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Hydrophobic natural deep eutectic solvents based on L-menthol as supported liquid membrane for hollow fiber liquid-phase microextraction of triazines from water and urine samples

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ABSTRACT

This work proposes the use of a hydrophobic natural deep eutectic solvent (NADES) as a supported liquid membrane (SLM) for hollow fiber liquid phase microextraction (HF-LPME) of triazines. NADES were prepared using L-menthol as hydrogen bond acceptor combined with different hydrogen bond donors of natural origin: carboxylic acids, alcohols and amines. Studies were carried out to determine whether the prepared NADES met the necessary requirements to be used as a SLM, such as stability in the HF and compatibility with HPLC. Then, the ability of each prepared NADES to extract 6 triazine herbicides by HF-LPME from aqueous samples was evaluated. Among them, the mixture L-menthol: formic acid (molar ratio 1:2) provided better extraction results and was selected as SLM. The influence the different parameters on extraction efficiency such as pH of both sample and acceptor solution, salting-out effect, extraction time and stirring rate on the extraction efficiency was carefully studied and optimized. The optimized HF-LPME procedure was applied to the analysis of aqueous samples such as artificial water containing humic acids, tap water, river water and urine, with excellent clean-up ability for all samples analyzed. Relative recoveries ranged from 68 to 128 %, and the LODs and LOQs obtained for the 6 triazines were 0.75–3.1 μg/L and 2.5–10.3 μg/L, respectively, depending on the analyte and the kind of sample. Additionally, according to the AGREEprep tool assessment, the proposed method appears as a greener approach compared to other microextraction methods reported in the literature for the analysis of triazines in water samples.

1. Introduction

At present, there is a growing concern within the scientific community on the effects of methods and procedures in the environment. The impact of human activity on the environment, especially scientific activity, was first highlighted in 1962 when Carson R. published "Silent Spring" [1]. In the 1990 s, Anastas and Warner introduced the term "Green Chemistry" as an approach to the synthesis, processing, and use of chemicals that considers the reduction of risks to humans and the environment [2]. Besides, Green Analytical Chemistry (GAC) emerged as one of the most active areas of green chemistry, with a focus on the design and development of new analytical methods that reduce the use and generation of hazardous substances at every stage of chemical analysis [3]. In this sense, Gałuszka *et al.* proposed a set of guidelines called the "12 Principles of Green Analytical Chemistry (GAC)" as a general approach to greening laboratory practices [4]. These principles prioritize avoiding sample treatment and the use of direct analytical techniques. However, although analytical instrumentation has dramatically improved sensitivity and selectivity, direct analysis of complex samples is not possible and thus sample preparation remains as one of the most important steps, with goals including using smaller initial sample sizes, improving extraction selectivity, increasing automation, reducing waste, and using small volumes or no organic solvents [5]. Recently, López-Lorente *et al.* introduced "The ten principles of Green

Abbreviations: ATZ, Atrazine; CZE, Capillary zone electrophoresis; DEA, Desethylatrazine; DES, Deep eutectic solvents; DIA, Desisopropylatrazine; GAC, Green Analytical Chemistry; GSP, Green Sample Preparation; HBA, Hydrogen bond acceptor; HBD, Hydrogen bond donor; HF, Hollow fiber; ILs, Ionic liquids; LLE, Liquid-liquid extraction; LPME, Liquid-phase microextraction; NADES, Natural deep eutectic solvents; PPZ, Propazine; SIM, Simazine; SLM, Supported liquid membrane; SUPRAS, Supramolecular solvents; TER, Terbutylazine; μPAD, Microfluidic paper-based analytical device.

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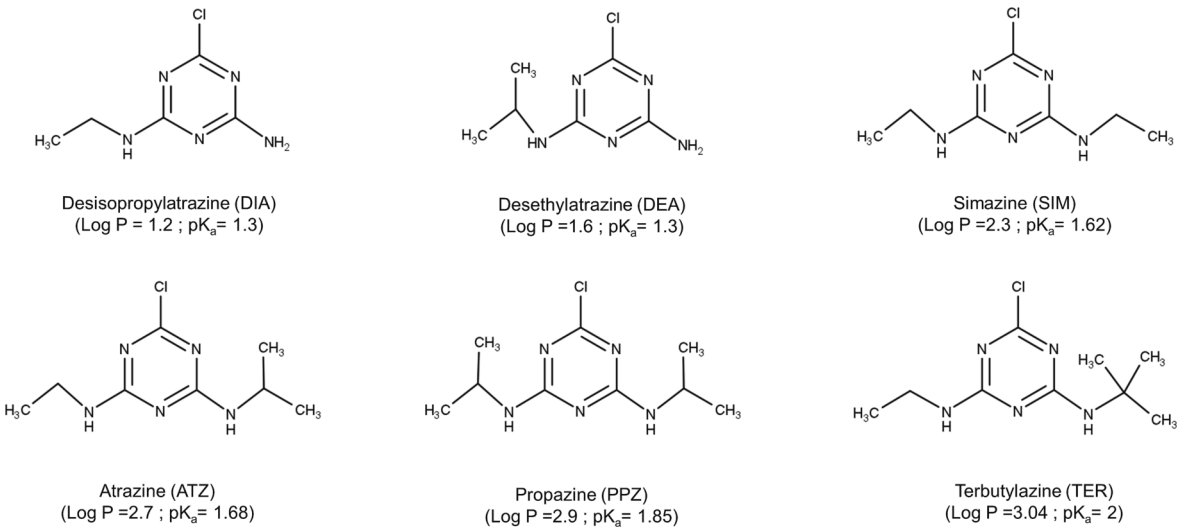

Fig. 1. Chemical structures and physicochemical properties of the 6 triazines studied.

Sample Preparation (GSP)" as a guide for the development of more environmentally friendly analytical methods by using safer solvents and reagents; minimizing waste generation; reducing energy consumption; increasing sample throughput through miniaturization; and guarantying operator safety, among others [6].

Consequently, new microextraction techniques, as liquid-phase microextraction (LPME), have been developed in the last decade. Initially, LPME consisted on the extraction of analytes from aqueous samples into a small drop of organic solvent [7]. Later, to increase the stability and reliability of LPME, a new technique, so called HF-LPME, was introduced. In HF-LPME, a water-immiscible organic solvent is immobilized as a thin supported liquid membrane (SLM) in the pores of a polypropylene hollow fiber (HF) [8]. In the three-phase HF-LPME configuration, analytes are first extracted into the SLM and then extracted into an acidic or alkaline aqueous solution placed in the lumen of the fiber. The aqueous extract can be finally analyzed with compatible techniques such as liquid chromatography (HPLC, UHPLC), capillary zone electrophoresis (CZE) or microfluidic paper-based analytical device (μPAD) [9–12]. HF-LPME requires only a small amount of organic solvent, typically 5–30 μL, making it not only an efficient technique for sample cleanup, but also more environmentally friendly [13].

According to the second GSP principle, the development and use of new environmentally friendly solvents as alternatives to toxic and volatile organic solvents is a key issue [14–16]. In the case of HF-LPME, greener solvents such as ionic liquids (ILs), deep eutectic solvents (DES) and supramolecular solvents (SUPRAS) have been investigated as suitable organic solvent alternatives. ILs, salts of organic cations and organic or inorganic anions, offer some advantages such as low vapor pressure, thermal stability, high conductivity and viscosity, among others [17]. However, the use of ILs in HF-LPME has often been limited by their high cost and their eventual toxicity and lack of biodegradability [18,19]. SUPRAS are nanostructured fluids formed by the self-assembly and coacervation of a colloidal suspension of amphiphilic surfactants. They are inexpensive, environmental friendly, and have the potential to be designer solvents with a wide variety of molecular interactions. However, the use of customized SUPRAS has been scarcely reported, and the poor compatibility of SUPRAS with mass spectrometry has posed an additional challenge to method development [20,21].

DES are mixtures of two (or more) compounds joined by hydrogen bonding, with one compound acting as a hydrogen bond acceptor (HBA) and the other as a hydrogen bond donor (HBD). The formation of hydrogen bonds causes the melting point of the mixture to be lower than the melting points of the individual components. The use of DES presents some advantages over traditional organic solvents and in addition some aspects as low cost, ease of preparation, biodegradability, and toxicity [20], making them ideal materials for the development of sustainable analytical methods. The first DES was prepared in 2003 using choline chloride as the HBA [22]. Despite its widespread use, choline chloride is quite polar and miscible in water, which limits its use in techniques such as Hf-LPME, where the solvent must be water-immiscible. The first hydrophobic DES was introduced in 2015 to broaden the range of applications [23], consisting of quaternary ammonium salts with long alkyl chains as HBA and fatty acids as HBD. In the same year, a hydrophobic DES based on DL-menthol and naturally occurring acids was described by Ribeiro *et al.* [24]. These new solvents derived from naturally occurring compounds and metabolites, so-called natural DES (NADES), are in line with the principles of green chemistry, as they are readily available, low-cost and highly biodegradable [25,26]. Since then, menthol-based NADES have been proposed as an alternative to traditional organic solvents used in common extraction techniques. Most examples are found in dispersive liquid–liquid microextraction, such as the extraction of benzophenone-type UV filters, phthalic acid esters, ketoprofen and diclofenac, triazines, sulfonamides, and parabens from a wide variety of samples [27–33]. Fewer examples can be found in solid–liquid extraction and dispersive solid–liquid microextraction, such as those used for the determination of phytocannabinoids and ergosterol [34,35]. To the best of our knowledge, there is only three publications on HF-LPME for the analysis of sulfonamides [36], steroidal hormones [37], and antiarrhythmic agents [38].

The aim of this work was to prepare a batch of menthol-based NADES using different HBDs and to evaluate their possible use as SLM in a HF-LPME process. In this regard, mixtures of L-menthol and natural carboxylic acids, alcohols, and amines at different molar ratios were prepared. Important requirements for their use as SLM, such as water immiscibility and stability on the HF during extraction, were studied. In addition, the ability of NADES to extract 6 triazines from aqueous samples by HF-LPME was investigated. Subsequently, HL-LPME using the selected NADES as SLM was optimized and successfully applied for the extraction of triazines from water and urine samples.

2. Materials and methods

2.1. Chemicals and reagents

Desisopropylatrazine (DIA), desethylatrazine (DEA), simazine (SIM), atrazine (ATZ), propazine (PPZ) and terbutylazine (TER) were purchased from Sigma-Aldrich (Madrid, Spain). Fig. 1 shows their chemical structures and some physicochemical properties. Stock standard

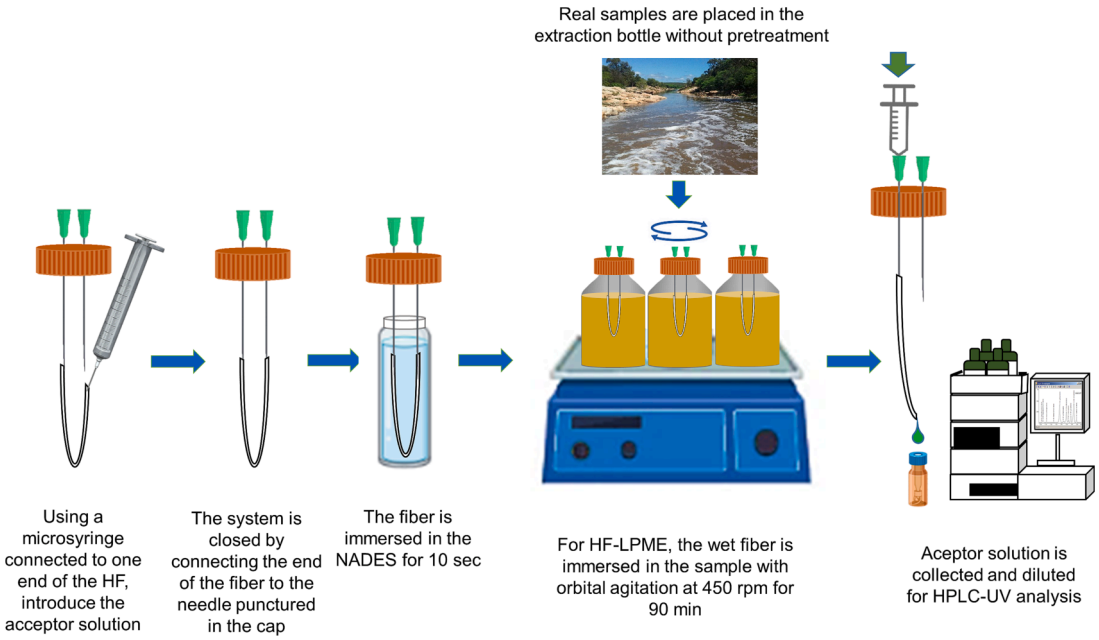

Fig. 2. HF-LPME procedure scheme.

solutions (1 g/L) were prepared in acetonitrile and kept at −22 °C. Formic acid, acetic acid, di-sodium hydrogen phosphate anhydrous (Na_2HPO_4), citric acid and hydrochloric acid (HCl) were obtained from Panreac (Barcelona Spain). L-Menthol, sodium chloride (NaCl), propanoic acid, isobutyric acid, butyric acid, ethanol, 2-propanol, triethylamine and 2-butylamine, humic acid sodium salt, and magnesium sulfate anhydrous were purchased from Merck (Madrid, Spain). HPLC grade acetonitrile (ACN), toluene and acetone were obtained from Honeywell (Seelze, Germany). Purified water was obtained from a Milli-Q purification unit supplied by Millipore (Madrid, Spain). To support the organic phase, Q3/2 polypropylene hollow fibers with a wall thickness of 200 μm, an inner diameter of 600 μm and pores of 0.2 μm were purchased from Membrana (Wuppertal, Germany).

2.2. Preparation of L-menthol based natural deep eutectic solvents

Each pair formed by HBA (L-menthol) with a given HBD was placed in a glass vial at different molar ratios. The vial was tightly closed and then vortexed and transferred to an incubator at a temperature of 60 °C equipped with a roller rotating at 24 rpm (Barloworld Scientific, Staffordshire, UK) for a total time of 15 min. Finally, prior to further use, obtained NADES were allowed to cool to room temperature.

2.3. Preparation of L-menthol: Formic acid (1:2) natural deep eutectic solvent

The L-menthol was stored in a desiccator prior to use, and the formic acid was used directly from the commercial bottle without further processing. For the synthesis of L-menthol: formic acid (1:2) NADES, 0.47 g of L-menthol (3 mmol) and 0.238 mL of formic acid (3 mmol), working as HBA and HBD respectively, were placed into 4.5 mL glass vials with screw cap. NADES was obtained at a temperature of 60 °C for 15 min as above described. After allowing the vial to cool to room temperature, two phases appeared and the NADES was collected and transferred to a new vial with the help of a Pasteur pipette. Any remaining water was eliminated by the addition of few milligrams of anhydrous magnesium sulfate. Selected NADES became cloudy at the end of the day, so it has to be prepared daily.

Optimum NADES was characterized by Fourier-transform infrared (FT-IR) spectroscopy on a Jasco FTIR-460 Plus spectrophotometer. All spectra were recorded by attenuated total reflectance between 4000 and 400 cm^{-1}, with the sample in the solid or liquid state without other treatment.

2.4. Sample preparation

Standard solutions of a mixture of 6 triazines in MilliQ water were prepared and used for NADES evaluation as SLM and further optimizations. An artificial water was prepared in the laboratory by dissolving humic acids in MilliQ water up to a concentration of 10 mg/L. Aqueous samples from the Manzanares River (Madrid, Spain), tap water from the laboratory (Madrid, Spain) and urine were collected to study the performance of the developed method in real samples. Urine samples were collected from volunteers and stored in the fridge until analysed, always within 48 h. No pre-treatment was performed on the real samples before analysis.

2.5. HF-LPME using L-menthol based NADES as SLM

An scheme of the HF-LPME procedure is shown in Fig. 2. Prior to use, polypropylene hollow fiber was cut into 8 cm pieces, washed with acetone, and dried. The setup for the HF-LPME is prepared as follows: two medical needles (21G × 1 1/2″ − 0.8 × 40 mm) were pierced through a plastic cap. Then, one of the needles was connected to one end of the hollow fiber and 25 μL of acceptor solution at pH 0.6 (HCl 0.25 M) was injected using a microsyringe. Finally, the system was closed by connecting the free end of the HF to the other needle, leaving the fiber in a U-shaped configuration. Then, the HF was dipped into the prepared NADES for 10 s to form the SLM in the pores of the HF. The as-prepared HF was then immediately transferred to 100 mL of aqueous sample for the extraction process, which was conducted under orbital agitation at 425 rpm for 90 min. Both the blank and spiked real samples (artificial water, tap water, river water and urine) were added directly to the extraction flask without pretreatment. Simultaneous treatment of 3 samples of 100 mL was possible in the orbital stirrer (Vibramax 100, Heidolph, Kelheim, Germany). After extraction, the fiber was removed from the sample and one side of the fiber was disconnected from the needle. The acceptor solution was transferred to a glass insert using a microsyringe. Finally, the extracts were diluted by adding 75 μL of a mixture citrate–phosphate buffer solution (0.15 M, pH 8.0): ACN (80:20,

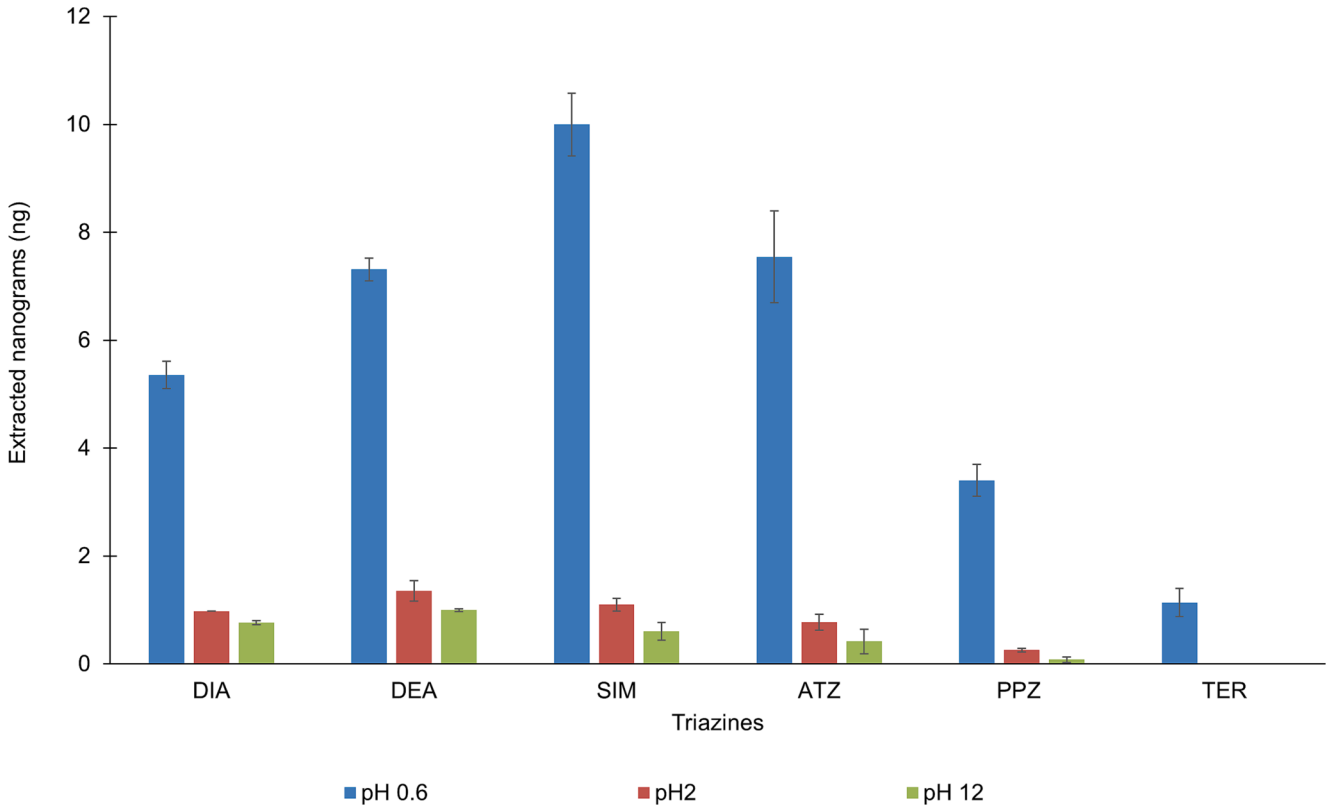

Fig. 3. Effect of acceptor phase pH on triazines extraction efficiency.

v/v) prior to chromatographic analysis. In order to prevent possible carryover effects, a fresh HF was used for each sample extraction.

2.6. HPLC-UV analysis

The extracts were analyzed by HPLC using an Agilent 1100 Series HPLC instrument (Agilent Technologies, Wilmington, DE) equipped with a gradient pump, an autosampler, and a programmable UV–visible detector. A sample volume of 75 μL was injected into a KromaPhase C18 HPLC column (100 × 4 mm, 3.5 μm) from Scharlab (Barcelona, Spain). Analytes were monitored at 220 nm with the column temperature set at 25 °C. The separation of the analytes was performed by gradient elution as follows: initial conditions of 85% A (water), 15% B (ACN). The gradient was programmed to change to 50 % A and 50% B in 19 min and then back to the initial conditions in 3 min, maintaining these conditions for another 3 min before starting the next run. Separation of triazines was achieved in 25 min at a flow rate of 1 mL/min.

3. Results and discussion

3.1. Preparation of L -menthol based natural deep eutectic solvents

For the synthesis of NADES, L-menthol acting as HBA and different HBDs of natural origin were mixed in a determined molar ratio. The carboxylic acids tested as HBDs were: formic acid, acetic acid, propanoic acid, butyric acid and isobutyric acid. In addition, two alcohols (ethanol and 2-propanol) and two amines (triethylamine and 2-butylamine) were tested as HBDs.

The list of NADES prepared, their molar ratio, their appearance after synthesis and their pH value are summarized in Table S1. For initial screening, each combination of HBA and HBD was prepared in a molar ratio of 1:1 (HBA: HBD). Other molar ratios (1:2, 1:4) were subsequently tested depending on the obtained results. Of the combinations studied, NADES prepared with formic acid showed two phases at room temperature and the rest showed a single transparent liquid phase. The pH values of the NADES obtained were in agreement with the pK_a values of carboxylic acid used in the synthesis.

3.2. Selection of the L-menthol-based NADES as membrane solvent for the HF-LPME of triazines

Solvents used as SLM for HF-LPME must be water-immiscible and non-volatile in order to become stable on the porous membrane of the HF during the extraction process. All prepared NADES were found to be immiscible with water and able to impregnate the HF and thus were evaluated as SLM for the extraction of triazines by HF-LPME under the following initial extraction conditions: 3.5 mL of aqueous sample solution spiked with 50 μg/L of a 6-triazine mixture, 25 μL of acceptor solution adjusted to pH 0.6 with HCl (0.25 M), and an extraction time of 30 min using orbital agitation at 1050 rpm. Extracts were collected and analyzed by HPLC.

Table S2 shows the stability of each NADES during extraction and the compatibility of the obtained extracts with subsequent HPLC analysis. During the extraction with the alcohol- and amine-based NADES, a white solid appeared in the sample solution, suggesting that these NADES were weaker and more unstable than those obtained with the carboxylic acids, likely due to their lower ability to form strong hydrogen bonds with L-menthol. This lack of stability would also explain the occurrence of dirty chromatograms caused by NADES leakage from the SLM into the acceptor solution [39]. The remaining NADES showed good stability as SLM during extraction.

In this study, a 3-phase HF-LPME configuration was selected for the extraction of triazines from aqueous samples, which comprises an aqueous donor phase, an aqueous acceptor phase, and an organic phase as SLM. In this configuration, the analytes, in their neutral form, are first extracted from the aqueous sample into the SLM. Subsequently, the analytes are re-extracted from the SLM into the acceptor solution. In order to prevent back diffusion of target analytes into the SLM, the pH of

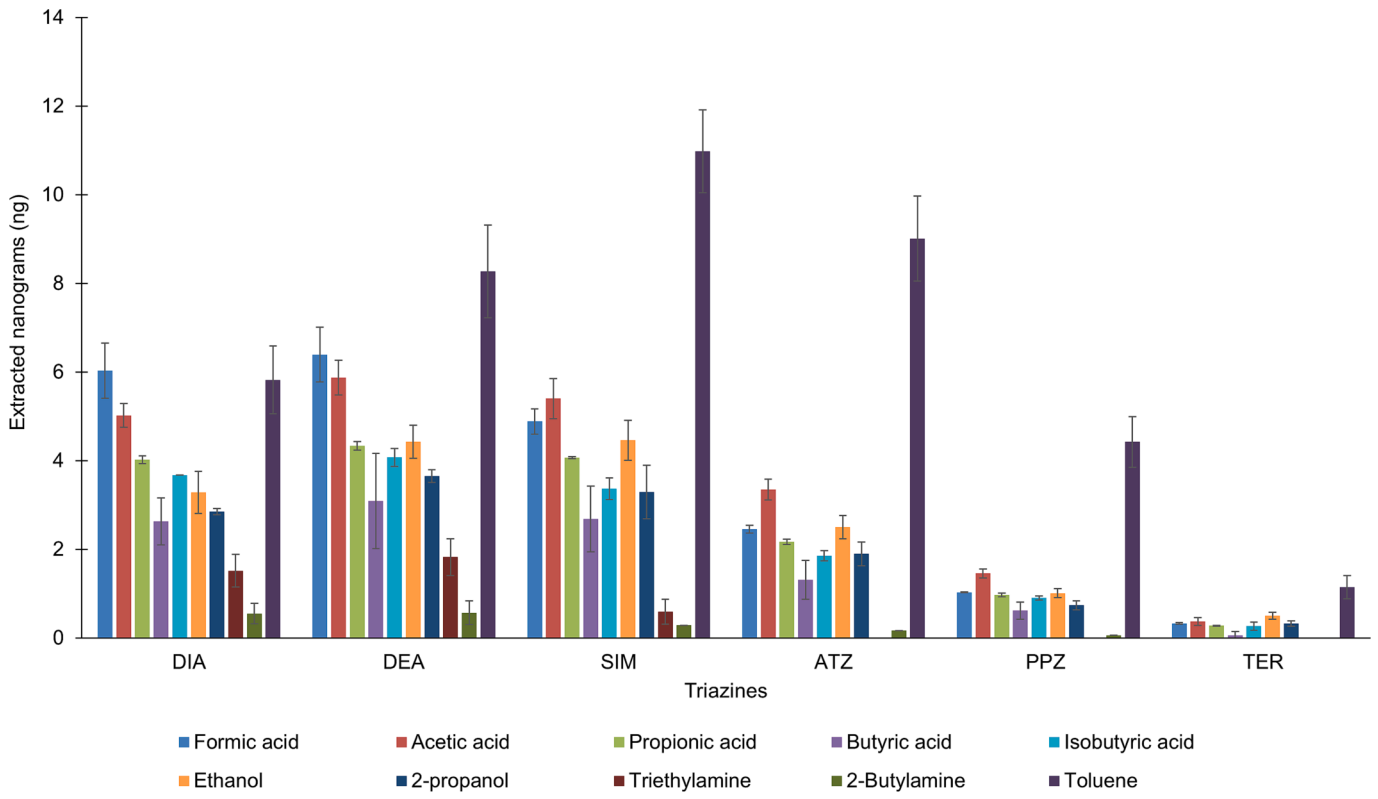

Fig. 4. Extracted nanograms of triazines using different L -menthol:organic acid-based NADES at molar ratio 1:1 and toluene as SLM.

the acceptor solution must guarantee the ionization of target analytes.

In the case of the triazines studied, which have pK_a values in the range of 1–2 (Fig. 1), a water sample pH of around 5–6 would be sufficient to have the triazines in their neutral form. Therefore, the pH of the acceptor solution should be investigated to ensure adequate ionization of the target analytes. For this study, the extractions were carried out in a volume of 3.5 mL of MilliQ water spiked with 50 μg/L of a mixture of 6 triazines, using toluene as a common solvent used as SLM. The acceptor solution was 25 μL of water at different pH values (0.6, 2, and 12.0) and the extraction time was fixed at 30 min under orbital

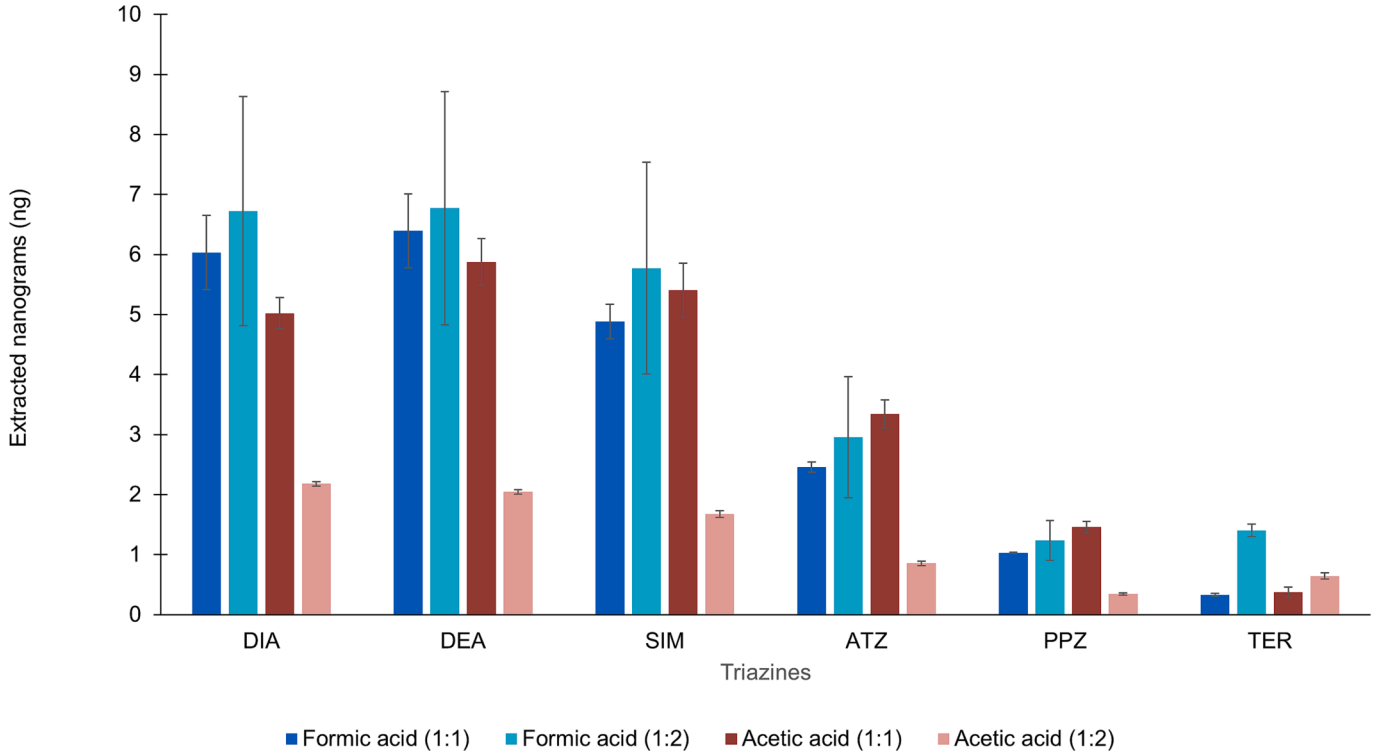

Fig. 5. Extracted nanograms of triazines using L-menthol: formic acid and L-menthol: acetic acid in molar ratios 1:1 and 1:2.

stirring at 1050 rpm. As seen in Fig. 3, the acceptor solution must have a very low pH (pH = 0.6) to keep the triazines ionized, since at higher pH values triazines were not extracted from the SLM. Accordingly, the aqueous donor sample could be analyzed directly without pH adjustment, and the pH of the acceptor solution was set to pH 0.6 (HCl, 0.25 M) for further experiments.

Fig. 4 shows the amount (ng) of triazines extracted with NADES prepared at a 1:1 M ratio as SLM and with toluene, a conventional organic solvent used in HF-LPME. Different extraction efficiencies were observed depending on the triazine, which could be related to its polarity, varying from a log P value of 1.15 (DIA) to 3.04 (TER) (Fig. 1). By shortening the length of the HBD, the resulting NADES would be more polar and thus extraction of DIA, DEA and SIM, the analytes with the lowest log P values, was favored. On the contrary, an increase in the alkyl chain length of the HBD would result in a more hydrophobic NADES favoring the extraction of PPZ and TER, since they display the highest log P values. However, it was found that the extraction efficiency obtained for PPZ and TER was unexpectedly low for the three groups of HBDs tested (carboxylic acids, alcohols, and amines) as well as for toluene. The low extraction of PPZ and TER suggests that the transfer from the SLM to the acceptor phase is poor, which could lead to compounds remaining in the SLM.

In summary, Fig. 4 shows that better extractions of the triazines were obtained with the NADES prepared with formic acid and acetic acid, followed by those prepared with ethanol. From literature, NADES are known to have high viscosity, which increases with increasing alkyl chain length, restricting the diffusion of analytes through them [40]. According to the results, the NADES that extracted better were precisely those with short alkyl chain, indicating that their lower viscosity favored triazines mass transfer through SLM.

Besides, since one of the objectives of this work is the substitution of conventional organic solvents used in HF-LPME, such as toluene, it is appropriate to compare the performance of selected NADES with toluene. As can be observed in Fig. 4, NADES prepared with formic acid and acetic acid provided an extraction efficiency for DIA and DEA similar to that obtained with toluene, but the remaining analytes were better extracted with toluene. However, the extraction efficiency was sufficient for trace enrichment of triazines from water samples. In addition, from the GSP perspective, the use of NADES prepared with formic acid has other advantages. It is a low volatile solvent and more

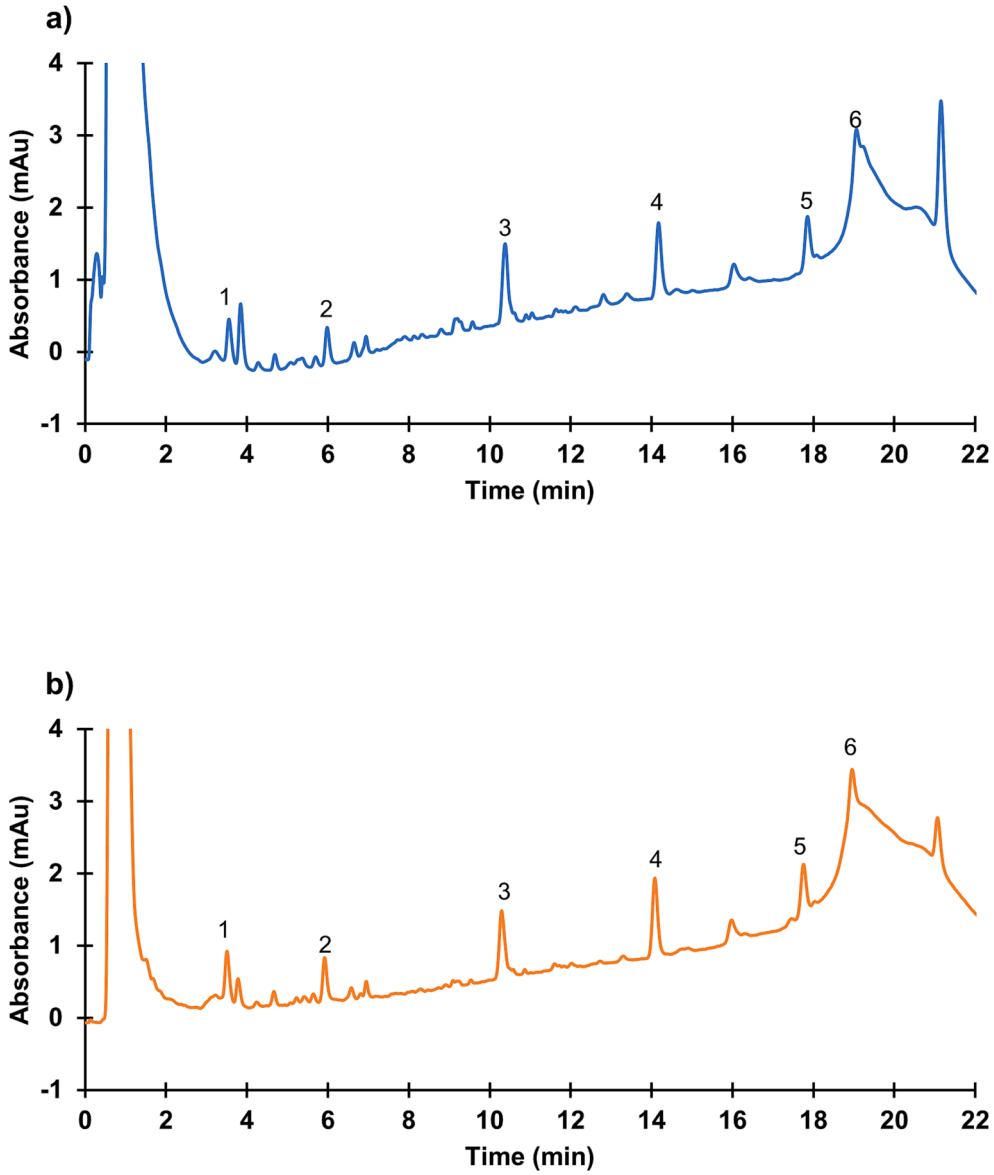

Fig. 6. Chromatograms obtained after HF-LPME using L-menthol: formic acid (1:2) NADES as SLM of the 6 triazines from MilliQ water sample (a) and artificial water (b), both spiked with 5 μg/L. Peak assignment: 1. DIA; 2. DEA; 3. SIM; 4. ATZ; 5.PPZ; 6.TER.

Table 1
Relative recoveries (RR, %) of the triazines herbicides real in samples after the proposed HF-LPME (n = 3).

	Tap water				River water				Urine			
	RR[a] (%)	SD	RR[b] (%)	SD	RR[a] (%)	SD	RR[b] (%)	SD	RR[a] (%)	SD	RR[b] (%)	SD
DIA	99	6	125	5	87	11	87	19	77	12	113	13
DEA	107	16	120	17	95	9	89	21	93	2	103	16
SIM	82	11	90	14	75	10	68	17	100	28	93	21
ATZ	106	16	114	6	91	5	91	7	103	27	111	29
PPZ	125	21	122	1	100	10	96	23	115	41	128	33
TER	111	11	113	11	70	6	78	11	98	25	121	27

[a] 2.5 μg/L.
[b] 5 μg/L.

stable as SLM than toluene, which tends to disappear from HF due to its high volatility. Therefore, the substitution of toluene by NADES results in a safer environment for the operator.

Finally, other molar ratios were studied for NADES prepared with formic acid, acetic acid and ethanol. When the amount of ethanol was increased to molar ratios of 1:2 and 1:4 (L-menthol: ethanol) and acetic acid was increased to 1:2 (L-menthol: acetic acid), a decrease in the extraction of triazines was observed. However, as can be seen in Fig. 5, a slight improvement in recovery was obtained by increasing the formic acid molar ratio to 1:2, especially for TER, which previously showed the lowest extraction at the 1:1 M ratio. Considering the results obtained, the NADES prepared with L-menthol:formic acid at a molar ratio 1:2 was selected as the SLM for the extraction of triazines.

FT-IR spectroscopy was used for the characterization of the selected NADES and their individual components (L-menthol and formic acid), and their corresponding spectra are shown in Fig. S1. Regarding the infrared spectra of NADES, an intense peak is observed at 1721 cm^{-1} corresponding to C = O stretching, which is shifted from the corresponding band at 1684 cm^{-1} observed in the infrared spectra of formic acid, indicating that hydrogen bonds are present in that area during NADES formation. Besides, the wide band from ~3400 to ~2500 cm^{-1} corresponds to the stretching of the O–H bond, which is characteristic of the existence of hydrogen bonding to carboxylic acid molecules or carboxylic acid dimers, suggesting again the interaction by hydrogen bonding between formic acid and menthol. In addition, the band at 3235 cm^{-1}, corresponding to O-H vibration in the menthol spectra, becomes imperceptible in the spectrum of the obtained NADES, thus indicating the formation of new hydrogen bonds.

3.3. Optimization of the HF-LPME procedure with L-menthol:formic acid (1:2) as SLM

Other factors that affect the extraction efficiency of the proposed method, such as salting-out effect, extraction time, and the agitation speed, were thoroughly examined and optimized.

3.4. Evaluation of salting-out effect

In many cases, increasing the salt concentration can increase the ionic strength of the sample, reducing the solubility of high molecular weight compounds in water and thus improving their extraction efficiency ("salting-out effect). Therefore, the effect of adding NaCl in several percentages (0, 5, 10, 20 and 25%, w/v) to 3.5 mL of MilliQ water spiked with 50 μg/L of a mixture of 6 triazines was investigated. The acceptor solution was 25 μL of water adjusted to pH 0.6 and the extractions were performed under stirring for 30 min at 1050 rpm. The results are shown in Fig. S2, which shows that the addition of NaCl at 25% (w/v) improved the extraction of DIA and DEA, but not the other analytes. In addition, increasing the salinity of the sample leads to increased irreproducibility, resulting in higher RSDs. In view of the benefits obtained, the addition of an extra sample preparation step was not worthwhile and the use of NaCl was therefore discarded.

3.5. Extraction time and agitation

In order to achieve proper enrichment factor for the extraction of triazines from environmental waters at realistic concentration levels, it is necessary to use a large sample volume. Accordingly, 100 mL of water sample was used in further experiments. Extraction time and stirring speed are key parameters in HF-LPME since might promote target analytes diffusion from the sample to the acceptor solution through the SLM and thus both parameters need to be properly optimized. A three-level factorial design was used to study the effects of the two factors (agitation and time) on the extraction efficiency. Thus, 9 treatment combinations with and additional central point (a total of 10 executions) were performed in the following conditions: 100 mL of sample spiked with a mixture of 6 triazines at 50 μg/L, L-menthol: formic acid (1:2) NADES as SLM and 25 μL of an acceptor solution of water at pH 0.6. Data analyses were performed using the statistical package Statgraphics Centurion XVII, release 17.2.00 (The Plains, Virginia, USA).

Fig. S3 depicts the Pareto and response surface charts generated from the experimental design on the effect of both parameters (agitation and extraction time) on the extracted amount (ng) of the 6 tested triazines. From the obtained results, both extraction time and agitation had a statistically significant positive effect (the vertical line defines the 95% confidence interval) on the extraction efficiency of the triazines with the highest log P values (PPZ, TER). For the remaining triazines, neither extraction time nor agitation had a statistically significant effect on extraction efficiency. Therefore, an extraction time of 90 min and stirring at 425 rpm were selected as optimal.

3.6. Analytical performance and sample application

The main advantages of HF-LPME are enrichment, sample clean-up and reduced solvent consumption. However, the presence of humic substances, co-extracted during sample preparation, causes a broad peak at the beginning of the chromatograms when performing environmental water analysis by HPLC-UV, especially at low UV wavelengths, which can prevent the correct determination of more polar analytes. Therefore, an artificial water sample was prepared by adding humic acids to MilliQ water at a concentration of 10 mg/L and subjected to the proposed HF-LPME method. Fig. 6 presents the chromatograms acquired after HF-LPME under optimal conditions of the Milli-Q water sample (Fig. 6a) and the artificial water sample (Fig. 6b), both of them fortified with the 6 triazines at a concentration level of 5 μg/L. As can be seen, the proposed method provided a clean final extract. The use of NADES as a membrane created a shield that was able to exclude the humic acids, and thus the baseline was similar to that obtained with MilliQ water. Hence, NADES proved to be a good alternative to conventional organic sorbents.

In order to demonstrate the applicability of the proposed method for the extraction of triazine herbicides from real samples, the proposed HF-LPME method was applied to the analysis of river water, tap water and urine samples. Samples were used without pretratement and were spiked with a mixture of 6 triazines in the concentration range from 2.5 to 20 μg/L and subjected to the proposed HF-LPME method in triplicate

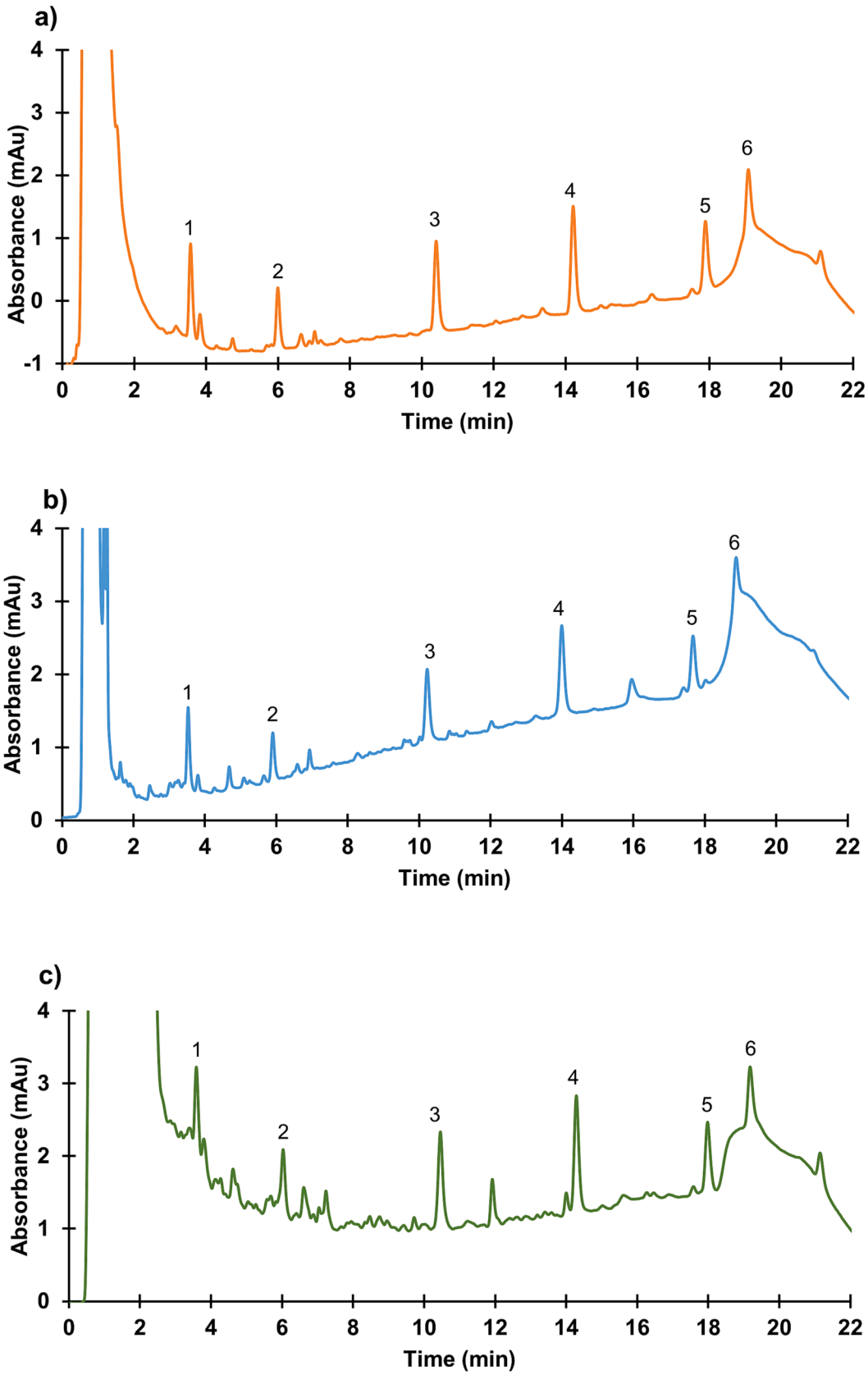

Fig. 7. Chromatograms obtained after HF-LPME using L-menthol: formic acid (1:2) NADES as SLM of the 6 triazines from tap water (a), river water (b) and urine (c), all spiked with 5 μg/L. Peak assignment 1. DIA; 2. DEA; 3. SIM; 4. ATZ; 5.PPZ; 6.TER.

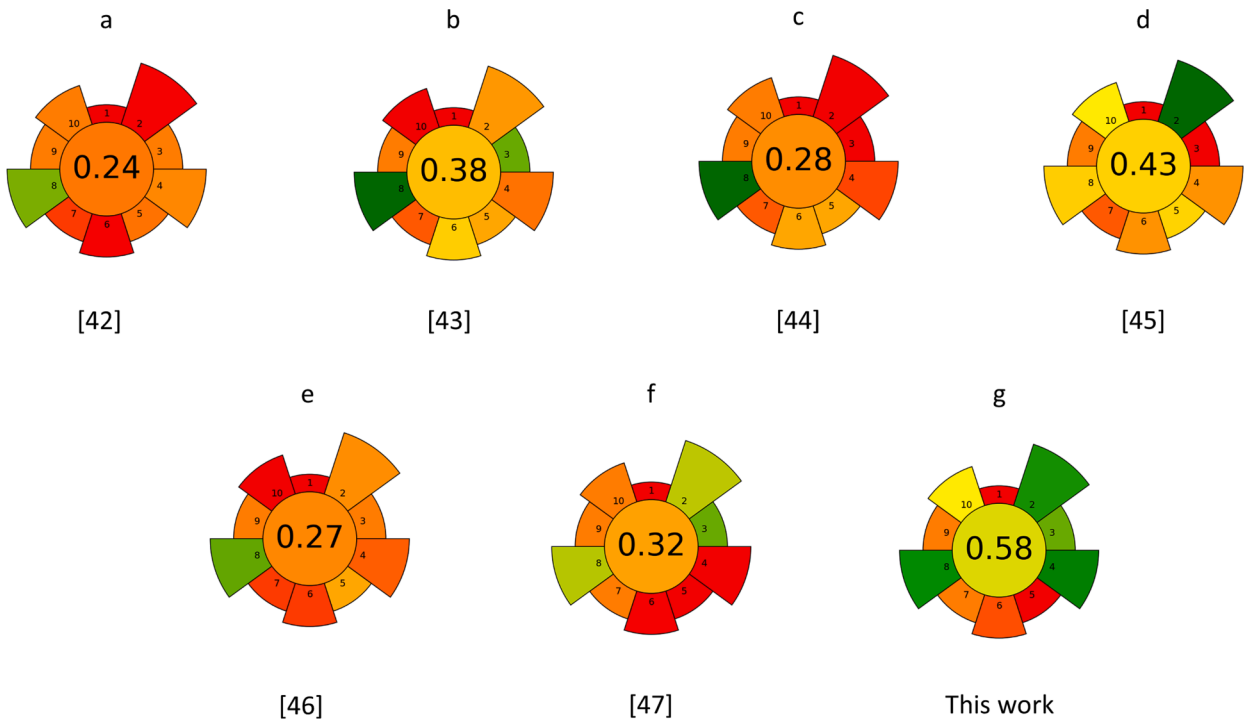

Fig. 8. Pictograms obtained using AGREEprep assessment of methods for the determination of triazines in environmental waters: (a) capsule phase microextraction [42]; (b) switchable hydrophilicity solvent homogeneous liquid–liquid microextraction [43]; (c) dispersive filter extraction [44]; (d) aqueous two-phase system [45]; (e) homogeneous ionic liquid microextraction combined with magnetical hollow fiber bar [46]; (f) hollow fiber liquid-phase microextraction [47], (g) the proposed method.

under optimal conditions. The calibration curves of the six triazines for each type of sample were obtained by plotting the peak area against the concentration of the fortified samples. Among the 18 calibrations prepared, a good linearity was found in the majority of the cases ($R^2 \geq 0.99$), with four exceptions where the lowest value was $R^2 = 0.975$.

The limits of detection (LODs) and quantification (LOQ) were determined as three and ten times, respectively, the standard deviation obtained in the analysis of spiked samples at the lower concentration level studied, divided by the slope of the calibration curve. The LODs and LOQs of the 6 triazines were 0.75–3.1 μg/L and 2.5–10.3 μg/L, respectively, depending on the analyte and the kind of sample.

Relative recoveries (RRs), used to evaluate the accuracy of the method, were calculated as the ratio of peak area measurements (n = 3) after HF-LPME of real samples and Milli-Q water, both spiked with triazines at two different concentrations (2.5 and 5 μg/L). RRs presented in Table 1 ranged from 68 to 128% depending on the analyte and sample type. The RSDs obtained for tap water were in the range of 1–17%, for river water from 5 to 25% and for urine from 2 to 40%. RSD values are particularly high for urine samples where, despite high purity extracts, the matrix would have caused some instability in the extraction process.

Chromatograms obtained after HF-LPME of tap water (a), river water (b) and urine (c) spiked with triazines at a concentration of 5 μg/L are shown in Fig. 7. A baseline similar to that of MilliQ water (Fig. 6a) was obtained for all samples tested, demonstrating that the NADES was able to act as a shield to prevent co-extraction of interferents from the matrix. This eliminates the need to perform a standard addition calibration for each sample type, as it would be possible to perform a Milli-Q water calibration to quantify triazines regardless of the sample type.

3.7. Comparison with other methods and greenness assessment of the proposed method

The analytical performance of the proposed method was compared with other published methods for the determination of triazines in environmental waters and was summarized in Table S3. As can be seen, the LODs provided by the proposed method were slightly higher than those reported in previous published methods. However, the herein proposed method presented advantages in terms of safety and environmental impact, which are also factors to be considered. In recent years, a metric tool called Analytical Greenness Metric for Sample Preparation (AGREEprep) has been developed specifically for assessing the greenness of analytical sample preparation [41]. In brief, a sample preparation method is assigned a score from 0 to 1 (1 represents the ideal system) for each of the 10 categories of impact (i.e. selection and use of solvents, materials and reagents, waste generation, energy consumption, sample size and throughput). The evaluation results in a pictogram that summarizes the overall greenness of the method and the pictograms for all the methods evaluated are summarized in Fig. 8.

This work obtained the highest score of all those compared methods (0.58) and stands out in 4 impact categories (criterion 2: use safer solvents and reagents; criterion 3: target sustainable, reusable and renewable materials; criterion 4: minimize waste; criterion 10: ensure safe procedures for the operator). This is mainly due to the substitution of organic solvents by NADES as the SLM, which is of natural origin and safer for the operator. In addition, there is no pre-treatment of the sample, which means it is not contaminated and less waste is generated.

4. Conclusions

In the present work, hydrophobic NADES based on L-menthol and different natural HBDs were prepared and evaluated to be used as SLM for HF-LPME of triazines in aqueous samples. Among the NADES evaluated, the combination of L-menthol: formic acid in a molar ratio of 1:2 was selected for its good stability as SLM and better triazine extraction ability. The factors that affect the extraction efficiency of the triazines, such as pH of the sample and acceptor solutions, salting-out effect, extraction time, and stirring speed, were carefully examined and optimized. The developed method was successfully applied to the

determination of 6 triazines in river water, tap water and urine. Excellent cleanup was achieved, avoiding matrix interferences such as humic acids, which allowed the determination of all the triazine studied with LODs of 0.75–3.1 μg/L, depending on analyte and sample.

The AGREEprep metric tool assessment confirmed that the proposed method is a greener approach addressing some of the principles of GAC and GSP such as the use of safer and sustainable reagents, reduction of chemicals, materials and waste, minimization of energy consumption, integration of steps and safer procedures for the operator.

CRediT authorship contribution statement

Myriam Díaz-Álvarez: Investigation, Writing – original draft. **Esther Turiel:** Conceptualization, Writing – review & editing. **Antonio Martín-Esteban:** Conceptualization, Writing – review & editing, Project administration, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

FT-IR spectra data associated to Fig. S1 and chromatographic data associated Fig. 6 and Fig. 7 are available at DIGITAL.CSIC repository (https://doi.org/10.20350/digitalCSIC/15433 and https://doi.org/10.20350/digitalCSIC/15434, respectively)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2023.109347.

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