Journal of Chromatography A, 1218 (2011) 4779–4787

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Sensitive determination of phenylarsenic compounds based on a dual preconcentration method with capillary electrophoresis/UV detection

Pingjing Li, Bin Hu*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, China

ARTICLE INFO

Article history:
Received 18 January 2011
Received in revised form 13 May 2011
Accepted 17 May 2011
Available online 27 May 2011

Keywords:
Hollow fiber liquid liquid liquid microextraction
Anion selective exhaustive injection
Phenylarsenic compounds
Capillary electrophoresis
Pig feed

ABSTRACT

A novel method based on off-line hollow fiber based liquid liquid liquid microextraction (HF-LLLME) combined with on-column anion selective exhaustive injection (ASEI)-capillary electrophoresis/ultraviolet (CE/UV) detection was proposed for the speciation of five phenylarsenic compounds including phenylarsonic acid (PAA), 4-aminophenylarsonic acid (4-APAA), 4-hydroxyphenylarsonic acid (4-HPAA), 4-nitrophenylarsonic acid (4-NPAA) and 3-nitro-4-hydroxyphenylarsonic acid (NHPAA) in this paper. In HF-LLLME, the target analytes were extracted from 5 mL aqueous samples (donor solution pH 2.15) through a thin phase of tributyl phosphate (TBP) inside the pores of a polypropylene hollow fiber and finally into an 18 μL 0.8 mmol/L Tris acceptor solution inside the lumen of the hollow fiber. Following HF-LLLME, the acceptor solutions were directly analyzed by ASEI-CE/UV. For ASEI, a large plug of water (91% length of total capillary) was introduced into the separation capillary before sample injection in order to prolong the sample injection time, and thus enhance the stacking efficiency. Under the optimized ASEI conditions, up to 236-fold of enrichment factor (EF) was obtained for the ASEI-CE/UV determination of target phenylarsenic compounds. By combining HF-LLLME with ASEI-CE/UV, EFs ranging from 155 to 1780-fold were achieved and the limits of detection (LODs) (at a signal-to-noise ratio of 3) were in the range of 0.68–6.90 μg/L for five phenylarsenic compounds; the relative standard deviations (RSDs) of corrected peak area were 5.6–11.8%. The proposed HF-LLLME–ASEI-CE/UV method was applied for the determination of five target phenylarsenic compounds in pig feed from a local pig farm, and storage pig litter, soil in agricultural field and lake water collected near this pig farm, the recoveries for the spiked samples were in the range of 85.7–104.5%, 66.7–96.2%, 28.9–46.9% and 86.9–107.8% for pig feed, pig litter, soil and lake water, respectively.

1. Introduction

Phenylarsenic compounds such as 3-nitro-4-hydroxyphenylarsonic acid (NHPAA, roxarsone) are anthropogenic organoarsenic compounds that are widely used as animal feed additives to promote growth or to control diseases in poultry farming [1]. Because of their highly water soluble nature, they can be excreted unchanged through animal litter. When the litter is stockpiled or used as fertilizer in agricultural sites, there will result in great risk of arsenic contamination on soils, surface water and ground water [2]. In addition, phenylarsenic compounds could be taken up and accumulated by plants growing on contaminated sites, and they are further subjected to a complex metabolization in the biosphere leading to various arsenic species which differ in their toxicity [3]. For a further investigation on the transportation mechanisms and potential toxic properties of phenylarsenic compounds, development of selective and sensitive methods for the analysis of different phenylarsenic compounds is of great importance.

Phenylarsenic compounds are nonvolatile and thermally stable, and could exist as charged compounds under certain pH conditions. Capillary electrophoresis (CE), which can rapidly separate the charged compounds based on their electrophoretic mobility within an electric field, is thus appropriate and has been applied for the analysis of various arsenic species including phenylarsenic compounds [4,5]. Application of CE to arsenic species analysis possesses several advantages such as nanoliters sample consumption, low running costs, and rapid separation. However, because of some general difficulties, CE is still limited in practice use. For instance, the concentration sensitivity of CE with UV detection is relatively low due to the short light path and small injection volume; sample matrix in real samples may cause irreversible adsorption onto the capillary wall, and lead to change of the migration times of analytes during CE analysis [5]. To overcome these limitations, application of sample pretreatment methods and on-column preconcentration techniques in CE to clean up sample matrix or enrich the target analytes are both very effective ways.

* Corresponding author. Tel.: +86 27 68752162; fax: +86 27 68754067.
E-mail address: binhu@whu.edu.cn (B. Hu).

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doi:10.1016/j.chroma.2011.05.058

Solid phase microextraction (SPME) [2,6–8], stir bar sorptive extraction (SBSE) [9] and liquid phase microextraction (LPME) [10,11] are simple, selective, time-efficient and relatively inexpensive miniaturized sample pretreatment preparation methods. Compared to SPME and SBSE, LPME has an additional advantage of no carryover effect. Given arsenic's affinity for forming strong bonds with sulfur, arsenic derivatization using various mercaptans, such as thioglycol methylate (TGM) [7,8] and 1,3-propanedithiol (PDT) [2] as derivatizing agent combined with SPME has been developed for the extraction of polar organoarsenical compounds including roxarsone, phenylarsonic acid (PAA), dimethylarsinic acid (DMA), and so on. However, derivatization procedure before SPME is time-consuming, tedious [2], and would perhaps lead to loss or transformation of arsenic species. Concerning the extraction of arsenic species by SBSE, only one research work was reported [9]. In Ref. [9], titania immobilized polypropylene hollow fiber as an extraction coating of SBSE was prepared to selectively extract phenylarsenic compounds and their possible transformation products. While LPME is widely applied for separation and determination of organic compounds [10–17], its application in extraction of inorganic analytes is only growing recently [18–21]. For the extraction of inorganic arsenite (As(III)) species, complexation with different chelating reagents such as ammonium pyrrolidine dithiocarbamate (APDC) [19,20] is commonly necessary to facilitate the transfer of target species into the organic phase in LPME. Hylton and Mitra [22] developed a microfluidic hollow fiber membrane extraction method for direct extraction of arsenic (V) by using alkyl phosphate as organic solvent. To the best of our knowledge, there is no report on the application of LPME for extraction of organic arsenic species including phenylarsenic compounds until the present time.

Besides the sample pretreatment techniques, an alternative way to improve the detection sensitivity in CE is to perform on-column preconcentration techniques, which are simple, versatile, and not necessary to modify the commercial instruments. To date, there have been four main kinds of on-column sample concentration techniques: sample stacking, sweeping, transient isotachophoresis, and dynamic pH junction [23]. Among them, sample stacking is the most experimentally simple approach, and can be applied in different formats, including normal stacking (NSM), large volume sample stacking (LVSS), field amplified sample injection (FASI), and selective exhaustive injection (SEI). In SEI, ionic analytes are electrokinetically injected and selectively focused by sample stacking for a much longer time than common FASI. Prolonged FASI can deplete the sample solution of analytes in the inlet vial, which is said to be exhaustive. As a result, higher enrichment factors could be expected in SEI than that obtained in common FASI. SEI can be divided into two categories including anionic selective exhaustive injection (ASEI) and cationic selective exhaustive injection (CSEI). In order to perform the sample stacking effectively, SEI requires the sample prepared in a very dilute solution or in a low-conductivity matrix. In addition, it was usually performed under suppressed electroosmotic flow (EOF), either by working under low-pH conditions [24] or by using a modified or coated capillary [25]. However, capillary coating method needs to be carefully optimized to ensure the reproducible analytical results; while under low-pH condition, a very long equilibration time is usually employed to equilibrate the inner wall of separation capillary in order to overcome the inefficient buffer capacity of low-pH buffer. Up to now, NSM [26], LVSS [26,27], and dynamic pH junction techniques [23] have been explored to on-column concentrate different arsenic species. Sun et al. [26] compared two different sample stacking methods including NSM and LVSS for sensitivity enhancement of eight arsenic species, and the results indicated that LVSS gave the lower LODs. Dynamic pH junction was investigated by Jaafar et al. [23], and an improvement in detection sensitivity by a factor of 100–800 were reported for roxarsone and its possible biotransformation species.

The purpose of this work is to develop a sensitive method by combining a suitable off-line LPME method with an efficient on-column stacking technique for the analysis of five phenylarsenic compounds in real samples by CE with UV detection. Based on passive diffusion by pH gradient and selective affinity between phenylarsenic compounds and tributyl phosphate, target phenylarsenic compounds were successfully extracted by a three phase-LPME mode named hollow fiber based liquid liquid liquid microextraction (HF-LLLME). Meanwhile, a new simple ASEI procedure was proposed as an on-column stacking technique to further improve the LODs of CE/UV for the analysis of target analytes. By integration of the good cleanup ability of HF-LLLME and high sensitivity enhancement of ASEI, a novel HF-LLLME–ASEI-CE/UV method was developed for the determination of five phenylarsenic compounds in pig feed from a local pig farm, storage pig litter, soil in agricultural field, and lake water collected near this pig farm as well.

2. Experimental

2.1. Reagents and materials

Table 1 shows the chemical structures of the target phenylarsenic compounds as well as some of their chemical properties. Phenylarsonic acid (PAA, 97%) and 4-aminophenylarsonic acid (4-APAA, 98%) was supplied by Alfar Aesar (Ward Hill, MA, USA) and Sigma–Aldrich (St. Louis, MO, USA), respectively. 4-Hydroxyphenylarsonic acid (4-HPAA, 98%) and 3-nitro-4-hydroxyphenylarsonic acid (NHPAA, 98%) were bought from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). 4-Nitrophenylarsonic acid (4-NPAA) was obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). Each stock solution of the phenylarsenic compounds was prepared in high purity water at a concentration of 1000 mg/L, and stored at 4 °C in refrigerator. Tributyl phosphate (TBP, 98.0%) was purchased from China Medicine Group, Sinopharmic Chemical Reagent Company (Shanghai, China). Other reagents used were of analytical reagent grade. High purity water obtained by a Milli-Q water purification system (18.2 MΩ cm, Millipore, Bedford, MA, USA) was used throughout the whole experiment.

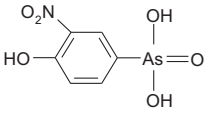
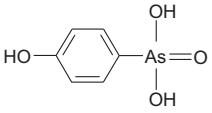
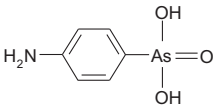
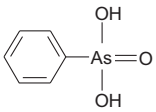
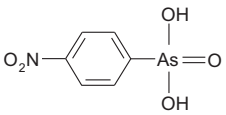
2.2. Instruments

All separation experiments were performed by Agilent 3D CE system (California, CA, USA) equipped with programmable, multiwavelength UV/visible detector. Fused-silica capillary of dimensions 48.5 cm (40 cm to the detector) × 50 μm i.d. × 360 μm o.d. (Yongnian Optical Fiber, Hebei, China) was used, and the capillary temperature was maintained at 25 °C. The detection wavelength used was 210 nm. A modified CE automatic sample vial as described in Ref. [28] was used for CE sample injection. Back ground electrolyte (BGE) of 25 mmol/L carbonate buffer (pH 9.75) was prepared by diluting the stock solutions of 200 mmol/L $NaHCO_3$ and 200 mmol/L Na_2CO_3 in a ratio of 1:1 (v/v). Prior to use, BGE was filtered through a 0.45 μm syringe filter and then ultrasonic degassed. In standard injection mode, the sample solution was injected hydrodynamically at 50 mbar for 5 s. Prior to use, the capillary was pretreated by flushing it sequentially, for 10 min each time, with 1 mol/L NaOH, high purity water, and the BGE. Between runs, the capillary was rinsed with BGE for 4 min.

2.3. HF-LLLME procedure

Referring to our previous work [28], HF-LLLME was carried out by U-shaped configuration. The 5 mL sample solution

Table 1
The names and structures of phenylarsenic compounds.

Name	Structures	pK_a [5]	Log $K_{O/W}$ [34]	WS[a] (mg/L) [34]
NHPAA roxarsone		3.9, 8.4, 10.1	−0.05	2.218e+004
4-HPAA (4-hydroxyphenylarsonic acid)		3.9	−0.45	3.234e+005
4-APAA (4-aminophenylarsonic acid)		2.0, 4.0, 8.6	−0.88	2.032e+005
PAA (phenylarsonic acid)		3.6, 8.8	0.06	3.796e+004
4-NPAA (4-nitrophenylarsonic acid)		3	−0.15	1.344e+004

[a] WS: water solubility.

adjusted to pH 2.15 by 2 mol/L H_2SO_4 was filled into a 7 mL vial. Eighteen microliters of acceptor solution (0.8 mmol/L tris(hydroxymethyl)aminomethane (Tris)) was injected into a 7.5 cm piece of Q3/2 Accurel polypropylene hollow fiber (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 0.2 μm. The hollow fiber, fixed on the end needles of two commercially conventional 10 μL microsyringes (Gaoge, Shanghai, China), was subsequently dipped in TBP solvent for 20 s, and then placed immediately into the sample solution. The sample solution was stirring at 1000 rpm for 25 min using an 85-2A constant temperature magnetic stirrer (Ronghua, Jiangsu, China). After extraction, the acceptor solution was withdrawn back into the syringes, and finally injected into the modified CE sample vial for CE analysis.

2.4. On-column preconcentration procedures

The combination of off-line preconcentration process with on-column preconcentration technique could further improve the detection sensitivity of CE-UV for the target analytes. Therefore, LVSS [26] and dynamic pH junction [23] were firstly tested for further enhancing the detection sensitivity of the five target phenylarsenic compounds after HF-LLLME procedures in this work. The extractant obtained by HF-LLLME was directly carried out for LVSS or dynamic pH junction analysis. Unfortunately, the separation and focusing of target analytes by dynamic pH junction were not successful by adjustment of BGE conditions such as pH and ion strength in our preliminary experiments, while LVSS could be feasible. The LVSS procedures for the analysis of five phenylarsenic compounds were optimized as follows: the separation capillary was firstly filled with BGE; after the standard solution was hydrodynamically injected into the capillary at 50 mbar for 90 s, a negative voltage (−5 kV) was applied to remove the sample matrix (0.8 mmol/L Tris solution as the acceptor solution in HF-LLLME). When the current reached 95% of the initial current value that was obtained by filling the capillary thoroughly with BGE at −5 kV, the applied voltage was switched to positive voltage (+30 kV) to start the subsequent separation.

Besides LVSS and dynamic pH junction, a new ASEI strategy was also proposed and adopted for effectively focusing of target analytes in this work. The main steps of proposed ASEI are illustrated in Fig. 1. After filling the capillary with BGE, a long water plug (91% length of separation capillary) was hydrodynamically injected into the capillary (Fig. 1A). Then, negative voltage (−10 kV) was applied with positive electrode at the capillary outlet, and the acceptor solution obtained by HF-LLLME was electrokinetically injected into the capillary at −10 kV for a long period of time (180 s) (Fig. 1B). Under the electric field, the target analytes anions moved rapidly into the capillary through the water plug and stacked at the boundary between the water plug and BGE zones. Once the anions reached the boundary, their velocities slowed down because the electric field strength decreased in the BGE zones. Meanwhile, the water plug was expelled from the inlet of the separation capillary (Fig. 1C).

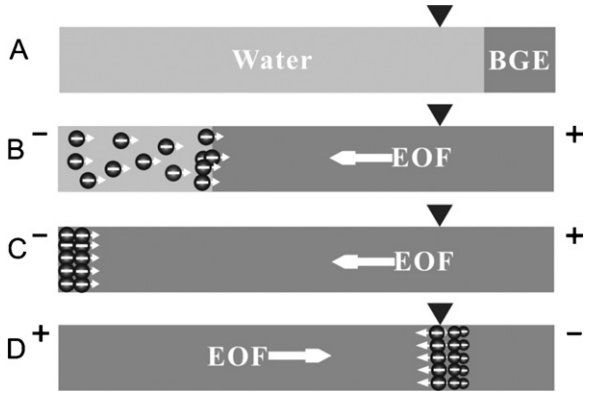


Fig. 1. Schematic illustration of the sample stacking mechanism of ASEI-CE/UV. ⊖ represents negative analyte.

When the current reached 95% of the initial current value that was obtained by filling the capillary thoroughly with the BGE at −10 kV, applied voltage was switched to positive potential (+30 kV) to start the subsequent separation (Fig. 1D).

2.5. Sample pretreatment

Pig feed sample, which was originally purchased from market, was obtained from a local pig farm (Wuhan, China). The storage pig litter, soil in agricultural field, and lake water were collected near this pig farm. The collected pig litter sample and soil sample were dried at 40 °C and ground to powder.

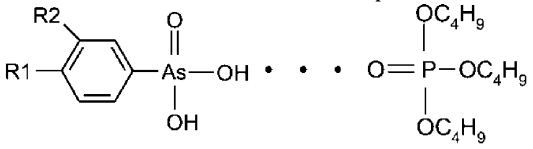
Because the target phenylarsenic species are water extractable, a water leaching procedure described by Rosal et al. [29] was employed. Briefly, 250 mg dried pig feed powder, 1.0 g storage pig litter sample and 1.0 g soil sample were weighed, respectively. Each weighed sample was suspended in 15 mL purified water, ultrasonicated for 1 h, and then centrifuged at 4000 rpm for 40 min. After that, the supernatant was filtered through a 0.45 μm nylon membrane and transferred into a 25 mL flask. The residue was extracted again as described above. The two supernatant portions were combined. After adding 2 g NaCl and adjusting to pH 2.15 by 2 mol/L H_2SO_4, the combined supernatant was diluted to 25 mL with high purity water.

The lake water sample with addition of appropriate amount of NaCl was adjusted to pH 2.15 using 2 mol/L H_2SO_4 and directly subjected to HF-LLLME process without filtration.

3. Results and discussion

3.1. Optimization of HF-LLLME conditions

In acidic solutions, the complexations of inorganic arsenic (V) with tributyl phosphate (TBP) and other esters of phosphoric acids such as dibutyl butylphosphonate, trioctylphosphine oxide (Cyanex 921) were known to be selective [22,30,31]. Phenylarsenic compounds are organic species of arsenic (V), in which one –OH group was substituted by different phenyl group, and they should have the similar complexation with TBP. Considering the properties and the structures of both target analytes and TBP, the formation of phenylarsenic compounds–TBP complexes were probably based on the hydrogen bonding and the hydrophobic interaction, and their structures could be expressed as:



In this study, the possibility of extraction of phenylarsenic compounds from acidic media by HF-LLLME using TBP as extraction solvent was investigated. Similar to extraction of inorganic As(V) with TBP, the transport mechanism of the phenylarsenic compounds through the hollow fiber could be schematically illustrated in Fig. 2. Transportation of phenylarsenic compounds results from the pH gradient between donor phase and acceptor phase. In donor phase, phenylarsenic compounds were existed as undissociated species in acidic media, and could form reversible complexes with TBP. Therefore, they were successfully extracted from donor phase into TBP solvent. Then, an alkaline solution was selected as acceptor phase. As a result, target analytes were back-extracted from TBP solvent into the acceptor solution, re-ionized, and concentrated.

The factors affecting on HF-LLLME, such as sample pH, acceptor phase concentration, stirring rate, ion strength, and extraction time were optimized in sequence.

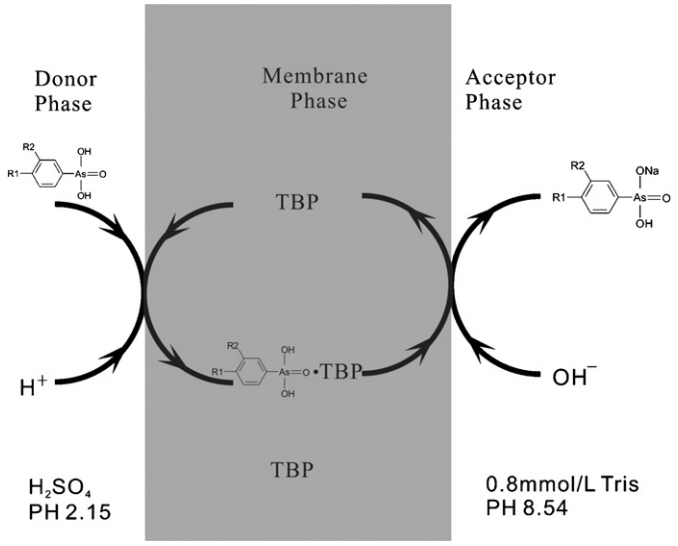


Fig. 2. Schematic representation of phenylarsenic compounds in HF-LLLME using TBP as extraction solvent. R1 = NH_2, OH, NO_3, H; R2 = NO_2.

3.1.1. Effect of acidic medium and sample pH

As mentioned above, pH gradient is the driving force in the transportation of five phenylarsenic compounds from the donor phase to the organic phase immobilized in the pores of hollow fiber in HF-LLLME. Therefore, the effect of different kinds of acidic media such as HCl, HNO_3, H_3PO_4 and H_2SO_4 at the same pH value on HF-LLLME was investigated, and the experimental results in Fig. 3 indicated that H_2SO_4 is slightly better than other acids. According to Ref. [32], inorganic arsenic (V) was found to be extracted into TBP with a simultaneous co-extraction of sulfuric acid by liquid liquid extraction (LLE). Similarly, in this study sulfuric acid in donor phase may not only provide an acidic media to convert the target phenylarsenic compounds to nonionic forms, but also participate in the extraction process by co-extraction with target phenylarsenic analytes as described in Eq. (1).

With H_2SO_4 as donor phase media, the effect of sample pH on the extraction efficiency of HF-LLLME was subsequently studied in the pH range of 1.65–3.10, and the results were shown in Fig. 4. It can be seen that the maximum extraction efficiency was obtained at pH 2.15 for all the target phenylarsenic compounds. As a result, pH 2.15 was selected as the optimal sample pH for the simultaneous

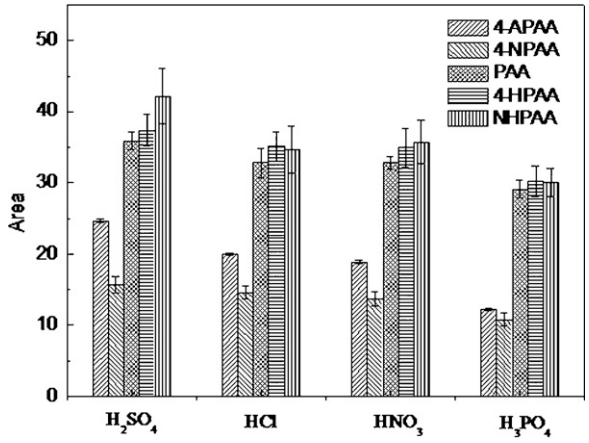


Fig. 3. Effect of different kinds of acid as medium of donor phase. Conditions: analytes concentration, 200 μg/L; sample pH was adjusted to 2.15 with 2 mol/L selected acid; organic solvent, TBP; stirring rate, 1000 rpm; acceptor phase, 18 μL 0.8 mmol/L Tris; extraction time, 15 min; NaCl (m/v), 8%.

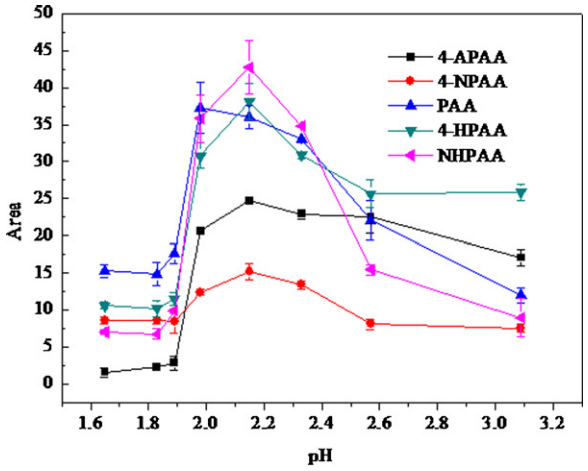


Fig. 4. Effect of sample pH on HF-LLLME. Conditions: analytes concentration, 200 μg/L; organic solvent, TBP; stirring rate, 1000 rpm; acceptor solution, acceptor phase, 18 μL 0.69 mmol/L Tris; extraction time 15 min; NaCl (m/v), 8%.

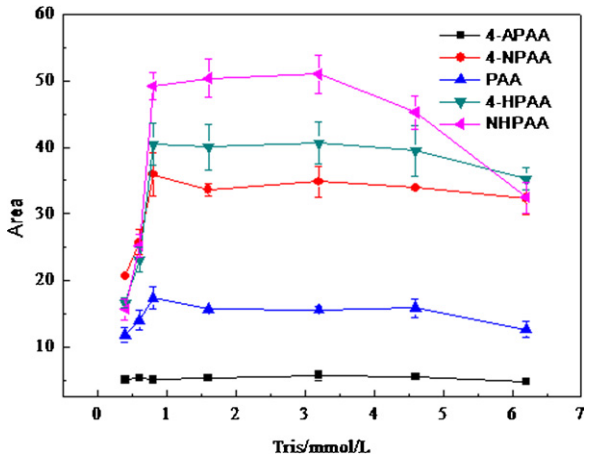
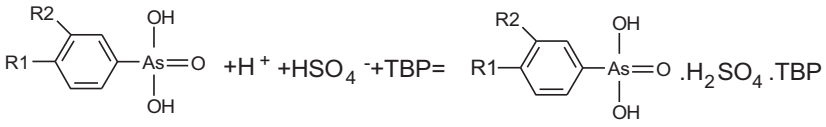


Fig. 5. Effect of the concentration of Tris in acceptor solution on HF-LLLME. Conditions: analytes concentration, 200 μg/L; sample solution, pH 2.15; organic solvent, TBP; stirring rate, 1000 rpm; extraction time, 15 min; NaCl (m/v), 8%.

extraction of five target phenylarsenic compounds by HF-LLLME in this work.



(1)

where R1 represented $-NH_2$, $-OH$, $-NO_3$, $-H$, and R2 represented $-NO_2$ and $-H$.

3.1.2. Effect of the concentration of acceptor solution

The acceptor solution in HF-LLLME would be finally subjected to ASEI analysis in the proposed HF-LLLME–ASEI-CE/UV method. Considering the extraction efficiency in HF-LLLME and compatibility with the subsequent ASEI system, acceptor solution should provide both high extraction efficiency for the target phenylarsenic compounds in HF-LLLME and low conductivity required in ASEI. For this purpose, commonly used pH-buffers are not appropriate. In our preliminary experiments, different alkaline solutions including NaOH, Na_2CO_3, Tris and $NH_3{\cdot}H_2O$ have been investigated as the acceptor solution. It was found that no ASEI could be obtained when NaOH and Na_2CO_3 were employed as the acceptor phase, probably due to their high conductivity. ASEI could be obtained when Tris or $NH_3{\cdot}H_2O$ was utilized as the acceptor phase, while the extraction efficiency obtained by Tris was better than that obtained by $NH_3{\cdot}H_2O$. In subsequent experiment, Tris solution without pH adjustment was employed as acceptor solution in this work.

The effect of Tris concentration on HF-LLLME was evaluated, and the dependence of extraction efficiencies on the Tris concentration varying from 0.4 to 6.2 mmol/L are shown in Fig. 5. It can be seen that the extraction efficiency was increased along with the Tris concentration increasing from 0.4 mmol/L to 0.8 mmol/L, and no obvious increasing of the extraction efficiencies were observed with further increase of the Tris concentration. Considering the higher conductivity of Tris solution within 1.6–3.2 mmol/L, 0.8 mmol/L Tris solution was employed as the acceptor solution for the subsequent experiment, despite slightly better extraction efficiencies obtained in the Tris concentration range of 1.6–3.2 mmol/L over that in 0.8 mmol/L.

3.1.3. Effect of NaCl and the stirring rate

Salt-out effect is widely used in different LPME modes to increase the extraction efficiency. Hydration spheres around the salt ions formed by water molecules could reduce the water concentration to dissolve the phenylarsenic compounds and be beneficial to extract phenylarsenic compounds into TBP solvent. Therefore, the effect of NaCl concentration up to 16% (m/v) in donor phase on the extraction of five target phenylarsenic compounds was examined and the results are shown in Fig. 6. As can be seen, the peak areas of 4-NPAA, 4-HPAA and NHPAA were increased with NaCl concentration increasing from 0% to 6% (m/v), and kept almost constant in the range of 6–12% (m/v), then decreased with the further increase of NaCl concentration up to 16% (m/v). The peak area of PAA kept increasing with the increase of salt content from 0 to 16% (m/v), while the effect of NaCl on CE peak area responses of 4-APAA was not significant. From a comprehensive view, the concentration of NaCl was adjusted to be 8% (m/v) in the donor phase.

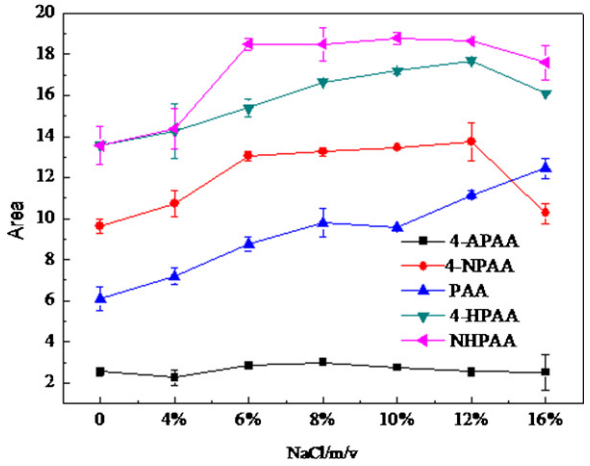


Fig. 6. Effect of NaCl concentration on HF-LLLME. Conditions: analytes concentration, 100 μg/L; sample solution, pH 2.15; organic solvent, TBP; stirring rate, 1000 rpm; acceptor phase, 18 μL 0.8 mmol/L Tris; extraction time, 15 min.

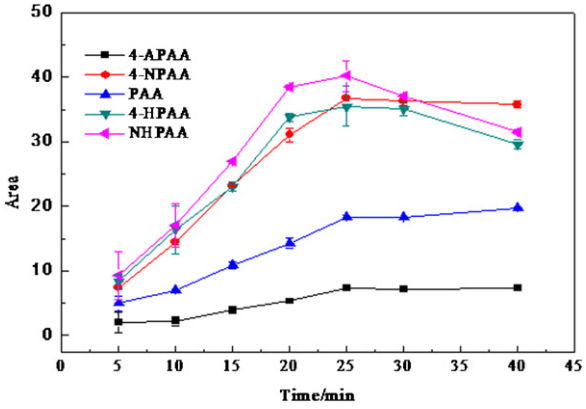


Fig. 7. Effect of extraction time on HF-LLLME. Conditions: analytes concentration, 200 μg/L; sample solution, pH 2.15; organic solvent, TBP; stirring rate, 1000 rpm; acceptor phase, 18 μL 0.69 mmol/L Tris; NaCl (m/v), 8%.

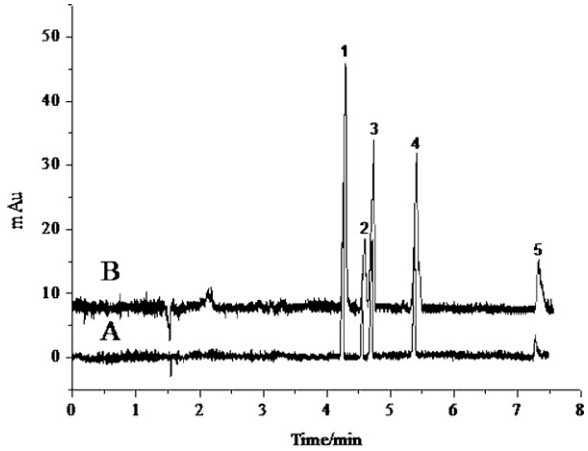


Fig. 8. Electropherograms of phenylarsenic compounds obtained by (A) standard injection at 50 mbar for 5 s; (B) ASEI at −10 kV for 180 s. Analytes: 1, 4-APAA; 2, 4-NPAA; 3, PAA; 4, 4-HPAA; 5, NHPAA. Analytes concentrations: (A) 20 mg/L 4-APAA, 20 mg/L 4-NPAA, 20 mg/L PAA, 20 mg/L 4-HPAA, 10 mg/L NHPAA, respectively; (B) 200 μg/L 4-APAA, 100 μg/L 4-NPAA, 200 μg/L PAA, 200 μg/L 4-HPAA, 50 μg/L NHPAA, respectively.

Generally, agitation can speed up the transfer of analytes from donor phase to organic solvent in HF-LLLME. However, in this study, investigation on the effect of agitation speed in the range of 600–1600 rpm showed no significant effect of stirring rate on CE peak area responses of target analytes. Finally, stirring rate at 1000 rpm was selected due to the good reproducibility.

3.1.4. Effect of extraction time

HF-LLLME is a nonexhaustive extraction approach, and the extraction efficiency is governed by partitioning of the analyte between donor phase and the immobilized organic solvent and by partitioning between the acceptor phase and the immobilized organic solvent. Generally, when the extraction is at the partitioning equilibrium, the maximum extraction efficiency would be attained. Because the extraction equilibrium of HF-LLLME is time-dependent, the effect of extraction time was examined in the range of 5–40 min in this work. As illustrated in Fig. 7, the peak areas of target analytes were increased rapidly with the increase of extraction time to 25 min. With the extraction time longer than 25 min, the analytical signals kept almost constant. It is implied that the extraction has reached the equilibrium at 25 min. To obtain high extraction efficiency, an extraction time of 25 min was selected.

3.2. Optimization of ASEI

In this experiment, a new strategy was utilized to realize ASEI stacking of phenylarsenic compounds. By introduction of a large plug of water into the separation capillary before sample injection, the electrokinetically injection time of ASEI was prolonged and thus a highly stacking efficiency of target analytes could be achieved. In order to obtain high sensitivity, good resolution and short analysis time for the target analytes in ASEI, parameters such as applied voltage, the length of water plug, and injection time were investigated.

Firstly, the effect of injection voltage on ASEI stacking efficiency was investigated with the injection voltage varying between −4 and −20 kV. When the injection voltage higher than −15 kV was applied, the electric current was observed to frequently drop to zero. This was probably caused by excessive Joule heating generated in the low-conductivity water plug during ASEI stacking. When the injection voltage was lower than −7 kV, a much longer injection time was required. Besides, peaks broaden and deterioration in detection sensitivity was observed. This phenomenon could be explained by band dispersion of the already stacked sharp sample zone during the long time sample injection process. Taking into consideration of above factors, an injection voltage of ASEI was selected at −10 kV, which gave rise to an efficient sample stacking and stable injection current during the whole CE analysis.

Pre-injection of a water plug before the sample injection has been proposed in FASI to increase sample stacking efficiency by providing proper electric field enhancement [24,33]. Usually, the length of water plug is kept short to achieve maximal analyte enrichment and best sensitivity. However, in our study, an almost full capillary volume of water plug pre-injected before sample introduction was found to be feasible to prolong the maximum allowable injection time of ASEI and increase the stacking efficiency accordingly. The maximum allowable injection time under a fixed injection voltage (−10 kV) was directly dependent on the length of the introduced water plug. Studies on the effect of the water plug length/injection time pairs of 20 cm/70 s, 28 cm/100 s, 36 cm/150 s and 44 cm/180 s on the CE peak area responses of target analytes indicated that the peak area increased with the increase of water plug length/injection time pairs. As a result, 44 cm water plug (91% length of separation capillary) was selected to obtain the maximum CE signal response without deterioration of separation resolution.

Additionally, like other stacking techniques with polarity switching, it is very essential to monitor the current variation very carefully during the sample electrokinetic injection in ASEI for a good reproducibility. At the beginning of sample injection, a large plug of water exists in capillary, the electric current increases slowly from 1 μA to 3 μA in 170 s. When most of water was expelled out of the capillary inlet by EOF, the electric current increases rapidly to a top value of 18 μA at −10 kV only in 10 s. At this time, the current should be carefully monitored, and once the current reached 95% of the top current value, applied voltage should be switched immediately to positive potential (+30 kV) to start the subsequent separation. Otherwise, some target anionic analytes may be lost.

Fig. 8(A) and (B) were electropherograms obtained by standard sample injection at 50 mbar for 5 s and by ASEI under the optimized ASEI conditions, respectively. It can be seen that even though the separation resolutions of target phenylarsenic compounds obtained by ASEI (Fig. 8(B)) are slightly worse than that obtained by standard sample injection (Fig. 8(A)), the five target analytes still could be baseline separated by ASEI.

Table 2
Comparison of the analytical performance obtained by LVSS-CE/UV and ASEI-CE/UV for the determination of phenylarsenic compounds.

Analytes	Linear range (μg/L)		R^2		RSD[a] (n = 5)/%			LOD (μg/L)		EF	
	LVSS	ASEI	LVSS	ASEI	LVSS	ASEI	SI[b]	LVSS	ASEI	LVSS	ASEI
4-APAA	150–3000	25–1000	0.9995	0.9995	4.26	6.39	1070	24	5.08	45	211
4-NPAA	150–3000	25–1000	0.997	0.9997	6.28	7.85	2080	41.6	8.82	50	236
PAA	150–3000	25–1000	0.9994	0.9977	7.63	4.61	940	26.6	8.57	35	110
4-HPAA	150–3000	25–1000	0.9994	0.9961	5.55	7.31	610	20.8	5.45	29	112
NHPAA	150–3000	25–1000	0.9986	0.9968	4.16	9.53	1210	38.5	6.12	31	198

[a] RSD: analytes concentration, 400 μg/L (as As) of 4-APAA, 4-NPAA, PAA, 4-HPAA, NHPAA for LVSS, respectively; and 100 μg/L of 4-APAA, 4-NPAA, PAA, 4-HPAA, NHPAA for ASEI. RSD was calculated by corrected peak (peak area/migration time).
[b] SI: standard injection, 50 mbar × 5 s.

Table 3
Analytical performance of HF-LLLME–ASEI–CE/UV for phenylarsenic compounds.

Analytes	Linear range (μg/L)	Linear equation	R^2	RSD[a]/% (n = 5)	LOD (μg/L)	EF
4-APAA	25–2000	$y = 0.283x + 2.279$	0.9945	8.3	6.9	155
4-NPAA	5–400	$y = 1.181x + 2.801$	0.9989	9.5	1.56	1333
PAA	20–800	$y = 0.437x + 1.909$	0.9986	11.8	2.96	317
4-HPAA	5–400	$y = 0.880x + 1.240$	0.9988	5.6	1.11	549
NHPAA	2.5–200	$y = 2.130x - 0.587$	0.9992	10.2	0.68	1779

[a] RSD: analytes concentration, 4-APAA 50 μg/L, 4-NPAA 10 μg/L, PAA 20 μg/L, 4-HPAA 10 μg/L, NHPAA, 5 μg/L.

3.3. Comparison of analytical performance of ASEI-CE/UV and LVSS-CE/UV

In order to evaluate the effectiveness of the ASEI technique, the performance and reliability of ASEI-CE/UV was investigated and critically compared with that obtained by LVSS-CE/UV. Table 2 was the analytical performance of ASEI-CE/UV for the analysis of five target phenylarsenic compounds and its comparison with LVSS-CE/UV. As can be seen, good correlation coefficients (R^2) ranging from 0.9961 to 0.9997 for ASEI and from 0.9970 to 0.9995 for LVSS were achieved, respectively, over the studied concentration range. The reproducibility, expressed by relative standard deviations (RSDs) of corrected peak areas which were obtained by five consecutive repeated injections, were below 9.53%, and 7.63% for ASEI-CE/UV and LVSS-CE/UV, respectively. Obviously, there was no significant difference in RSDs between the two techniques, although the reproducibility of LVSS-CE/UV was a little bit better than that of ASEI-CE/UV. The LODs, based on an S/N of 3, were 5.08–8.82 μg/L for ASEI-CE/UV and 20.8–41.6 μg/L for LVSS-CE/UV, respectively. The sensitivity enhancement factor (EF) was evaluated by EF_{LODs}, which is defined as the ratio of LODs obtained by standard injection (at 50 mbar for 5 s) CE/UV to that obtained by ASEI-CE/UV or LVSS-CE/UV. The obtained EFs were 110–136 and 29–50-fold for ASEI-CE/UV and LVSS-CE/UV, respectively, indicating that ASEI offered the higher enhancement in sensitivity in comparison with LVSS.

In conclusion, ASEI as an on-column preconcentration method for the improvement of detection sensitivity of target phenylarsenic compounds is simple, reliable and more effective than LVSS.

3.4. Analytical performance of HF-LLLME–ASEI-CE/UV

For HF-LLLME–ASEI-CE/UV analysis of five phenylarsenic compounds, the extraction process was operated as described in the section of HF-LLLME procedure. The post-extraction phase (acceptor phase) of HF-LLLME was directly injected into capillary for further ASEI-CE/UV analysis. All the validation data of HF-LLLME–ASEI-CE/UV method are shown in Table 3. As could be seen, good linearity with correlation coefficients ranging from 0.9945 to 0.9992 was obtained for the five target phenylarsenic compounds. The precision of this method was determined by analyzing standard solution at the same concentration for five times continuously, and the RSDs were 5.6–11.8%. The LODs based on an S/N of 3 were in the range of 0.68–6.9 μg/L. In comparison to standard injection mode (at 50 mbar for 5 s), EFs ranging from 155 to 1780-fold were achieved.

Table 4 is the comparison of analytical performance of the present method to that obtained by several other approaches for phenylarsenic compounds analysis. As could be seen, the LODs obtained by the proposed method are lower than those reported in Refs. [26,29], and comparable with that in Ref. [23]. It is worth being mentioned that the proposed method using an inexpensive commercial UV detector could provide lower LODs than that obtained in Ref. [29], which employed an expensive, sensitive ICP/MS detector.

3.5. Sample analysis

To evaluate the practical applicability of the HF-LLLME–ASEI-CE/UV method, the developed system was applied to the analysis

Table 4
Comparison of detection limits for phenylarsenic compounds found in the literatures following different analytical approaches.

Analytes	Method	Detection	LOD (μg/L)	EF	Sample	Ref.
NHPAA, As(III), As(V), MMA, DMA, PAA	Dynamic pH junction	CE/UV	0.34–1.93	100–800	Chicken litter[a]	[23]
NHPAA, 4-NPAA, 3-APAA, As(III), As(V), MMA, DMA, PAA	LVSS Co-EOF NSM	CE/UV	4–100 18.1–573	34–62 12–16	Water	[26]
NHPAA, AHPAA, HPAA, MMA, DMA, As(III), As(V)		CE-ICP/MS	1–3		Chicken manure	[29]
4-APAA, 4-NPAA, PAA, 4-HPAA, NHPAA	HF-LLLME–ASEI	CE/UV	0.68–1.9	155–1779	Water, feed, pig litter, soil	This work

PAO: phenylarsine oxide; AHPAA: 3-amino-4-hydroxyphenylarsonic acid.
[a] Clean up with C_{18} cartridge, recovery for NHPAA obtained was 71.9%.

Table 5
The concentrations of phenylarsenic compounds in environmental samples detected by HF-LLLME–ASEI-CE/UV ($n = 3$).

Analytes	Pig feed		Lake water		Pig litter		Soil	
	Found (ng/g)	Recovery[a] (%)	Found (μg/L)	Recovery[a] (%)	Found (ng/g)	Recovery[a] (%)	Found (ng/g)	Recovery[a] (%)
4-APAA	N.D.[b]	85.9	N.D.	107.8	N.D.	66.7	N.D.	38.9
4-NPAA	N.D.	99.8	N.D.	102.6	N.D.	78.4	N.D.	28.9
PAA	N.D.	88.7	N.D.	86.9	N.D.	67.9	N.D.	40.5
4-HPAA	N.D.	96.9	N.D.	95.7	N.D.	87.9	N.D.	36.5
NHPAA	337.2 ± 39.5	104.5	N.D.	102.5	N.D.	96.2	N.D.	46.9

[a] Recovery, 100 μg/L 4-APAA, 25 μg/L 4-NPAA, 50 μg/L PAA, 50 μg/L 4-HPAA, 25 μg/L NHPAA (as As) were spiked in the sample, respectively. Recovery = (the amount found in the spiked sample − the amount found in the sample) × 100%/the amount added.
[b] N.D., not detected.

of five phenylarsenic compounds in different samples including pig feed from a local pig farm, and storage pig litter, soil in agricultural field, and lake water collected near this pig farm as well. The recoveries of the HF-LLLME–ASEI-CE/UV method were determined with the addition of the standard solution of target analytes into the real sample solution after sample leaching. Peaks were first identified by migration time, and their identity was subsequently confirmed by spiking experiments. Table 5 listed the analytical results of five target phenylarsenic compounds in pig feed, storage pig litter, soil and lake water obtained by HF-LLLME–ASEI-CE/UV. As could be seen, only NHPAA (roxarsone) was found at a level of 337.2 ± 39.5 ng/g in the pig feed sample, and other four phenylarsenic species were not detected in this sample. The determined value of roxarsone in the feed sample is lower than the maximum allowed level recommended by the Food and Drug Administration of USA (FDA) (50 mg/kg roxarsone) [4]. Recovery for the spiked pig feed sample was in the range of 85.9–104.5% for the five phenylarsenic compounds. Fig. 9 shows the electropherograms of the pig feed and the spiked pig feed samples.

No target phenylarsenic compounds were found in the other samples including storage pig litter, soil in agricultural field, and lake water collected near this pig farm. The results indicated that the area near this pig farm is not contaminated by phenylarsenic compounds. However, because the phenylarsenic compounds may ultimately decompose to produce inorganic arsenate during storage of poultry litter and after land application to soil, it is promising to develop a method for simultaneous determination of phenylarsenic and inorganic arsenate species in environmental samples in our further work. For the spiking experiments, the recoveries of 86.9–107.8% were obtained for all phenylarsenic species in lake water sample. The recovery in pig litter sample was found to be 96.2% for NHPAA, 87.9% for HPAA, 78.4% for NPAA, 67.9% for PAA and 66.7% for APAA, respectively. The recovery for the spiked soil sample was not satisfactory, very low recoveries of less than 50% for all five phenylarsenic compounds were obtained. The electropherograms with a very noisy background trace were found for the soil samples (the electropherograms were not given). The reasons could be attributed to the following two aspects: one is the very complex matrix in water leaching solution of soil, and the other is the very low detection wavelength of 210 nm employed in CE/UV. To the best of our knowledge, there is no published work applying HF-LLLME for enrichment of target analytes from soils until now. It is presumable that the extraction of target analytes by HFLLLME could be a problem for soil matrix.

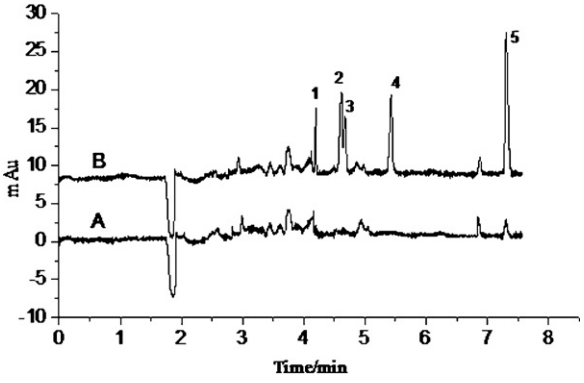


Fig. 9. Electropherograms of phenylarsenic compounds obtained by HF-LLLME–ASEI-CE/UV in (A) the pig feed sample; (B) the spiked pig feed sample. Analytes: 1, 4-APAA; 2, 4-NPAA; 3, PAA; 4, 4-HPAA; 5, NHPAA.

The above real samples analysis results indicated that the proposed HF-LLLME–ASEI-CE/UV method can be reliably adopted for the analysis of five phenarsenic compounds in the pig feed and lake water with satisfactory recoveries, but is difficult in analysis of the storage pig litter, and especially soil sample due to the very complex matrix. Considering a non-specific UV detector was employed for the determination of target analytes in this work, it is reasonable to believe that if the developed HF-LLLME–ASEI-CE system was combined with an element-specific detector (e.g. ICP/MS), it will have great potentiality to quantitatively analyze phenylarsenic species in pig litter and soil samples. Further studies are under way to explore this potentiality in our laboratory.

4. Conclusions

In the present study, a HF-LLLME system based on passive diffusion by pH gradient was established for the extraction of five phenylarsenic compounds from sulfuric acid media by using TBP as a selective extraction solvent. To further improve the determination sensitivity in CE/UV, a simple ASEI strategy without suppression of EOF was applied for on-column enriching target analytes. In ASEI, a very large plug of water was introduced before sample injection, thus the sample can be injected for a long time of 180 s at −10 kV, and a high enhancement factors up to 236-fold were obtained without HF-LLLME procedure. Owning to the compatibility of extractant obtained by HF-LLLME to ASEI, a new HF-LLLME–ASEI-CE/UV method for the analysis of phenylarsenic compounds was successfully established and applied to the analysis of target analytes in different samples. The spiking experiments demonstrated that the satisfactory recoveries for all five phenylarsenic species could be obtained by the analysis of pig feed and lake water samples, indicating that HF-LLLME–ASEI-CE/UV is a promising approach for analysis of phenylarsenic species in these samples at low levels. However, it is difficult to analyze pig litter sample, and especially soil sample in agricultural field.

Acknowledgements

Financial supports from the Science Fund for Creative Research Groups of NSFC (No. 20621502, 20921062), National Nature Science Foundation of China (20775057) and MOE of China (NCET-04-0658) are gratefully acknowledged.

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