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7 1 **Hollow fiber–liquid phase microextraction for trace enrichment of the**
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9 2 **residues of atrazine and its major degradation products from environmental**
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11 3 **waters and human urine samples**
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Abstract

A sample preparation technique using a hollow fiber–liquid phase microextraction (HF-LPME) coupled to the liquid chromatography has been developed for selective extraction and analysis of atrazine (ATZN) and its major degradation products including desethylatrazine (DEA), desisopropylatrazine (DIA), didealkylatrazine (DDA) and hydroxyatrazine (ATOH) from environmental water and human urine samples. Extraction conditions have been optimized as follows: membrane solvent, di-*n*-hexylether; acceptor pH, 0.0; donor pH, 7.0; sample volume, 200 mL; extraction time, 5 h; stirring rate, 150 rpm; and ionic strength, in terms of NaCl added, 20% (*w/v*). The analytical method developed has been validated both in reagent water and environmental water and human urine samples. Trace level detection as well as linearity, with coefficient of determinations (r^2) ranged from 0.994 to 0.999, was obtained for the triazine compounds over a wide range of analyte concentrations between 10 and 500 $\mu\text{g L}^{-1}$, using peak area as the response variable. The repeatability and reproducibility of the method were less than 11 and 17%, respectively, at the concentration of 50 $\mu\text{g L}^{-1}$ for each analyte. Limits of detection and quantification ranged from 0.03 to 1.12 $\mu\text{g L}^{-1}$ and 0.10 to 3.73 $\mu\text{g L}^{-1}$, respectively, using HPLC with UV detection. This confirms reliability of the developed technique for further application in trace level enrichment of the residues of the target analytes and other polar, ionizable and structurally related contaminants in environmental waters and human urine samples.

Keywords: Liquid phase microextraction; Hollow fiber, Trace enrichment; Atrazine; Degradation products; Environmental waters; Human urine

1. Introduction

The use of chemical pesticides for various purposes such as forestry and railway management, protection against infections with parasites transmitted to humans by and against insects and weeds in agriculture is very common all over the world today. Effective use of pesticides for agricultural purposes, in particular, has been known to improve the quality and quantity of food. Advances in pesticide technology have increased the ability to sustain and improve the health and well-being of the ever growing human population. However, most of the pesticide residues that are released to the environment are known and reported to be toxic to humans, aquatic lives and inhabitants of the ecosystems.¹ There are many pathways for exposure: in drinking water from contaminated well, in food from household pesticide use and from residues on plants as they are picked, or machinery as they are being handled or repaired, from pesticide drift as it is being sprayed, from spills during transport and from dermal exposure during mixing, loading and application.²

Out of the several classes of pesticides known to be in use frequently for various purposes, are herbicides. They are mainly utilized either for killing or severely injuring weeds and have been applied for elimination of unwanted plant growth or killing the plant pests since the mid-twentieth century. Symmetrical (*s*-) triazines, introduced in the 1950s, are one of the largest classes of agrochemicals produced and are also among the most commonly used herbicides.³ A report from world pesticide market indicated that about 30% of the herbicides produced are triazines.⁴ They are used extensively for selective pre- and post- emergence control of leafy and grassy weeds in different agricultural crops including corn, soya bean, wheat, maize, sugar cane and barely.⁵⁻⁷

Herbicides applied to the environment are usually transformed into their degradation products which are mainly more polar than the parent compounds and thus having greater tendencies to stay in aquatic media and the particles of soil.⁸ The half-lives of *s*-triazines in different environmental compartments, for example, vary from few weeks to several months^{5,7-9} which may be caused by various biochemical processes such as dealkylation, dechlorination, hydroxylation, deamination and ring cleavage of the parent compounds.¹⁰ Atrazine, for example, degrades in soil through both biotic and abiotic reaction mechanisms to the dealkylated

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7 1 degradation products desethylatrazine (DEA), desisopropylatrazine (DIA) and the hydroxylated
8 metabolite (ATOH).¹⁰⁻¹² Further dealkylation of these compounds has also been reported
9 resulting in the opening of the triazine ring and eventual mineralization to carbon dioxide and
10 ammonia.¹¹
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13 5 Trace quantities of *s*-triazine residues and their degradation products, whose environmental
14 fates and effects have not yet been sufficiently studied, are found in various complex matrices of
15 environmental, biological, food, etc origins, at low concentration levels. Consequently and due to
16 the occurrences of the residues and their transformed products at trace levels, their analysis
17 mainly require the use of pre-concentration and clean-up techniques in order to bring their
18 concentrations, in the extracts, to a level detectable by the available conventional instruments.¹³
19 Furthermore, overall detection levels of such trace pollutants seem to depend more on the
20 isolation and enrichment procedures chosen than the final quantitative determination method
21 employed.¹³⁻¹⁵
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23 14 While methods for separation and determination of low concentration levels of the
24 untransformed compounds are available, methods for selective enrichment of the metabolites in
25 the presence of their parent compounds are limited.⁵ Some of the methods reported in the
26 literatures, in the past few years, used for selective separation of the mixture of these compounds
27 from various sample matrices include: classical liquid-liquid extraction (LLE),¹⁶ supported
28 liquid membrane extraction (SLM),^{5,17} solid-phase extraction (SPE),^{18,19} supercritical fluid
29 extraction (SFE),²⁰ dispersive liquid-liquid microextraction (DLLME),^{21,22} liquid phase
30 microextraction (LPME),²³ solid phase-microextraction (SPME)²⁴ and coupled extraction
31 techniques.^{25,26}
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34 23 Hollow fiber-liquid phase microextraction (HF-LPME) has several advantages over the
35 other membrane techniques used for trace and ultratrace level enrichment of pollutants in various
36 sample matrices.²⁷ Extensive applications of the technique, in a variety of analysis areas and its
37 friendliness, both for safety of the environment as well as in operation, have been reviewed by
38 several contributors²⁷⁻³⁰ with the major focus on the pesticide pollutants. However, use of the
39 HF-LPME method for simultaneous extraction and enrichment of atrazine and varieties of its
40 degradation products is limited. One such application was reported by Peng *et al* (2007),²¹ for
41 extraction of atrazine and two of its metabolites, DIA and DEA, in environmental water samples.
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6 1 However, the major hydrolysis product, ATOH, and also DDA, which are also available in
7 2 various samples as successive degradation products of DIA and DEA, have not been considered
8 3 in their study. Similarly, Megersa *et al.* (2001)⁵ have reported an automated supported liquid
9 4 membrane (SLM) extraction procedure for trace enrichment of *s*-triazine compounds and a
10 5 number of their metabolites in environmental and biological sample matrices. The method
11 6 offered selective sample preparation and clean-up, in a continuous flow system, and exhibited
12 7 low detection limits for both the parent compounds and their degradation products. However, it
13 8 required a specially automated SLM set-up with three pumps and four valves, not commonly
14 9 available in the laboratories of the developing world. It has been noted that no literature report on
15 10 simultaneous extraction and clean-up of atrazine and its most common degradation products
16 11 utilizing HF-LPME in the matrices considered.

12 12 Thus, in the current study, polypropylene based porous hollow fibers impregnated with di-
13 13 *n*-hexylether in the micropores of the fiber and filling its lumen with 1 M HCl, as the acceptor
14 14 phase, forming a very simple and stable extraction device was developed. Major experimental
15 15 parameters influencing the efficiencies of the extraction processes have also been investigated
16 16 and optimized. The proposed miniaturized technique was successfully applied for efficient and
17 17 quantitative extraction of trace level residues of atrazine and its major degradation products from
18 18 samples of environmental and biological origins.

19 2. Experimental

20 2.1. Chemicals and materials

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22 22 The *s*-triazine herbicide and their degradation products, used in this study, include atrazine
23 23 (ATZN), desethylatrazine (DEA), desisopropylatrazine (DIA), didealkylatrazine (DDA) and
24 24 hydroxyatrazine (ATOH). Relevant physicochemical properties of the target analytes are given
25 25 in Table 1. All standards of the herbicides were reference materials for residue analysis
26 26 purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Organic solvents used for
27 27 immobilizing into the hollow fiber membrane pores were *n*-undecane, di-*n*-hexyl ether and 1-
28 28 octanol; all obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Humic acid (Aldrich,
29 29 Germany) and sodium chloride (Labmerk Chemicals PVT Ltd., India) were used during

1 optimization of the developed technique. Other chemicals used include phosphoric acid, obtained
2 from Sigma Chemical Co. (St. Louis, USA); potassium dihydrogen phosphate, the product from
3 Fluka Chemie AG (Buchs, Switzerland); dipotassium hydrogen phosphate from Alanar® BDH
4 laboratory supplies (Poole, England), and HPLC-grade solvents; acetonitrile and methanol, used
5 as mobile phase, were purchased from Techno Pharmchem (Haryana, India). In addition,
6 analytical grade NaOH and HCl were used to adjust the sample pH. All reagents and solvents
7 used in the study were either of analytical or HPLC grade.

8
9 *Table 1 suggested here*

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11 Polypropylene hollow fiber membranes (50/280 Accurel® PP) tubing (50 µm wall
12 thickness, 280 µm inner diameter, 0.1 µm pore size) was obtained from Membrana GmbH
13 (Wuppertal, Germany). BD MicroFine Syringes (with needle of 0.30 mm outer diameter and 8
14 mm length, 0.5 mL prepared for U-100 insulin injection) ordered from BD Consumer Healthcare
15 (Franklin Lakes, USA) were used to fill into the lumen of the hollow fiber (the acceptor) and to
16 flush out the acceptor solution into a small glass vial (200 µL) after extraction.³¹

17 18 **2.2 Water and urine samples collection and pretreatment**

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20 Tap water samples were collected from three sampling sites within the surrounding of Addis
21 Ababa University campus, Addis Ababa, with geographical locations: latitude 9°02'02.11"N,
22 longitude 38°45'45.79"E and at elevation of 2,448 m above sea level. Samples of river water
23 were collected from Awash River; located at about 125 km southwest of Addis Ababa, with
24 geographical locations: latitude 8°55'12"N, longitude 40°02'33.65"E and at elevation of 2,007 m
25 above sea level. Urine samples were obtained from non-exposed volunteers of our research
26 group.

27 Except for filtration and pH adjustment, no further pretreatment was carried out on tap
28 water samples. Actually, the samples were taken after allowing the tap to flow for about ten min,
29 to discharge soluble cations which can possibly be collected at the tip of the faucet. Samples of
30 the river water were all filtered, mainly to remove suspended impurities and particulate matters,

1 followed by rinsing of the filter paper, repeatedly with distilled deionized water in order to
2 facilitate quantitative transfer of the target analytes to the filtrate. Then, The filtered water
3 samples were kept in the refrigerator when not immediately extracted.⁷ Urine samples, on the
4 other hand, were stored in the refrigerator, at 4 °C, for at least 48 h to ensure sedimentation of the
5 precipitates formed, may be as a result of the chemical interaction between the various species
6 found in the complex matrices of the urine samples. Finally, the upper clean portion (in a 2.5 L
7 bottle) was membrane filtered and rinsed as above. All extractions have been carried out at
8 ambient temperature; 20 ± 2 °C.

2.3 Instrumentation

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12 The HPLC system used for the analysis was Agilent 1200 series equipped with Quaternary
13 Pump, Agilent 1200 Series Vacuum Degasser, Agilent 1200 series Autosampler and Agilent
14 1200 Series Diode Array detector Purchased from Agilent Technologies (Waldbronn, Germany).
15 Chromatographic separation of the compounds was performed on a C₁₈ analytical column
16 (Techsphere 5ODS, 25 cm x 4.6 mm ID; HPLC Technology, Macclesfield, Cheshire, UK). The
17 samples were shaken using HT Infors orbital shaker from Infors AG (Bottmingen, Switzerland).
18 A pH meter, Hanna Instruments (Portugal) was used to adjust the sample and buffer pH and data
19 manipulation was carried out by B.02.0x revision Agilent ChemStation software.

2.4 Preparation of the standard solutions

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22 Standard stock solutions, 100 mg L⁻¹, were prepared from the standards of *s*-triazine herbicide
23 and its degradation products as follows: Atrazine, DEA and DIA were dissolved in acetonitrile,
24 and DDA was dissolved in 5 mL acetonitrile and 5 mL reagent water. Hydroxy product of
25 atrazine (ATOH) was first dissolved in 1 mL of 1.0 M HCl and then in the mixture of the solvent
26 used for DDA.⁵ All the resulting solutions were diluted to the final required volume with
27 acetonitrile. All stock standard solutions were stable and stored at 4 °C when not in use.

28 The working standard solution of 20 mg L⁻¹, containing each of the target analytes, was
29 prepared every week by mixing appropriate amount of the stock solutions and diluting with
30 reagent water to the required volume. A series of concentrations of the standard solutions for

1 calibration were prepared in the concentration range of 10 to 500 $\mu\text{g L}^{-1}$, at five points. A
2 mixture containing 0.5 mg L^{-1} of each analyte was prepared from the 20 mg L^{-1} standard
3 solutions for spiking. Evaluation of precision was based on triplicate injections and peak area
4 was taken as instrumental response, which was finally converted to enrichment factor for
5 comparison. The acceptor solution was prepared from 37% HCl acid, in 50 mL, and dilute to the
6 final volume with reagent water. The donor sample solution was prepared in phosphate buffer at
7 pH 7.0¹⁴.
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9 2.5 Extraction procedures

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11 The extraction procedure followed is briefly described as follows:³¹ the hollow fiber was cut
12 manually into approximately 20 cm length and the two ends were looped together to give
13 appropriate shape, leaving both ends free for subsequent use. Then, the lumen of the hollow fiber
14 was flushed and filled with the acceptor solution using the BD Micro-Fine syringe. Afterwards,
15 the fiber was dipped into the organic solvent for few minutes to impregnate the pores of the
16 hollow fiber wall forming the organic liquid membrane. The lumen of the fiber was then slowly
17 flushed with more acceptor solution, composed of 1.0 M HCl to remove any organic solvent left
18 in the lumen and also remove air bubbles from the lumen and thus filling it completely. The two
19 ends of the fiber were folded and enveloped with a strip of aluminum foil and inserted into a
20 small piece of glass tubing. Then, the filled and sealed fiber was rinsed with reagent water. The
21 HF-LPME device, which was ready for use at this stage, was transferred to 200 mL sample
22 solution. After shaking the whole set-up, using an orbital shaker at 150 rpm for 3 and 5 h, the
23 acceptor solution containing the extracted analytes was collected into vials with 200 μL inserts.

24 Collection of the extract was performed as follows: one end of the sealed fiber was cut and
25 connected to a needle of a retracted syringe and the other end was then cut and put into the vial.
26 Then, the syringe plunger was pushed in to dispense the acceptor solution, containing the
27 analytes, into the vial. The collected acidic extract, which was 1.0 M with respect to HCl, was
28 neutralized by 7.0 M NaOH solution to pH 7.0. It is to be noted that precise measurement of the
29 volume of NaOH solution, has got a decisive effect in minimizing the variability in the replicate
30 analyses. After capping, the vial was kept on the autosampler of the HPLC for injection.

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6 1 Approximately, 10–12 μL extract solution was collected and 5 μL was injected to the HPLC
7 system for analysis.
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10 3 11 4 **2.6 Chromatographic conditions**

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13 6 Chromatographic separations were carried out employing gradient elution composed of 3.5 mM
14 7 aqueous phosphate buffer at $\text{pH} = 7$ and acetonitrile, as mobile phase. The flow rate was 1 mL
15 8 min^{-1} and the column temperature was maintained at 25 $^{\circ}\text{C}$. The injected extract was eluted using
16 9 gradient program for a total of 28 min runtime. The gradient program was as follows: 10%
17 10 acetonitrile was increased to 15% during 5 min and then kept constant until 10 min. The
18 11 percentage of acetonitrile was further increased to 70% until 33 min and again kept constant until
19 12 35 min. Thereafter, the composition was restored to 10% acetonitrile, initial condition, during 2
20 13 min followed by a 3 min equilibration time. Quantification of the analytes was achieved using
21 14 UV-DAD detection at the wavelength of 235 nm.
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32 16 **3 Results and discussion**

33 17 34 18 **3.1 Performance of the extraction system**

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36 20 It has been experimentally verified that with the membrane extraction very high concentration
37 21 enrichment factor can be achieved.³² The same also holds true for HF-LPME since the acceptor
38 22 volume in the lumen, is generally much lower than the volume of the bulk sample solution.
39 23 Analytes are enriched into the stagnant acceptor phase particularly when the chemical conditions
40 24 can be appropriately worked out in order to selectively and irreversibly trap the target analytes in
41 25 question. Experimental parameters governing the attainment of maximum enrichment also need
42 26 to be optimized which include the membrane solvent, solution pH for both phases, extraction
43 27 time, shaking speed, ionic strength and presence of the humic substances. The extent to which
44 28 the target analytes quantitatively transferred and accumulated in the acceptor solution is often
45 29 evaluated by using the enrichment factor, E_c .^{7,33–37} It is defined as the ratio of the concentration
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1 of the target analyte enriched and collected from the acceptor phase (C_a) to the concentration in
2 the bulk of the extraction sample solution (C_s), and given by the following equation:

$$E_e = C_a/C_s$$

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3.1.1. Selection of the membrane solvent. One of the prior crucial steps, during optimization of the membrane extraction process, is the choice of organic solvent that is used to immobilize into the lumen of the hollow fiber. Selection of the solvent is mainly based on the proper immobilization of the pores of the fiber, immiscibility with water and the solvent stability³³ and analytes permeability through the fiber depends on the physical properties and chemical nature of the solvents. Extensive discussions on selection of the organic solvent, physical parameters and other similar important requirements the solvent of choice should fulfill are documented in several literatures.³²⁻³⁴

14 In the present study, three organic solvents; *viz.*, di-*n*-hexylether, *n*-undecane and 1-octanol were tested for use in the pores of the fiber wall. Extraction of the analytes was performed from 0.5 mg L⁻¹ standard aqueous solution of each compound utilizing the three solvents. Extractions were carried out for 3 h, during the initial stages, at the shaking speed of 100 rpm and the acceptor acidic solution of 1.0 M HCl. Atrazine and its major degradation products, used as model compounds, were not appreciably enriched to any detectable levels when 1-octanol and *n*-undecane were used as the membrane solvent. However, all the compounds of interest have been quantitatively enriched better in the acidic acceptor when di-*n*-hexylether was utilized, due to the relatively better polarity that allows improved permeation of the analyte compounds through the lumen. The results obtained in this study are in good agreement with those documented in the literature for pesticide compounds of similar chemical properties.^{17,35}

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3.1.2 Effect of the acceptor and sample solution pH. Detailed theoretical and quantitative treatments of the membrane extraction for ionizable analytes have been provided by Jonsson *et al.* (1993)³⁶ along with the conditions for attaining maximum enrichment factors, particularly for the pH of both the donor and acceptor phases. Accordingly, for efficient extraction of basic compounds, the acceptor trapping solution pH should be at least 3.3 units below the lowest pK_a of the compounds in the mixture.^{32,37} The compounds investigated, in the current study, have pK_a values ranging from 1.30 to 5.15, Table 1.¹⁷ This means that the acceptor solution ideally should have a pH of at least less than -2.0. However, pH below 0.0 was not fully met for some of the compounds and they could therefore not completely be trapped. On the other hand, in one of our earlier studies, it has been described that even under incomplete trapping situation significant maximum enrichment factors can be attained.³² Based on these facts, the effect of pH of the acidic acceptor solution was studied in the solutions consisting 50 mM, 100 mM, 250 mM, 500 mM and 1000 mM HCl, results of which are shown in Fig. 1. As can be seen from Fig. 1, the highest signal responses were obtained when 1000 mM (or 1.0 M) HCl was used. Therefore, 1.0 M HCl was used as the acceptor solution was utilized throughout the study, giving a pH of approximately 0.0. The resulting extract was then neutralized to pH 7.0 using 7 M NaOH.

Fig. 1 suggested here

The extraction efficiency in LPME of the weak organic bases and acids also depends on the pH of the sample solution.^{14,36} The sample solution pH was thus varied in appropriate alkalinity to deionize atrazine and its degradation products in order to facilitate efficient extraction. Accordingly, 200 mL of the sample solution containing 0.5 mg L⁻¹ each of the compounds was spiked with 5 mM phosphate buffer, ranging in pH from 2.0 to 8.0.¹⁴ The buffers were prepared from H₃PO₄/KH₂PO₄ (pH=2), KH₂PO₄ (pH=4) and KH₂PO₄/K₂HPO₄ (pH=6.0–8.0). The enrichment factor exhibited rapid increasing tendency within the pH ranges of 2–4 and then the increase was gradual up to pH 7.0, and then started declining, Fig. 2. Therefore, pH 7.0 was chosen as the optimum sample solution pH for the subsequent studies. The decreasing tendencies of the enrichment factors beyond pH 7.0 may be attributed to the degradation of the target

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1 analytes at extreme pHs.⁹ Similar observations have also been noted by other workers for
2 compounds possessing similar chemical natures.^{7,14,17,32,35}

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4 *Fig. 2 suggested here*

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6 **3.1.3 Effect of the sample volume.** Findings of the earlier studies signify that for both
7 two-phase and three-phase LPME, high analyte enrichments can be achieved, since the volume
8 ratios of the acceptor and donor phases, i.e., V_D/V_A , respectively, are normally high.³⁷ Thus,
9 sample volume is one of the major factors, making LPME very attractive, especially for
10 relatively small sample volumes, as similar enrichment may not be obtained with SPE or LLE.³⁸
11 In order to investigate the effect of sample volume on the enrichment factor, four different
12 volumes, viz., 50, 100, 200 and 500 mL, using phosphate buffer solution, (pH=7 adjusted by 5
13 mM phosphate buffer) was employed and extracted in the acceptor solution of 1.0 M HCl for 3 h
14 while shaking at 150 rpm. The enrichment factor was found to increase with increasing sample
15 volume up to 200 mL, and then begins to decline. The decrease in the enrichment factor, after
16 200 mL, may be attributed to the lowered flux diffusion through the large volume of the sample
17 solution.³¹ The sample solution was thus adjusted to a constant volume of 200 mL for all the
18 subsequent extractions, in order to enrich maximum amounts of the analytes under study.

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3.1.4 Effect of shaking speed and extraction time. Once the parameters governing maximum enrichment in the donor and acceptor phases have been optimized, it may be reasonable to consider investigation of the influence of hydrodynamic conditions. One dynamic process which may critically affect the enrichment factor of the extraction process is the speed at which the sample solutions are agitated. Agitation can be done either by stirring or shaking. A preliminary study was carried out comparing static, stirring and shaking conditions. Extraction efficiencies were compared and found to be the highest when the samples were agitated using orbital shaker. Enrichment gain during shaking may be caused by movement of the fiber through the acceptor solution in the lumen, which as result facilitates efficient transfer of the analytes to the acceptor phase. On the other hand, very low enrichment factor has been obtained when the static mode was employed, as movement of the analytes may greatly be slowed down. As shaking gives the best enrichment, within the specified time interval, the effect of shaking speed was investigated by varying the speed up to 150 rpm. It was observed that with increasing shaking speed, the enrichment factor also increases up to a certain maximum value. This is so because the diffusion in the aqueous phase increases with increasing agitation rate and furthermore faster agitation rate decreases the diffusion layer in the aqueous phase around the surface of the membrane. This may be resulted in increase of the mass transfer which also facilitating continuous exposure of the extraction membrane surface to the fresh aqueous sample. Sufficiently higher enrichment factor was obtained when shaking speed is adjusted to 150 rpm. Although higher enrichments could be expected with increasing the shaking speed, the experiment was not continued for higher speed because of the instrumental limitation, i.e., 150 rpm is the highest speed attained. However, this was compensated by increasing extraction time, at the speed of 150 rpm, since under diffusion controlled conditions, it is possible to reduce shaking speed by increasing extraction time to obtain maximum enrichment factor.³¹

HF-LPME is a three-phase extraction system with two liquid–liquid interfaces; as a result, the analyte molecules require sufficient time to diffuse through each phase and cross all interfaces to get into the acceptor phase. Therefore, optimizing the extraction time is critical when working in the kinetic regime. The amount of analytes extracted increases with longer extraction time before equilibrium is attained until a maximum enrichment may be obtained near equilibrium. Hence, the influence of extraction time on the enrichment factor of the five

1 compounds was studied. Fig. 3 shows the effect of extraction time on the enrichment factor of
2 the compounds in HF-LPME. For the current study, 5 h extraction time, which is also in the
3 linear range of the enrichment curve, was observed to be the optimum time and thus chosen for
4 the subsequent experiments. The requirements of using longer extraction time is not unusual with
5 similar membrane materials,³¹ particularly when the mass transfer processes are slow. To this
6 end, extraction time was extended to 7 h, in order observe the behavior of the curves. However,
7 the curves were found to level off after 5h, may be indicating that equilibrium has already been
8 attained around 5 h for the analyte compounds studied, as can also be seen in Fig. 3. It is evident
9 that though the extraction time, in this regard, is relatively longer, with the type of set up used in
10 current study, which are simpler and available in common laboratories, it is possible to arrange
11 several parallel extractions within the specified time.

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13 *Fig. 3 suggested here*

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15 **3.1.5 Effects of the ionic strength and humic acid.** In most conventional extraction
16 processes, analytes enrichment can be enhanced or retarded by addition of salts³⁹, depending on
17 the nature of analytes. Similarly, in this study, various amounts of sodium chloride were added to
18 the sample solution to investigate the effect of ionic strength on extraction. This was performed
19 by varying the amounts of sodium chloride added to the sample solutions, from 0, 5, 10, 15, 20,
20 25 and 30% (in w/v). Fig. 4 depicts the ionic strength variation effect on the enrichment factors,
21 for the five target compounds in reagent water. Enrichments of the more hydrophobic analytes;
22 atrazine and hydroxy-atrazine, were increased significantly during the initial extraction period
23 and gradually declined as more salt was added, the optimum being 20% sodium chloride. This
24 could be due to the increased electrostatic interactions between the salt molecules and the
25 analytes as the salt concentration increased further, which may contribute towards the diminished
26 availability of the analytes moving to the fiber.

27 Humic acids are dissolved organic carbon (DOC) often present in natural waters at various
28 concentrations.³⁹ The presence of such compounds might affect and sometimes complicate the
29 extraction processes. The influence of three different concentrations of humic acid, on the
30 enrichment factors of the target compounds, was studied in the concentration range of 0–50%

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6 1 (w/v). The experimental results obtained have also evidenced that addition of humic acid has not
7 2 significantly affected the enrichment factor. A one way ANOVA test also revealed that there is
8 3 no statistically significant difference between the mean enrichment factors of each analyte from
9 4 one level of humic acid concentration to another in the range tested at 95% confidence level.⁴⁰
10 5 This may be because humic acid, ($pK_a=5.5$),²¹ occurs mainly in ionized form at pH 7.0, and thus
11 6 its transfer through the hollow fiber supported liquid membrane device is significantly
12 7 prohibited. Moreover, ionization of the humic acid also lowers its binding ability to the analytes
13 8 which is again a favorable condition for its insignificant effect on attainment of maximum
14 9 enrichment.

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23 10 *Fig. 4 suggested here*

24 25 11 **3.2 Optimum values**

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29 13 Upon optimizing the experimental parameters of the HF-LPME of the model compounds, the
30 14 following optimum values have been obtained: membrane solvent, di-*n*-hexylether; an acceptor
31 15 pH of 0.0; a donor pH of 7.0; sample volume of 200 mL; extraction time of 5 h; a stirring rate of
32 16 150 rpm; and ionic strength, in terms of NaCl added, of 20% (w/v). These values were employed
33 17 in preconcentration and extraction of the target analytes from environmental waters and human
34 18 urine samples.

35 36 37 38 39 40 41 42 20 **3.3 Method validation**

43 44 45 46 22 *Linearity, limits of detection and quantification, repeatability and reproducibility*

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49 24 Analytical performances of the developed membrane technique was studied in reagent water, in
50 25 order to investigate the characteristics such as linearity, limit of detection (LOD), limit of
51 26 quantification (LOQ), repeatability and reproducibility. The results are shown in Table 2.

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53 27 Limit of detection is the concentration derived from the smallest response or signal that can
54 28 be detected with reasonable certainty for a given analytical procedure.⁴⁰ In chromatographic

1 analyses, LOD and LOQ are usually defined as concentrations that give a signal to noise ratio of
2 3:1 and 10:1, respectively, and are expressed as the concentration of the analytes.^{37,40} In the
3 current study, the LOD and LOQ values were calculated by analyzing blank spiked samples,
4 obtained from peak height measurements, as this was convenient at lower concentrations. For the
5 target analytes, the values obtained were found to vary from 0.03 to 0.75 $\mu\text{g L}^{-1}$ for LOD and
6 0.10 to 2.50 $\mu\text{g L}^{-1}$ for LOQ.

7 Linearity is the proportional relationship between the amount of the extracted analyte and
8 its initial concentration in the sample matrix. Linearity is determined by calculating the
9 regression line using the mathematical treatment of the results (*i.e.*, least mean squares) versus
10 the analyte concentration.⁴¹ The linear regression with proportional weighting was thus
11 calculated for the plot of the peak height versus concentrations of the analytes. To this effect,
12 linearity of the method was tested by running five replicate extractions, containing the analytes
13 with concentrations ranging between 10 and 500 $\mu\text{g L}^{-1}$, in reagent water. All the analytes
14 exhibited good linearity with coefficient of determinations (r^2) ranged from 0.994 to 0.999.

15
16 *Table 2 suggested here*
17

18 Reproducibility and repeatability studies were conducted in order to evaluate the precision
19 of the extraction method. Repeatability (intra-day precision) of the method, expressed as relative
20 standard deviation (RSD), was investigated by extracting the reagent water spiked with the
21 standard solution containing 50 $\mu\text{g L}^{-1}$ of each analyte. Three replicate extractions were carried
22 out in the same manner, during the same day. The RSD values obtained were below 11%, which
23 is fairly acceptable. On the other hand, reproducibility (inter-day precision) of the developed
24 analytical technique was also evaluated using spiked sample solutions, prepared as described
25 above, and extractions and analyses were performed during four consecutive days. The RSD
26 values determined were below 17%. Relatively larger deviations observed in the reproducibility
27 studies which could possibly be originated from the differences in wall thickness and pore size as
28 well as manual handling of the fibers. Besides, the possible dilution effect, while working in
29 microliter range, during pH adjustment of the resulting extracts may also contribute towards the
30 observed variability of the RSD values, as has also been reported by other workers.^{5,31} The

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6 1 method showed very good repeatability and reasonable reproducibility at such low concentration
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8 2 of the analytes, Table 2.
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13 4 **3.4 Applications**

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15 6 The optimized and validated HF-LPME method was applied to the extraction of parent *s*-triazine,
16 7 atrazine and its major degradation products (DEA, DIA, DDA, and ATOH) in environmental
17 8 water samples; tap water, river water and human urine samples.

18 9 It was observed that the chromatographic signals or peaks obtained for the matrices spiked
19 10 with the target compounds, peaks representing the possibly co-extracted interferents were not
20 11 eluted, confirming that the resulting peaks of the analytes were similar to those of the extracts of
21 12 the reagent water. As a consequence, the differences of the resulting enrichments were found
22 13 insignificant, compared to the extracts obtained from spiked reagent water, analyzed under
23 14 similar conditions. These findings have coined further experiments to be considered which
24 15 enable comparison of the parameters governing the validity of the extraction technique; both for
25 16 the extracts of the reagent water and that of the tap water, river water and urine samples.
26 17 Accordingly, linearity, limit of detection and limit of quantification of the method were
27 18 compared with that of the reagent water, Table 3. The results obtained for both extracts were not
28 19 significantly differing from each other, as has also been depicted in Tables 2 and 3. Thus,
29 20 reasonable conclusions could be drawn from the overall observations describing that the
30 21 analytical HF-LME method developed is reliable and can efficiently be applied for trace level
31 22 enrichment of the residues of the target compounds considered in this study and other polar,
32 23 ionizable and structurally related contaminants in environmental waters and human urine
33 24 samples.
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35 26 *Table 3 suggested here*
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3.5 Selectivity of the proposed analytical method

According to the latest IUPAC recommendation, 'selectivity refers to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other compounds of similar chemical behaviors.⁴² To experimentally demonstrate and come up with high selectivity, membrane extraction has a clear advantage over other sample preparation techniques, as the compounds that reach the analytical extraction systems have to selectively cross the membrane and enriched in the acceptor phase. Enhanced selectivity has been achieved since the technique offers both enrichment and clean-up, during any one extraction, as all charged species are rejected from entering the membrane.^{5,7}

The selectivity of the membrane extraction techniques further depends primarily on the membrane material (physical state, morphology, structure and polarity), on the properties of the donor and acceptor phases (pH value, polarity, etc) and on the properties and concentration of the analytes.⁴³ Utilizing the optimized parameters, validation results, satisfactory enrichments and appreciable separation of the target analytes, the matrices considered in this study were spiked with known concentrations of the target compounds. The retention time for each target analyte obtained from chromatographic signals of the various extracts is given in Table 2, for the analytes eluted under gradient programs, similar to the reported works^{5,21}. Absence of the interfering peaks, around the retention times of the compounds, in the matrices considered, confirmed the potentials of the proposed extraction method to be utilized as attractive alternative analytical technique in trace level analysis of the target analytes and other pollutants having similar physicochemical properties.

4 Conclusions

The HF-LPME technique has been developed for selective extraction and quantitative determination of trace level pollutants of atrazine and its major degradation products in drinking and environmental water samples as well as the urine samples. Experimental parameters influencing the extraction efficiency have been optimized and applied to samples containing complex matrices. The advantages observed include usage of minimum organic solvent

1 requirements, low cost and simplicity for use compared to other liquid membrane extraction
2 techniques. The only limitation of the method is the long extraction time. However, this aspect
3 could offset by the cheap materials and simple handling, permitting the extraction of many
4 samples in parallel.

5 The developed method was validated and found to exhibit good linearity, low values of
6 LOD and LOQ thus reliable for trace analyses, good repeatability and reasonable reproducibility
7 at trace level concentrations of the analytes considered in the present study. Based on the
8 experimental findings, general conclusion could be drawn concerning the suitability of the
9 proposed technique for selective extraction of contaminants from environmental and biological
10 samples. Furthermore, by modifying the technique or using automated-online HF-LPME
11 extraction, which is the recent trends in sample preparation and offering several advantages, it
12 could also be possible to obtain even better selective extraction with higher enrichment factor
13 and much lower detection and quantification limits.

14 Acknowledgements

15 Continued assistance of the Department of Chemistry, Addis Ababa University, is appreciated
16 for providing the required laboratory facilities. The consumables, solvents and pesticide
17 standards were provided by the Center for Analysis and Synthesis of the Lund University,
18 Sweden. Financial support from the International Science program (ISP) through the “Trace
19 Level Pollutants Analysis” project (ETH:04) is also gratefully acknowledged.

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Figure Captions

Fig. 1

Effect of the acceptor pH on enrichment factor of the target analytes. Experimental conditions: concentration of each analyte extracted, 0.5 mg L^{-1} ; shaking speed, 150 rpm; sample volume, 200 mL; and, extraction time, 3 h. Error bars are expressed in terms of standard deviation, SD.

Fig. 2

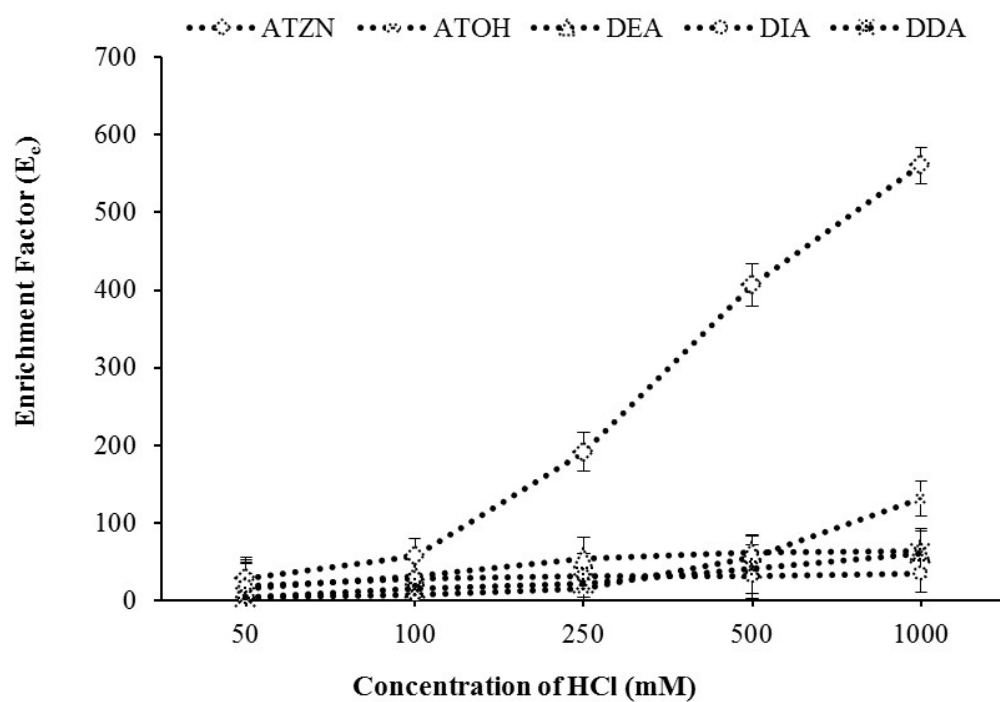
Effect of the sample solution pH on enrichment factor of the target analytes. Experimental conditions: concentration of each analyte extracted, 0.5 mg L^{-1} ; shaking speed, 150 rpm; extraction time, 3 h; acceptor solution, 1 M HCl. Error bars as in Fig. 1.

Fig. 3

Effect of the extraction time on enrichment factor of the target analytes. Experimental conditions: concentration of each analyte extracted, 0.5 mg L^{-1} ; extracted sample volume, 200 mL; shaking speed, 150 rpm; acceptor solution, 1.0 M HCl. Error bars as in Fig. 1.

Fig. 4

Effect of the ionic strength of the extraction solution on enrichment factor of the target analytes. Experimental conditions: concentration of each analyte extracted, 0.5 mg L^{-1} ; shaking speed, 150 rpm; extraction sample volume, 200 mL; extraction time, 5 h; acceptor solution. 1.0 M HCl. Error bars as in Fig. 1.



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Fig. 1

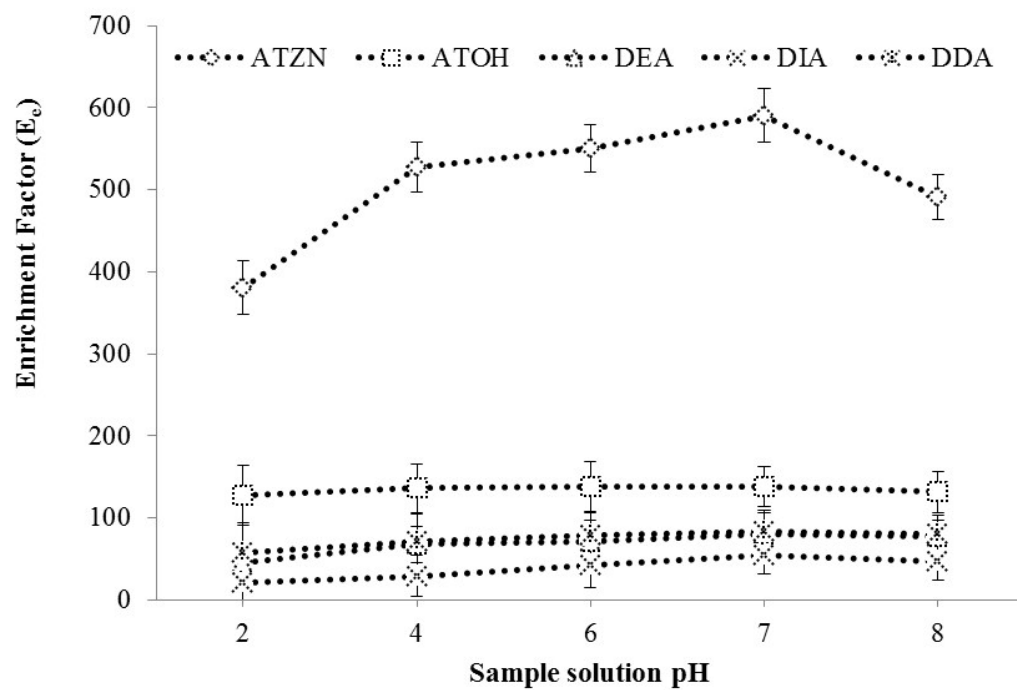


Fig. 2

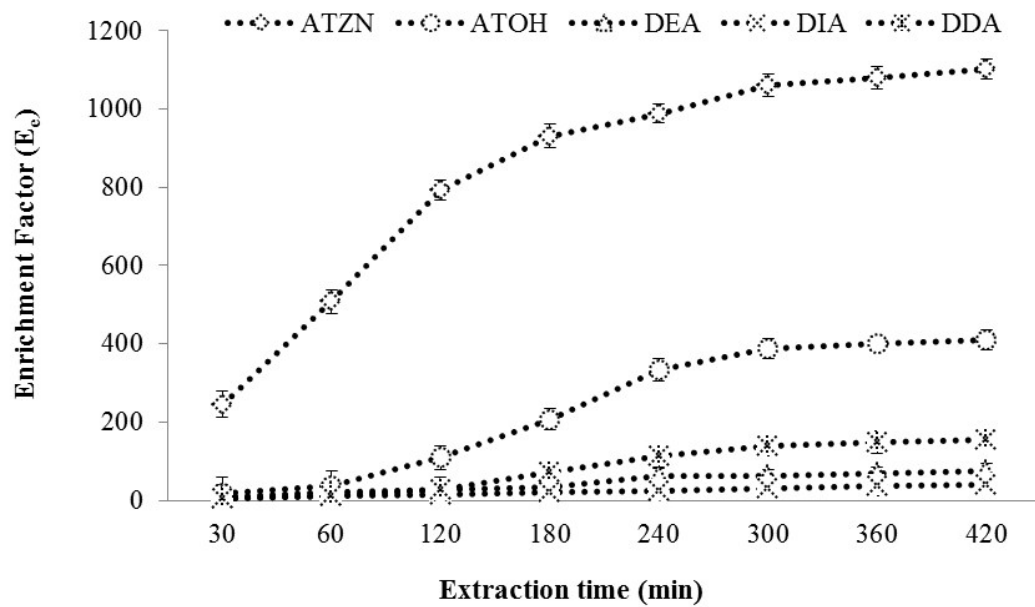
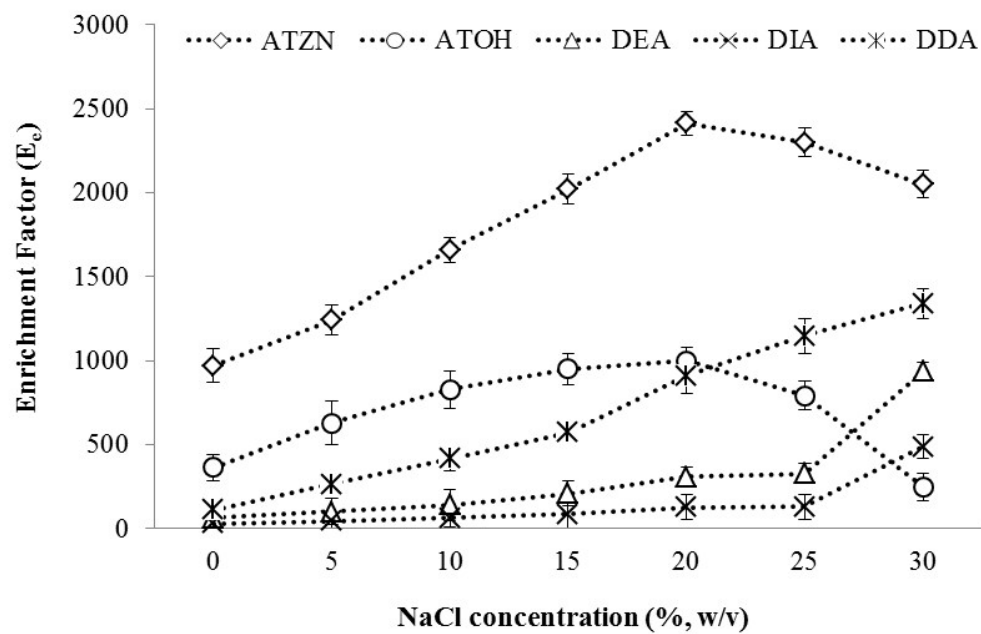
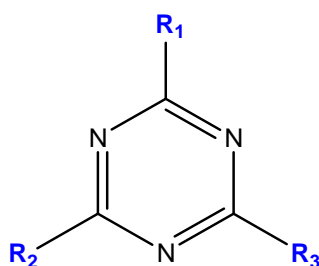


Fig. 3

**Fig. 4**

1 **Table 1** Representative physicochemical properties of the target compounds^{5,9}



| Analyte | R ₁ | R ₂ | R ₃ | Mol. wt, g mol ⁻¹ | Solubility, mg L ⁻¹ (22°C) | Log K _{ow} ^a | pK _a |
|---------|----------------|---------------------------------------|--------------------------------------|---------------------------------|--|----------------------------------|-----------------|
| DDA | -Cl | -NH ₂ | -NH ₂ | 145.6 | NA ^b | 0.32 | 1.5 |
| DIA | -Cl | -NH ₂ | -NH-CH ₂ -CH ₃ | 173.60 | 3200 | 1.15 | 1.30-1.58 |
| DEA | -Cl | -NH-CH(CH ₃) ₂ | -NH ₂ | 187.63 | 670 | 1.52 | 1.30-1.65 |
| ATOH | -OH | -NH-CH(CH ₃) ₂ | -NH-CH ₂ -CH ₃ | 197.24 | 5.9 | 1.4 | 5.15 |
| ATZN | -Cl | -NH-CH(CH ₃) ₂ | -NH-CH ₂ -CH ₃ | 215.7 | 33 | 2.5 | 1.68 |

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4 ^a log K_{ow}: *n*-octanol–water partition coefficients, defined as the ratio of the equilibrium concentrations of a dissolved substance in two immiscible solvents; ^bNot available

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Table 2 Validation of the performance of the HF-LPME developed for the model compounds in reagent water including their retention time

| Analyte | Retention time, min | LOD, $\mu\text{g L}^{-1}$ | LOQ, $\mu\text{g L}^{-1}$ | r^2 , ^a | Rept. ^b , 50 $\mu\text{g L}$ (% , n = 3) | Repd. ^c , 50 $\mu\text{g L}$ (% , n = 4) |
|---------|---------------------|---------------------------|---------------------------|----------------------|---|---|
| DDA | 4.7 | 0.18 | 0.60 | 0.997 | 3.1 | 15.9 |
| DIA | 9.2 | 0.75 | 2.50 | 0.998 | 6.2 | 13.8 |
| DEA | 15.1 | 0.40 | 1.33 | 0.999 | 8.5 | 16.1 |
| ATOH | 16.8 | 0.08 | 0.27 | 0.999 | 10.5 | 16.2 |
| ATZN | 25.5 | 0.03 | 0.10 | 0.996 | 4.6 | 11.3 |

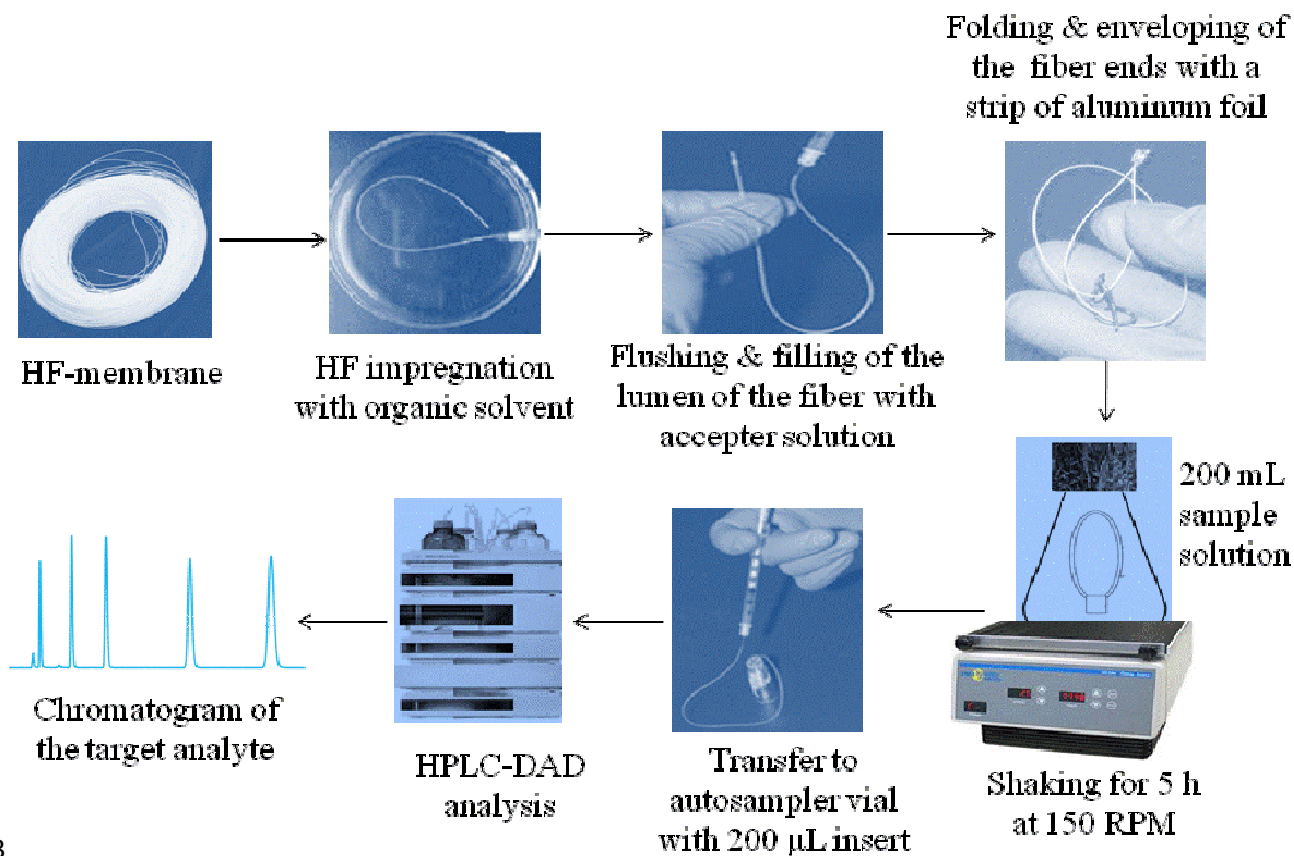
^a regression coefficient; ^b repeatability; ^c reproducibility; LOD & LOQ - limit of detection and quantification, respectively.

Table 3 Linearity, limit of detection and quantification of the optimized analytical method for extraction of the *s*-triazine compounds in tap water, river water and human urine samples

| Matrices | Analytes | Conc. range ($\mu\text{g L}^{-1}$) | LOD ($\mu\text{g L}^{-1}$) | LOQ ($\mu\text{g L}^{-1}$) | Correlation coefficient | Enrichment Factor (E_e) | % RSD ($n = 3$) |
|-------------|----------|---|---------------------------------|---------------------------------|----------------------------|--------------------------------|----------------------|
| Tap water | DDA | 10-500 | 0.20 | 0.67 | 0.9991 | 982 | 5.8 |
| | DIA | 10-500 | 0.82 | 2.70 | 0.9990 | 106 | 9.1 |
| | DEA | 10-500 | 0.44 | 1.47 | 0.9994 | 298 | 8.6 |
| | ATOH | 10-500 | 0.09 | 0.30 | 0.9986 | 1261 | 11.1 |
| | ATZN | 10-500 | 0.03 | 0.10 | 0.9924 | 2343 | 5.2 |
| River water | DDA | 10-500 | 0.20 | 0.67 | 0.9993 | 982 | 8.9 |
| | DIA | 10-500 | 0.84 | 2.80 | 0.9991 | 101 | 6.1 |
| | DEA | 10-500 | 0.45 | 1.50 | 0.9998 | 291 | 10.5 |
| | ATOH | 10-500 | 0.09 | 0.30 | 0.9986 | 1256 | 7.0 |
| | ATZN | 10-500 | 0.03 | 0.10 | 0.9950 | 2351 | 5.9 |
| Human urine | DDA | 10-500 | 0.27 | 0.90 | 0.9997 | 983 | 13.4 |
| | DIA | 10-500 | 1.12 | 3.73 | 0.9961 | 103 | 11.6 |
| | DEA | 10-500 | 0.48 | 1.60 | 0.9978 | 291 | 9.9 |
| | ATOH | 10-500 | 0.12 | 0.40 | 0.9992 | 1265 | 13.5 |
| | ATZN | 10-500 | 0.05 | 0.17 | 0.9907 | 2351 | 6.1 |

1 **Graphical Abstract**

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