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# Synthesis of mixed coating with multi-functional groups for in-tube hollow fiber solid phase microextraction–high performance liquid chromatography–inductively coupled plasma mass spectrometry speciation of arsenic in human urine

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ABSTRACT

A novel method based on in-tube hollow fiber-solid phase microextraction (in-tube HF-SPME) on-line coupled with ion pair reversed phase high performance liquid chromatography (IP-RP-HPLC)–inductively coupled plasma mass spectrometry (ICP-MS) was developed for arsenic speciation. Partially sulfonated poly(styrene) (PSP) and mixed-sol of 3-mercapto propyltrimethoxysilane (MPTS) and N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPTS) were prepared and immobilized in the pores and the inner surface of polypropylene hollow fiber (HF). The prepared MPTS–AAPTS/PSP immobilized HF was characterized by FT-IR spectroscopy and scanning electron microscope (SEM). With arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA), dimethylarsenic acid (DMA), arsenobetaine (AsB) and arsenocholine (AsC) as model arsenic species, a series of factors that influence the extraction of target arsenic species by in-tube HF-SPME, including pH value, sample volume and flow rate, elution conditions and interference of co-existing ions were investigated in details, and the conditions for subsequent HPLC–ICP-MS determination were also optimized. Under the optimal conditions, the sampling frequency was 6.5 $h^{-1}$, the detection limits for six target arsenic species were in the range of 0.017–0.053 $\mu g\,L^{-1}$ with the relative standard deviations ($c_{As(V),MMA}$ = 0.1 $\mu g\,L^{-1}$, $c_{As(III),DMA,AsB,AsC}$ = 0.5 $\mu g\,L^{-1}$, $n$ = 5) ranging in 3.1–8.7%, and the enrichment factors were varied from 4 to 19-fold. To validate the accuracy of this method, certified reference materials DORM-2 (dogfish) and CRM No. 18 (human urine) were analyzed, and the determined values were in good agreement with the certified values. The proposed method was also successfully applied for arsenic speciation in human urine samples, and the recoveries for the spiked samples were in the range of 92.6–107%. The self-designed in-tube HF-SPME–HPLC–ICP-MS system shows high efficiency and good stability, and the proposed method is sensitive and suitable for simultaneous speciation of organic and inorganic arsenic species (including anions and cations) in biological samples.



## 1. Introduction

Non-metallic element arsenic is widely distributed in nature as various species [1] which can enter and accumulate in human body to cause the toxic effect. The toxicity of arsenic highly depends on its chemical forms and oxidation state [2]. Inorganic arsenic species such as arsenite (As(III)) and arsenate (As(V)) are considered as strong toxic and carcinogenic compounds. The toxicity of monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA) is much lower than that of inorganic arsenic, while arsenobetaine (AsB) and arsenocholine (AsC) are considered as non-toxic arsenic compounds. Recent researches indicated that trivalent methylated arsenic is highly toxic, and may be more toxic than some inorganic arsenic compounds [3]. Therefore, total arsenic determination cannot effectively reveal the toxicity of arsenic and its chemical behavior in organism, the speciation analysis of arsenic has become one of the hot research topics in analytical community [4,5].

At present, hyphenated techniques by combining high efficient separation techniques with sensitive element-specific detectors are most widely used for arsenic speciation [6–8]. Among the element-specific detection techniques [6,9–11], ICP-MS [6,12–14] is widely applied due to its merits of high sensitivity, wide linear range, and easy to couple with various separation techniques. Separation techniques for arsenic speciation could be classified into chromatography methods [15–20] and non-chromatography methods [21,22]. With a variety of separation modes, good precision, and easy to interface with the subsequent detectors such as

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ICP-MS, high performance liquid chromatography (HPLC) is widely applied for arsenic speciation, especially ion-pair reversed phase (IP-RP)–HPLC [12,13,17] and ion exchange chromatography (IEC) [18–20] which are suitable for polar compounds separation.

For elemental speciation in real world, in most cases the concentrations of some species are at or below the detection limits of HPLC–ICP-MS, and the sample matrix is often complex which will affect the accuracy of the analytical results. Therefore, it is of great significance to apply a sample pretreatment method to effectively separate the matrix and preconcentrate the target arsenic species prior to HPLC–ICP-MS analysis [23]. However, it is a challenge for analytical chemists to develop a method that can simultaneously enrich various arsenic species with different physical and chemical properties, and has the merits of simplicity, fast speed, easy automation, and compatibility to subsequent separation/detection techniques. Up to now, the used sample pretreatment methods for arsenic speciation include solvent extraction [24], solid phase extraction (SPE) [25], microwave assisted extraction [26], in-tube solid phase microextraction (SPME) [27], matrix solid-phase dispersion (MSPD) [28], stir bar sorptive extraction (SBSE) [29] and so on, but most of them involve multi-steps process and are difficult to automate.

In-tube SPME (also called capillary microextraction (CME)), which can be used as an on-line preconcentration technique coupling to HPLC–ICP-MS, has demonstrated great potential in the arsenic speciation due to its merits of multiple-coating options, low-cost, simple operation, rapidness, good reproducibility, low sample/reagents consumption, and easy atomization. Pawliszyn et al. [27] had successfully employed in-tube SPME coupled to HPLC/electrospray ionization mass spectrometry (ESI-MS) for the speciation of four organic arsenic species (MMA, DMA, AsB, and AsC). The extraction efficiency and enrichment factor for four target arsenic species were in range of 3.42–22.01% and 2.28–14.67-fold, respectively. However, no inorganic arsenic species were included in this work probably due to the limitation of the employed coatings. On the other hand, the amount of the coatings on the inner surface of the fused-silica capillary is limited, which will lead to a low adsorption capacity and restrict the application of in-tube SPME. Compared with in-tube SPME based on the support of fused-silica capillary, porous membrane supported SPME shows relatively large specific surface area, high extraction rates and extraction efficiency, but this mode cannot be easily automated or on-line coupled to HPLC [30–33].

The purpose of this approach is to develop a novel in-tube hollow fiber (HF)-SPME and on-line couple it to HPLC–ICP-MS for both inorganic and organic arsenic speciation in human urine samples. For extraction of different arsenic species including As(III), As(V), MMA, DMA, AsB and AsC, partially sulfonated poly(styrene) (PSP)/3-mercapto propyltrimethoxysilane (MPTS)-N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPTS) immobilized polypropylene hollow fiber (HF) was prepared, and a series of factors that influence the extraction of target arsenic species by in-tube HF-SPME were investigated in details. The developed method was validated by the speciation of arsenic in biological samples.

## 2. Experimental

### 2.1. Apparatus

The HPLC system consisted of a LC-10AD high-pressure pump, a CTO-10A column oven, and a DGU-12A degasser (Shimadzu, Japan). CAPCELL PAK C18 column (250 mm × 4.6 mm, 5 μm) was used for the separation of six target arsenic species. As an on-line detector, ICP-MS (Agilent 7500a, Japan) with a Babington nebulizer was interfaced to HPLC via a minimum length piece of Telfon tubing (i.d. 0.5 mm, length 30 cm) with a finger-tight PEEK fitting. The operating conditions for HPLC–ICP-MS are given in Table 1.

**Table 1**
Operating conditions for the analytical instrument.

| | |
|---|---|
| *HPLC* | |
| Stationary phase | CAPCELL PAK C18 |
| Sample loop volume | 200 μL |
| Mobile phase | 2.5 mmol $L^{-1}$ sodium butanesulfonate, 4 mmol $L^{-1}$ malonic acid, 0.5% (v/v) methanol |
| Flow rate | 1.0 mL $min^{-1}$ |
| Column temperature | 25 °C |
| *ICP-MS plasma* | |
| Rf power | 1150 W |
| Rf matching | 1.5 V |
| Sampling depth | 6.8 mm |
| Carrier gas | 1.1 L $min^{-1}$ |
| *Time-resolved data acquisition* | |
| Scanning mode | Peak-hopping |
| Dwell time | 100 ms |
| Integration mode | Peak area |
| Detected isotope | $^{75}As$ |

An IFIS-C flow injection system (Ruimai Tech. Co. Ltd., Xi'an, China) was used for on-line coupling in-tube HF-SPME to HPLC–ICP-MS. The Accurel Q3/2 polypropylene hollow fiber membrane (i.d. 600 μm, 200 μm wall thickness, 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). A Mettler Toledo 320-s pH meter (Mettler Toledo Instruments Co. Ltd., Shanghai, China) with a combined electrode was used to control the pH. TS2-60 Multi-Syringe pump (Baoding Longer Precision Pump Co. Ltd., Baoding, China) was used to pump the coating materials into the hollow fiber. The WX-3000 microwave accelerated system was obtained from EU Chemical Instruments Co. Ltd. (Shanghai, China), and ECH-1 temperature control heating panel was purchased from Sineo Microwave Chemistry Technology Co. Ltd. (Shanghai, China).

The structure of MPTS–AAPTS/PSP coating was characterized by 170SX FI-IR (NICOL ET, USA). And the scanning electron micrograph (SEM) of the MPTS–AAPTS/PSP immobilized HF was obtained using an X-650 scanning electron microscope (Hitachi, Tokyo, Japan) at an acceleration voltage of 25 kV.

### 2.2. Standard solutions and reagents

Stock solutions (1.000 g $L^{-1}$ as As) of As(III), As(V), MMA, DMA, AsB and AsC were prepared by dissolving a certain amount of $NaAsO_2$ (>90%, Wako, Japan), $Na_2AsO_7{\cdot}H_2O$ (>99%, Wako, Japan), $CH_3AsO_3Na_2$ (98.5%, J&K Chemical Ltd., China), $C_2H_6AsO_2Na{\cdot}H_2O$ (>98.5%, Genebase Bioscience Co., Ltd., China), $C_5H_{11}AsO_2$ (95%, Wako, Japan) and $C_5H_{14}AsBrO$ (95%, Wako, Japan) in high purity deionized water, respectively. Tetramethoxysilane (TMOS), 3-mercapto propyltrimethoxysilane (MPTS) and N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPTS) were purchased from Organic Silicon Material Company of Wuhan University (Wuhan, China). And poly(styrene) (PS) (Alfa Aesar, MA, USA) was used to synthesize partially sulfonated poly(styrene) (PSP). Acetic anhydride, 1,2-dichloroethane (DCE) and malonic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), butanesulfonate was obtained from Tianjin Aoran Fine Chemical Research Institute (Tianjin, China).

All reagents used were of analytical reagent grade. High purity deionized water obtained by a Milli-Q Element system (18.2 MΩ cm, Millipore, Molsheim, France) was used throughout this work. All laboratory ware was cleaned by soaking in 10% (v/v) nitric acid for at least 24 h.

### 2.3. Preparation of in-tube HF-SPME coatings

#### 2.3.1. Synthesis of MPTS–AAPTS silica sol

1 mL TMOS was added dropwise in 4 mL ethanol, and the mixed solution was stirred at ambient temperature for 30 min. And then 0.25 mL MPTS was added dropwise in the solution followed by another 15 min stirring at ambient temperature. After that, the solution was put in an ice bath, and 0.25 mL AAPTS was dropwise added into the solution followed by further 30 min stirring.

### 2.4. Synthesis of PSP

PSP was prepared as described in literature [34]. Firstly, acetyl sulfate was freshly prepared. 10 mL acetic anhydride was mixed with 20 mL DCE in a round flask under argon atmosphere. Then the round flask was put in an ice bath to cool the solution to ~0 °C. After that, 4 mL sulfuric acid in stoichiometric amount with respect to the desired virtual degree of sulfonation in the polymer was added. Finally, the test tube is capped and the resulting acetyl sulfate in DCE solution was ready to be used.

Then 5 g of atactic PS was dissolved in 80 mL DCE. The flask was capped and the solution, which was maintained under the argon throughout the procedure, was heated to 80 °C with reflux condensation. Then the prepared acetyl sulfate was syringed into the flask from the cap, and the sulfonation reaction was sustained for 2 h under stirring until 10 mL of 2-propanol was added. The resulting solution was cooled down and then concentrated by removing ~50 mL of DCE through rotary evaporator under vacuum. The concentrated solution was added dropwise into 1.5 L high purity water by a syringe, and the PSP was precipitated. The PSP was filtered, and washed for 2 h in high purity water under reflux condensation, and filtered again. Finally, PSP was dried under vacuum at 60 °C for 2 days.

#### 2.4.1. Preparation of MPTS–AAPTS/PSP immobilized HF

Hollow fibers were cut into segments with a uniform length of 20 cm. The HF segments were sonicated for 2 min in acetone to remove the contaminants in the fiber, and dried prior to use.

Firstly, 1 mL MPTS–AAPTS silica sol was withdrawn in a syringe which was then fixed at a syringe pump. Then the MPTS–AAPTS silica sol was pumped into the HF with the flow rate of 0.1 mL $min^{-1}$, and maintained in HF for 1 h to fully evaporate the solvent. The process was repeated once. Afterwards, the MPTS–AAPTS immobilized HF was heated to 80 °C and maintained in an oven for 12 h to age the coating. Secondly, 100 mg PSP was dispersed in 1 mL N,N-dimethylformamide (DMF), and the mixed solution was injected into the MPTS–AAPTS immobilized HF by a syringe pump with the flow rate set at 0.1 mL $min^{-1}$. The mixed solution was maintained in the MPTS–AAPTS immobilized HF for 1 h to allow DMF completely evaporated, and the modified HF was again heated to 80 °C and maintained in the oven for 12 h.

#### 2.4.2. Preparation of MPTS–AAPTS coated fused-silica capillary

For comparison, MPTS–AAPTS coated fused-silica capillary was prepared as reported previously with minor modifications [35]. Briefly, the fused-silica capillary (i.d. 320 μm) was activated by rinsing subsequently with 1 mol $L^{-1}$ NaOH for 2 h, high purity water for 30 min, 1 mol $L^{-1}$ HCl for 2 h, and high purity water for another 30 min. Then the fused-silica capillary was dried at 160 °C while purged with argon for 5 h. After that, MPTS–AAPTS silica sol was passed through the fused-silica capillary for 1 h, and the residual sol was removed by passing argon. This process was repeated twice. Then the MPTS–AAPTS coated fused-silica capillary was heated at 120 °C for 8 h in a muffle furnace. Afterwards, the MPTS–AAPTS coated fused-silica capillary was cut into 40 cm segments prior to use.

### 2.5. Procedure

A schematic diagram of in-tube HF-SPME–HPLC–ICP-MS system was illustrated in Fig. 1. A flow injection instrument (FI), consisting of two pumps (A and B) and an eight-way valve, was used for on-line coupling in-tube HF-SPME and HPLC–ICP-MS. As shown in Fig. 1, two MPTS–AAPTS/PSP immobilized HFs (HF1 and HF2) were parallelly placed between FI pumps and eight-way valve. The designed programs included eight steps, as listed in Table 2. Initially, the FI valve was in position 0 and the six-port valve was in INJECT position. In steps 1–4, pump A pumped sample solution, water and eluent at a fixed flow rate for a certain time (see Table 2) into the HF1 continuously for extraction, flush and elution, respectively; at the same time pump B pumped 0.15 mol $L^{-1}$ $HNO_3$ and water at a fixed flow rate for a certain time (see Table 2) into the HF2 continuously for regeneration and conditioning, respectively. At step 4, the six-port valve was switched to LOAD position, the eluent passed through the HF1 was collected in the peek loop. Then, the six-port valve was immediately directed to INJECT position for subsequent HPLC–ICP-MS analysis. During steps 5–8, the FI valve was automatically switched to position 1. At this time, 0.15 mol $L^{-1}$ $HNO_3$ and water were driven continuously by pump A to regenerate and conditioning the HF1, respectively; while sample solution, water and eluent were driven continuously by pump B to pass through the HF2 for extraction, flush and elution, respectively. At step 8, the six-port valve was again moved to LOAD position, and the extracted arsenic species desorbed from HF2 were pumped into the peek loop. Thereafter, the six-port valve was simply switched to INJECT position for another HPLC–ICP-MS analysis.

It is noteworthy that this "parallel" in-tube HF-SPME–HPLC–ICP-MS effectively reduced analytical time from 18.2 min (9.2 min for extraction, flush and elution and 9 min for separation) to 9.2 min.

### 2.6. Sample preparation

For arsenic species extraction from DORM-2 dogfish (NRCC, Canada), 10 mL high purity deionized water was added to 0.25 g of dogfish sample in a 10 mL centrifuge tube. Then the mixture solution was ultrasonicated for 20 min. After that, the suspension was centrifuged at 3500 rpm for 20 min, and the supernatant was transferred into a 25 mL flask. The residue was extracted with 5 mL high purity deionized water for 3 times as described above. The four supernatant portions were combined and diluted to 25 mL with high purity deionized water.

For CRM No. 18 human urine sample (NIES, Japan), 9.57 g of high purity deionized water was added to dissolve the freeze-dried urine powder with gently swirling prior to use.

Human urine samples were obtained from Zhongnan Hospital of Wuhan University (Wuhan, China). After sampling, the urine samples were directly used for the determination of target arsenic species without further pretreatment.

For total arsenic determination, portions of DORM-2 dogfish (0.1 g), CRM No. 18 human urine dissolved solution (1 mL), and human urine samples (1 mL) were transferred into PTFE vessel, respectively. After 1.5 mL concentrated $HNO_3$ was added, the microwave digestion was performed according to the following heating programs: 3 atm for 1 min, 8 atm for 2 min and then 10 atm for 3 min. After digestion, the PTFE vessels were put on the ECH-1 temperature control heating panel, and the samples were heated to remove the superfluous acid. Then the digest was diluted to 5 mL with high purity deionized water prior to pneumatic nebulizer (PN)-ICP-MS detection. The blank sample of the same amount of acid was processed by the same procedure without dogfish or urine samples.

**Table 2**
Program for in-tube HF-SPME–HPLC–ICP-MS process.

| Step | | Event | Channel | Pump speed (μL min$^{-1}$) | Time (min) | FI valve position | Six-port valve |
|---|---|---|---|---|---|---|---|
| 1 | A | Begin extraction | Sample | 200 | 1.5 | 0 | INJECT |
| | B | Regeneration | $HNO_3$ | 100 | | | |
| 2 | A | Continue extraction | Sample | 200 | 6 | 0 | INJECT |
| | B | Conditioning | Water | 200 | | | |
| 3 | A | Flush | Water | 200 | 0.5 | 0 | INJECT |
| | B | Conditioning | Water | 200 | | | |
| 4 | A | Elution | Eluent | 50 | 1.2 | 0 | LOAD |
| | B | Conditioning | Water | 200 | | | |
| 5 | A | Regeneration | $HNO_3$ | 100 | 1.5 | 1 | INJECT |
| | B | Begin extraction | Sample | 200 | | | |
| 6 | A | Conditioning | Water | 200 | 6 | 1 | INJECT |
| | B | Continue extraction | Sample | 200 | | | |
| 7 | A | Conditioning | Water | 200 | 0.5 | 1 | INJECT |
| | B | Flush | Water | 200 | | | |
| 8 | A | Conditioning | Water | 200 | 1.2 | 1 | LOAD |
| | B | Elution | Eluent | 50 | | | |

## 3. Results and discussion

### 3.1. Preparation and characterization of MPTS–AAPTS/PSP coating

The HF used was made of hydrophobic polypropylene. It was found in our experiments that the aqueous solution cannot permeate through the pores in the wall of HF when the flow rate was lower than 2 mL min$^{-1}$.

#### 3.1.1. Preparation of MPTS–AAPTS/PSP coating for in-tube HF-SPME

In in-tube SPME, coating material plays an important role in the improvement of the sensitivity and selectivity of the method. In

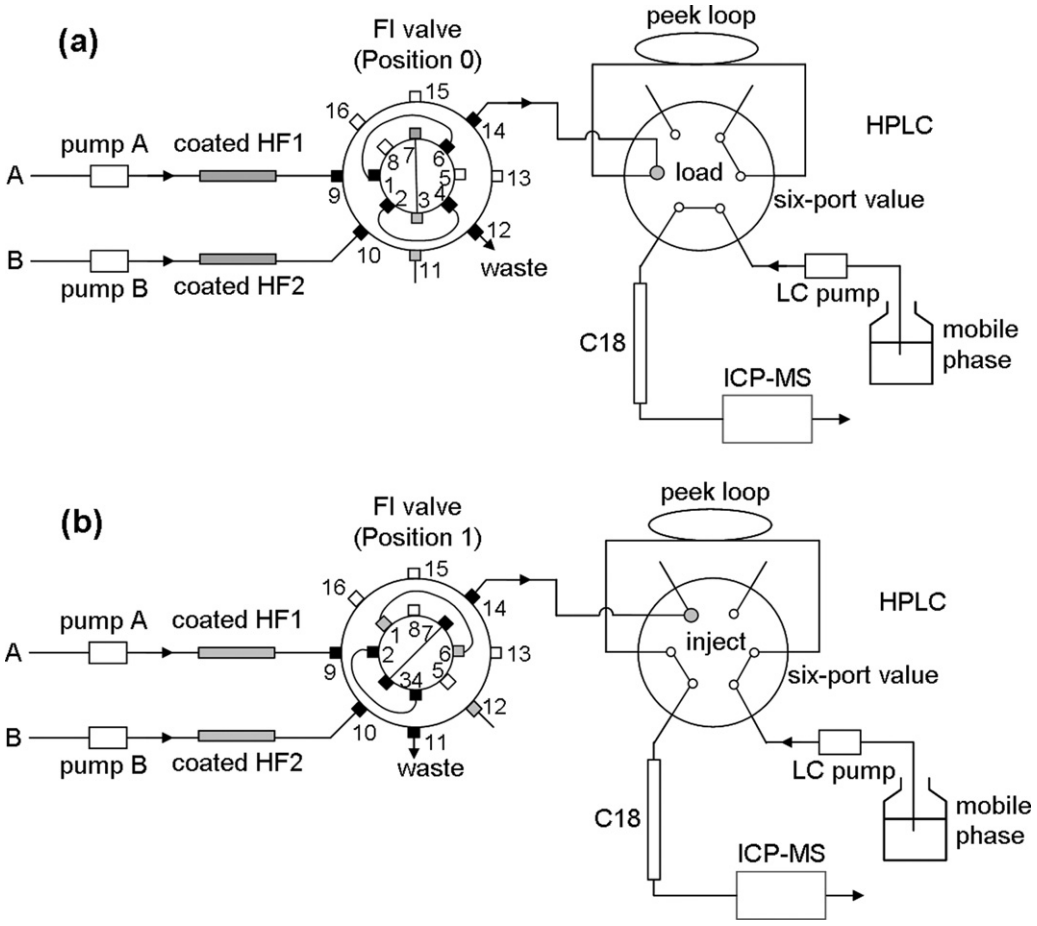


**Fig. 1.** Schematic diagram of on-line HF-in-tube SPME–HPLC–ICP-MS. (a) Step 4 listed in Table 2; (b) step 5 listed in Table 2.

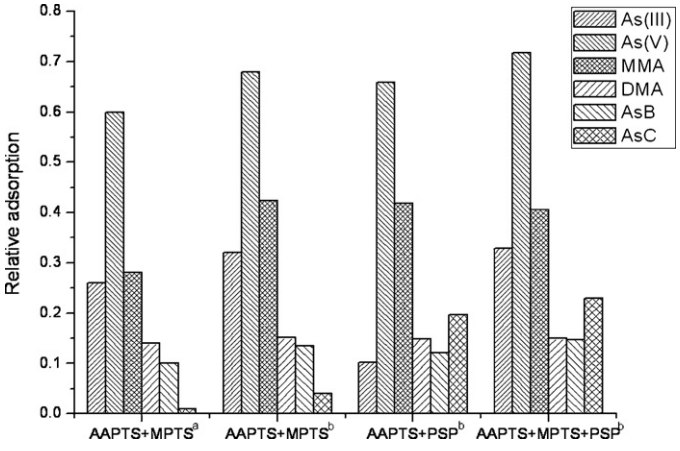


**Fig. 2.** Effect of different coatings on the extraction of six target arsenic species. (a) Coatings on fused-silica capillary (length 40 cm); (b) coatings on HF (length 20 cm). ($c_{As(III),As(V),MMA,DMA,AsB,AsC}$ = 5 μg $L^{-1}$, pH = 6, sample volume: 1 mL).

this work, six target arsenic species are all hydrophilic compounds, but they have different charge and p$K_a$ value (see Table S1 in Supplementary material) in the aqueous solution. When pH is around 7, As(III) is neutral, As(V) and MMA are negative charged, DMA and AsB are amphoteric, while AsC is positive charged, therefore, it is hard to find an extraction material with single functional group to simultaneously adsorb all these target arsenic species. Yu et al. [25] investigated the effect of various SPE cartridges on the retention behaviors of arsenic species, they found that inorganic and organic arsenic species could not or would not be completely adsorbed on the non-polar cartridges (e.g. $C_8$, $C_{18}$), As(V) and MMA could be adsorbed on strong anion exchange cartridge, while DMA, AsB, and AsC could be adsorbed on strong cation exchange cartridge well. This gives us a hint that different arsenic species might be simultaneously extracted if a suitable extraction material with multiple functional groups is employed. In this work, MPTS–AAPTS, AAPTS/PSP, MPTS–AAPTS/PSP immobilized HF, and MPTS–AAPTS coated fused-silica capillary were prepared, and the extraction performance of these coatings for the target arsenic species were investigated. As could be seen in Fig. 2, the addition of MPTS with mercapto group greatly improved the adsorption of neutral As(III), and an improvement of the adsorption of the positively charged AsC was observed when PSP was added in the mixed coatings, while negatively charged As(V) and MMA and amphoteric DMA and AsB had a constant adsorption on these four mixed coating. In addition, because PSP could not be stably coated on the fused-silica capillary, both HF and fused-silica capillary were used as the solid phase support for the MPTS–AAPTS coating, and the adsorption of target arsenic species was investigated. As could be seen in Fig. 2, the adsorption of all the target arsenic species on MPTS–AAPTS immobilized HF was higher than that on MPTS–AAPTS coated fused-silica capillary probably due to the relatively large specific surface area of the porous membrane. Therefore, MPTS–AAPTS/PSP immobilized HF was used for simultaneous extraction of different arsenic species in this work.

Then the effect of the coating components on the extraction of target arsenic species was examined. By fixing the volume of TMOS and ethanol as 1 and 4 mL, respectively, the effect of the amount of AAPTS and MPTS in the mixed sol was investigated, and the results obtained by MPTS–AAPTS/PSP immobilized HF were shown in Fig. S1 in Supplementary material. As could be seen, good adsorption and reproducibility to all the target arsenic species were obtained when 0.25 mL AAPTS and 0.25 mL MPTS were used. The effect of the amount of PSP was also investigated, and the results demonstrated that the adsorption efficiency of AsC was increased with the increase of the amount of PSP in the coating. But when the concentration of PSP in DMF was higher than 100 g $L^{-1}$, it might lead to a blockage of HF due to high viscosity. Finally, 0.25 mL AAPTS and 0.25 mL MPTS were added in the mixed sol, and the concentration of PSP in DMF was optimized as 100 g $L^{-1}$.

**Table 3**
Preparation reproducibility for MPTS–AAPTS/PSP immobilized HF.

| Reproducibility | As(III) | As(V) | MMA | DMA | AsB | AsC |
|---|---|---|---|---|---|---|
| Within one batch ($n$ = 5) | 6.5% | 7.9% | 7.7% | 4.6% | 5.9% | 7.4% |
| Batch to batch ($n$ = 5) | 9.2% | 15% | 14% | 10% | 13% | 10% |

$c_{As(V)}$ = 5 μg $L^{-1}$, $c_{As(III),MMA,DMA,AsB,AsC}$ = 10 μg $L^{-1}$.

### 3.1.2. Characterization of MPTS–AAPTS/PSP coating

The mixed coating of MPTS–AAPTS/PSP was characterized by FT-IR spectroscopy and SEM. As shown in IR spectra (Fig. 3), the mercapto group peak and the amino group peak were clearly observed at 2430 $cm^{-1}$ and 1597 $cm^{-1}$, respectively. The adsorption bands at 1042 $cm^{-1}$ and 1188 $cm^{-1}$ were characteristic peaks of sulfonic group, and the adsorption band in the region of 1400–1500 $cm^{-1}$ was attributed to benzene ring skeleton stretching vibration of PSP. All these results demonstrated successful preparation of MPTS–AAPTS/PSP coating. Fig. 4 shows the SEM of HF and MPTS–AAPTS/PSP immobilized HF. As could be seen from the cross-sectional image of HF shown in Fig. 4(a), there were tracks left when cut by scissors due to the toughness of polypropylene HF. While no tracks by scissors were observed in Fig. 4(b) because the MPTS–AAPTS/PSP immobilized HF became harder and crisper than that of HF. Compared with the textural image of inner surface of HF and MPTS–AAPTS/PSP immobilized HF (Fig. 4(c) and (d)), more porous was observed on the MPTS–AAPTS/PSP immobilized HF which would benefit the mass transfer of the target analytes in the extraction process.

The prepared MPTS–AAPTS/PSP immobilized HF shows good reproducibility and regeneration capability. As shown in Table 3, the RSDs were in the range of 4.6–7.9% within one batch and 9.2–15% among different batches, respectively. And the immobilized HF could be reused for 30 times without obvious loss of extraction efficiency under the optimized conditions.

## 3.2. In-tube hollow fiber-solid phase microextraction

### 3.2.1. Effect of pH

An appropriate pH value can improve the adsorption and reduce interference from the matrix as well. The effect of pH on the extraction of target arsenic species was investigated, and the results were shown in Fig. 5. As could be seen, As(III) was adsorbed on the MPTS–AAPTS/PSP immobilized HF at the maximum amount within pH 3–9 due to its high affinity to the mercapto group of MPTS. With the increase of pH from 2 to 5, the adsorption of As(V) and MMA on MPTS–AAPTS/PSP immobilized HF was increased obviously and then kept unchanged with the pH further increasing from 5 to 9. Within pH 5–9, As(V) and MMA are negatively charged, and the amino group of AAPTS is positively charged, the electrostatic interaction between As(V) or MMA with AAPTS leads a constant retention of As(V) and MMA on the MPTS–AAPTS/PSP immobilized HF. For amphoteric DMA and AsB, a constant adsorption was obtained in the pH range of 2–10 and 5–10, respectively. The adsorption of AsC was increased with increasing pH due to a cation exchange interaction between positively charged AsC and the sulfonic group of PSP. These results clearly showed that simultaneous extraction of all the target arsenic species could be realized in the pH range of 6–8, therefore, a physiological pH of 7.4 was selected in subsequent experiments.

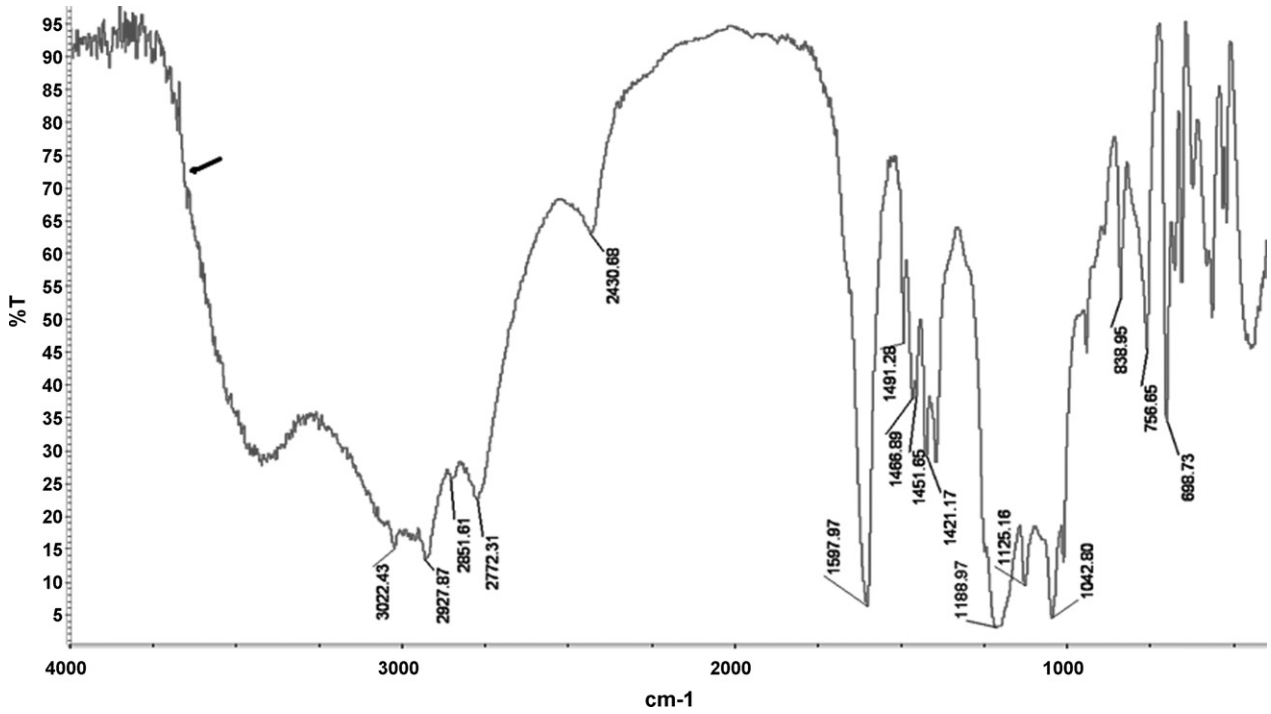


**Fig. 3.** Infrared spectra of MPTS–AAPTS/PSP coating.

*3.2.2. Effect of sample flow rate and volume*

The effect of sample flow rate on the extraction of target arsenic species was studied in the range of 0.05–0.5 mL min$^{-1}$. As shown in Fig. S2, no obvious impact of sample flow rate on the extraction was observed when the sample flow rate was less than 0.3 mL min$^{-1}$, so 0.2 mL min$^{-1}$ was applied for the subsequent experiments. By fixing the amount of As(V) at 5 ng and other arsenic species each at 10 ng, the effect of sample volume on the extraction of target arsenic species was investigated with sample volume varying in the range of 1–5 mL. The results in Fig. S3 showed no obvious influ-

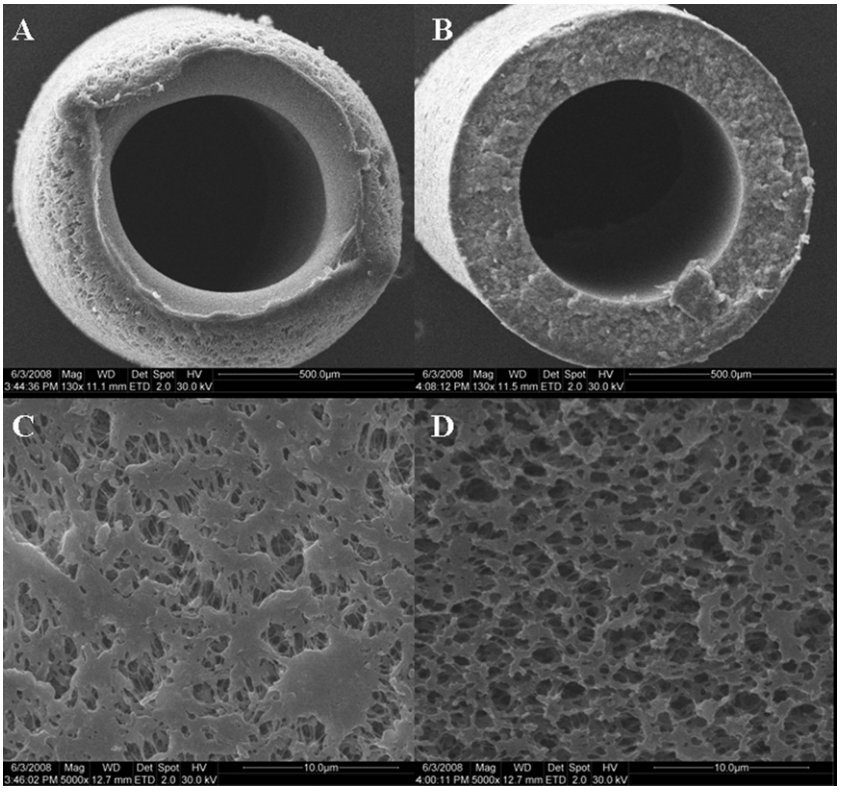


**Fig. 4.** SEM images of polypropylene hollow fiber and MPTS–AAPTS/PSP immobilized hollow fiber. (a) Cross-sectional image of polypropylene hollow fiber; (b) cross-sectional image of MPTS–AAPTS/PSP immobilized hollow fiber; (c) textural image of inner surface of polypropylene hollow fiber; (d) textural image of inner surface of MPTS–AAPTS/PSP immobilized hollow fiber.

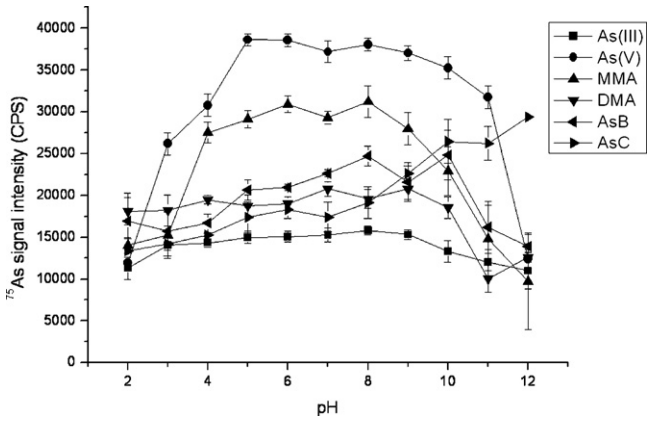


**Fig. 5.** Effect of pH on the extraction of six target arsenic species. ($c_{As(V)}$ = 5 μg L$^{-1}$, $c_{As(III),MMA,DMA,AsB,AsC}$ = 10 μg L$^{-1}$, sample flow rate: 100 μL min$^{-1}$, sample volume: 1 mL, eluent: 0.1 mol L$^{-1}$ $HNO_3$, elution flow rate: 50 μL min$^{-1}$, elution volume: 100 μL).

ence of sample volume on the extraction of target arsenic species. Although larger sample volume would result in higher enrichment factors, 1.5 mL sample solution with a flow rate of 0.2 mL min$^{-1}$ was employed for further experiments to improve the sample throughput and match the analytical time between in-tube HF-SPME process and online HPLC–ICP-MS determination.

### 3.2.3. Optimization of elution conditions

The adsorption of target arsenic species was poor when pH < 3 as shown in Fig. 5, thus nitric acid was selected as the eluent. The effect of acidity, elution volume and flow rate were investigated with orthogonal design. For this purpose, a $L_9$ ($3^4$) orthogonal form was applied and the assignments of factors and levels were depicted in Table S2. The peak areas of the target arsenic species were considered as the experimental response, and their mean analysis results obtained by Statistical Product and Service Solutions (SPSS) software were listed in Table 4. As could be seen, 0.15 mol L$^{-1}$ $HNO_3$ used as eluent could desorb all the target arsenic species completely but AsC. Considering $HNO_3$ at high concentration might oxidize the mercapto group of MPTS and destroy C18 column, 60 μL 0.15 mol L$^{-1}$ $HNO_3$ at the flow rate of 50 μL min$^{-1}$ was applied for the elution in this work.

Due to insufficient desorption of AsC, the regeneration of the MPTS–AAPTS/PSP immobilized HF was studied by continuously passing 0.15 mol L$^{-1}$ $HNO_3$ through the modified HF, and the target arsenic species in several effluents (each 60 μL) were determined by HPLC–ICP-MS. As could be seen in Fig. S4, the residual AsC could be cleaned up by 120 μL 0.15 mol L$^{-1}$ $HNO_3$. Finally, 150 μL 0.15 mol L$^{-1}$ $HNO_3$ was selected as clean-up solution.

### 3.3. Optimization of chromatographic separation conditions

IP-RP-HPLC with appropriate ion-pair reagents added in the mobile phase was well suitable for simultaneous separation of anionic, cationic and neutral molecules, resulting in successful separation of various arsenic species [6]. In this method, sodium butanesulfonate were employed as ion-pair reagents, malonic acid was used to adjust the pH of the mobile phase, and the effect of their concentration on the separation of six target arsenic species were studied separately by applying standard solution of six target arsenic species prepared in high purity water.

The effect of the concentration of sodium butanesulfonate on the separation of six target arsenic species was investigated, and the results were shown in Table S3. As could be seen, with the increase of the concentration of sodium butanesulfonate, the capacity factor ($k$) of positively charged AsC were obviously increased due to the ion-pair formed between AsC and sodium butanesulfonate, and little influence on the $k$ of neutral As(III) and amphoteric DMA and AsB was observed because of their negligible interaction with sodium butanesulfonate, while the $k$ of negatively As(V) and MMA were slightly decreased owing to a competitive effect between these two arsenic species and sodium butanesulfonate. Taking into consideration of both resolution and separation time, 2.5 mmol L$^{-1}$ sodium butanesulfonate was used for subsequent experiments.

Malonic acid can not only adjust the mobile phase pH, but also interact with the target arsenic species and thus affect their retention behavior. The effect of malonic acid concentration on the separation of six target arsenic species was studied, and the results were listed in Table S4. As could be seen, with the increase of malonic acid concentration, the $k$ of As(V), MMA, DMA and AsB were increased, the $k$ of AsC were decreased, and the retention behavior of neutral As(III) remained unchanged. To get a good resolution and a fast separation, 4 mmol L$^{-1}$ malonic acid was selected in this work. Finally, the mobile phase consisting of 2.5 mmol L$^{-1}$ sodium butanesulfonate, 4 mmol L$^{-1}$ malonic acid and 0.5% (v/v) methanol was employed, and all the target arsenic species were well separated by isocratic elution, the chromatogram was shown in Fig. 6. It should be mentioned that when the target arsenic species were prepared in the elution (0.15 mol L$^{-1}$ $HNO_3$) and injected in HPLC–ICP-MS, the retention time of As(V) was shifted from 130 s to 149 s, while the retention times for others were not obviously changed, and all the six target arsenic species could still be well separated.

### 3.4. Effect of coexisting ions

Under the optimized conditions, the interference of common coexisting ions such as $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$, $Fe^{3+}$, $Zn^{2+}$, $Cl^-$ and $NO_3^-$ was studied. As could be seen in Table S5, the tolerance concentration of the coexisting ions obtained in this method were higher than their reported concentration range in urine

**Table 4**
Means analysis results obtained by SPSS software.

| Parameters | | As(III) | As(V) | MMA | DMA | AsB | AsC |
|---|---|---|---|---|---|---|---|
| Acidity (mol L$^{-1}$) | 0.10 | 131196.4 | 22415.12 | 104355.6 | 80085.17 | 46379.61 | 36653.86 |
| | 0.15 | 164372.8 | 26591.08 | 116899.3 | 111840.7 | 94025.13 | 52520.61 |
| | 0.20 | 167098.9 | 27893.53 | 130319.9 | 124347.9 | 105361.2 | 117945.9 |
| Volume (μL) | 40 | 122456.0 | 19813.76 | 27907.39 | 64616.95 | 38663.18 | 13816.31 |
| | 50 | 150549.6 | 17547.59 | 91967.86 | 94240.18 | 62308.74 | 43444.73 |
| | 60 | 189662.3 | 97370.77 | 282199.6 | 157416.7 | 124793.0 | 149859.3 |
| Flow rate (μL min$^{-1}$) | 20 | 140775.2 | 8991.664 | 87789.61 | 91039.75 | 66499.33 | 49052.92 |
| | 50 | 157108.1 | 66058.13 | 156612.0 | 105719.5 | 80626.58 | 93940.35 |
| | 100 | 164784.7 | 11849.94 | 157673.2 | 119514.6 | 78639.00 | 64127.10 |

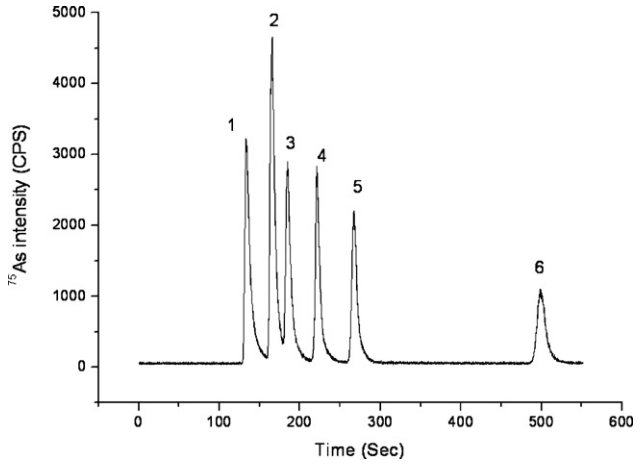


**Fig. 6.** HPLC–ICP-MS chromatogram of target arsenic species. (mobile phase: 2.5 mmol $L^{-1}$ sodium butanesulfonate, 4 mmol $L^{-1}$ malonic acid, 0.5% (v/v) methanol, flow rate: 1.0 mL $min^{-1}$, column temperature: 25 °C, target arsenic species: (1) As(V), (2) As(III), (3) MMA, (4) DMA, (5) AsB, (6) AsC; $c_{As(V),MMA,DMA,AsB,AsC}$ = 100 μg $L^{-1}$, $c_{As(III)}$ = 150 μg $L^{-1}$).

[36,37], indicating a good anti-interference capability and application potential of the proposed method in urine samples.

### 3.5. Analytical performance

The analytical performance was validated under the optimized conditions, and the results were given in Table 5. According to the IUPAC recommendation, the limits of detection (LODs, evaluated as the concentration corresponding to three times the standard deviation of 11 runs of the blank solution) were calculated to be 0.017–0.053 μg $L^{-1}$ with the relative standard deviations (RSDs) ranging in 3.1–8.7% ($c_{As(V),MMA}$ = 0.1 μg $L^{-1}$, $c_{As(III),DMA,AsB,AsC}$ = 0.5 μg $L^{-1}$, $n$ = 5) and 1.5–5.2% ($c_{As(V)}$ = 5 μg $L^{-1}$, $c_{As(III),MMA,DMA,AsB,AsC}$ = 10 μg $L^{-1}$, $n$ = 7), respectively. The chromatograms for the six target arsenic species with concentrations of 0.1 and 0.5 μg $L^{-1}$ by in-tube HF-SPME–HPLC–ICP-MS was shown in Fig. S5. The enrichment factors (EF, defined as the ratio of the calibration curve slope after and before the in-tube HF-SPME) for target arsenic species were varied from 4.0 to 19-fold. The analytical time was sharply reduced to 9.2 min for one single run due to the integrity of extraction, elution, separation and detection, resulting in a sample throughput up to 6.5 $h^{-1}$. A comparison of the LODs obtained in this work with that obtained by several other approaches [13,14,17–20,27,29,38] was shown in Table 6. As could be seen, the developed method was one of the most sensitive methods for arsenic speciation. The LODs of the proposed method were lower than that of HPLC–ICP-MS [17–19] and CE–ICP-MS [14] except ion chromatography-high resolution field sector–ICP-MS [20]. Compared with hydride generation ICP-MS methods [13,38], the LODs were comparable, but the arsenic species of AsB as the target analyte in this work could not be analyzed by hydride generation methods. Compared with SBSE [29], in-tube HF-SPME is easier to on-line combine with HPLC–ICP-MS. Compared with literature [27], the developed method was more sensitive and could be applied for simultaneous analysis of inorganic and organic arsenic species (including anions and cations).

The proposed method was validated by the speciation of arsenic in certified reference materials DORM-2 dogfish and CRM No. 18 human urine samples, and the analytical results were shown in Table 7. As could be seen, the determined results were in good agreement with the certified values, which indicated that the proposed method is suitable for arsenic speciation in biological samples such as urine and fish tissue.

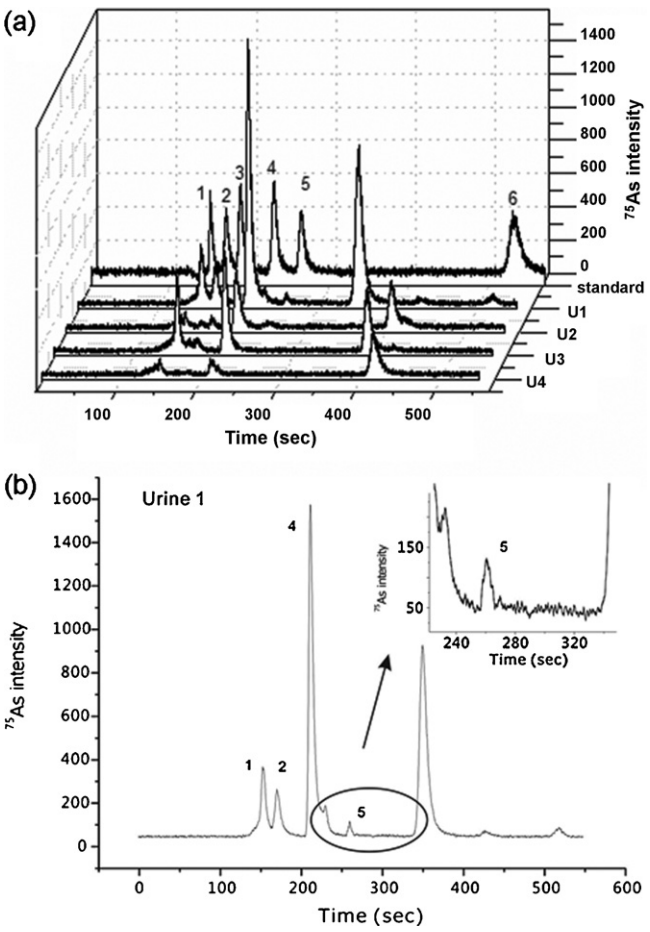


**Fig. 7.** Chromatograms of arsenic species in real samples (a) and the enlarged chromatogram for Urine 1 (b). (1. As(V), 2. As(III), 3. MMA, 4. DMA, 5. AsB, 6. AsC; U1, U2, U3 and U4 represent four individual human urine samples after in-tube HF-SPME–HPLC–ICP-MS; standard represents the arsenic standard solution after in-tube HF-SPME–HPLC–ICP-MS, $c_{As(III),DMA,AsB,AsC}$ = 10 μg $L^{-1}$, $c_{As(V),MMA}$ = 2.5 μg $L^{-1}$).

### 3.6. Sample analysis

The developed method was applied to arsenic speciation in human urine samples obtained from Wuhan, China, external calibration was used for quantification. The chromatograms of various arsenic species for four individual urine samples obtained by in-tube HF-SPME–HPLC–ICP-MS were shown in Fig. 7(a), and the enlarged chromatogram for Urine 1 was shown in Fig. 7(b). As could be seen, in human urine there are As(III), As(V), MMA, DMA, AsB and unknown arsenic species, in which DMA is one of the main arsenic metabolites in human body from Wuhan. The analytical results in Table 8 demonstrate the total concentrations of As are higher than the sum concentrations of the known As species, which indicated that the surplus amount referred to the unknown As species. Table 8 also listed the recoveries of six target arsenic species for real samples, the recoveries were in the range of 92.6–107%. Arsenosugars and their metabolites were not investigated because marine algae rich in arsenosugars are not on the diet of the volunteers from Wuhan (inland area of China). Besides, it is reported that thio-dimethylarsinate (thio-DMA) is a common metabolite in urine samples from arsenic-exposed women in Bangladesh, and might be overlapped with other arsenic species in HPLC–ICP-MS [39]. However, As(III)-thio species or other unknown arsenic species were not identified here because ESI-MS is not available currently in our lab. Some further research works should be done to study the toxicity and the metabolism of arsenic in organism.

**Table 5**
Analytical performance for target arsenic species obtained by in-tube HF-SPME–HPLC–ICP-MS.

| Target arsenic species | LOD ($\mu g\,L^{-1}$) | RSD | | EF | Linear range ($\mu g\,L^{-1}$) | Linear equation | $r^2$ |
|---|---|---|---|---|---|---|---|
| | | a | b | | | | |
| As(III) | 0.042 | 5.1% | 4.9% | 5.4 | 0.5–1000 | $y = 3150.7x - 287.45$ | 0.9995 |
| As(V) | 0.018 | 3.1% | 4.2% | 19 | 0.1–1000 | $y = 18735x + 8104.0$ | 0.9992 |
| MMA | 0.017 | 7.4% | 5.2% | 13 | 0.1–1000 | $y = 10458x + 3368.9$ | 0.9983 |
| DMA | 0.040 | 8.7% | 1.5% | 4.2 | 0.5–1000 | $y = 5249.1x + 2729.4$ | 0.9987 |
| AsB | 0.053 | 5.4% | 2.6% | 4.0 | 0.5–1000 | $y = 3149.3x + 2914.4$ | 0.9998 |
| AsC | 0.047 | 4.0% | 2.1% | 4.6 | 0.5–1000 | $y = 4082.8x + 6745.6$ | 0.9997 |

a: $c_{As(V),MMA} = 0.1\ \mu g\,L^{-1}$, $c_{As(III),DMA,AsB,AsC} = 0.5\ \mu g\,L^{-1}$ ($n = 5$), b: $c_{As(V)} = 5\ \mu g\,L^{-1}$, $c_{As(III),MMA,DMA,AsB,AsC} = 10\ \mu g\,L^{-1}$ ($n = 7$).

**Table 6**
Comparison of LODs found in the literature for arsenic speciation.

| Analytical methods | LODs ($\mu g\,L^{-1}$) | | | | | | Ref. |
|---|---|---|---|---|---|---|---|
| | As(III) | As(V) | MMA | DMA | AsB | AsC | |
| IP-RP HPLC–ICP-MS | 0.09 | 0.10 | 0.15 | 0.17 | 0.16 | 0.15 | [17] |
| Anion exchange HPLC–ICP-MS | 1.2 | 1.0 | 0.9 | 1.7 | 0.4 | 0.6 | [18] |
| Cation exchange HPLC–ICP-MS | 0.24 | 069 | 0.49 | 0.47 | – | – | [19] |
| Narrow bore IC[a]-HRFS[b]-ICP-MS | 0.005 | 0.005 | 0.005 | 0.005 | 0.010 | – | [20] |
| CE-ICP-DRC[c]-MS | 0.9 | 0.9 | 0.6 | 1.8 | 0.7 | – | [14] |
| IP-RP HPLC–HG[d]-ICP-MS | 0.032 | 0.042 | 0.051 | 0.029 | – | – | [13] |
| FI-HG-ICP-MS | 0.05 | 0.09 | – | – | – | – | [38] |
| SBSE[e]–HPLC–ICP-MS | 0.065 (iAs[f]) | | 0.020 | 0.013 | – | – | [29] |
| Polypyrrole-coated in-tube SPME HPLC/ESIMS | – | – | 1.25 | 0.54 | 0.18 | 0.25 | [27] |
| In-tube HF-SPME-HPLC–ICP-MS | 0.042 | 0.018 | 0.017 | 0.040 | 0.053 | 0.047 | This work |

[a] Ion chromatography.
[b] High resolution field sector.
[c] Dynamic reaction cell.
[d] Hydride generation.
[e] Stir bar sorptive extraction.
[f] Inorganic As.

**Table 7**
Analytical results for arsenic speciation in certified reference materials (mean ± s.d., $n = 3$).

| Certified materials | | As(III) | As(V) | MMA | DMA | AsB | AsC | Total As |
|---|---|---|---|---|---|---|---|---|
| DORM-2 | Determined(mg As $kg^{-1}$) | N.D.[a] | N.D. | N.D. | 0.35 ± 0.02 | 16.2 ± 0.2 | N.D. | 17.9 ± 0.4 |
| | Certified (mg As $kg^{-1}$) | – | – | – | – | 16.4 ± 1.1 | – | 18 ± 1.1 |
| CRM No. 18 | Determined ($\mu g$ As $L^{-1}$) | 3.28 ± 0.2 | 2.64 ± 0.4 | 2.57 ± 0.5 | 30.9 ± 3.5 | 71.9 ± 6.9 | N.D. | 139 ± 7.5 |
| | Certified ($\mu g$ As $L^{-1}$) | – | – | – | 36 ± 9 | 69 ± 12 | – | 137 ± 11 |

[a] Not detected.

**Table 8**
Analytical results (mean ± s.d., $n = 3$) for arsenic speciation in human urine samples.

| | Urine-1 | | | Urine-2 | | | Urine-3 | | | Urine-4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Added ($\mu g\,L^{-1}$) | Found ($\mu g\,L^{-1}$) | Recovery (%) | Added ($\mu g\,L^{-1}$) | Found ($\mu g\,L^{-1}$) | Recovery (%) | Added ($\mu g\,L^{-1}$) | Found ($\mu g\,L^{-1}$) | Recovery (%) | Added ($\mu g\,L^{-1}$) | Found ($\mu g\,L^{-1}$) | Recovery (%) |
| As(III) | 0 | 4.99 ± 0.24 | – | 0 | 1.55 ± 0.08 | – | 0 | 1.45 ± 0.08 | – | 0 | 0.58 ± 0.08 | – |
| | 10 | 14.7 ± 0.67 | 98.1 | 10 | 11.9 ± 0.71 | 103 | 10 | 12.0 ± 0.35 | 105 | 2 | 2.48 ± 0.14 | 96.1 |
| As(V) | 0 | 1.88 ± 0.07 | – | 0 | 0.73 ± 0.05 | – | 0 | 2.08 ± 0.08 | – | 0 | 0.80 ± 0.03 | – |
| | 5 | 6.49 ± 0.36 | 94.3 | 5 | 5.83 ± 0.15 | 102 | 5 | 7.15 ± 0.36 | 101 | 2 | 2.99 ± 0.10 | 107 |
| MMA | 0 | N.D.[a] | – | 0 | 0.65 ± 0.04 | – | 0 | 0.75 ± 0.02 | – | 0 | 0.19 ± 0.02 | – |
| | 2 | 1.98 ± 0.11 | 99.0 | 2 | 2.52 ± 0.26 | 95.1 | 2 | 2.91 ± 0.17 | 106 | 2 | 2.27 ± 0.15 | 104 |
| DMA | 0 | 24.8 ± 2.3 | – | 0 | 9.87 ± 0.93 | – | 0 | 15.6 ± 0.63 | – | 0 | 2.47 ± 0.17 | – |
| | 25 | 51.2 ± 3.8 | 103 | 10 | 18.4 ± 0.84 | 92.6 | 25 | 43.0 ± 0.12 | 106 | 10 | 11.5 ± 0.30 | 92.2 |
| AsB | 0 | 2.50 ± 0.11 | – | 0 | 0.84 ± 0.05 | – | 0 | N.D. | – | 0 | N.D. | – |
| | 5 | 7.42 ± 0.30 | 98.9 | 5 | 5.59 ± 0.16 | 95.8 | 5 | 5.35 ± 0.34 | 107 | 2 | 2.09 ± 0.10 | 105 |
| AsC | 0 | N.D. | – | 0 | N.D. | – | 0 | N.D. | – | 0 | N.D. | – |
| | 5 | 4.69 ± 0.40 | 93.8 | 5 | 5.22 ± 0.09 | 104 | 5 | 4.99 ± 0.37 | 99.8 | 2 | 2.02 ± 0.15 | 101 |
| Sum[b] | | 34.17 | | | 13.64 | | | 19.88 | | | 4.04 | |
| Total As[c] | | 41.57 | | | 22.27 | | | 26.91 | | | 17.22 | |

[a] N.D.: not detected.
[b] The sum concentration of As(III), As(V), MMA, DMA, AsB and AsC obtained by in-tube HF-SPME–HPLC–ICP-MS.
[c] Total As was determined by PN-ICP-MS after digestion.

## 4. Conclusions

A novel method based on in-tube HF-SPME on-line coupled to HPLC–ICP-MS for arsenic speciation in urine and fish tissue was developed. With MPTS–AAPTS/PSP as mix coating for in-tube HF-SPME, six inorganic and organic arsenic species were effectively extracted simultaneously. Compared with other manual sample pretreatment techniques applied for arsenic speciation, the proposed in-tube HF-SPME is easy to automate, which is benefit to avoid the possible transformation of arsenic species and the sample loss during the procedure. This self-designed on-line system by combining in-tube HF-SPME and HPLC–ICP-MS shows high efficiency, good sensitivity, precision, sample clean-up and anti-interference ability, which can be directly applied for arsenic speciation in real urine samples.

## Acknowledgements

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.12.086.

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