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Supramolecular nano solvent-based hollow fiber liquid phase microextraction as a novel method for simultaneous preconcentration of acidic, basic and amphiprotic pollutants

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Abstract

The coextraction of acidic, basic and amphiprotic pollutants from various matrixes is a considerable and disputable concept in sample preparation strategies. In this study, for the first time, coextraction of acidic, basic and amphiprotic pollutants was performed using supramolecular nano solvent-based hollow fiber liquid phase microextraction (SS-HF-LPME) as an efficient method followed by high performance liquid chromatography-photo diode array detection. The supramolecular solvent (SUPRAS) is formed through coacervation of decanoic acid aqueous vesicles in the presence of tetrabutylammonium hydroxide. The results revealed that 40% SUPRAS in 1-decanol has the best extraction efficiency for three selected model analytes (4-nitrophenol, 3-nitroaniline and 1-amino-2-naphthol). The extraction process was accomplished in two phase mode and the unique interactions between the solvent and polar analytes (hydrophobic, electrostatic, hydrogen bonding and π-cation interactions) resulted in elevated coextraction efficiency. Central composite design methodology combined with desirability function approach was applied to develop predictive models for simulation and optimization of SS-HF-LPME procedure. The optimized conditions were: pH of the sample, 9.0; percentage of SUPRAS in 1-decanol, 40%; extraction time, 30 min; salt concentration, 20% w/v; stirring rate, 1250 rpm. Under the optimum conditions, detection limits and linear dynamic ranges were achieved in the range of 0.1-0.2 μg L^{-1} and 0.5-400 μg L^{-1}, respectively. The percent of extraction recoveries and relative standard deviations (n = 5) were in the range of 56.1-71.1 and 4.1-6.9, respectively. Finally, the applicability of this method was successfully confirmed by analyzing rain, snow, river, dam and wastewater samples.

Keywords: Supramolecular nano solvent; Hollow fiber liquid phase microextraction; Coextraction; Central composite design; Desirability function; Pollutants.

1. Introduction

It has been reported that aniline, phenol and their derivatives are acute environmental pollutants and they are classified as the hazardous wastes and priority toxic pollutants by Environmental Protection Agency of America [1,2], moreover, they have been suspected to be carcinogenic agents [2-4]. They are consumed in diverse manufacturing processes such as pesticides and herbicides, pharmaceuticals, plastics, dyestuff, pigments, wood preservatives, rubber chemicals, and explosives [5-7]. Anilines and phenols can easily permeate through soil and contaminate ground water due to their high solubility in water [2]. Herein, coextraction of these pollutants is in a point of view.

Nitrophenols are a class of the most important pollutants present in the environment. Nitrophenols are formed in the atmosphere through the photochemical reaction of benzene with nitrogen monoxide in highly polluted air [2,8]. For instance, 4-nitrophenol (4-NP) is one of the 129 organic pollutants listed by the United States Environmental Protection Agency as carcinogens and hazardous to human beings as well as the environment [9]. Furthermore, 4-NP damages mitochondria and inhibits energy metabolism in human and animals [5,9]. Hence, exploring a simple, rapid, sensitive, environmentally friendly and cost effective method for 4-NP determination is crucial.

Azo dyes are synthetic organic colorants generally produced by coupling a diazonium compound with an aromatic amine or a phenol and they are utilized in various areas such as nutrition, cosmetics, paper, pharmaceutical, printing ink, textile and tanning industries [10]. Several azo dyes used as colorants for food, drugs and cosmetics can be reduced by cell suspensions of predominant intestinal anaerobes [11], therefore, it can be assumed that the ingestion of certain azo dyes is a risk for human health indeed. In this sense, 1-amino-2-

naphthol (1-A2N), produced by the reduction of Acid Orange 7, has been reported to induce bladder tumors [12]. The high toxicity of 1-A2N (EC_{50} 0.1±0.03 mg L^{-1}) is probably due to its high solubility in lipids [13].

Nitroaniline isomers such as 3-nitroaniline (3-NA), as nitro-substituted derivatives of aromatic amines, have become more and more significant in environmental science due to their high toxicity and their suspected carcinogenic properties [14,15]. These pollutants are mainly used as intermediates in the synthesis of dyestuff, pharmaceuticals, pesticides, and herbicides [6,15], and then they are released in the environment directly as industrial wastes or indirectly as breakdown products of pesticides and herbicides [15-17].

Several analytical methods such as high-performance liquid chromatographic (HPLC) method with ultraviolet [2,8], mass spectrometry [18] or electrochemical detection [19], gas chromatography with flame ionization [20], or mass spectrometry detection [21], and capillary zone electrophoresis have been utilized for the determination of phenol, aniline and their derivatives [22,23]. All the named methods have been successfully applied for the routine analysis of each category, but none of them afford simultaneous quantification of the mentioned acidic, basic and amphiprotic pollutants in a single step.

Sample preparation procedures play a dominant role in chemical analyses. Extensive sample cleanup procedures are usually required to remove matrix components which may interfere with the analysis [24]. Liquid-liquid extraction and solid phase extraction are commonly applied as sample pretreatment techniques in analytical chemistry [15,25,26]. However, these methods are time-consuming, generally labor-intensive, and require large quantities of expensive, toxic and environmentally unfriendly organic solvents [27]. The solvent microextraction techniques, which are commonly faster and simpler than conventional methods, effectively overcome these problems by reducing the amount of organic solvent

consumption [28]. Moreover, extraction, preconcentration, and sample introduction to the analytical instrument are performed in a single step [29,30]. In 1999, a novel and efficient liquid phase microextraction technique based on applying hollow fiber membrane (HF-LPME) was developed [31]. Using this microporous hollow fiber membrane provides the merits of the protection of the acceptor phase as well as efficient sample microfiltration through the pores of the hollow fiber [32,33]. HF-LPME can be done either in two or three-phase configuration. In the two phase sampling configuration (HF-LPME), the analytes of interest are extracted from an aqueous sample to a water immiscible extraction solvent which is immobilized in the pores and lumen of the hollow fiber. In contrast, in the three phase sampling configuration (HF-LLLME), limited to ionizable analytes, the analytes are extracted from an aqueous sample through the water immiscible extractant which is immobilized in the pores of the hollow fiber and ultimately back extracted into an acceptor aqueous phase inside the lumen of the hollow fiber [28,34].

Various extractants, including common solvents (i.e. long chain aliphatic alcohols, long chain hydrocarbons, ethers) [28,29,31,33], ionic liquids [35,36], and supramolecular nanosolvents (SUPRASs) [32,37] have been applied in HF-LPME. However, SUPRASs are of interest due to their unique properties. SUPRASs, also referred to as coacervates [38], which are used in surfactant liquid-liquid phase separation [39], are nanostructured liquids constructed from three dimensional aggregates of amphiphilic compounds. The supramolecular solvent produced from coacervation of decanoic acid aqueous vesicles in the presence of tetrabutylammonium (Bu_4N^+) cation has been utilized as an extraction solvent in numerous literatures [32,37,40-44]. Two characteristics give the alkyl carboxylic acid-based coacervates a high potential for analytical extraction processes. First, the polar region of molecular aggregates comprise protonated and deprotonated carboxylic groups and ammonium groups; hence, various types of interactions (e.g., electrostatics, π-cation,

hydrogen bonds, formation of mixed aggregates, etc.) can be established with the analytes of interest, in addition to the hydrophobic interactions in the hydrocarbon region [37]. Second, vesicles have a number of available solubilization moieties; therefore, high concentrations of polar and non-polar analytes can be solubilized in each aggregate [32,37,41].

In this context, the aim is to develop HF-LPME method based on applying supramolecular nanosolvent for coextraction and determination of some priority acidic, basic and amphiprotic pollutants in various samples for the first time. To the best of our knowledge, there is no report on the coextraction of acidic, basic and amphiprotic pollutants using supramolecular solvent-based hollow fiber liquid phase microextraction method. The unique properties of this solvent made the coextraction of the analytes of interest feasible. Although direct extraction with the supramolecular solvent may be easier and faster than SS-HF-LPME method, the selectivity and repeatability of SS-HF-LPME can greatly be improved due to the protection of the acceptor phase as well as efficient sample microfiltration through the pores of the hollow fiber. Central composite design (CCD) in combination with desirability function (DF) approach has been utilized to develop a predictive model for simulation and optimization of SS-HF-LPME method. Finally, the optimized procedure was applied to determine the analytes in various real samples satisfactorily.

2. Experimental

2.1. Chemicals and reagents

4-NP, 3-NA, 1-A2N, Acid red 88, alizarin yellow GG and methylene blue were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Diphenhydramine and sodium diclofenac was kindly donated by Darou Pakhsh (Tehran, Iran) and used without further purification. Decanoic acid (DA), tetrabutylammonium hydroxide ($(Bu)_4A^+$), ammonium hydroxide (28%

w/v), NaCl, 1-octanol, 1-nonanol, 1-decanol, 1-undecanol, *n*-hexadecane, which all were of analytical-grade were supplied by Merck (Darmstadt, Germany). HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from Caledon (George-town, Ont., Canada). Ultrapure water was prepared using a milli-Q system from millipore (Bedford, MA, USA). Rain and snow water samples were collected during April 2013 and February 2014, respectively. River water sample was collected from Karaj River (Karaj, Iran). Wastewater sample was obtained from a pharmaceutical factory (Tehran, Iran) and dam water sample was collected from Ilam Dam (Ilam, Iran).

2.2. Equipment

2.2.1. Chromatographic conditions and equipment

Analysis of the standard and test samples was performed by Shimadzu SCL-10AVP HPLC instrument from Shimadzu Company (Tokyo, Japan) combined with an LC-10AVP pump, SPD-M10AVP diode array detector (DAD), a Rheodyne 7725i (PerkinElmer, USA) injector, along with a 20 µL sample loop. The LC-solution program for LC was used to perform data processing. A capital HPLC column (Scotland, UK) ODS-H C_{18} (250 mm × 4.6 mm, i.d. 5 µm) was employed for all separations. The mobile phase was a mixture of deionized water and acetonitrile (50:50, v/v) for 12 min and 100% acetonitrile for 3 min at the flow rate of 1 mL min^{-1} with the detector wavelength set at 230, 240 and 315 nm for 3-NA, 1-A2N and 4-NP, respectively. The pH of solutions was adjusted by using a methrohm digital pH meter 827 equipped with a glass calomel electrode. In the extraction procedure, a 8.5 mL sample vial, and a MR 3001 heating-magnetic stirrer from Heidolph Company (Kelheim, Germany) were used. EBA 20 Hettich centrifuge (Oxford, England) and a 50 µL Hamilton HPLC syringe (Reno, NV, USA) were employed, too.

2.2.2. Dynamic light scattering measurements

Dynamic light scattering (DLS) measurements were carried out with a Malvern Zetasizer Nano ZS using Dispersion Technology.

2.3. Preparation of standard solutions and real samples

Stock solutions of pollutants, dyes and drugs (1000 mg L^{-1}) were prepared in HPLC grade methanol and stored in a fridge at 4 ºC and brought to ambient temperature just prior to use. Mixed working solutions of the analytes at different concentrations were prepared by dilution with ultra-pure water or deionized water containing various NaCl concentrations. The water samples were filtered through a Millipore 0.22-μm cellulous acetate filter before the extraction process. 8 mL spiked/non-spiked rain water sample was used without any dilution.

2.4. Preparation of the supramolecular solvent

SUPRAS was prepared by mixing 5.15 g of DA and 15.6 mL of tetrabutylammonium hydroxide in 200 mL distilled water at pH 7 ± 0.1. The mixture was stirred at 1200 rpm for 10 min to dissolve DA [32,37]. Phase separation was performed by centrifugation of the mixture at 4000 rpm for 5 min and the obtained SUPRAS was used for further experiments.

2.5. SS-HF-LPME procedure

The Accurel Q3/2 polypropylene hollow fiber membrane (200 μm wall thickness, 600 μm I.D. and 0.2 μm pore size) was obtained from Membrana Company (Wuppertal, Germany) and used for all experiments. Hollow fibers were ultrasonically cleaned with acetone for 5 min. Each dried fiber was cut manually into 10.0 cm segments, which may approximately accommodate 27 μL of the receiving phase. Afterward, 8.0 mL of the sample solution (pH, 9.0 adjusted with a dilute NaOH solution; NaCl concentration, 20% w/v) containing 0.1 mg

L^{-1} of the target analytes was transferred into a 8.5 mL vial (48 mm height × 7.5 mm diameter) with a 4 mm × 7 mm magnetic stir bar. The sample vial was placed on the magnetic stirrer and a 50 μL Hamilton microsyringe (Bonaduz, Switzerland) was used to introduce the receiving phase (40% SUPRAS in 1-decanol) into the hollow fiber. 35 μL of the receiving phase was then withdrawn into the microsyringe and its needle was inserted into the lumen of the hollow fiber. Thereafter, the fiber was inserted in the organic phase (40% SUPRAS in 1-decanol) for 90 s and the excess of the organic phase was carefully removed by washing the outside of the hollow fiber with ultrapure water. Subsequently, the receiving phase was injected into the lumen of hollow fiber and the end of the hollow fiber was sealed by a piece of aluminum. The U-shape hollow fiber was immersed into the sample solution. The extraction was performed at room temperature and the sample was stirred at 1250 rpm during the extraction for 30 min. After extraction, the fiber was removed from the sample vial, the end of the hollow fiber was opened, and the receiving phase was retracted into the microsyringe. Finally, 20 μL of receiving phase was injected into the HPLC-PDA system for subsequent analysis.

2.6. Response surface methodology and desirability function

Traditional optimization methods with successive variations in variables such as a one-factor-at-a-time (OVAT) approach are still used, although it's well accepted that they are relatively time-consuming and expensive for a large number of variables and frequently fail to predict the optimum condition [45,46]. The major drawback of OVAT approach is the lack of inclusion of the interactive effects among variables [47]. Therefore, in order to optimize the preconcentration of the model analytes by the proposed method, a central composite design (CCD) in combination with desirability function (DF) was employed. It's worth to note that for an experimental design involving four variables expressed by CCD, linear, quadratic and

cross terms can be involved. The precise optimum point can be obtained by the aid of response surface methodologies, exhibiting relationships between variables and responses graphically [48].

Finding optimum conditions for a single response is usually relatively simple, but in practice the problems are often more complex and the studied phenomena are described by a number of responses. Certain responses can oppose one another; changes in a factor which promote one response may have a suppressing effect on the others, *etc* [49]. To solve this problem, in 1980, Derringer and Suich applied an overall response to optimize multiple responses by developing DF [50]. Therefore, in the case of multiple responses optimizations, the Derringer function or DF can be employed, since it's the most critical and most widely applied multi-criteria methodology in analytical procedures [51]. At first, in DF approach, each predicted response is transformed to a dimensionless desirability value (d) and then all transformed responses are combined into one particular response. The scale of the individual DF ranges between 0-1, while for the most desirable response d is equal to 1 and for a completely undesired response d is 0 [52]. Different transformations on data may be implemented depending on whether the response is optimum when it is maximized, minimized, or at a predefined value [53].

In this work, the experimental design matrix and data analysis were carried out by the Design-Expert statistical software program (7.0.0 trial version).

3. Results and discussion

3.1. Size determination of SUPRAS

The size and morphology of the nano-sized aggregates was explored by DLS technique. The DLS size distribution of aggregates is depicted in Fig. 1S (Electronic Supplementary Data).

The peak centered at approximately 1-2 nm corresponds to aqueous micelles. The peaks appeared at 28-59 and 342-531 nm are related to vesicles. Moreover, the results revealed that vesicles are the dominant type of aggregate in the SUPRASs.

3.2. Optimization of SS-HF-LPME parameters

Before confining any specific limits for performing CCD, some pilot experiments should be carried out to evaluate the approximate domains for each factor. The factors influencing the extraction capability of the proposed method such as pH of sample, membrane solvent, percentage of SUPRAS, extraction time, salt content of sample solution and stirring rate were investigated and optimized. Out of these six factors, membrane solvent and stirring rate were selected using one variable at a time method. Stirring rate was fixed at 1250 rpm, since observations exhibited that by increasing stirring rate up to 1250 rpm, the extraction of the target analytes was increased as well. The volume and shape of the vial was suitable, so no air bubble was formed at such a high speed and extraction kinetics would be promoted. The optimization of the four other factors was performed using central composite design in combination with desirability function approach (CCD-DF).

3.2.1. Selection of membrane solvent

Compatibility with the lipophilic polypropylene hollow fiber, low water solubility to prevent dissolution into the aqueous phase, affinity for target compounds, reasonable higher solubility of analytes in the organic phase than in the aqueous phase and low volatility which will restrict solvent evaporation during extraction, are several important criteria for the selection of organic solvent as a liquid membrane to achieve the highest enrichment factor [28,54]. Based on the required characteristic, it was observed (Fig. 1) that 1-decanol containing SUPRAS was more appropriate not only for less risk of solvent loss in longer extraction time

but also due to the unique interactions between the solvent and polar analytes (hydrophobic, electrostatic, hydrogen bonding and π-cation interactions) that results in elevated coextraction efficiency. Besides, in the case of 1-decanol the results were more reproducible than the other solvents. It's worth to note that all tested solvents contain 50% SUPRAS. The viscosity of 1-decanol (voscosity = 12.05 cP, polarity index = 0.37) is higher than 1-octanol (viscosity = 7.77 cP, polarity index = 0.54) and 1-nonanol (11.7 cP, polarity index = 0.41) and is lower than 1-undecanol (viscosity = 17.2 cP, polarity index = 0.27). It can be claimed that the higher viscosity of 1-decanol leads to its stability during the extraction process. Moreover, the polarity of 1-decanol is higher than undecanol. However, most of the target analytes have low partition coefficients, so there was no possibility to have good extraction capability with non-polar solvents such as *n*-hexadecane (viscosity = 3.45, polarity index = 0.21).

3.2.2. Central composite design and desirability function

In the next step, the affecting factors were selected based on preliminary experiments and optimized by a CCD experiment. In other words, CCD was utilized to optimize the effect of four factors (pH of sample, extraction time, percentage of SUPRAS and salt content of sample solution). According to the experiment equation obeying CCD; $N = 2^f + 2f + C_0$, where f is the number of variables and C_o is the number of center points, f and C_0 were set at 4 and 6, respectively, which mean that 30 trials should be performed [55].

The following equation is implemented in order to find the best joint response acquisition (DF), also named as geometric mean (Geo mean).

$$D = (d_1^{r_1} x\, d_2^{r_2} x \ldots\ldots x\, d_n^{r_n})^{\frac{1}{\sum r_i}} = \left(\prod_{i=1}^{n} d_i^{r_i}\right)^{\frac{1}{\sum r_i}} \qquad \text{Eq. 1}$$

where r_i is the importance of each variable relative to the others. A matter of the utmost importance is maximization of DF in the optimization procedure, i.e. when DF (ranging from 0 to 1) is a non-zero value, all the variables which are simultaneously optimized can be supposed to have a desirable value [48]. Obtaining an appropriate set of conditions that will meet all the determined criteria is the main goal of an optimization procedure and achieving a DF=1 is not a purpose. The results of CCD were investigated according to the criteria assigned based on desirable levels of factors and responses (Table 1) in order to find the best extraction conditions. To get the desired extraction efficiency as an objective function, Geo mean as an indicator of extraction efficiency was maximized. It's worth noting that an initial data preprocessing, i.e., normalizing the related responses of each analyte is necessary before data analysis. Subsequently, the obtained DF would be an input value for CCD [56].

The experimental data presented a good accordance with the quadratic polynomial equation (Table 2). Analysis of Variance (ANOVA) was used to evaluate the significant terms in the model for each response and the related significances were judged by the F-statistic calculated from the data (Table 1S, Electronic Supplementary Data). The model F-value of 6.49 (p-value = 0.0004) implies that the model is significant and there is only a 0.04% chance that a model F-value of 6.49 could occur due to noise. The p-value for lack of fit (LOF) in the ANOVA table was higher than 0.05 that confirms the LOF is not significant relative to the pure error [56].

Two dimensional (2-D) color maps are depicted in Fig. 2, representing high desirability with warm ‘‘red’’ and low desirability with cold ‘‘blue’’ colors. The optimum point can be selected from the constructed design space by visual examination which is in accordance with the highest desirablity value condition. In consequence, the highest D value of 0.916 was obtained at pH = 9.0, extraction time = 30 min, SUPRAS percentage = 40% v/v in decanol and salt content = 20% w/v as the optimum conditions.

318 The sample pH determines the form of analytes in aqueous solution which plays an important
319 role in the coextraction of target analytes. In pH 9.0, 4-NP exists in anionic form and the
320 other analytes are in their neutral forms. Therefore, 4-NP can interact with SUPRAS and 1-
321 decanol through hydrophobic, electrostatic (between negative charge of 4-NP and positive
322 sites of TBA), hydrogen bonding and π-cation interactions [37,43,57]. 3-NA and 1-A2N can
323 interact through hydrophobic interaction, hydrogen bonding and π-cation interaction. These
324 mixed mode mechanisms and multiple binding sites would provide a good solubilisation of
325 the model analytes in SUPRAS, thus assisting efficient extraction of the analytes. The
326 extraction efficiencies of target analytes were improved dramatically by increasing SUPRAS
327 content from 0 to 40% and then decreased that may be due to an increase in solvent viscosity
328 which decreases the mass transfer rate [57]. Furthermore, the results exhibited that the
329 coextraction of the analytes is possible in acidic medium. The extraction of positively
330 charged (protonated) species can be a result of ion pair formation between decanoate and
331 protonated 3-NA and 1-A2N species [37]. However, the best extraction efficiency was
332 obtained at basic medium. The extraction efficiency of target analytes was augmented
333 dramatically by increasing extraction time from 10 to 30 min and then a decrease may be due
334 to the solvent loss and air bubbles formation, which would suppress the extraction efficiency.
335 The extraction efficiency of the analytes increased by addition of NaCl to the aqueous
336 solution up to 20% w/v. According to the salting-out effect, the solubility of analytes in the
337 aqueous phase will be decreased and their partitioning into the organic phase will be
338 increased. In higher NaCl concentrations, the viscosity of the aqueous solution may act as a
339 hindrance in the mass transfer process and leads to lower extraction efficiency of the analytes
340 [61].
341 Through the statistical processes, the response surfaces obtained for the global desirability
342 function based on the design and modeled CCD are depicted in Fig. 2, in which some of the

surfaces obtained for the different factor combinations are presented. As can be appreciated, the global desirability function value was about 0.916, for all these possible experimental conditions. According to the overall results of the optimization study, pH = 9.0, extraction time = 30 min, SUPRAS percentage = 40% v/v in decanol and salt content = 20% w/v were selected as the optimum values.

3.3. Applicability of SS-HF-LPME method for coextraction of other compounds

Under the optimized conditions, the performance of the proposed method was explored for simultaneous extraction of some other basic and acidic compounds. For this purpose, diphenhydramine (DPH, pK_a = 9.0) as a basic drug and sodium diclofenac (DIC, pK_a = 4.2) as an acidic drug were extracted under the optimized conditions (obtained for 4-NP, 3-NA and 1-A2N) and acceptable results were achieved. Under this condition, preconcentration factors of 75 and 110 for DPH and DIC were obtained, respectively. Moreover, the applicability of this method for extraction of acidic (acid red 88 and alizarin yellow GG) and a basic dye (methylene blue) was explored. As depicted in Fig. 3, a color change was observed before and after the extraction process, indicating that the dyes were successfully extracted into the acceptor phase. For more clarity, it's worth to note that, 25 mg L^{-1} of each dye was subjected to the extraction protocol. All the obtained results confirmed the applicability of SS-HF-LPME for coextraction of various compounds which is due to the mixed mode mechanisms and multiple binding sites of SUPRAS.

3.4. Analytical figures of merit of SS-HF-LPME

The analytical performance of the proposed method is tabulated in Table 3. Quality features of the current method were evaluated under the final optimized conditions. Under the optimized conditions, limit of detection (LOD), regression equation, correlation of

368 determination (r^2), dynamic linear range (DLR), preconcentration factor (PF), and extraction
369 recovery (R%) of each analyte were evaluated. LOD values were calculated at the signal to
370 noise ratio of 3. Repeatability (within day RSDs, n = 5 sample, at 30 µg L^{-1} level of the
371 analytes) and reproducibility (between day RSDs, n = 3 day, at 30 µg L^{-1} of the analytes) of
372 the method for the determination of the target analytes were equal or less than 6.9% and
373 12.9%, respectively. Enrichment factor (EF) values were calculated as the ratio of the slopes
374 of the calibration curves before and after preconcentration. The extraction recoveries were
375 calculated by the following equation [28]:

376 $$ER\% = EF \times \frac{V_f}{V_i} \times 100 \qquad \text{Eq. 2}$$

377 where EF is enrichment factor and V_f and V_i are the organic phase and aqueous sample
378 volume, respectively.

379

380 3.5. *Analysis of real samples*

381 To evaluate the accuracy and also applicability of the mentioned procedure for complicated
382 samples, the coextraction of the aforementioned model compounds in real water samples
383 (snow water, rain water, river water, dam water and pharmaceutical wastewater) was
384 performed. Fig. 4 and 2S represent the chromatograms of the rain, snow, river, dam and
385 wastewater samples analysis before and after spiking. Nitrophenols such as 4-NP are formed
386 in the atmosphere from the photochemical reaction of benzene with nitrogen monoxide in
387 highly polluted air. Hence the presence of 4-NP in snow and rain water samples in highly
388 polluted areas is expected, in contrast to the river water that may be polluted or not due to
389 firstly, probably originating from a not polluted area, secondly, probably containing 4-NP
390 even lower than the LOD of the method. Table 4 exhibits that the results of the three replicate
391 analyses of each real sample obtained by the proposed method, are in good agreement with
392 the spiked levels.

393

394 *3.6. Comparison of SS-HF-LMPE with other alternative methods*

395 Table 5 compares the figures of merit of SS-HF-LPME method and the alternative methods
396 for the extraction of the target analytes in various matrices. The comparison results
397 demonstrated that the current method involves wide linear dynamic range and low detection
398 limit and also entails the advantage of the coextraction of acidic, basic and amphiprotic
399 compounds over most of the other methods. Besides, this method required only a very small
400 amount of an environmentally friendly organic solvent. Utilizing fresh acceptor phase and
401 discarding the hollow fiber after each extraction, eliminates the possibility of sample
402 carryover and ensures repeatability and reproducibility.

403

404 **4. Conclusion**

405 In the current method, for the first time, a novel strategy for coextraction of acidic, basic and
406 amphiprotic pollutants (with different polarities) using supramolecular nano solvent-based
407 hollow fiber liquid phase microextraction was proposed. The polar region of the nano solvent
408 is comprised of protonated and deprotonated carboxylic groups and ammonium groups;
409 therefore, various type of interactions (e.g., electrostatics, π-cation, hydrogen bonds,
410 formation of mixed aggregates, etc.) can be established with analytes of interest, in addition
411 to hydrophobic interactions in the hydrocarbon region. Moreover, vesicles have a number of
412 available solubilization moieties; therefore, high concentrations of polar and non-polar
413 analytes with different nature (acidic, basic or amphiprotic) can be solubilized in each
414 aggregate. The mentioned method is simple, fast and cheap. Regarding few microliters of
415 organic solvent consumption and environmentally friendly nature of it, this strategy can be
416 considered as a green technique. Utilizing fresh acceptor phase and discarding the hollow

417 fiber after each extraction has led to high reproducibility and repeatability of the method as
418 well as avoiding the carryover problems.
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533 **Figure Legends**

534 **Fig. 1:** Effect of organic solvent on the extraction efficiency, conditions: sample volume: 8.0
535 mL; stirring rate: 1250 rpm; extraction time: 45 min; concentration of analytes: 0.5 mg L^{-1};
536 pH of sample: 10; 50% v/v SUPRA, without salt addition.

537 **Fig. 2:** 2-D model depicts overall desirability function and the response surfaces obtained for
538 the global desirability function.

539 **Fig. 3:** Photographs of dye preconcentration under the optimal conditions: (a,b) acid red 88
540 (c,d) alizarin yellow GG and (e,f) methylene blue; a, c and e are before the extraction
541 initiation; b, d and f are after extraction time of 30 min.

542 **Fig. 4:** The chromatograms of (A): snow water sample (a) before spiking, (b) spiked at 10 µg
543 L^{-1} of each analytes (B): rain water sample (a) before spiking, (b) spiked at 10 µg L^{-1} of each
544 analytes, and (C): river water sample (a) before spiking, (b) spiked at 10 µg L^{-1} of each
545 analytes after SS-HF-LPME under optimized conditions.

546

Fig. 1

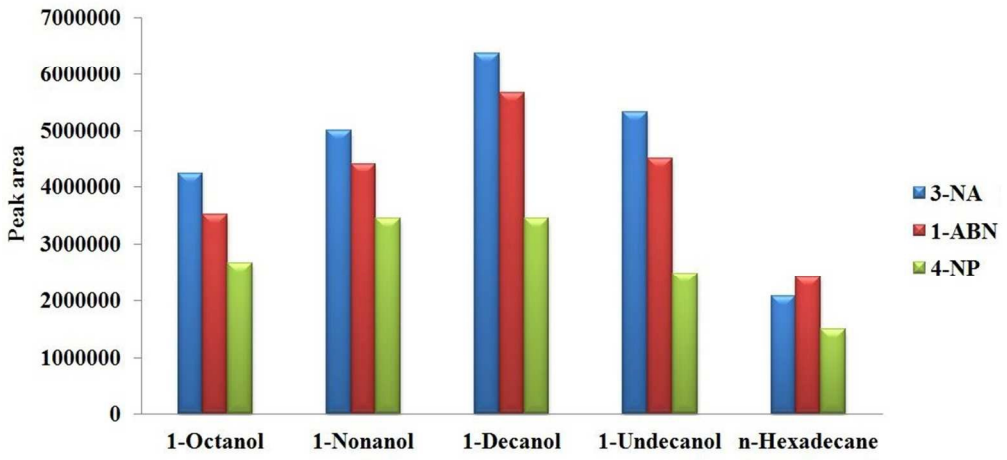

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Fig. 2

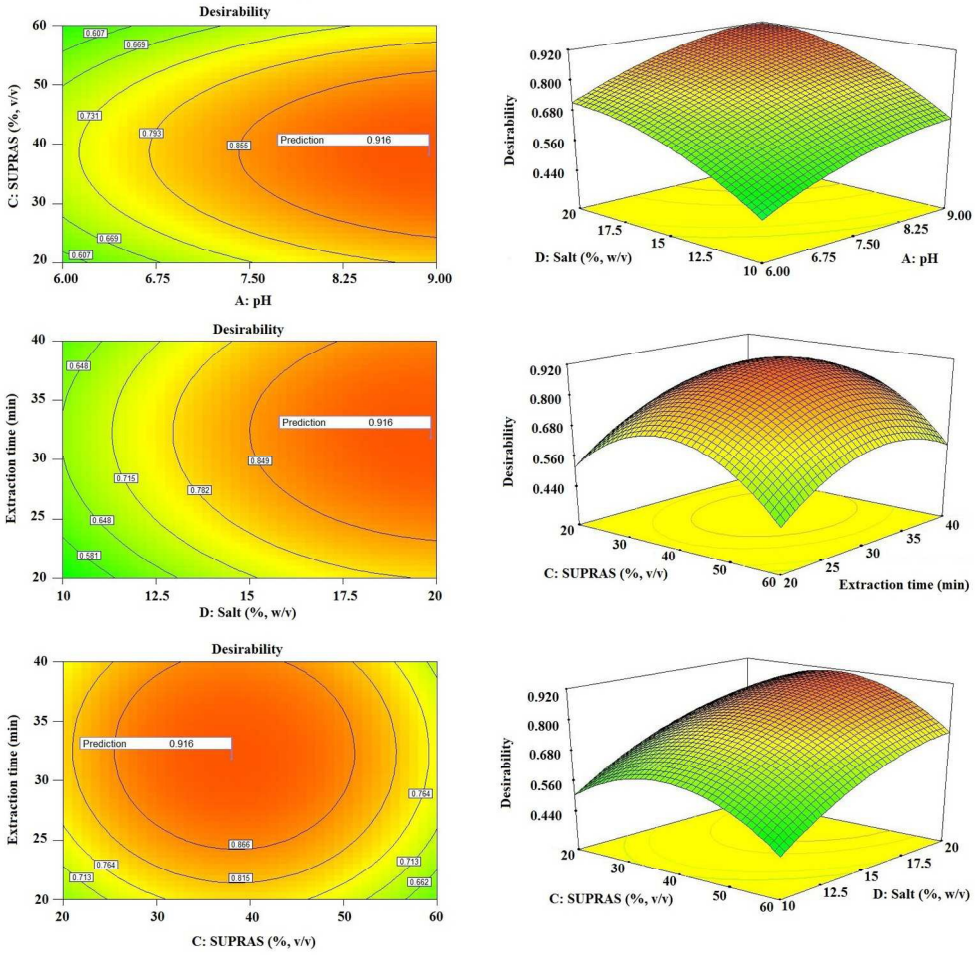

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Fig. 3a-d

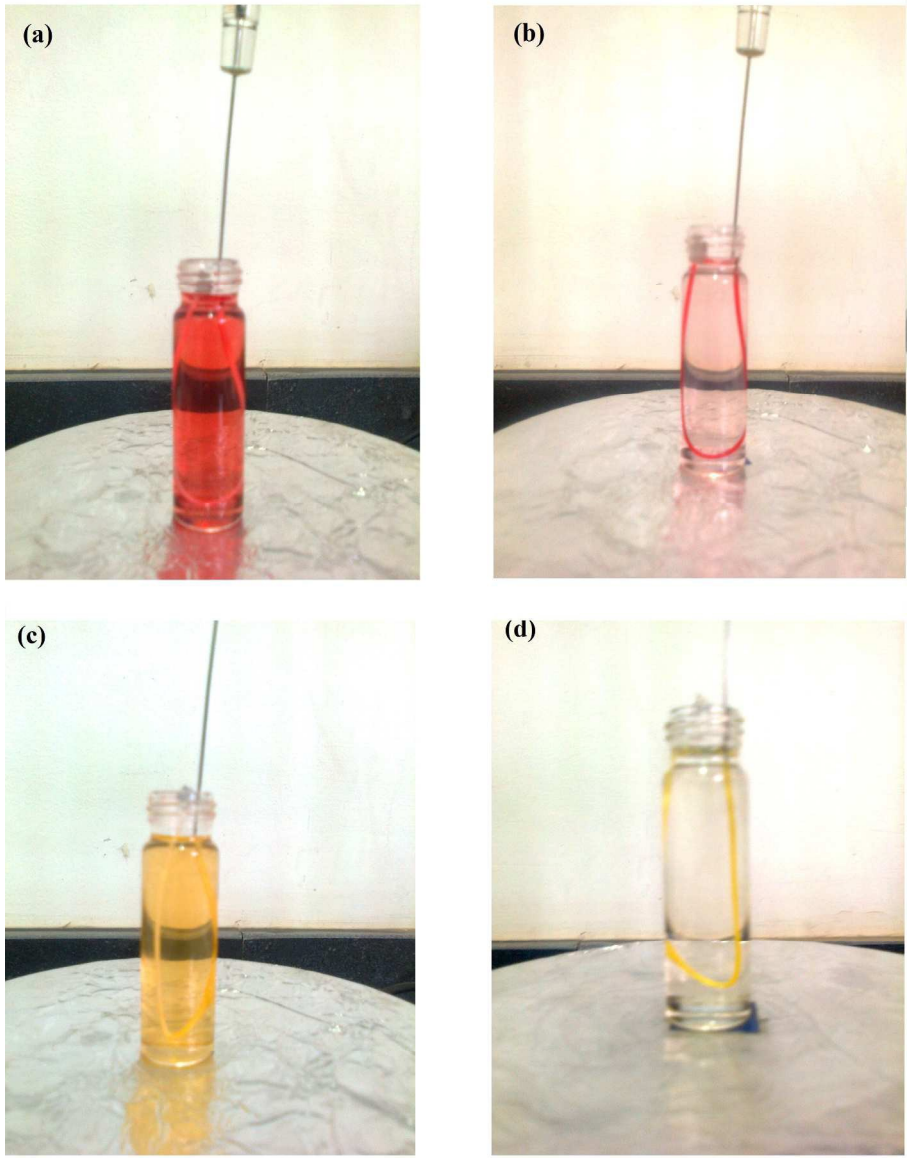

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Fig. 3e-f

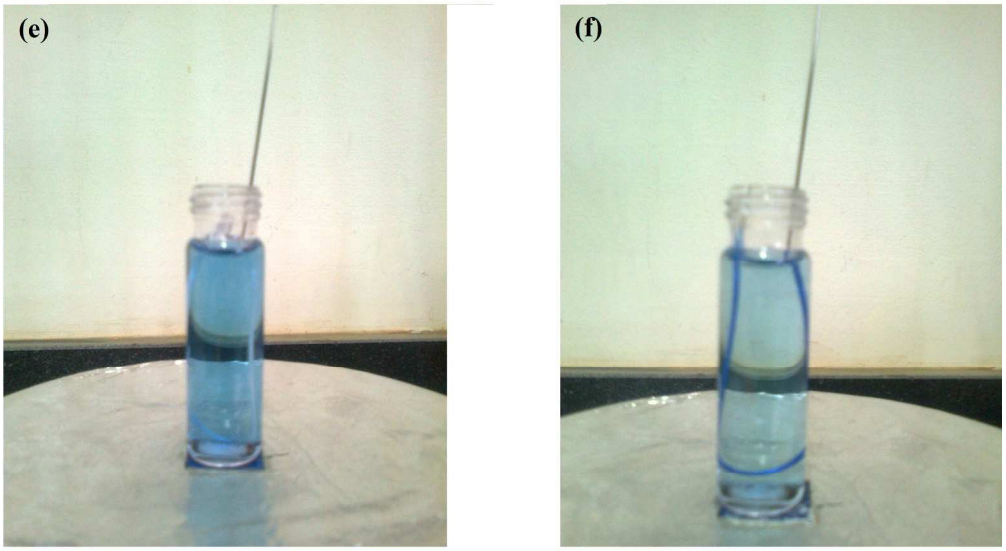

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Fig. 4a

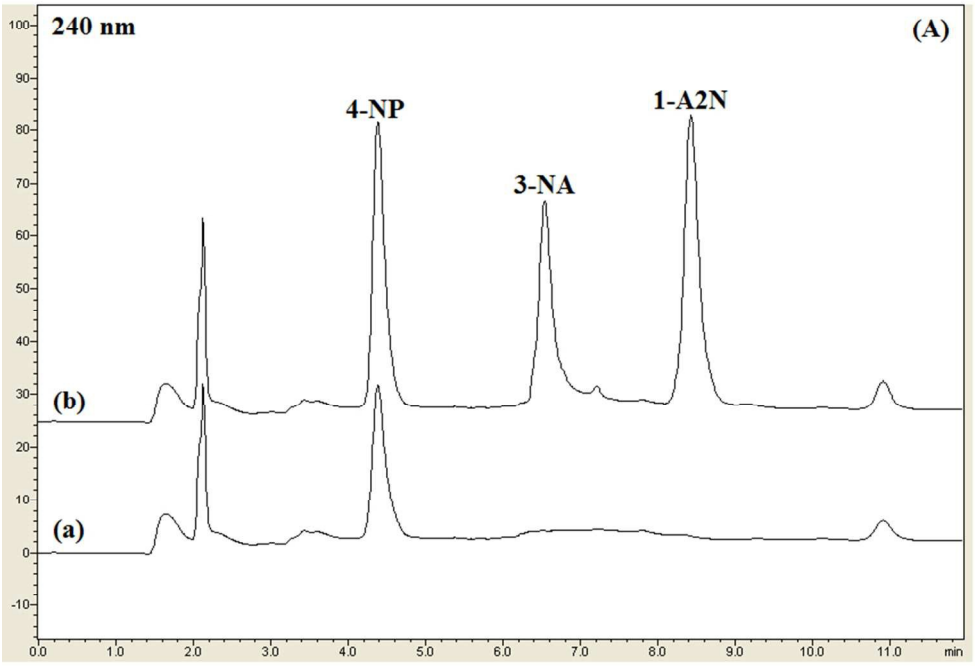

Fig. 4b

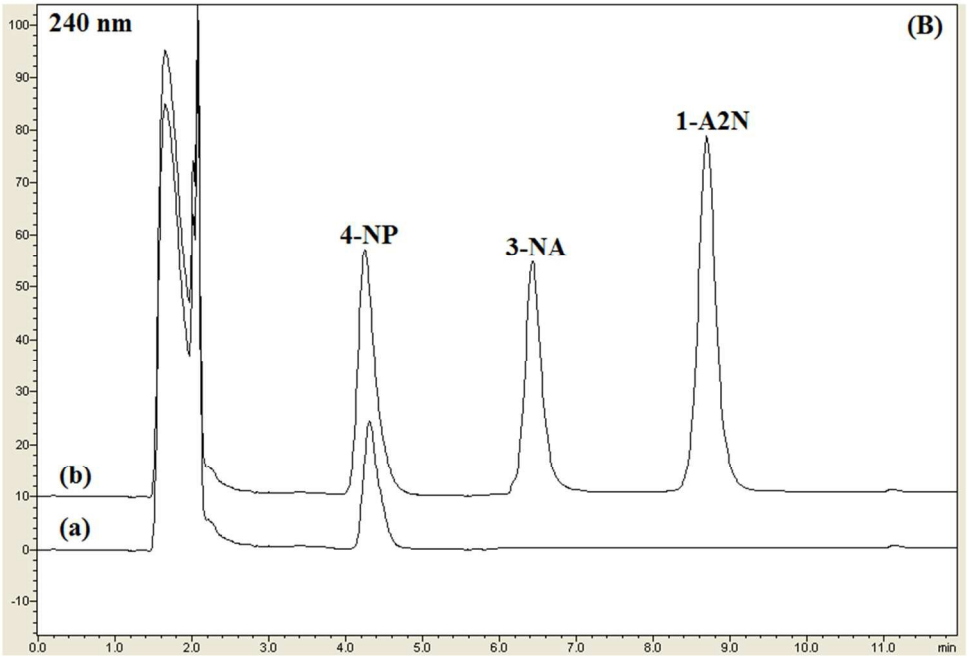

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Fig. 4c

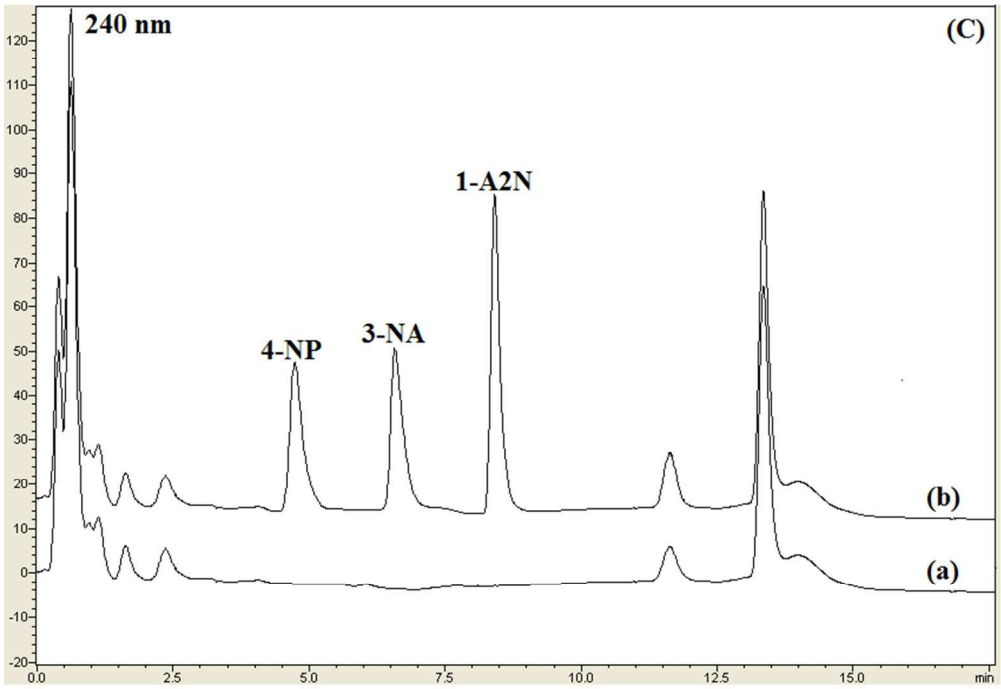

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Table 1
Experimental variables and levels of the central composite design (CCD).

	Level			Star points (α = 2)	
	Lower	Central	Upper	-α	+α
A: pH	6.0	7.5	9.0	4.5	10.5
B: Extraction time (min)	20	30	40	10	50
C: SUPRAS (%, v/v)	20	40	60	0	80
D: Salt content (%, w/v)	10	15	20	5	25

Table 2
Sequential Model Sum of Squares.

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F	
Mean vs Total	16.47	1	16.47			
Linear vs Mean	0.17	4	0.042	4.58	0.0065	
2FI vs Linear	1.608E-004	6	2.679E-005	2.200E-003	1.0000	
Quadratic vs 2FI	0.17	4	0.044	11.51	0.0002	Suggested
Cubic vs Quadratic	0.012	8	1.500E-003	0.23	0.9706	Aliased
Residual	0.045	7	6.411E-003			
Total	16.87	30	0.56			

Table 3
Analytical figures of merit of SS-HF-LPME method.

Analyte	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	DLR ($\mu g\ L^{-1}$)	Regression equation	R^2	EF[a]	ER[b] (%)	RSD (%)[c] (within day)	RSD (%)[c] (between day)
4-NP	0.20	0.5	0.5-400	y = 20473 C [d] – 4736.5	0.9998	166	56.1	6.9	12.9
3-NA	0.15	0.5	0.5-400	y = 23590 C + 1857.5	0.9986	178	60.2	5.4	9.1
1-A2N	0.10	0.5	0.5-400	y = 33802 C + 6254.2	0.9995	211	71.1	4.1	8.5

[a] Enrichment factor for each analyte was calculated as the ratio of the slopes of the calibration curves with and without preconcentration.
[b] Extraction recovery.
[c] Relative standard deviation (n = 5 samples for within day and n = 3 days for between day) was obtained at 30 $\mu g\ L^{-1}$ level of the analytes.
[d] Concentration in $\mu g\ L^{-1}$.

Table 4
Determination of the target analytes in various matrices.

Sample	Analyte	C_{added}	C_{found}	RR [a] (%)	RSD (%) (n = 3)
Snow water	4-NP	-	11.2	-	7.0
		10.0	21.9	107	8.8
	3-NA	-	n.d.	-	-
		10.0	9.7	97	6.4
	1-A2N	-	n.d.	-	-
		10.0	9.0	90	7.0
Rain water	4-NP	-	7.8	-	6.0
		10.0	16.9	91	6.8
	3-NA	-	n.d.	-	-
		10.0	10.9	109	7.5
	1-A2N	-	n.d.	-	-
		10.0	10.2	102	5.3
River water	4-NP	-	n.d.	-	-
		10.0	9.5	95	4.6
	3-NA	-	n.d.	-	-
		10.0	8.9	89	6.4
	1-A2N	-	n.d.	-	-
		10.0	10.4	104	5.0
Dam water	4-NP	-	n.d.	-	-
		10.0	10.6	106	7.1
	3-NA	-	n.d.	-	-
		10.0	9.3	93	5.8
	1-A2N	-	n.d.	-	-
		10.0	9.7	97	4.1
Wastewater	4-NP	-	n.d.	-	-
		10.0	8.6	86	8.0
	3-NA	-	n.d.	-	-
		10.0	9.1	91	6.3
	1-A2N	-	n.d.	-	-
		10.0	9.5	95	7.5

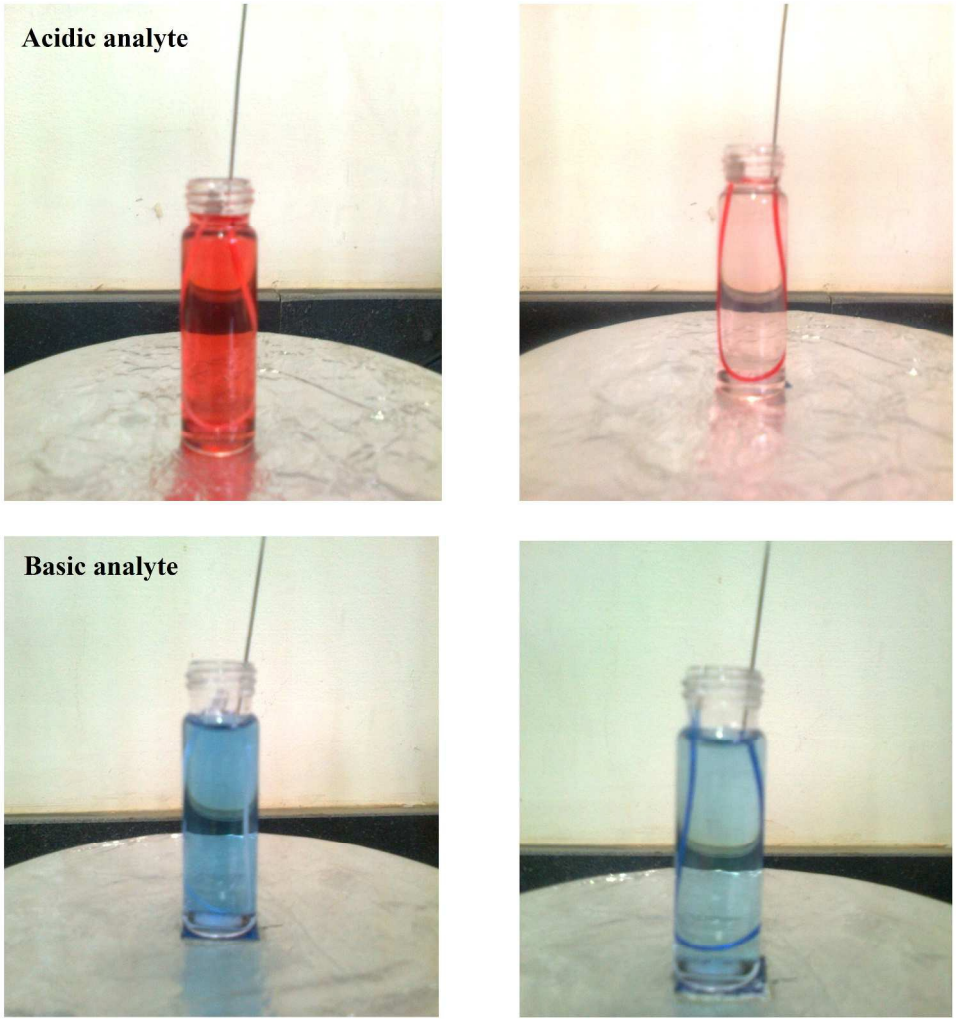
All concentrations are based on μg L^{-1}.
[a] Relative recovery

Table 5
Comparison of SS-HF-LPME with alternative methods used for the extraction and determination of the target analytes.

Analytes	Method	Sample	DLR	LOD	LOQ	RSD (%)	Ref.
4-NP	IP-LPME [a]-HPLC-DAD	Tap, mineral and rain water	0.2-75	0.1	-	≤6.3	[2]
4-NP	HF-LPME-CLC [b]	Sea water	1-200	0.5	-	≤6.2	[8]
3-NA, 4-NP 1-A2N	D-μ-SPE [c]-HPLC-DAD	Rain, snow and river water	0.5-600	0.1-0.25	0.5-1	≤8.5	[56]
4-NP	MSPE [d]-HPLC-UV	Tap, river and rain water	0.75-100	0.3	0.75	4.9	[58]
3-NA	HF-LPME-HPLC-UV	Tap, river and ground water	1-1000	0.1	1	4.1	[59]
3-NA	DSD-LLLME [e]-HPLC-UV	Tap, river and ground water	5-1500	1.0	5	4.9	[60]
3-NA, 4-NP 1-A2N	SS-HF-LPME-HPLC-DAD	Rain, snow, river, dam and wastewater	0.5-500	0.1-0.2	0.5	≤6.9	Current method

[a] Ion pair based surfactant assisted microextraction.
[b] Capillary liquid chromatography.
[c] Dispersive-micro solid phase extraction.
[d] Magnetic solid phase extraction.
[e] Directly suspended droplet liquid-liquid-liquid microextraction.
All concentrations are based on μg L^{-1}.

Graphical abstract



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