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Two-step hollow fiber-based, liquid-phase microextraction combined with high-performance liquid chromatography: A new approach to determination of aromatic amines in water

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Abstract

A novel method for the extraction of aromatic amines present in water samples is produced here coupling two-step liquid-phase microextraction with high performance liquid chromatography by using a monolithic column.The hydrophobic porous polypropylene membranes were used as the interface between the donor water sample and the acceptor aqueous solution. In the first step, the analytes were extracted from a sample solution (pH 13) as donor phase into the organic phase, benzyl alcohol-ethyl acetate (80–20%, v/v) immobilized in the pores of a polypropylene dish and further into an acidified acceptor phase (pH 2) inside the polypropylene membrane.This step had about 100% relative recovery with an enrichment factors of over 59.9. For the second step, using a single piece of polypropylene hollow fiber, was shaped with a star liked profile as the acceptor phase. The acceptor solution in the first step was the donor phase for the second step. This solution was adjusted again to pH 13 with NaOH solution. Five microlitres of HCl solution (pH 2) as the acceptor phase was added to the hollow fiber, star liked profile, as the acceptor phase. This acceptor solution, after the second extraction step, was subsequently withdrawn into a micro syringe and directly injected into an HPLC system for analysis. With this two-step microextraction, total enrichment factors of >6000 could be obtained and detection limits of $\leq$250.0 ng/l ($S/N = 3$)could be achieved. The calibration curves for analytes were linear within the range of 20.0 ng/l to 300 μg/l. All expreriments were carried out at room temperature, $22 \pm 0.5\,^{\circ}C$.

Keywords: Aromatic amine; Two-step hollow fiber based; Liquid-phase microextraction; Water; HPLC; Monolithic column

1. Introduction

It is well known that the aromatic amine carcinogens had been thought to induce some tumors in humans. But most of them are required to be metabolized to exert their genotoxicity in specific organs or tissues [1] and furthermore, with the growing use of these compounds in different industries such as dyes, cosmetics, pesticides, pharmaceuticals and as intermediates in many chemical syntheses [2]. Their residues have become significant contaminants in environmental waters due to their toxicity and biological activity. These compounds are of most concern if released into the environment [3,4].

Recently analytical techniques based on reversed-phase high performance liquid chromatography (RP-HPLC) have become the procedures of choice for determining aromatic amines in environmental analysis [5,6]. Because of low concentration of these amines in environmental sample pretreatment and a pre-concentration step is generally required for determination of trace aromatic amines as the pollutants [7]. Sample preparation is traditionally carried out by liquid–liquid extraction (LLE) or by solid-phase extraction (SPE), while in most cases, the final analysis is accomplished by either high-performance liquid chromatography (HPLC) or capillary gas chromatography (GC). Since both of LLE and SPE techniques need a substantial amount of organic solvents. Therefore, several efforts have been reported [8,9] such as solid phase microextraction (SPME) [10,11]. This has

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been a widely used microextraction technique and is a solvent free technique, but SPME, suffers from sample carry-over between the runs [12].

Solvent microextraction (SME) is a technique that involves suspending a drop of organic solvent from a microsyringe tip into the sample, as described by Jeanet and Cantwell [13,14]. Although SME has several advantages over the previously extraction methods, but at high stirring rates the drop is instable [15]. To overcome this limitation, the hollow fiber membrane solvent microextraction method allows high sample agitation, reducing the Nernst diffusion layer and improvement the extraction efficiency. Additionally, a large volume of acceptor solution can be used. This improves the rate of analyte transfer across the membrane [16]. In one kind of these miniaturised supported liquid membrane extraction techniques (SLME), aqueous donor phase samples containing basic analytes were adjusted through the outside of a porous hollow fiber and the analytes were extracted in a three-phase system through an organic solvent immobilized in the pores and into an aqueous acidic acceptor phase inside the lumen of the hollow fiber [17,18]. In this work we used a very simple, cheap, quick and robust two-step fiber membranes microextraction method as a novel method for analysis of aromatic amines. We combined two liquid–liquid–liquid phase microextraction steps together for achieving a high enrichment factors and low detection limits. For this purpose we used in the first step, a polypropylene round dish with large surface area and low depth (24.9 cm i.d. and 2 cm height) and in the second step, a piece of polypropylene hollow fiber (0.12 cm i.d. and 1.5 cm length), which was shaped with a star liked profile in the usual format of hollow fiber solvent microextraction [19]. The main factors which influence the extraction procedure such as: the composition of the acceptor phase the nature of the donor phase, extraction times, organic solvent and stirring rate were investigated. The optimized conditions were applied to real water analysis coupled with RP-HPLC.

2. Experimental section

2.1. Chemicals and reagents

Analytical reagents grade 3-chloroaniline (3CA), 3-bromoaniline(3BA), benzyl alcohol, ethyl acetate, *n*-hexane, *iso*-octane, cyclohexane, were purchased from Merck (Darmstadt, Germany). 2-Nitroaniline (2NA) and 4-nitroaniline (4NA) were obtained from Riedel-De Haenga (Hannover, Switzerland) methanol and butyl acetate was purchased from Fluka (Buchs, Switzerland). All of these compounds were HPLC grade.

Sodium hydroxid and hydrogen chloride were obtained from Merck. Deionized water and real water were purified in a Milli-Q filtering system (Millipore). Stock solutions of the analytes (2.0 mg/ml) were prepared separately in methanol and they were stored at 4 °C. The standard sample containing

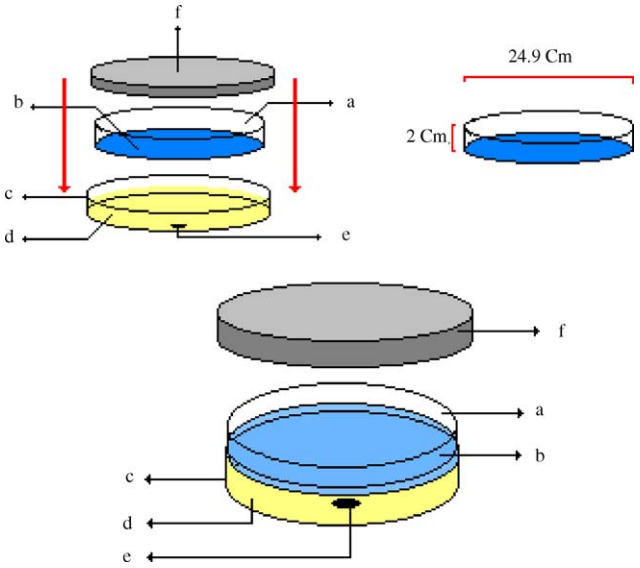

Fig. 1. First step LLLME extraction device; (a) polypropylene dish; (b) acceptor phase (pH 2.0); (c) glass dish; (d) donor phase (pH 13.0); (e) stirring bar; (f) aluminium cover.

all aromatic amines at 2.0 μg/ml was prepared by diluting the stock solutions with a pH 2.0 (HCl, 0.01 N).

Daily working solutions were prepared by combining aliquots of each stock solution and diluting to appropriate concentrations with pH 13.0 (NaOH, 0.1 N) and stored in a refrigerator.

The polypropylene sheets (150 μm wall thickness, 0.2 μm nominal wall pore size) was purchased from the Sahand Co. Mashhad, Iran, and was made edged dish with round bottom (24.9 cm i.d. and 2 cm height of edges) for the first step (Fig. 1) and in the second step, it was formed tubular with 1.5 cm height and shaped with a star liked profile and end sealed with heat. All modifications were done only with heat press in our lab.

2.2. HPLC system

The HPLC system used in this work was a Waters (Millipore. Co, Milford, MA, U.S.A) and consisted of a Waters (488) Tuneable absorbance detector and a Waters 746 integrator.

Limitation in the performance of a packed column is well recognized based on the pressure limit of column efficiency and the pressure drop. One approach to overcome the problems is to fabricate a monolithic column made of one piece of a porous solid with small-sized skeletons and relatively large through-pores which could provide both high column efficiency and low pressure drop [20–22]. The monolithic silica columns were evaluated in reversed-phase HPLC. These showed lower plate heights and much lower pressure drops [23,24] than the conventional columns packed with the 5 μm C_{18} silica particles. Therefore, we used of a Chromolith performance RP-18e column (4.6 mm diameter 100 mm length, 2 μm macropore size and 13 nm mesopore size) from Merck

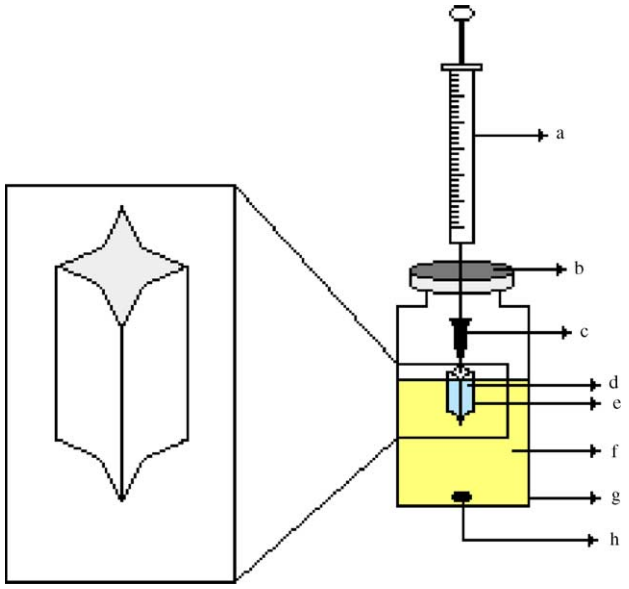

Fig. 2. Second-step LLLME extraction device; (a) HPLC syringe; (b) vial cover; (c) conical guide; (d) acceptor phase (pH 2.0); (e) hollow fiber with star liked profile; (f) donor phase (pH 13.0); (g) glass vial; (h), stirring bar.

(Darmstadt Germany). A RP-18 guard column was fitted upstream of the analytical column.

The mobile phase was methanol–water optimized on (45:55, v/v) was delivered by a Waters LC-600 HPLC pump. The flow rate of the mobile phase was 3 ml/min and the UV detection wavelength was set at 254 nm.

2.3. Two-step solvent microextraction

The experimental setup is illustrated in Figs.1 and 2. In the first step, 5.0 ml of organic solvent was added to the polypropylene dish for 5 min to immobilize the pores, and then the excess of organic solvent was removed. In the second step, polypropylene hollow fiber was plunged into the organic solvent for 5 min and then the excess of the solvent was removed.

For the first step, the sample solution (first phase) was held in a glass dish (25 cm i.d., 2.5 cm height). The polypropylene dish (24.9 cm i.d. and 2 cm height), impregnated with the organic solvent (second phase) was adjusted and fitted perfectly, into the glass dish as outer bottom surface of the polypropylene container was in contact with the surface of the sample solution. 3 ml of acceptor phase solution (third phase) was added into the polypropylene container. For the second step, 1.50 cm length of hollow fiber (1200 μm i.d., a wall thickness of 150 μm, a pore size of 0.2 μm and a porosity of 70%) was used. This fiber was shaped with a star liked profile using heat press as shown in Fig. 2. This shape was selected for increasing the contact area of the hollow fiber with donor and acceptor solutions, decreasing the volume of the fiber and the length was reduced to 1.50 cm for 5 μl of acceptor solution and the reduced length improved compatibility with small sample volumes, which are highly relevant in some analytes in the biomedical and environmental applications. In addition, a conical guide was placed on the top of the fiber to ensure that the microsyringe needle was effectively guided into the fiber. A 25 μl micro-syringe, with a cone tip (0.49 mm o.d.) (Hamilton, Reno, NV, USA) was used for delivery and removal of the acceptor phase, and this concept is much more compatible with modern auto-samplers. Before each extraction, the syringe was rinsed with acetone and then with deionised water for 10 times to avoid the analyte carry-over and air bubble formation. Prior to use the fiber was dept in acetone for 3 h to remove the contaminations. Because of the fiber is very inexpensive, we used from any fiber in one period of extraction, thus was avoided the sample carry-over.

2.4. LPME procedure

pH Values of the donor and acceptor solutions are very important parameters in three-phase LPME. As our previous studies pH 13 (with 0.1 M, NaOH) and pH 2 (with 0.01 M, HCl) were chosen for the donor and acceptor phases, respectively [25].

In general, depending on the nature of the contaminants, addition of salt to the sample solution can decrease their solubility and consequently increase their hydrophobicity [26]. This is due to the salting-out effect where fewer water molecules are available for dissolving the analyte molecules, preferably forming hydration spheres around the salt ions [27].

As was described, after the first step of extraction, acceptor solution (acidified with HCl) was transferred into a 10-ml sample vial and NaOH was added to adjust the pH of the solution to pH 13. This was the donor solution for the second step of the extraction. Thus, NaCl was naturally formed in the new donor solution and enhanced the extraction efficiencies by salting-out effect [28].

For the first step as described, 300 ml of sample solution (adjusted on pH 13 with NaOH) was held in the glass dish and the impregnated polypropylene container (with organic solvent) was adjusted into the glass dish. 3.0 ml of the acceptor phase (adjusted on pH 2.0 with HCl) was added to the polypropylene dish, An aluminium foil was used to cover the dishes during extraction to prevent the evaporation of the organic phase. The solution was agitated with a stirring rate of 1000 rpm. A stirring bar, measuring (1.4 cm × 0.4 cm) was used to facilitate the mass transfer process. A Ruhro (Retsch, Germany) R021 heater and magnetic stirrer was used to stir the extraction mixture. After 30 min, the acceptor phase was transferred into a 10 ml sample vial. A 2.0 ml acceptor solution was added into the polypropylene dish for washing and then flashed again into the same vial. NaOH solution was used as to adjust the pH of the solution to 13. This solution was used as the donor phase for the second step of the extraction. The magnetic bar (0.5 cm length, 0.3 cm width), was placed into the solution. The hollow fiber star-liked as described was flam-sailed at the one end. Subsequently, 5.0 μl of the acceptor solution (HCl, pH 2.0) was injected into the short fiber

with HPLC syringe. The fiber was impregnated by dipping for 1.0 min. into the organic solvent (benzyl alcohol–ethyl acetate, 80–20%) and then the fiber and HPLC syringe placed into the donor solution of the sample in vial that was placed in the magnetic plate stirrer for stirring during the extraction as in the first step.

Agitation of the sample enhances extraction and reduces the time to thermodynamic equilibrium. Increasing the stirring speed of the donor phase enhances extraction by aiding diffusion of analytes present in the donor phase through the interfacial layer of the hollow fiber and into the acceptor solution. In LPME, the hollow fiber protects the acceptor solution, and consequently high agitation speeds can be applied and improve the repeatability of the extraction method [29], but under great agitation air bubbles generated on the surface of the hollow fiber and solvent dissolution was promoted reducing which leads to reduced analytical signals for each analyte and decrease the precision of the method [30,31]. Therefore, the stirring speed were selected 900 rpm, in both extraction steps.

After extraction, the final acceptor phase was with-drown into a syringe and injected into the HPLC system.

3. Results and discussion

3.1. Basic principal of extraction

Liquid–Liquid–Liquid phase microextraction techniques involves three phases. Firstly, analyte is extracted from the aqueous sample solution (donor phase) into the organic phase immobilized within the pores of the hollow fiber, then analyte is back-extracted into the aqueous acceptor phase inside the hollow fiber. As an example for an analyte such as *A*, the extraction process is:

$$A_{(\text{donor phase})} \leftrightarrow A_{(\text{organic phase})} \leftrightarrow A_{(\text{acceptor phase})} \quad (1)$$

And the initial amount of analyte *n*, is equal to the sum of individual amounts of analyte present in the all phases during the whole extraction process.

$$n_i = n_d + n_{org} + n_a \quad (2)$$

In which n_d, is the amount of analyte in the donor phase (sample), n_{org}, is the amount of analyte in the organic phase and n_a, is the amount of analyte in the acceptor phase, respectively.

At the equilibrium condition, Eq. (2) can be written as:

$$C_i V_d = C_{eq.d} V_d + C_{eq.org} V_{org} + C_{eq.a} V_a \quad (3)$$

C_i, is the initial concentration of analyte, $C_{eq.d}$, $C_{eq.org}$ and $C_{eq.a}$ are analyte concentrations in the donor, organic and acceptor phases at equilibrium condition, respectively. V_d, V_{org} and V_a, are the volumes of the donor, organic and acceptor phases, respectively.

In the LLLME system, partition coefficients between the phases are:

$$K_{org/d} = \frac{C_{eq.org}}{C_{eq.d}} \quad (4)$$

$$K_{org/a} = \frac{C_{eq.org}}{C_{eq.a}} \quad (5)$$

And

$$K_{a/d} = \frac{C_{eq.a}}{C_{eq.d}} = \frac{K_{org/d}}{K_{org/a}} \quad (6)$$

By rearrangement the Eq. (2) at equilibrium [29], can be written as:

$$n_{eq.a} = \frac{K_{a/d} V_a C_i V_d}{K_{a/d} V_a + K_{org/d} V_{org} + V_d} \quad (7)$$

The *relative recovery* can be expressed as:

$$R = \frac{100 n_{eq.a}}{C_i V_d} = \frac{100 K_{a/d} V_a}{K_{a/d} V_a + K_{org/d} V_{org} + V_d} \quad (8)$$

The enrichment factor (EF), can be calculated as follows:

$$EF = \frac{C_a}{C_i} = \frac{V_d R}{100 V_a} \quad (9)$$

Or

$$EF = \frac{1}{(K_{org/a}/K_{org/d}) + (K_{org/a} V_{org})/V_d + (V_a/V_d)} \quad (10)$$

In the LLLME, the volume of the organic solvent immobilized in the pores of the hollow fiber (V_{org}) is small, and (Eq. (10)) may be simplified to:

$$EF = \frac{1}{1/K_{a/d} + V_a/V_d} \quad (11)$$

According to these calculation and experimental data (Tables 2 and 3), *K* values of 100 are required for the analyses in order to obtain high enrichment factors. In addition, the V_d/V_a should not be below 100 in order to obtain high enrichment factor and appropriate analyte pre-concentrations [17,32].

4. Optimization of the method

4.1. Organic solvent

Choosing the most suitable organic solvent is very important for achieving a good selectivity of the target compounds. The chose of solvent should be based on comparison of selectivity, extraction efficiency, and the level of toxicity. This factor is very critical for three-phase microextraction. In addition, the polarity of the organic phase should be similar to that of the polypropylene fiber so that it can be easily immobilised within the pores of the fiber. This function greatly affects the

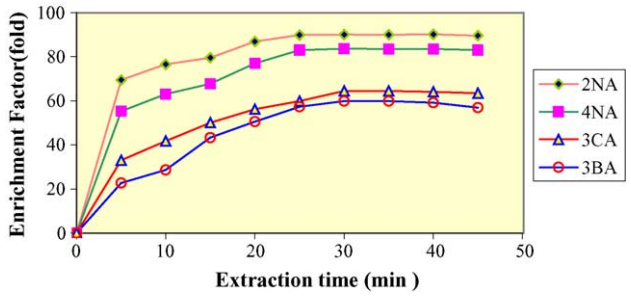

Fig. 3. Effect of extraction time on enrichment factor for the first step of extraction: 100.0 μg/l, concentration for each analyte, stirring speed, 900 rpm, temperature, 22 ± 0.5 °C.

performance of hollow fiber LPME since extraction occurs on the surface of the immobilised solvent [33,34].

Six different organic solvents were investigated namely; *n*-hexane, *iso*-octane, cyclohexane, butyl acetate, ethyl acetate and benzyl alcohol, and as we described in the previous work [25] benzyl alcohol, ethyl acetate (v/v. %benzylalcohol, 80) was optimised as the most suitable organic solvent. The same solvent mixture was used in both extraction steps.

4.2. Extraction times

LPME is not an exhaustive extraction technique. Although maximum sensitivity is attained at equilibrium, complete equilibrium needs not to be attained for accurate and precise analysis. Extraction times usually matched to the chromatography run, thus allowing maximum sample throughput. However, when choosing an extraction time profile, precise timing becomes essential for good precision [34,35]. Therefore, the extraction time is one of the most important factors influencing the extraction efficiency. With the polypropylene impregnated with benzyl alcohol–ethyl acetate (v/v, %benzylalcohol, 80), NaOH solution (pH 13.0) in the donor solution, and HCl (pH 2.0) as the acceptor phase, the extraction times for two-steps was investigated for aniline derivatives as evident from Figs. 3 and 4. The range of extraction times investigated was between 5 and 45 min. The enrichment factor was increased with increasing the extraction times (T_1, time of extraction for step 1 and T_2, time of extraction for step 2) from 5 to 30 min for step 1 and 5 to 20 for step 2 and it was reached to maximum when the samples were stirred for 30 min for step 1 and 20 min for step 2 (with stirring rate, 900 rev/min), but after this optimum times, the efficiency showed a soft decline because of the loss of the ethyl acetate due to it's evaporation and solvation in water. The optimized extraction time, T_1 was 30 min and T_2 was 20 min.The experiment were performed at room temperature (22 ± 0.5 °C).

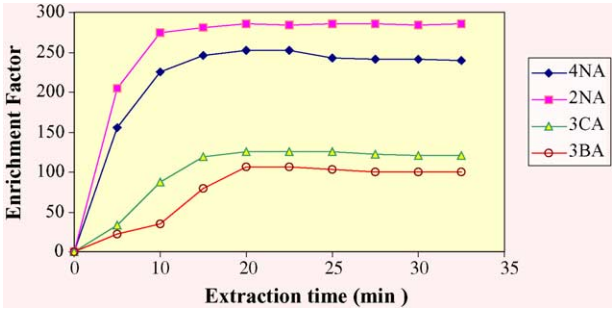

Fig. 4. Effect of extraction time on enrichment factor for the second step of microextraction. Stirring speed; 900 rpm; temperature; 22 ± 0.5 °c.

4.3. Phases volume

In the present work, the phase volume of donor and acceptor solutions was optimized. According to Eq. (11), the enrichment factor can be improved by the increasing the volume ratio of donor and acceptor phases [36–38]. The results, however, indicate that the best extraction efficiency was obtained when the donor acceptor ratio was more than 100-fold. As the two steps of the extraction are based on the same principles we can simplify the optimization experiments based on the second step of the extraction [39].

According to (Eq. (6));

$$C_{\mathrm{eq.a}} = K_{\mathrm{a/d}} C_{\mathrm{eq.d}} \tag{12}$$

And we can write:

$$C_{\mathrm{eq.a}} = \frac{n_{\mathrm{a,eq}}}{V_{\mathrm{a}}} \tag{13}$$

And

$$C_{\mathrm{eq.d}} = \frac{n_{\mathrm{d,eq}}}{V_{\mathrm{d}}} \tag{14}$$

So,

$$\frac{n_{\mathrm{a}}}{V_{\mathrm{a}}} = \frac{K_{\mathrm{a/d}} n_{\mathrm{d}}}{V_{\mathrm{d}}} \tag{15}$$

If there is recovery in the first step about 100%, this means that:

$$n_{\mathrm{d,initial}} = n_{\mathrm{a,final}} = n_{\mathrm{a,eq}} \tag{16}$$

Thus, $n_{\mathrm{d,eq}}$ is very small.

$$K_{\mathrm{a/d}} = n_{\mathrm{a,eq}} \left(\frac{V_{\mathrm{d}}}{V_{\mathrm{a}}} \right) \tag{17}$$

We used of this method for determination of trace amount of analyses, thus:

$$K_{\mathrm{a/d}} \sim \frac{V_{\mathrm{d}}}{V_{\mathrm{a}}} \tag{18}$$

We experimented the recoveries for all of the analyses in the first step by changing the $V_{\mathrm{d}}/V_{\mathrm{a}}$ values and as shown in the Table 1, for $V_{\mathrm{d}}/V_{\mathrm{a}}$ ratio of 100, we considered the best recovery for all of analyses. Thus, $V_{\mathrm{d}}/V_{\mathrm{a}}$ was adjusted at 100 in each of the steps.

Table 1
Optimisation of phase volumes from the first step of microextraction[a]

Volume of the acceptor phase (ml)	V_d/V_a[b]	Relative recovery (%)			
		4NA	2NA	3CA	3BA
4	75	98.1	99.9	95.9	92.3
3	100	98.7	99.5	96.9	95.4
2	150	90.3	91.5	88.9	85.4
1	300	71.0	73.5	59.9	50.6

[a] Extraction conditions: volume of donor phase, 300 ml. Initial concentration of each analyte, 100 μg/l. Extraction time, 30 min. Stirring speed, 900 rpm. Temperature, 22 ± 0.5 °C. All extractions were performed in triplicate.
[b] Phase volumes ratio, V_d is volume of the donor phase (sample solution), and V_a is volume of the acceptor phase.

4.4. Quantitative aspects

The enrichment factors, linear range, precision (RSD) and detection limits (LOD) for the second step are given in Table 2. The total enrichment factors were between 6091.83 and17093.77. The linearity of this method for analyzing standard solution has been investigated between the ranges 0.02–300 μg/l. The precision of method was studied for a set of five replicate measurments. The RSD ranged from 8.98 to 15.91%. LODs were based on a signal to noise ratio of 3, calculated for seven replicate runs and were in the range of 10–250 ng/l.

4.5. Real water analysis

Potable water from the Mashhad water-supply network, spiked with all of the aromatic amines was extracted using two-step LLLME under optimal conditions. The results are shown in Fig. 5. Because of the matrix effect on the pH adjustment, more concentrated NaOH (6 M) was needed to adjust the pH of sample at 13.0, we used a spiked sample to test because no aromatic amines were detected in this real water sample. The recoveries of the analytes from this real sample were higher than 86% compared with that of spiked pure water. This indicate that the matrix effect dose not have any significant effect on the extraction efficiency.

The performance of this method was also tested by analyzing real water from the dye and plastic industries, from Mashhad, Iran and also Caspian Lake water sample. As shown in Table 3, 2NA, 4NA, 3CA and 3BA, are present as contaminations in the samples in low concentration (see Fig. 6)

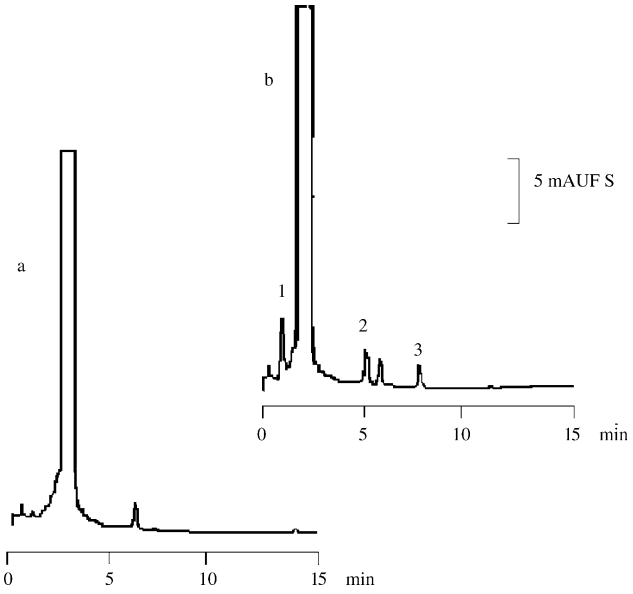

Fig. 5. Chromatogram of potable water from the Mashhad water-supply network, after two-step LLLME: (a) water sample and (b) the same sample spiked with 1 ng/ml of aromatic amines under optimum conditions. Peaks: 1; 4-nitroaniline (4NA), 2; 3-chloroaniline (3CA), 3; 3-bromoaniline(3BA).

Table 3
Concentration (μg/l) of aromatic amines detected in real waters using Two-Step LLLME-HPLC

Compound	Caspian sea	Dye plant	Plastic industries
2NA	0.039	Trace	Trace
4NA	0.018	0.016	Trace
3CA	Trace	0.100	0.130
4BA	Trace	Trace	Trace

Table 2
Performance of the two-step method

Compound	Enrichment factor (step 1)	Enrichment factor (step 2)	Total enrichment factor	RSD% ($n = 5$)	Linear range (μg/l)	Correlation coefficient (r^2)	LOD ng/l ($n = 7$)	Relative recovery (%)
4NA	83.60	251.73	17093.77	10.57	0.05–100	0.9996	30	84.5
2NA	90.10	286.33	14907.55	8.98	0.02–100	0.9989	10	86.8
3CA	64.50	126.00	7325.91	13.89	0.20–300	0.9979	100	79.5
3BA	59.90	106.43	6091.83	15.91	0.50–300	0.9945	250	74.9

Conditions: At the first step; 300 ml aqueous sample with PH 13 as the donor phase; organic phase, benzyl alcohol–ethyl Acetate (2: 1); 3.0 ml aqueous solution with PH 2 as the acceptor phase; $T_1 = 30$ min. Stirring speed 900 rpm. At the second step; 5.0 ml aqueous sample with PH 13 as the donor phase; organic phase, benzyl alcohol–ethyl acetate (2: 1); 5.0 μl aqueous solution with PH 2 as the acceptor phase; $T_2 = 20$ min.; Stirring speed 900 rpm; temperature 22 ± 0.5 °C.

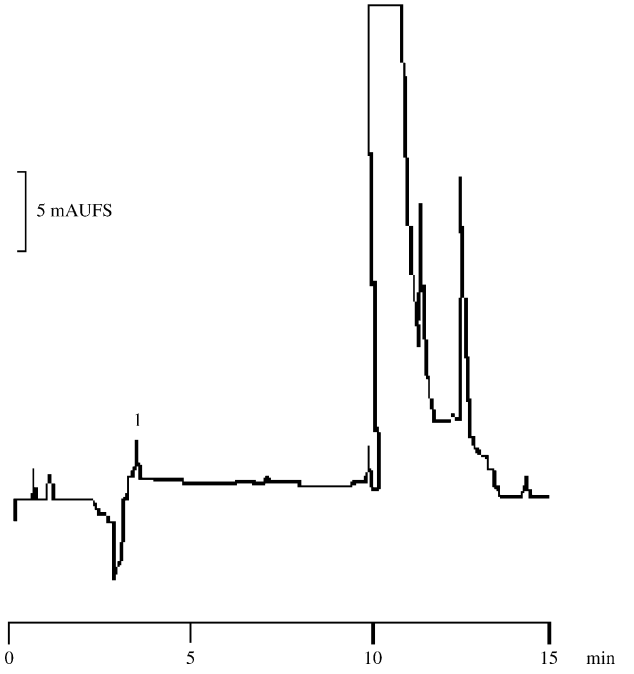

Fig. 6. Chromatogram of Caspian sea water from Iran, after two-step LLLME under optimum conditions: Peak 1; 2-nitroaniline (2NA).

5. Conclusion

In present study, a two-step liquid-phase microextraction technique coupled with HPLC by using a monolithic column has been developed to quantify trace levels of aromatic amines in water samples. The cost, sample preparation time as well as consumption of toxic organic solvents were minimized without affecting the sensitivity of the method.

The results obtained in this work indicate the development and application of a novel LLLME approach of extracting aniline derivatives from water samples, using polypropylene membranes with the obvious advantages of higher enrichment factors and lower detection limits. Because the principles of the two-step are similar, the optimization of the entire process focused on the second step. This technique was applied for analysis of environmental water samples. The cost of each extraction unite was low and each unit was a disposable device utilized only for a single extraction. This was a major advantage because memory effect, carry-over effect and cross-contamination were totally eliminated.

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