of quantification (LOQ) were calculated following the International Union of Pure and Applied Chemistry (IUPAC) recommendation as 3.3 S_y/b and 10 S_y/b; respectively, where b is the slope of the regression curve and S_y the standard error of the blank [30,31] The LOD and LOQ values were in a range from 0.7 to 2.9 ng mL^{-1} and 2.2–8.7 ng mL^{-1}, respectively. These figures of merit resulted to be similar, or even better, than others reported in the literature [32–34].

Average relative standard deviations (RSD (%)) values were from 4.3% to 6.7% achieved at concentration levels from 0.5 to 50 ng mL^{-1} for all of the compounds. The EF values obtained according to Eq. (1) were in the range from 130 to 136 folds.

One disadvantage of ESI-MS/MS ionization/detection is that the process is susceptible to matrix signal suppression or enhancement [35,36]. The liquid chromatography/mass spectrometry response obtained from the standards may differ considerably from matrix samples. In this work, the effect of the natural water matrices was assessed by comparing the analytical response of the HAAs in pure solvent with the signals in the sample matrices. The matrix effect (ME)

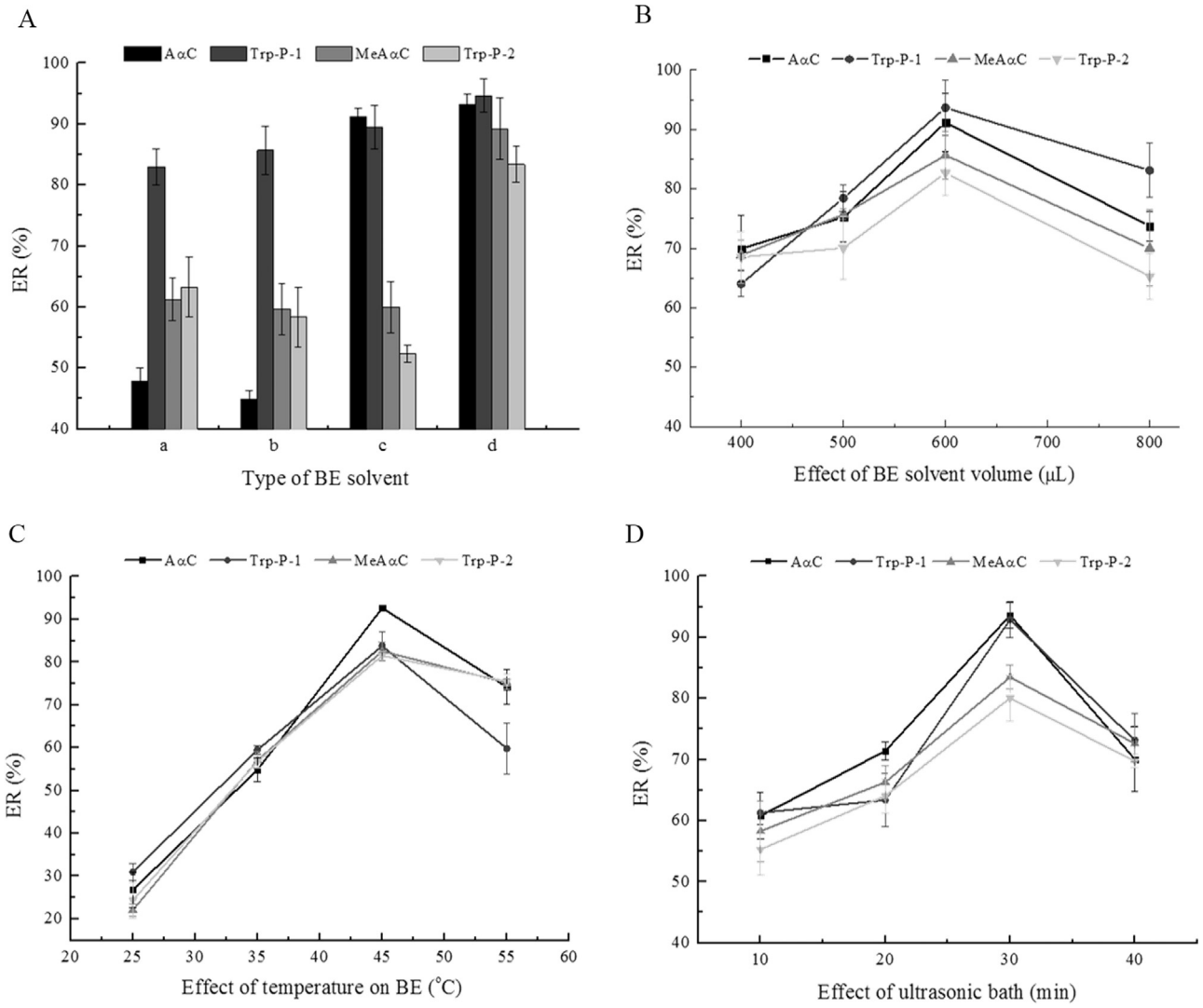

Fig. 5. Effects of the experimental parameters on BE efficiency: **(A)** Type of BE solvent. Conditions: a) ACN/H_2O (0.014 mol L^{-1} NaOH); b) ACN/H_2O (0.1% (v/v) formic acid); c) ACN (0.014 mol L^{-1} NaOH); d) ACN (0.1% (v/v) formic acid). **(B)** Effect of BE solvent volume. **(C)** Effect of temperature. **(D)** Effect of ultrasonic bath. Extraction conditions: 10 mL sample volume; 75 μL of 1-dodecanol (extraction solvent); 100 μL of methanol (dispersive solvent); 45 °C; 5 min centrifugation time, 3000 rpm. Back-extraction conditions: 30 min ultrasonic bath time; 45 °C; 5 min centrifugation time, 3000 rpm.

was evaluated by comparing the slopes of the calibration curves of standards in both pure solvent 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 according to Eq. (4).

$$ME = 100 - (\text{b spiked/b } solvent \times 100) \quad (4)$$

The obtained results exhibited that the matrix under analysis did not significantly affect the analytical performance of the studied compounds after applying the DLLME-SFO-UABE.

The RR (%) values were obtained by addition of known amounts of the four HAAs at different concentration levels, 0–50 ng mL^{-1}), to blank real sample matrices and then they were processed according to Eq. (2). The RR (%) values for the four studied HAAs ranged from 92% to 106% as shown in Table 3. From the previous results, it was possible to conclude that the developed analytical method is fast, reproducible, has good precision and satisfactory recoveries.

3.4. Application to real samples

The natural water samples were pretreated as described in Section 2.3, extracted and preconcentrated using DLLME-SFO-UABE associated to

Table 2
Linearity, linear range, limits of detection and quantification, enrichment factor and relative standard deviation of the DLLME-SFO-UABE method coupled to UPLC-(+)-ESI-MS/MS.

Compounds	r^2	LR (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	EF	ER	RSD (%) (n = 3)
AαC	0.996	2.2–50	0.7	2.2	132.4	97.6	5.6
MeAαC	0.999	3.9–50	1.3	3.9	131.9	97.2	6.2
Trp-P-1	0.994	2.9–50	0.9	2.9	130.5	96.0	6.7
Trp-P-2	0.999	8.7–50	2.9	8.7	136.5	100.6	4.3

Table 3
Recovery study for the analysis of spiked natural water samples by applying the proposed methodology.

		Tap water				Lake water				
HAAs	Sample concentration (ng mL^{-1})	Concentration added (ng mL^{-1})	Concentration determined (ng mL^{-1})	RR (%)	RSD (%) $n = 3$	Sample concentration (ng mL^{-1})	Concentration added (ng mL^{-1})	Concentration determined (ng mL^{-1})	RR (%)	RSD (%) $n = 3$
AαC	N.D.*	0				*	0			
	*	5	5.0	100.0	6.6	*	5	4.9	98.0	5.9
	*	10	10.0	100.0	3.3	*	10	10.4	104.0	7.5
	*	20	20.0	100.0	1.9	*	20	20.0	100.0	4.5
	*	50	49.6	99.2	1.5	*	50	49.6	99.2	1.9
MeAαC	*	0				*	0			
	*	5	5.0	100.0	5.0	*	5	5.2	104.0	1.5
	*	10	9.3	93.0	2.7	*	10	9.6	96.0	8.9
	*	20	20.2	101.0	8.1		20	19.4	97.0	7.6
	*	50	49.5	99.0	3.9	*	50	50.0	100.0	1.8
Trp-P-1	*	0				*	0			
	*	5	4.6	92.0	2.9	*	5	4.8	96.0	2.2
	*	10	9.8	98.0	6.1	*	10	9.8	98.0	2.1
	*	20	20.8	104.0	6.0	*	20	19.6	98.0	3.3
	*	50	51.0	102.0	4.5	*	50	50.9	101.8	1.9
Trp-P-2	*	0				*	0			
	*	5	5.0	100.0	5.8	*	5	4.9	98.0	2.6
	*	10	10.3	103.0	2.8	*	10	10.2	102.0	2.5
	*	20	21.2	106.0	1.8	*	20	20.8	104.0	2.8
	*	50	49.5	99.0	6.5	*	50	49.8	99.6	3.2

N.D. *: not detected.

UPLC-(+)-ESI-MS/MS. Any of the studied amines were detected in the studied real samples under the established experimental conditions. The obtained concentration values for the spiked samples with the four studied non-polar HAAs are gathered in Table 3. Thus considering the obtained LODs, it could be concluded that perhaps an ultra trace exposure of the inhabitants to the studied pollutants through natural waters, such as lake and tap water, could be taken place.

It is considered that this study adds important information on the quality of natural and drinking water in Argentina since, to the best of our knowledge, it reports for the first time an analytical methodology to determine four HAAs (AαC, MeAαC, Trp-P-1 and Trp-P-2).

4. Conclusions

A sensitive, selective and precise analytical methodology based on DLLME-SFO-UABE coupled with UPLC-(+)-ESI-MS/MS was developed for the simultaneous determination of four non-polar HAAs in natural water sales. In addition, excellent characteristics of solvent usage reduction and high enrichment factors were demonstrated. Moreover, the methodology can be considered environment-friendly since reduced volumes of low toxicity solvents were employed. This is the first report of BE combined with the DLLME-SFO technique applied to any determination of HAAs. The addition to the UABE step to the DLLME-SFO strategy made feasible the coupling to the UPLC-MS/MS system.

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