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Analysis of aromatic amines in water samples by liquid–liquid–liquid microextraction with hollow fibers and high-performance liquid chromatography

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Abstract

Liquid–liquid–liquid microextraction (LLLME) with hollow fibers in high-performance liquid chromatography (HPLC) has been applied as a rapid and sensitive quantitative method for the detection of four aromatic amines (3-nitroaniline, 4-chloroaniline, 4-bromoaniline and 3,4-dichloroaniline) in environmental water samples. The preconcentration procedure was induced by the pH difference inside and outside the hollow fiber. The target compounds were extracted from 4-ml aqueous sample (donor solution, pH~13) through a microfilm of organic solvent (di-*n*-hexyl ether), immobilized in the pores of a hollow fiber (1.5 cm length×0.6 mm I.D.), and finally into 4 μl of acid acceptor solution inside the fiber. After a prescribed period of time, the acceptor solution inside the fiber was withdrawn into the microsyringe and directly injected into the HPLC system for analysis. Factors relevant to the extraction procedure were studied. Up to 500-fold enrichment of analytes could be obtained under the optimized conditions (donor solution: 0.1 *M* sodium hydroxide solution with 20% sodium chloride and 2% acetone; organic phase: di-*n*-hexyl ether; acceptor solution: 0.5 *M* hydrochloric acid and 500 m*M* 18-crown-6 ether; extraction time of 30 min; stirring at 1000 rev./min). The procedure also served as a sample clean-up step. The influence of humic acid on the extraction efficiency was also investigated, and more than 85% relative recoveries of the analytes at two different concentrations (20 and 100 μg/l) were achieved at various concentration of humic acid. This technique is a low cost, simple and fast approach to the analysis of polar compounds in aqueous samples.

Keywords: Water analysis; Liquid–liquid–liquid microextraction; Extraction methods; Hollow fibers; Amines, aromatic

1. Introduction

Aromatic amines are widely used in industry for making dyes, cosmetics, and medicines, and as intermediates in many chemical syntheses [1,2]. Many amines are proven or suspected to be carcinogenic, and have been implicated in inducing cancer of the bladder [3,4]. Therefore, the monitoring of their levels in environmental waters is important for the protection of health and the environment. These compounds have been included in the US Environmental Protection Agency (EPA) list of priority pollutants [5,6].

The most popular techniques for the analysis of aromatic amines in environmental waters are gas chromatography (GC) and high-performance liquid chromatography (HPLC) [7]. However, these compounds are thermolabile and polar compounds, and a

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derivatization step is often required to obtain good GC performance [1,8,9]. Hence, HPLC analysis seems to be a good alternative to GC analysis since there are no derivatization requirements. A preconcentration step is necessary, however, in trace analysis owing to the relatively low sensitivity of HPLC detectors suitable for these compounds.

Both classical liquid–liquid extraction (LLE) and solid-phase extraction (SPE) have been employed for the extraction of these amines from environment aqueous samples [7,10]. However, both techniques require the evaporation of solvent to dryness and the reconstitution of the dry residue in a suitable solvent for HPLC or capillary electrophoresis (CE). These manual procedures are normally tedious and prone to loss of analytes through evaporation and reconstitution. Solid-phase microextraction (SPME) has also been applied to the preconcentration of aniline [11–13] in various matrixes in combination with GC–MS. When SPME is coupled to HPLC, a special SPME–HPLC interface device has to be used for solvent desorption to recover all sorbed analytes and to avoid carry-over. In comparison with SPME, LLE at the miniaturized scale can overcome this problem since the final extract is in liquid form at a very small volume and no solvent desorption process is required before HPLC analysis.

Ma and Cantwell demonstrated simultaneous forward and back-extraction (SME/BE) across a microliter-size organic liquid membrane [14,15]. Pedersen-Bjergaard and Rasmussen demonstrated a liquid-phase microextraction (LPME) method for preconcentration of drugs from samples [16–20] prior to CE or HPLC analysis. The technique utilizes a porous polypropylene hollow fiber, immobilized with organic solvent (1-octanol), as a barrier between two aqueous phases (donor phase outside and acceptor phase inside) adjusted at different pH values. Under stirring, the analytes were extracted from the donor phase through the organic solvent in the pores of the hollow fiber, and finally into the acceptor solution inside the fiber. With a high volume ratio between the donor solution (1–2 ml) and the acceptor phase (10–25 μl), high enrichment factors (>50-fold) were achieved. Our group developed further this technique by decreasing the volume of acceptor phase to only several microliters (2 μl) [21]. We termed it liquid–liquid–liquid microextraction (LLLME) and applied it to the analysis of nitrophenols. Since the ratio between the donor phase and the acceptor phase was increased largely, the extraction efficiency was improved significantly, and up to 380-fold enrichment of analytes could be obtained.

In the present work, we applied LLLME combined with HPLC to the analysis of aromatic amines in aqueous samples. Different aspects affecting the extraction efficiency (organic solvent selection, phase ratio between donor phase and acceptor phase, extraction time, composition of donor and acceptor solutions (Table 1)) were investigated. In addition, the influence of humic acid on the extraction efficiency was studied and applications to practical water samples were carried out. The current study provides a simple, fast and economical approach to the detection of aromatic amines in aqueous samples.

Table 1
Effect of composition of donor and acceptor solutions[a]

Target compounds	0.1 *M* HCl as acceptor solution[b]				0.1 *M* NaOH as donor solution[c]			
	0.001 M NaOH	0.01 M NaOH	0.1 M NaOH	1 M NaOH	0.001 M HCl	0.01 M HCl	0.1 M HCl	0.5 M HCl
3-NA	18	20	24	29	1	6	24	53
4-CA	179	199	231	204	17	128	231	229
4-BA	189	211	241	205	12	114	241	270
3,4-DCA	50	54	65	65	1	17	65	196

[a] Enrichment factor varied within 10% RSD ($n=4$) for working solution at a concentration of 100 μg/l of each analyte.
[b] The concentration of HCl in the acceptor solution is fixed, and the concentration of NaOH in the donor solution is varied.
[c] The concentration of NaOH in the donor solution is fixed, and the concentration of HCl in the acceptor solution is varied.

2. Experimental

2.1. Standards and reagents

Four aromatic amines [3-nitroaniline (3-NA), 4-chloroaniline (4-CA), 4-bromoaniline (4-BA) and 3,4-dichloroaniline (3,4-DCA)] were purchased from AccuStandards (New Haven, CT, USA). Isoamyl ether, 1-octanol and isooctane were obtained from Merck (Darmstadt, Germany). Di-*n*-hexyl ether was from Tokyo Chemical Industry (Tokyo, Japan). The 18-crown-6 ether ($C_{12}H_{24}O_6$) was purchased from Fluka (Buchs, Switzerland). Humic acid (sodium salt) was from Aldrich (Milwaukee, WI, USA). HPLC-grade methanol was brought from Mallinckrodt (Paris, KY, USA). The water used was purified on a Milli-Q ultrapure water purification system (Millipore, Bedford, MA, USA).

Each analyte was dissolved in methanol to obtain a stock solution with a concentration of 1 mg/ml. They were stored at 4 °C. A separate 10.0 mg/l standard solution containing the four target compounds was prepared in water from stock solutions and used as working solutions.

Groundwater was used as the practical sample. The water was stored at 4 °C. Tap water samples (directly potable) were collected freshly from our laboratory.

The Q3/2 Accurel polypropylene hollow fiber was bought from Membrana (Wuppertal, Germany). The inner diameter of the hollow fiber was 600 μm, the thickness of the wall was 200 μm and the pore size was 0.64 μm.

2.2. Instrumentation

The HPLC system consisted of a Shimadzu (Tokyo, Japan) LC-6A pump, a Rheodyne 7010 injector equipped with 20-μl sample loop, a Shimadzu SPD-6AV UV–Vis detector and a Shimadzu C-R6A integrator. A Chrompack Inertsil ODS-2 (250×2 mm) column was used for separations. The mobile phase was a mixture of methanol–pure water–(0.5 *M* sodium acetate–glacial acetic acid buffer, pH 3.40) (60:35:5). The mobile phase flow-rate was 0.2 ml/min and UV detection was at 254 nm.

2.3. Extraction process

The basic experimental apparatus was shown has been previously described [21]. Briefly, 4 ml of aqueous sample was placed in a 4 ml sample vial, along with a 12×4 mm magnetic stirring bar. The sample vial was clamped to fix its position above the magnetic stirrer (Heidolph, Kelheim, Germany). A 10-μl HPLC syringe (SGE Scientific, Sydney, Australia) was used to introduce the acceptor solution into the hollow fiber, and to support fiber. It also served as a sample introduction device for the HPLC. The syringe was clamped on a retort stand during the extraction procedure.

A hollow fiber was cut to produce 2-cm segments. The approximate internal volume of this segment was ~4.5 μl. One of the ends of the hollow fiber segment was flame-sealed. The hollow fiber segments were sonicated for 2 min in HPLC-grade acetone to remove any contaminants in the fiber. After sonication, the fibers were removed from the acetone, and the solvent was allowed to evaporate completely. These hollow fiber segments were used for subsequent extractions.

A 4 μl volume of acceptor solution was withdrawn into the HPLC microsyringe and the needle of the syringe was inserted into a hollow fiber segment with a sealed end. The acceptor solution was introduced carefully. Attention should be paid that the acceptor solution did not penetrate the wall of hollow fiber; otherwise it would be lost during the extraction process. The hollow fiber was then immersed in the organic solvent for 5 s for impregnation of its pores. Then, the fiber (together with the syringe needle) was placed into the aqueous sample and the syringe was fixed on the retort stand. The magnetic stirrer was switched on to start the extraction. After a prescribed time, the syringe was removed from the sample and the magnetic stirrer was switched off. The acceptor solution in the hollow fiber was withdrawn back into the syringe. Part of extractant was discarded to remove possible solvent contamination, and only 2-μl of aliquot was left for injection. An aliquot (3-μl) of pure water was withdrawn into the syringe to combine with the 2-μl of acceptor phase, and the entire solution in the needle was injected into the HPLC system for analysis. The extraction performance was evaluated by HPLC

signals (peak area) and the enrichment factor (EF), defined as ratio of the peak of the analytes obtained after and before LLLME.

3. Results and discussion

3.1. Basic principle

Since the LLLME involves three phases, donor phase, organic microfilm and acceptor phase, for an analyte *i*, the extraction process may be represented by the equation

$$i_{a_1} \leftrightarrow i_o \leftrightarrow i_{a_2} \quad (1)$$

where the subscript a_1 represents the aqueous donor phase, o represents the organic phase within the pores of the hollow fiber, and a_2 the aqueous phase.

At equilibrium, the distribution ratios for the analyte *i* in the three-phase system are

$$K_1 = C_{o,eq}/C_{a_1,eq}$$

and

$$K_2 = C_{o,eq}/C_{a_2,eq}$$

then, the mass balance in the three-phase system can be expressed as [14,16]

$$C_{a_1,\,initial} = \frac{K_2 C_{a_2,eq}}{K_1} + \frac{K_2 C_{a_2,eq} V_o}{V_{a_1}} + \frac{C_{a_2,eq} V_{a_2}}{V_{a_1}} \quad (2)$$

where $C_{a_1,initial}$ is the initial concentration of *i* in the donor phase, $C_{a_2,eq}$ is the equilibrium concentration of *i* in the acceptor phase, and V_{a_1} is the volume of the donor phase, V_o is the volume of solvent in the pores of the hollow fiber, and V_{a_2} is the volume of the acceptor phase.

The EF, defined as the ratio $C_{a_2,eq}/C_{a_1,initial}$, can be calculated as [16]:

$$EF = \frac{1}{(K_2/K_1) + (K_2 V_o/V_{a_1}) + (V_{a_2}/V_{a_1})} \quad (3)$$

Since V_o is very small, Eq. (3) can be simplified to

$$EF = \frac{1}{(1/K) + (V_{a_2}/V_{a_1})} \quad (4)$$

where

$$K = K_1/K_2 = C_{a_2,eq}/C_{a_1,eq} \quad (5)$$

From Eq. (4), it is obvious that a large *K* and phase ratio V_{a_1}/V_{a_2} will result in a higher enrichment factor. To have a large *K*, it is necessary to convert the analytes in the acceptor phase by such reactions as protonation, complexation, etc. to species that have very slight affinity for the organic phase [14]. It is also to prevent the reverse extraction from the acceptor phase to the donor phase. In the current study, we use both protonation and complexation to obtain large *K* and to prevent the reverse extraction.

3.2. Optimization of LLLME

To achieve the optimum extraction efficiency, various parameters were investigated and are discussed respectively.

3.2.1. Solvent selection

The type of organic solvent used in LLLME is an essential consideration for efficient analyte preconcentration. Generally speaking, there are several requirements for the organic solvent selected. Firstly, it should be easily immobilized in the hollow fiber pores, and be non-volatile. This is to prevent solvent loss during the extraction. Secondly, the organic solvent should be immiscible with water because it serves as a barrier between two aqueous phases, the donor and the acceptor. Lastly, the solubility of analytes in the solvent should be higher than that in the donor phase and be lower than that in the acceptor phase. This is in order to promote analyte migration from the donor phase through the pores of the hollow fiber and finally to the acceptor. Based on the above considerations, 1-octanol, di-*n*-hexyl ether and diamyl ether were evaluated as solvents for LLLME. Evaluation was accomplished by 20 min of LLLME from standard solutions of 100 μg/l anilines dissolved in 0.1 *M* sodium hydroxide (NaOH), and 0.1 *M* hydrochloric acid (HCl) was used as acceptor phase. All of these solvents were easily immobilized in the hollow fiber by 5 s soaking in the solvent. The results show that di-*n*-hexyl ether can provide the best extraction performance, and it was selected for the rest of the work.

3.2.2. Phase ratio between donor and acceptor phase

In the present work, the phase ratio of donor and acceptor solutions was changed in the range of 2000:1 to 800:1, by changing the volume of the acceptor phase whilst the volume of donor phase was kept constant at 4 ml. According to Eq. (4), the enrichment factor can be improved by the increase in the volume ratio of donor and acceptor phase. The results, however, indicate that the best extraction efficiency was obtained when 4-μl of acceptor phase was used (i.e. donor:acceptor ratio of 1000:1). We observed that air bubbles were produced during extraction when a small volume of acceptor solution was used; the extraction efficiency could be influenced by this. We thus selected 4-μl acceptor solution and 4-ml donor solution for subsequent experiments.

3.2.3. Extraction time

As other techniques of microextraction, LLLME is a type of equilibrium extraction. Once sufficient extraction time has elapsed for equilibrium to be established, a further increase in extraction time does not affect the amount of analyte extracted.

The range of extraction times investigated here was 5–60 min. The experiments were accomplished with the hollow fiber impregnated with di-*n*-hexyl ether, 0.1 *M* NaOH in the donor phase, and 0.1 *M* HCl as the acceptor solution. The stirring speed was set at 1000 rev/min. The results indicate that the respective equilibria for 3,4-DCA and 3-NA were established after 30 min, while the HPLC signals of the other two analytes stabilized after 50 min. Since it is unnecessary to wait until the equilibria for all analytes to be established, 30 min was chosen as the extraction time for the rest of the study.

We also examined the effect of stirring speed on the time required for equilibrium to be established. The selected extraction states were static (no stirring), light agitation (200 rev./min) and vigorous agitation (1000 rev./min), respectively. 3,4-DCA was selected as a representative of aromatic amines to show their behavior under the different extraction status (Fig. 1).

According to the theoretical model of mass transfer for solvent microextraction [22], as stirring speed was increased the thickness of the diffusion film in the aqueous phase decreased causing an increase in mass transfer coefficient. Our results of LLLME support this explanation too. From Fig. 1, it can be seen that the equilibrium of 3,4-DCA was established after 20 min when 1000 rev./min of stirring was applied. When 200 rev./min of stirring was applied, the change of HPLC signals become steady only after 50 min, while extraction in the static solution was still far away from equilibrium even after 60 min, and the increase of HPLC signals with elapsed extraction time was very slow. Hence, agitation can not only increase the extraction efficiency, but also shorten the time required for equilibrium to be established. On the basis of our observation, 1000 rev./min of stirring speed was used for subsequent experiments.

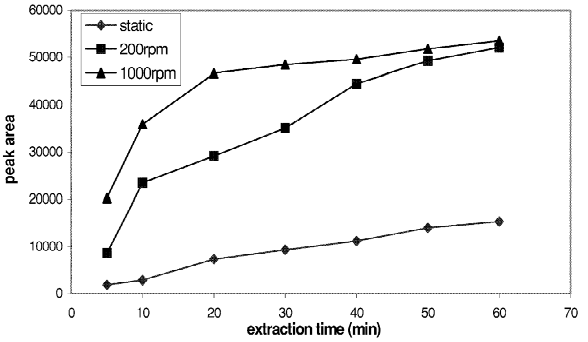

Fig. 1. The effect of stirring speed on the time needed for establishment of equilibrium using 3,4-DCA as a representative example of aromatic amines. Extraction conditions: di-*n*-hexyl ether as the immobilized solvent in 2-cm hollow fiber; 0.1 *M* NaOH in the donor phase and 0.1 *M* HCl in the acceptor phase.

3.2.4. Compositions of donor and acceptor solutions

As we discussed previously, it is necessary to convert the analytes in the acceptor phase by some means to get a large K and thus prevent reverse extraction. Therefore, it is critical to adjust the composition of the donor and acceptor phases.

For the reactions used in LLLME to promote microextraction, protonation is the most common one to date [16–20]. Normally, the donor solution is adjusted to deionize the analytes and the acceptor phase adjusted to ionize them. The difference in pH between the donor and acceptor phases can promote the analytes extracted from the former phase to the

latter. Since our target compounds are weak basic compounds with low pK_a {3-NA (pK_a = 2.47), 4-CA (3.98), 4-BA (3.86), 3,4-DCA (2.97) [32]}, protonation is also an important reaction. We firstly investigated the donor phase, which was adjusted to deionize aromatic amines by alkaline (NaOH) concentration varying from 0.001 to 1 *M*. To make sure that analytes could be extracted, 0.1 *M* HCl was used as acceptor phase. The results, shown in Table 1, indicate that increase of NaOH concentration does provide some but not significant enhancement of extraction efficiency. 0.1 *M* NaOH was selected to obtain better extraction efficiency.

While the pH adjustment in the donor solution was not a critical factor, the extraction efficiency was more sensitive to pH adjustment in the acceptor phase. As shown in Table 1, the HCl concentration was studied in the range of 0.001 to 0.5 *M*. The higher HCl concentration was not used to avoid possible problems with the Inertsil ODS-2 column with a highly acidic sample. It is obvious that the influence of pH on the EF for every analyte is dramatic, especially for 3-NA and 3,4-DCA which have relatively lower pK_a values. Finally, 0.5 *M* HCl was used because it provided highest EF for each compound. One precaution must be mentioned. In practice, rinsing the HPLC injector with pure water after each injection should be carried out to prevent possible acid erosion of the stainless steel injector.

The addition of a salt can often improve extraction recovery when conventional extraction methods are used. Sodium chloride (NaCl) is normally used for this purpose [23]. In the current work, NaCl was added into the donor solution in the range of 2% to saturation. The results, shown in Fig. 2, indicate an initial increase in the extraction EFs with an increase in salt concentration, with a maximum being reached at 20%, followed by a decrease in EFs with further increase in salt concentration to saturation point. This phenomenon can be explained by two simultaneously occurring processes [24]. At the beginning, analyte EF was enhanced due to 'salting out', a fact that water molecules form hydration spheres around the ionic salt molecules. These hydration spheres reduce the amount of water available to dissolve analytes in water [25]. On the other hand, polar molecules may participate in electrostatic interactions with the salt ions in solution [26], thereby reducing their ability of mass transfer. Initially, the predominant process is the interaction of salt with water. As salt concentration increases further, salt molecules begin to interact with analyte molecules. Thus the initial improvement in analytes extracted with salt addition was followed by a decrease when

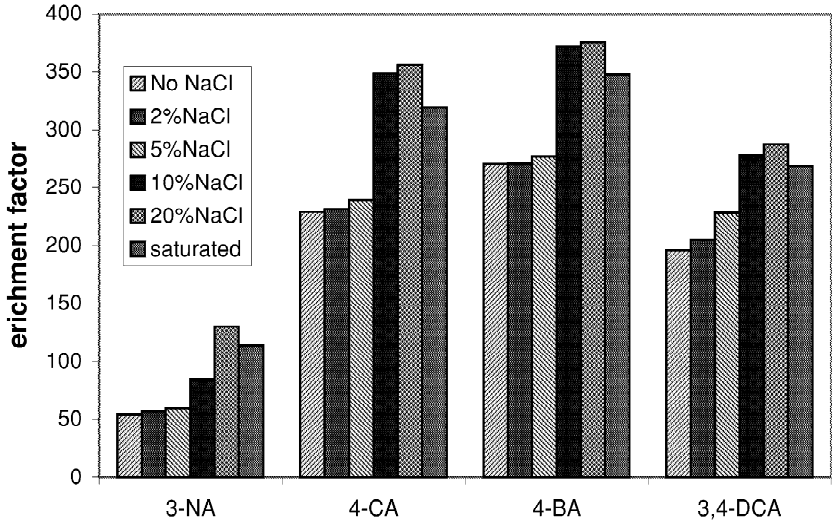

Fig. 2. The effect of salt added in the donor phase on the extraction efficiency for aromatic amines. Extraction conditions: di-*n*-hexyl ether as the immobilized solvent in 2-cm hollow fiber; 0.1 *M* NaOH in the donor phase and 0.5 *M* HCl in the acceptor phase; extraction time 30 min with stirring speed 1000 rev./min.

salt concentration was increased further. A 20% NaCl addition in the donor phase was selected since it provided the best EFs for all the analytes.

When solvent microextraction was used to extract polycyclic aromatic hydrocarbons in soils [27], organic solvent was added into the soil solution to promote extraction. Here we studied whether an organic additive in the donor phase was beneficial for LLLME. Methanol and acetone were selected and compared in the range 2–10% (v/v). The results (Table 2) show that solvent additive in the donor phase did enhance the extraction efficiency. This effect was most significant when 2% acetone was added. The results are expressed as relative response in Table 2. The possible reason for the observation is that a small proportion of the organic solvent existing in the water can help the dissolved analyte molecules transfer to the organic solvent in the pores of hollow fiber.

In this work, besides protonation, we also investigated the influence of analyte complexation on LLLME. It has been reported that protonated aniline can complex with crown ether in solution [28]. We used 18-crown-6 ether to study the effect of complexation on extraction. The 18-crown-6 ether was added in 0.5 *M* HCl of the acceptor phase and the concentration was varied from 100 to 500 m*M*. The results of crown ether addition indicate that no significant effect on extraction was obtained at lower concentrations; however, increases were achieved at higher concentration of 18-crown-6 ether (400–500 m*M*), especially for 4-BA and 3,4-DCA (20–30% improvement in EFs). Thus, 500 m*M* 18-crown-6 ether in the acceptor phase was used to enhance the extraction.

To summarize, the optimum composition of donor phase was 0.1 *M* NaOH solution with 20% NaCl and 2% acetone; while that of the acceptor phase was 0.5 *M* HCl and 500 m*M* 18-crown-6 ether.

3.2.5. Extraction efficiency

Based on the experiments discussed above, the optimal extraction efficiency on aromatic amines was obtained by utilizing a 2.0 cm long porous hollow fiber immobilized with di-*n*-hexyl ether, 4 μl of 0.5 *M* HCl and 500 m*M* 18-crown-6 ether solution as the acceptor phase, 4 ml of 0.1 *M* NaOH solution with 20% NaCl and 2% acetone as the donor phase, stirring speed of 1000 rev./min and an extraction time of 30 min. A practical demonstration of the enrichment attainable with LLLME is presented in Fig. 3. Chromatogram (a) was generated by an injection of 2.0 μl aqueous standard solution containing 10 mg/l (10 ppm) of each analyte. Chromatogram (b) was obtained by injecting 2.0 μl of acceptor solution after LLLME. The concentration in the donor solution of analytes was 20 μg/l each. It is clear that EF of several hundred-fold can be achieved by LLLME under the optimal conditions.

3.3. Quantitative analysis

The repeatability, linearity and limits of detection under the optimal extraction condition were investigated, and the results are shown in Table 3.

Inter-day and intra-day repeatability in peak areas were studied for six replicate experiments for 1 day and over different days. The relative standard deviations (RSDs) of the four anilines were lower than 4.83% for inter-day experiments and 7.26% for intra-

Table 2
Effect of organic solvent addition in donor solution on the extraction efficiency for aromatic amines (100 μg/l each) by LLLME from aqueous sample[a]

Analyte	3-NA	4-CA	4-BA	3,4-DCA
No solvent addition	100.0	100.0	100.0	100.0
2% MeOH addition	86.32	93.61	91.09	85.93
5% MeOH addition	84.87	101.8	102.6	101.1
10% MeOH addition	66.34	85.72	88.53	94.22
2% Acetone addition	146.4	125.8	119.7	108.2
5% Acetone addition	141.5	114.1	107.7	91.20
10% Acetone addition	100.3	88.57	95.41	86.84

[a] The data were expressed as mean relative HPLC response based on three replicates, RSD<10%.

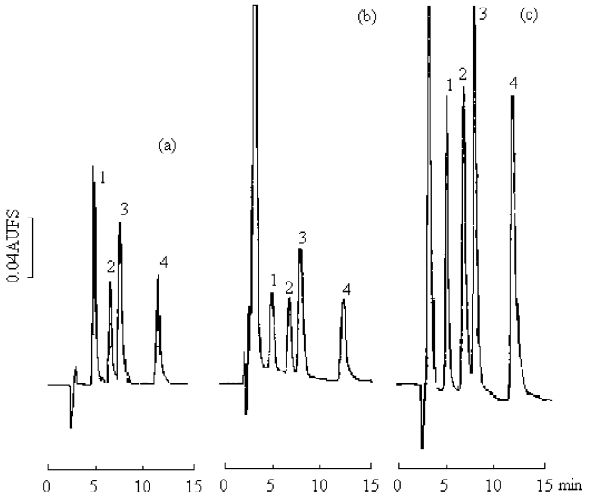

Fig. 3. Liquid chromatograms of a mixture of: (1) 3-nitroaniline; (2) 4-chloroaniline; (3) 4-bromoaniline; and (4) 3,4-dichloroaniline: (a) 2.0 μl injection of 10.0 mg/l standard solution without extraction; (b) 2.0 μl of acceptor solution after extraction from 20.0 μg/l donor solution under the optimal extraction conditions; (c) 2.0 μl of acceptor solution after extraction from surface water donor solution spiked with 100.0 μg/l each analyte under the optimal extraction conditions. The mobile phase was a mixture of methanol–pure water–(0.5 *M* sodium acetate–glacial acetic acid buffer, pH 3.40) (60:35:5). Flow rate: 0.2 ml/min, UV detection: 254 nm. Extraction conditions are given in the text.

day experiments. It demonstrates that the repeatability of extraction was better than those of other drop-based microextraction [29,30] since the use of the hollow fiber can enhance the stability of microextraction by protecting the organic solvent plug.

The linearity was evaluated within the range 1–500 μg/l except for 4-BA (0.5–500 μg/l) (Table 3). Each analyte exhibited good linearity with correlation coefficient $r^2 > 0.9917$. The limits of detection (LODs) were estimated based on a signal-to-noise ratio of 3. The wide linearity range, good repeatability and low detection limits indicate a high potential for monitoring aromatic amines by LLLME in water samples.

3.4. Real water analysis

In real environmental sample analysis, the extraction efficiencies are generally lower than those of pure water samples due to the existence of dissolved organics (largely humic acid) in the sample [31]. In this work, we selected humic acid as one of the matrix factors to access its effect on the extraction efficiencies on LLLME by adding humic acid (sodium salt) into the donor phase. Relative recovery, which is defined as the ratio of HPLC peak areas of spiked real water extracts or humic acid addition extracts to spiked Milli-Q water extracts, was employed for evaluation. Table 4 shows that higher than 85% relative recoveries were obtained for all the analytes at different humic acid concentration, indicating that as high as 200 mg/l humic acid in water had little influence on the extraction efficiency of LLLME.

In addition, tap water and surface water were spiked with aromatic amine standards at various concentrations to assess matrix effects. To examine the influence of some particles existing in surface water on LLLME, the sample was directly used for LLLME without any pretreatment (for example, filtration). Chromatogram (c) in Fig. 3 was obtained by injecting 2.0 μl of the acceptor solution after LLLME from the surface water donor solution. The concentration in the donor solution of analytes was 100 μg/l each. It is obvious that the chromatogram

Table 3
Quantitative results of LLLME

Analyte	RSD (%)[a] ($n=6$)		Linearity range (μg/l)	Correlation coefficient (r^2)	Limit of detection (μg/l)	Enrichment factor (EF)
	Inter-day	Intra-day				
3-NA	4.28	5.63	1.0–500.0	0.9988	0.10	240±5
4-CA	4.83	7.26	1.0–500.0	0.9998	0.08	470±11
4-BA	3.92	5.12	0.5–500.0	0.9988	0.05	510±10
3,4-DCA	3.84	4.89	1.0–500.0	0.9917	0.10	460±9

[a] Repeatability was investigated at a concentration of 50 μg/l for each analyte.

Table 4
Relative recoveries of aromatic amines to show the effect of donor solution matrix on the extraction efficiency[a]

Analyte	3-NA	4-CA	4-BA	3,4-DCA
20 mg/l humic acid[b]	96.73	94.88	93.36	95.70
50 mg/l humic acid[b]	94.37	96.65	96.71	97.86
100 mg/l humic acid[b]	87.52	85.28	91.03	93.16
200 mg/l humic acid[b]	90.60	89.60	91.18	89.85
Surface water spiked with 100 μg/l	96.60	97.27	101.5	98.10
Tap water spiked with 100 μg/l	105.4	97.48	98.63	99.48
Surface water spiked with 10 μg/l	88.29	88.50	96.81	91.04
Tap water spiked with 10 μg/l	103.7	92.90	105.3	92.16

[a] RSD<10% for three replicates.
[b] Donor solutions were prepared at 100 μg/l of each analyte with different humic acid concentrations.

shows no interferences. The data in Table 4 shows that good relative recoveries were achieved at different spiking concentrations with no obvious interferences in the analysis.

The above results demonstrate that LLLME with hollow fiber provides a potential alternative to trace enrichment of aromatic amines from real water samples.

4. Conclusion

The potential for LLLME has been demonstrated as a sample preparation technique prior to HPLC for aromatic amines analysis in water samples. LLLME provided extracts with high enrichment factors along with excellent sample clean-up. Furthermore, there was no need for evaporation of solvent and reconstitution of analytes prior to injection into the HPLC. Disposable extraction hollow fiber segments eliminated the possibility of carry-over, and the cost of the extraction was low. From the results of our experiments, LLLME combined with HPLC has been illustrated to be viable, easy to use, rapid and economical technique for analysis of aromatic amines in aqueous samples.

Acknowledgements

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