



# Selectively Adsorptive Extraction of Phenylarsonic Acids in Chicken Tissue by Carboxymethyl $\alpha$ -Cyclodextrin Immobilized $\text{Fe}_3\text{O}_4$ Magnetic Nanoparticles Followed Ultra Performance Liquid Chromatography Coupled Tandem Mass Spectrometry Detection

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## Abstract

Carboxymethyl  $\alpha$ -cyclodextrin immobilized  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles (CM- $\alpha$ -CD- $\text{Fe}_3\text{O}_4$ ) were synthesized for the selectively adsorptive extraction of five phenylarsonic acids including *p*-amino phenylarsonic acid, *p*-nitro phenylarsonic acid, *p*-hydroxy phenylarsonic acid, *p*-acylamino phenylarsonic acid and *p*-hydroxy-3-nitro phenylarsonic acid in chicken tissue. Using ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS), a highly sensitive analytical method was proposed for the determination of five phenylarsonic acids. It was shown that CM- $\alpha$ -CD- $\text{Fe}_3\text{O}_4$  could extract the five phenylarsonic acids in complex chicken tissue samples with high extraction efficiency. Under the optimal conditions, a high enrichment factor, ranging from 349 to 606 fold, was obtained. The limits of detection (LODs) (at a signal-to-noise ratio of 3) were in the range of 0.05–0.11  $\mu\text{g}/\text{kg}$  for the five phenylarsonic acids. The proposed method was applied for the determination of five target phenylarsonic acids in chicken muscle and liver samples. Recoveries for the spiked samples with 0.2  $\mu\text{g}/\text{kg}$ , 2.0  $\mu\text{g}/\text{kg}$  and 20  $\mu\text{g}/\text{kg}$  of each phenylarsonic acids were in the range of 77.2%–110.2%, with a relative standard deviation (RSD) of less than 12.5%.

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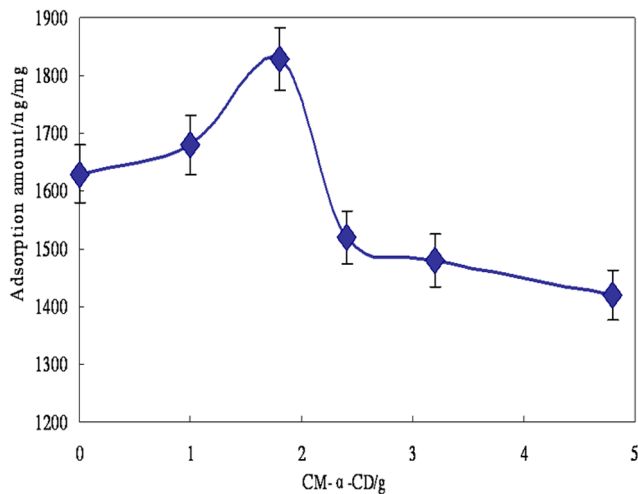
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## Introduction

4-Hydroxy-3-nitrobenzenearsonic acid, also known as roxarsone (ROX), has been used since 1944 as a feed additive in the poultry industry to promote growth and to control coccidiosis, a parasitic disease that infects the intestinal tract of poultry [1]. Besides ROX, some other organic arsenic compounds, including *p*-amino phenylarsonic acid (*p*-APAA), *p*-nitro phenylarsonic acid (*p*-NPAA), *p*-hydroxy phenylarsonic acid (*p*-HPAA) and *p*-acylamino phenylarsonic acid (AAPAA) (**Fig. 1**) have been successively employed for the same purposes. Their slight structural difference, i. e. different substituent groups on the aromatic ring, results in different growth-promoting and disease-controlling effects [2]. Phenylarsonic acids have been approved as feed additives by many countries at levels of 25–50 mg/kg [3]. Recent studies showed that phenylarsonic acids in the environment might be converted into elemental arsenic and other inorganic arsenic compounds, which are known to be strongly carcinogenic [4]. Some countries in EU strictly control the use of

phenylarsonic acid additives, while in the U.S., Tyson Foods, the country's largest poultry producer, stopped the use of arsenic compounds in 2004. After the release of 2011 FDA report of elevated inorganic arsenic in the livers of chickens treated with ROX, Pfizer Animal Health, the US manufacturer of ROX, quickly suspended ROX sales [5]. In order to monitor the residues of phenylarsonic acid in animal products, it is of great significance to establish a convenient, sensitive and reliable method to analyze the organic arsenic in samples [6].

Several analytical methods for the determination of phenylarsonic acids in the environment have been reported, including liquid chromatography (LC) coupled to atomic absorption spectroscopy (AAS) [7], atomic emission spectroscopy (AES) [8] or atomic fluorescence spectrometry (AFS) [9] as well as gas chromatography-mass spectrometry (GC-MS) [10], capillary electrophoresis (CE) coupled to ultraviolet and visible light detector [11] or inductively coupled plasma-mass spectrometry (ICP-MS) [12]. LC has been demonstrated to be the most effective method in arsenic separation, and ICP-MS can provide low



**Figure 1. Effect of CM- $\alpha$ -CD amount used in modification on the adsorption efficiency of phenylarsonic acids (ROX). (n = 3, with RSD < 6.5%).**

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detection limit. Therefore, LC-ICP-MS is one of the most powerful research means for analysis of organic arsenic in complex samples [2,13]. The LC-MS/MS is a powerful separation and detection platform in multi residues analysis. Pergantis et al [14] have developed a stable method for determination of 5 phenylarsonic acids including ROX, p-APAA, p-NPAA, AAPAA, p-HPAA and other organic arsenics using LC-MS/MS in positive ionization mode. The LODs of the developed method achieved sub ng/g level, whereas the analytical time was too long. Furthermore, compared with other arsenic speciation methods, the LC-MS/MS could provide more structural information of the phenylarsonic acids.

After feeding, nearly all phenylarsonic acids are excreted unchanged to the environment through the disposal of poultry litter, so the residues in animal tissues are very low [15,16]. Some studies indicated that the approved conditions of use mandate a 5-day withdrawal period from the medicated feed before animals are slaughtered, and limits are in place for total residues of combined arsenic (As) in meat from ROX treated animals [ $0.5 \text{ mg kg}^{-1}$  As in muscle tissue and eggs, and  $2 \text{ mg kg}^{-1}$  As in liver and kidney] [17]. To further lower the detection limits of phenylarsonic acids in complex biological samples, nano-materials have been used to selectively extract and concentrate arsenic compounds. The most commonly used nano-materials for the adsorption of arsenic compounds include goethite [18], titania [19], iron oxide [20,21], Fe<sub>3</sub>O<sub>4</sub> nanoparticles [22,23] or modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles [24]. Among these nano-materials, Fe<sub>3</sub>O<sub>4</sub> nanoparticles are well suited for arsenic analysis due to the following advantages. First, they can be easily isolated from solutions by applying an external magnetic field [25], which ensures simplified sample adsorption and elution processes. Second, Fe<sub>3</sub>O<sub>4</sub> nanoparticles have been demonstrated to have higher affinity toward arsenic element than other nano-materials, resulting in higher arsenic extraction efficiency. Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles have been used for removing inorganic arsenic ions in water sample. For example, Fe<sub>3</sub>O<sub>4</sub> nanoparticles dispersed in chelating resins or coated with adequate chelating agents have been used for the removal of a wide range of metal ions from wastewater, overall displaying higher adsorption capacity than traditional materials [26–29]. More recently,  $\beta$ -CD coated Fe<sub>3</sub>O<sub>4</sub> nanoparticles have been

successfully applied for the removal of methylene blue and copper ions [30,31]. It is found that the cavity of cyclodextrin and its surface hydroxyl group can impart better binding capability and chemical stability to the magnetic particle [32].

In this work, a highly sensitive determination method was established to monitor five phenylarsonic acids in chicken tissues. In our first attempt, it was found that the adsorption of phenylarsonic acids by Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles was not as good as inorganic arsenic compounds. It was then decided to use CM- $\alpha$ -CD to couple on the surface of Fe<sub>3</sub>O<sub>4</sub> providing CD cavities to fit the benzene rings in the structures of phenylarsonic acid compounds. In addition, hydrogen bonding and electrostatic interactions between hydroxyl/carboxyl groups of modified CM- $\alpha$ -CD and amino/nitro of phenyl arsenic acids were also increased. The adsorption properties of modified Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles to phenylarsonic acids were studied, and the interactions between nanoparticles and phenylarsonic acids were examined. The synthesized materials were successfully applied in the sample clean-up and pre-concentration of phenylarsonic acids in chicken muscle and liver samples, which were subsequently separated and detected by UPLC-MS/MS.

## Materials and Methods

### Apparatus

ICP-MS (Agilent7500Ce, USA) was used to study the adsorption properties of synthesized material for phenylarsonic acids. The optimum operation parameters of ICP-MS were selected by tuning. The power was 1550W, the flow rates of cooling air, auxiliary air and carrier were 15.0 L/min, 1.0 L/min and 1.12 L/min, respectively. The sample rate of ascension by using peristaltic pump was set as 1.0 mL/min. The integration time for arsenic was 0.3 s/isotope. The operating parameters of UPLC-MS/MS (Waters Xevo TQ, USA) were as follows: capillary voltage = 2.8 kv, desolvation temperature = 450°C, desolvation gas flow rate = 600 L/Hr. The mobile phase was a mixture of acetonitrile (solvent A) and water containing 0.1% formic acid (solvent B) at a flow rate of 0.3 mL/min. All chromatographic separations were carried out in linear gradient mode as follows. In the first minute, solvent A was maintained at 98% (v/v). Solvent B quickly dropped to 30% from 1 to 3 min, followed by dramatic increase back to 98% from 3 to 5 min. MS parameters of UPLC-MS/MS are showed in **Table 1**.

The closed microwave digestion system (CEM MARS, American) was used to digest samples for the determination of the total arsenic in the crude samples. The homogenizer (IKA, Germany) was applied to sample pretreatment and rocking hammock bed was from Zhicheng, Shanghai, China.

### Standard solutions and reagents

Five phenylarsonic acids (98%) were obtained from the Chinese Academy of Agricultural Sciences. The standard stock solutions were prepared by dissolving each arsenic species in pure water at an arsenic concentration equivalent to that of 1 mg/mL phenylarsonic acids and stored at 4°C in the dark.

Reagents for preparing magnetic nanoparticles: FeCl<sub>2</sub>·4H<sub>2</sub>O and FeCl<sub>3</sub>·6H<sub>2</sub>O (analytical reagent grade) were purchased from Tianjin Guangfu Fine Chemical Research Institute. Sodium chloroacetate (98%) was bought from Alfa Aesar.  $\alpha$ -cyclodextrin (98%) was purchased from Beijing Dilang Biochemical Technology Co., Ltd., China. Other reagents including methanol, ethanol, acetone, toluene, formic acid and sodium hydroxide were of analytical reagent grade and all bought from Beijing Chemical

**Table 1.** Analytical parameters of MS/MS.

Compounds	Molecular weight	Precursor Ion (m/z)	Product Ion (m/z)	Cone voltage (V)	Collision voltage (V)
p-APAA	217	218	92	27	21
			109	27	16
p-HPAA	218	219	110	28	18
			201	28	13
p-NPAA	247	248	202	30	18
			230	30	14
AAPAA	260	261	244	29	14
ROX	263	264	246	33	14

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Plant. The water used throughout the experiment was purified using a Milli-Q water purification system (Millipore, Germany).

### Preparation of CM- $\alpha$ -CD stabilized magnetic nanoparticles

**Synthesis of CM- $\alpha$ -CD.** CM- $\alpha$ -CD was prepared according to the following procedures.  $\alpha$ -CD (3.55 mmol) and NaOH (90.2 mmol) were first dissolved into 20 ml water. The solution was then heated at 90°C for 5 min, followed by the addition of 74.6 mmol sodium chloroacetate. The solution was heated for 3 h at 90°C under stirring. Once cooled to room temperature, the pH of the solution was adjusted to 6–7 by hydrochloric acid. The nearly neutral solution obtained was then poured into about 500 mL methanol. CM- $\alpha$ -CD was precipitated out as white solids, which was filtered and washed with methanol for a few times and then dried under vacuum for 3 d at 50°C and 0.085 MPa. The melting point of the CM- $\alpha$ -CD product was about 245.5°C as determined by micro melting point apparatus.

**Synthesis of Fe<sub>3</sub>O<sub>4</sub> nanoparticles.** Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles were prepared according to the conventional coprecipitation method [33]. A mixture of 2.0 g FeCl<sub>2</sub>·4H<sub>2</sub>O and 5.2 g FeCl<sub>3</sub>·6H<sub>2</sub>O was added into a 500 mL conical flask containing 200 ml 0.05 M HCl. After dissolution of the solids, 250 ml 0.75 M NaOH solution was poured into the flask under a blanket of N<sub>2</sub>. The mixture was stirred for another 2 h at 80°C. The Fe<sub>3</sub>O<sub>4</sub> nanoparticles were then obtained in the form of black precipitates, which were separated with a magnet and washed subsequently by water (3 times) and ethanol (twice). It should be noted that both the HCl and NaOH solutions were degassed by a sonicator for 20 min before use.

**CM- $\alpha$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles (CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub>).** The Fe<sub>3</sub>O<sub>4</sub> nanoparticles prepared in the previous step were added into 60 ml PBS buffer solution (pH = 6.6) containing 1.6 g of CM- $\alpha$ -CD. The suspension was sonicated for 3 min and then stirred for 3 h at 80°C. After cooling to room temperature, the nanoparticles were washed several times by PBS buffer solution to remove excess CM- $\alpha$ -CD. The CM- $\alpha$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles were then dried at 80°C in a vacuum oven.

### Adsorption procedure

**Static adsorption.** In a 10 mL centrifuge tube, 5 mg CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles were mixed with 8 mL standard solution of phenylarsonic acid with a given concentration. The centrifuge tube was placed on a rocking hammock bed at a rate of 270 rpm. After equilibrating for 30 min, the magnetic nanoparticles were separated from the solution with external magnetic field. The

nanoparticles were rinsed twice with ethanol and dried in N<sub>2</sub>. To desorb target compounds, 1.0 mL pure water was added to the nanoparticles followed by equilibration for 10 min. The aqueous solution containing target compounds was filtered through a 0.22  $\mu$ m Poly (ether sulfones) (PES) syringe filter and analyzed by ICP-MS. Standard phenylarsonic acid solutions of other concentrations were analyzed in the same way.

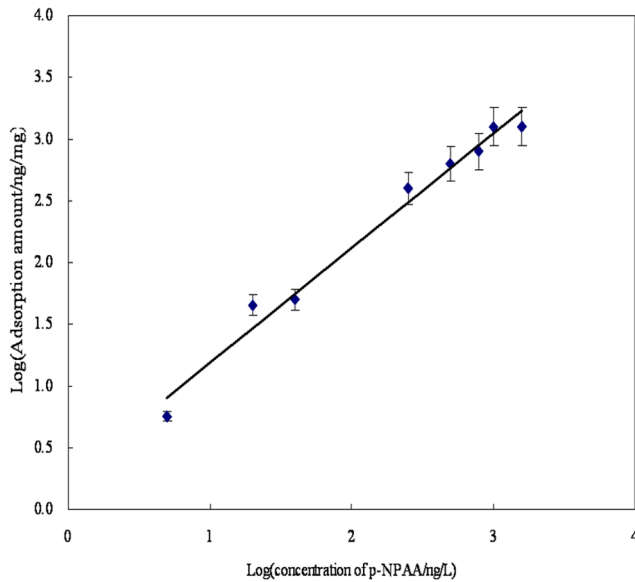
**Dynamic adsorption.** In a 10 mL centrifuge tube, 5 mg CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles were mixed with 8 mL standard solution of phenylarsonic acid with a given concentration. A number of centrifuge tubes were prepared in this manner for a given concentration of standard. The centrifuge tubes were placed on an orbital shaker at a rate of 270 r/min. At different time points, one tube was removed and the magnetic nanoparticles contained in the tube were separated from the solution with external magnetic field. The following washing, desorption and ICP-MS analysis procedures were the same as those in the static adsorption step. Standard phenylarsonic acid solutions of other concentrations were analyzed in the same way.

### Sample analysis

Chicken tissues including meat and liver were bought from a supermarket in Beijing. Chicken meat and chicken liver samples were pulverized and freeze-dried for 24 h. The freeze-dried sample was homogenized by grinding and frozen until analysis. In a 50 mL centrifuge tube, 5.0 g freeze-dried chicken tissue sample and 10 mL ethanol were added. The extraction was repeated twice, each lasting 30 min. The combined extracts were equilibrated with 5 mg CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles for 10 min. Then the magnetic nanoparticles which adsorbed target analytes were separated under external magnetic field, the analytes adsorbed on the nanoparticles were then desorbed with 2 mL deionized water. The aqueous solution containing target compounds was filtered through a 0.22  $\mu$ m PES syringe filter and analyzed by UPLC-MS-MS.

### Determination of total arsenic

The total amount of arsenic in chicken tissue samples was determined by microwave digestion ICP-MS according to reference [34]. Each digestion can containing 0.5 g chicken tissues samples was added 5 mL 65% nitric acid. Stages digestion method by controlling temperature was used. The obtained digestion solution was diluted until the concentration of nitric acid fell below 5% and then subjected to ICP-MS analysis.

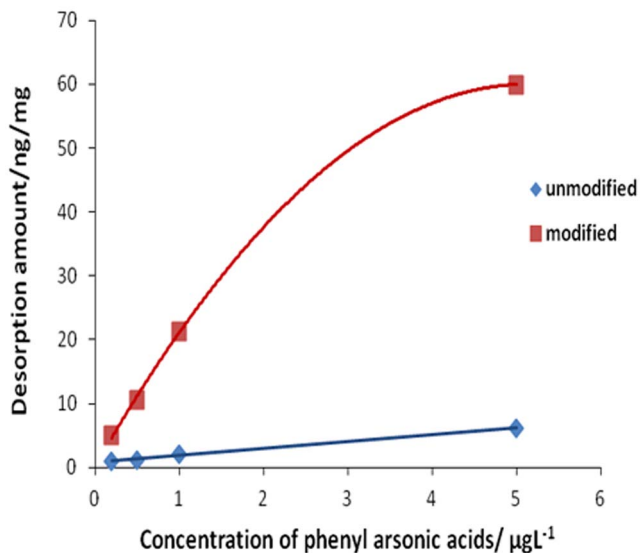


**Figure 2. Recovery of different concentrations of phenylarsonic acids (ROX). (n = 3, with RSD < 5.0%).**  
doi:10.1371/journal.pone.0107147.g002

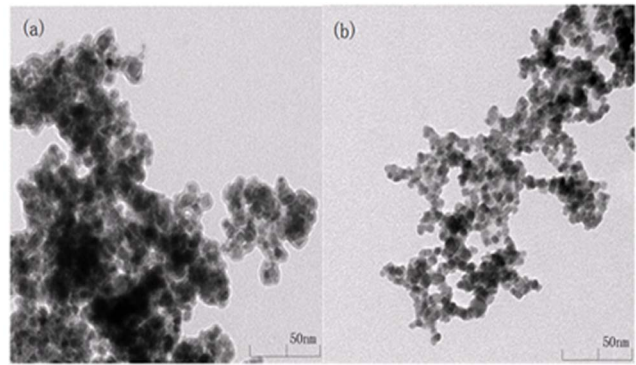
## Results and Discussion

### Optimization of adsorption efficiency

For optimal adsorption efficiency, the amounts of CM- $\alpha$ -CD for the modification of Fe<sub>3</sub>O<sub>4</sub> nanoparticles were varied in six dosages of 0.8, 1.6, 2.4, 3.2, and 4.8 g to obtain 450 mL of Fe<sub>3</sub>O<sub>4</sub> suspension as described in section 2.3.2. The adsorption was carried out by equilibrating 5 mg CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> in 8 mL ROX standard solution at a concentration of 1  $\mu$ g/L. The adsorption efficiency was then determined by ICP-MS. As shown in Fig. 1, the highest adsorption efficiency corresponds to 1.6 g CM- $\alpha$ -CD. Fig. 2 shows the adsorption behaviors of ROX at different concentrations by CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub>. The amount of



**Fig. 3 Comparison of Fe<sub>3</sub>O<sub>4</sub> nanoparticles (blue diamond) and CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles for adsorption of ROX (red square).**  
doi:10.1371/journal.pone.0107147.g003



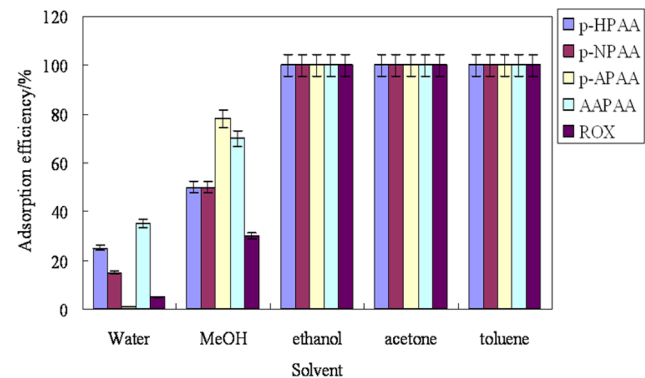
**Figure 4. TEM images of magnetic Fe<sub>3</sub>O<sub>4</sub> nanoparticles (a) and CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles (b).**  
doi:10.1371/journal.pone.0107147.g004

adsorbed ROX is linear over a large concentration range of ROX. To verify the important role of CM- $\alpha$ -CD in the adsorption of phenylarsonic acid, the adsorption efficiencies of modified and unmodified Fe<sub>3</sub>O<sub>4</sub> nanoparticles for p-NPAA were compared. As shown in Fig. 3, modified material was obviously superior to unmodified material.

### Characterization of magnetic CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub>

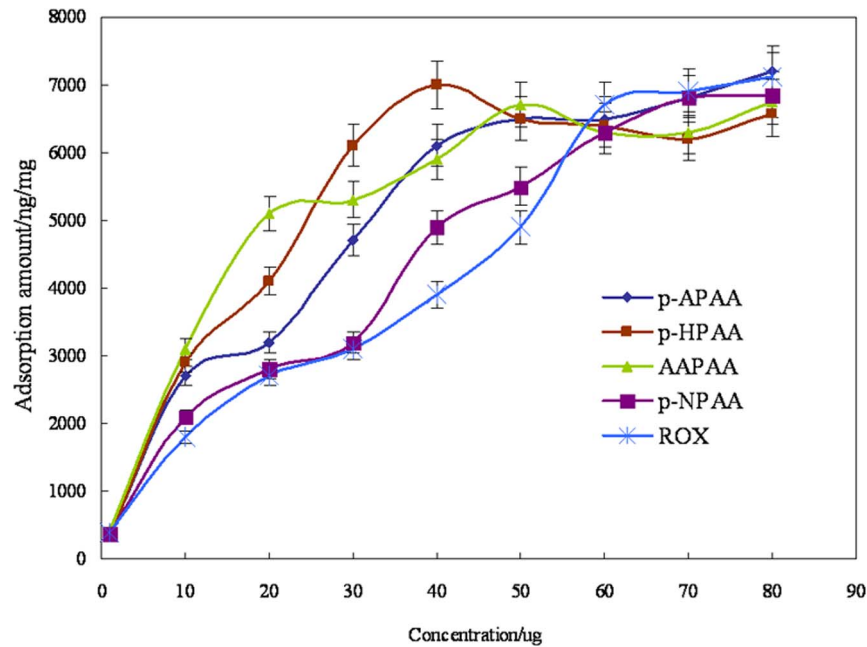
The transmission electron microscope (TEM), fourier transform infrared spectrometry (FTIR) and thermo gravimetric analyzer (TGA) were used to characterized the magnetic nanoparticles. The TEM images of Fe<sub>3</sub>O<sub>4</sub> and CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles are shown in Fig. 4 (a) and Fig. 4 (b), respectively. Unmodified Fe<sub>3</sub>O<sub>4</sub> nanoparticles, approximately 10 nm in diameter, tend to aggregate because of size effect of Fe<sub>3</sub>O<sub>4</sub> nanoparticles. On the other hand, CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles shown in Fig. 4 (b) are much better dispersed in an aqueous solution with diameters of about 5 nm, because the Fe<sub>3</sub>O<sub>4</sub> nanoparticles were modified with CM- $\alpha$ -CD and the surface of Fe<sub>3</sub>O<sub>4</sub> nanoparticles was protected by CM- $\alpha$ -CD. It can be observed in Fig. 4 (b) that the composite of modified nanoparticles was more compacted and displayed roughly spherical shapes.

The FTIR spectrums of Fe<sub>3</sub>O<sub>4</sub>, CM- $\alpha$ -CD and CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles respectively were scanned (See Fig. S1). In all three samples, a strong characteristic O-H absorption band at



**Figure 5. Effect of adsorption solvent on the adsorption efficiency of five phenylarsonic acids.** The concentration of each phenylarsonic acids was 50 ng/mL. Adsorption time was 30 min. 5 mg adsorbent was used. (n = 3, with RSD < 4.5%).  
doi:10.1371/journal.pone.0107147.g005





**Figure 6. Adsorption saturation curve of CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles to five phenylarsonic acids.** 5 mg adsorbent was used for each solution. Adsorption time was 30 min. Desorption was in water for 10 min. (n = 3, with RSD < 5.7%). doi:10.1371/journal.pone.0107147.g006

around 3400 cm<sup>-1</sup> is clearly visible. There is a strong Fe-O absorption peak at 580 cm<sup>-1</sup> for both Fe<sub>3</sub>O<sub>4</sub> and CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub>, suggesting the intactness of the Fe-O bond during the modification process. Compared with the innate Fe<sub>3</sub>O<sub>4</sub> nanoparticles, new characteristic peaks at 1100 and 2900 cm<sup>-1</sup> appeared in the spectrum of CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles, corresponding to C-O-C and C-H groups of CM- $\alpha$ -CD, which are also visible in the spectrum of pure CM- $\alpha$ -CD. This confirms the success of the modification of CM- $\alpha$ -CD to Fe<sub>3</sub>O<sub>4</sub> nanoparticles.

The amount of CM- $\alpha$ -CD grafted on the surface of Fe<sub>3</sub>O<sub>4</sub> and the number of CM- $\alpha$ -CD molecules immobilized on a single Fe<sub>3</sub>O<sub>4</sub> nanoparticle can be estimated from the TGA results. It is known that CM- $\alpha$ -CD decomposes completely above 600°C. The TGA curves of Fe<sub>3</sub>O<sub>4</sub> and CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> exhibit two steps of weight loss. In both cases, the first step can be attributed to the loss of residual water. The amounts of CM- $\alpha$ -CD coated on the surface of Fe<sub>3</sub>O<sub>4</sub>, calculated on the basis of the second-step loss, are 5.3% and 9.6% for Fe<sub>3</sub>O<sub>4</sub> nanoparticles and CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles, respectively. The increase of the weight loss can be ascribed to CM- $\alpha$ -CD grafted on Fe<sub>3</sub>O<sub>4</sub> nanoparticles (about 43 mg/g). According to Y. P. Wu [35], the number of CM- $\alpha$ -CD molecules immobilized on a single Fe<sub>3</sub>O<sub>4</sub> can be calculated in

equation (1):

$$N = \frac{4 \times (W_{CD} - W_{Fe_3O_4}) \times N_{A\rho\pi R^3}}{3 \times M_{CD} \times (1 - W_{CD}) \times 10^{21}} \quad (1)$$

where N is the number of CM- $\alpha$ -CD molecules immobilized on each Fe<sub>3</sub>O<sub>4</sub>, R is the mean radius of CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> (5.0 nm based on the TEM results),  $\rho$  is the density of the nanoparticle (4.8 g/cm<sup>3</sup>), N<sub>A</sub> is Avogadro's number, W<sub>CD</sub> and W<sub>Fe<sub>3</sub>O<sub>4</sub></sub> are the weight losses of the CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>3</sub>O<sub>4</sub> respectively, M<sub>CD</sub> is the molar mass of CM- $\alpha$ -CD immobilized on CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub>. The calculated number of CM- $\alpha$ -CD molecules immobilized on each CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> is about 70.

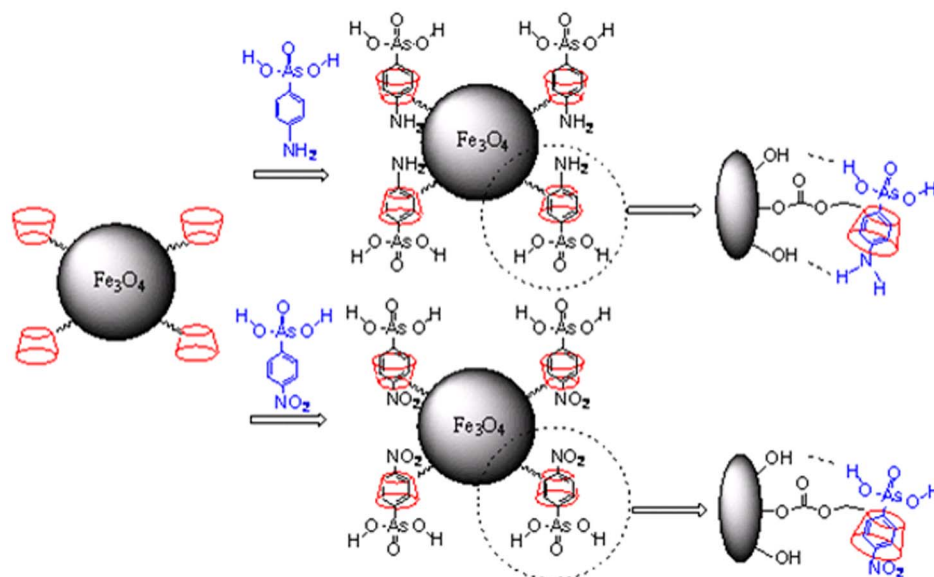
### Selection of adsorption solvent

The solvent for phenylarsonic acid solutions is an important factor affecting the adsorption efficiency. Five candidate solvents, i.e., water, methanol, ethanol, acetone and toluene, were compared (Fig. 5). The adsorption efficiency of all target arsenic species could reach 100% in ethanol, acetone and toluene. Ethanol was selected as the solvent in the following experiments

**Table 2. Enrichment factors of CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> for 0.1  $\mu$ g/L of each phenylarsonic acids in toluene solvent.**

Phenylarsonic acids	Theory concentration ( $\mu$ g/L)	Experimental concentration ( $\mu$ g/L)	Enrichment factor	Enrichment Efficiency (%)
p-APAA	50.0	41.4	414	82.8
AAPAA	50.0	42.6	426	85.2
ROX	50.0	58.1	581	116.2
p-HPAA	50.0	60.6	606	121.2
p-NPAA	50.0	34.9	349	69.8

doi:10.1371/journal.pone.0107147.t002



**Figure 7. The likely interactions between CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> and the acids p-APAA and p-NPAA.**

doi:10.1371/journal.pone.0107147.g007

due to the relatively low toxicity. In water and methanol, the adsorption efficiencies of the five target compounds are very poor, which might be because of the water and methanol possess higher polarity than ethanol, acetone and toluene.

### Static adsorption

**Saturation of adsorption.** The adsorption saturation curve is shown in **Fig. 6**. The point of saturation was reached at 40  $\mu$ g for p-HPAA, p-APAA, and AAPAA, whereas it was 60  $\mu$ g for p-NPAA and ROX, corresponding to about 0.2  $\mu$ mol of each phenylarsonic acid.

The amount of CM- $\alpha$ -CD coated on the surface of Fe<sub>3</sub>O<sub>4</sub> could be estimated from the saturation of adsorption. Since one phenylarsonic acid molecule was supposed to fit one CD cavity, there should be approximately equal amount of CD and phenylarsonic acid. Thus, the amount of CM- $\alpha$ -CD might be calculated through its molecular mass of 1029 according to equation (2). The estimated amount of grafted CM- $\alpha$ -CD was about 41.2 mg/g, which was similar to 43 mg/g calculated by TGA in 3.2.3.

$$m_{\text{CM-a-CD}} = \frac{n_{\text{saturated}} \times M_{\text{CM-a-CD}}}{m_{\text{adsorbent}}} \quad (2)$$

**Enrichment factors of low concentration.** The lowest concentrations of phenylarsonic acids that could be absorbed by CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> as well as enrichment factors were investigated. Ultimately, the enrichment factors of p-APAA, AAPAA, ROX, p-HPAA and p-NPAA were 414, 426, 581, 606 and 349 (theoretical enrichment factor was 500) at the concentration of 0.1  $\mu$ g/L. Enrichment efficiency was 69.8%-121.2% and experimental results are shown in **Table 2**.

### Dynamic adsorption

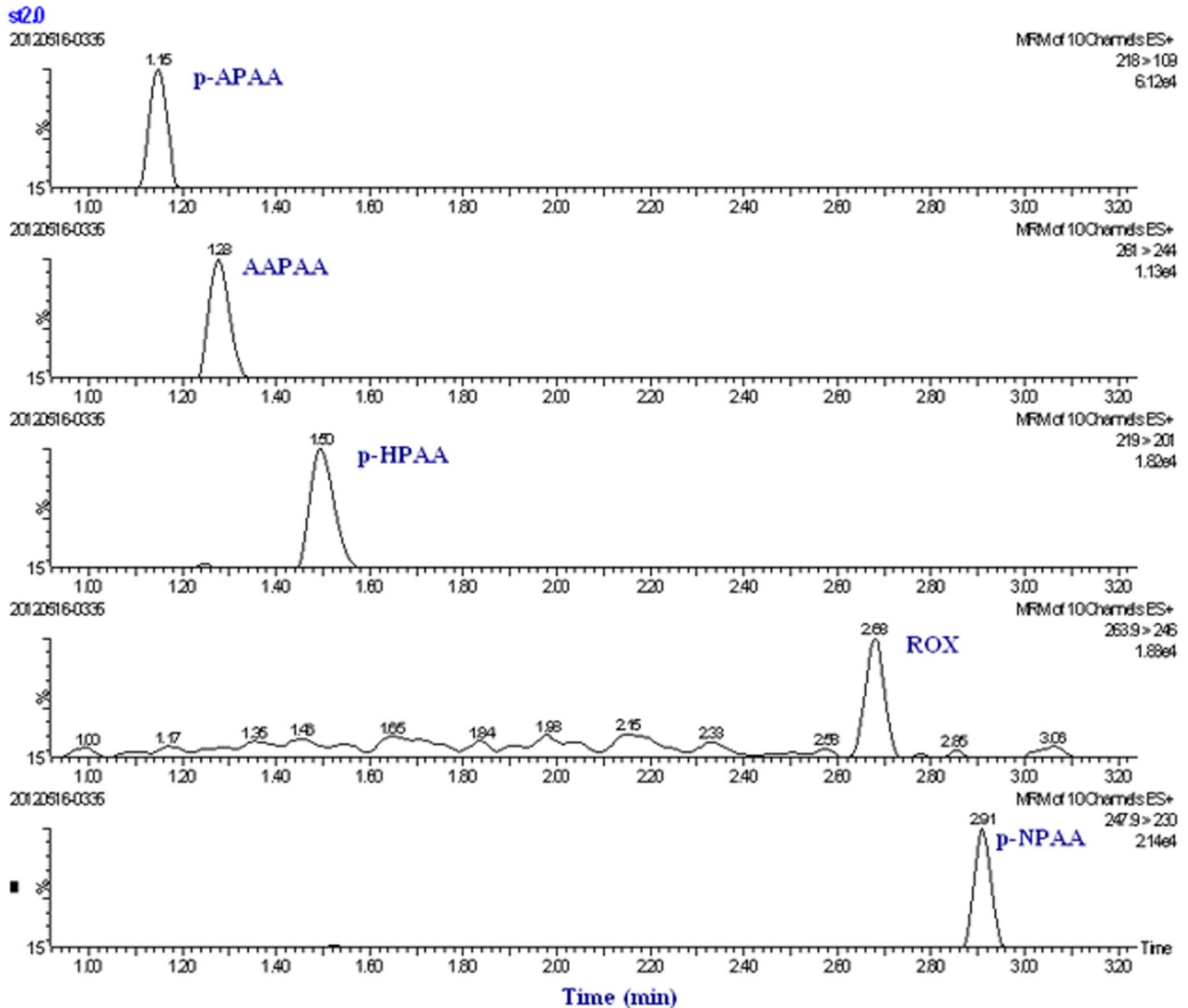
**Optimization of adsorption time and desorption time.** The water was selected as desorption solvent. The adsorption efficiency was optimized by varying the adsorption time in the range of 1–40 min with all other parameters held constant. The adsorption efficiency increased with the adsorption time from 1 to 30 min followed by a plateau. The rate of adsorption was so high that 5 min was enough to adsorb the target compounds. Similarly, desorption efficiency was optimized by varying desorption time in the range of 1–10 min. The desorption efficiency increased with desorption time till 10 min, at which point it plateaued. Therefore, 10 min was selected as desorption time and desorption efficiency was achieved above 75%.

**The tolerance of coexisting inorganic ions and organic analogues.** By fixing the concentration of each phenylarsonic acids at 50  $\mu$ g/L and 12 coexisting inorganic ions including Mn<sup>2+</sup>, Cu<sup>2+</sup>, K<sup>+</sup>, Zn<sup>2+</sup>, Ba<sup>2+</sup>, Fe<sup>3+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Pb<sup>2+</sup>, Cr<sup>3+</sup>, Cd<sup>2+</sup>, and

**Table 3.** Concentration ( $\mu$ g/L) of coexisting analogues when tolerance factor less than 5%.

	phenol	benzoic acid	p-hydroxybenzoic acid	m-hydroxybenzoic acid
AAPAA	500	500	500	500
p-HPAA	500	250	250	250
p-NPAA	50	500	50	250
p-APAA	>500	500	500	250
ROX	250	500	500	250

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**Figure 8. MRM chromatogram of five arsenical compounds standards at 2.0  $\mu\text{g/L}$ .**  
doi:10.1371/journal.pone.0107147.g008

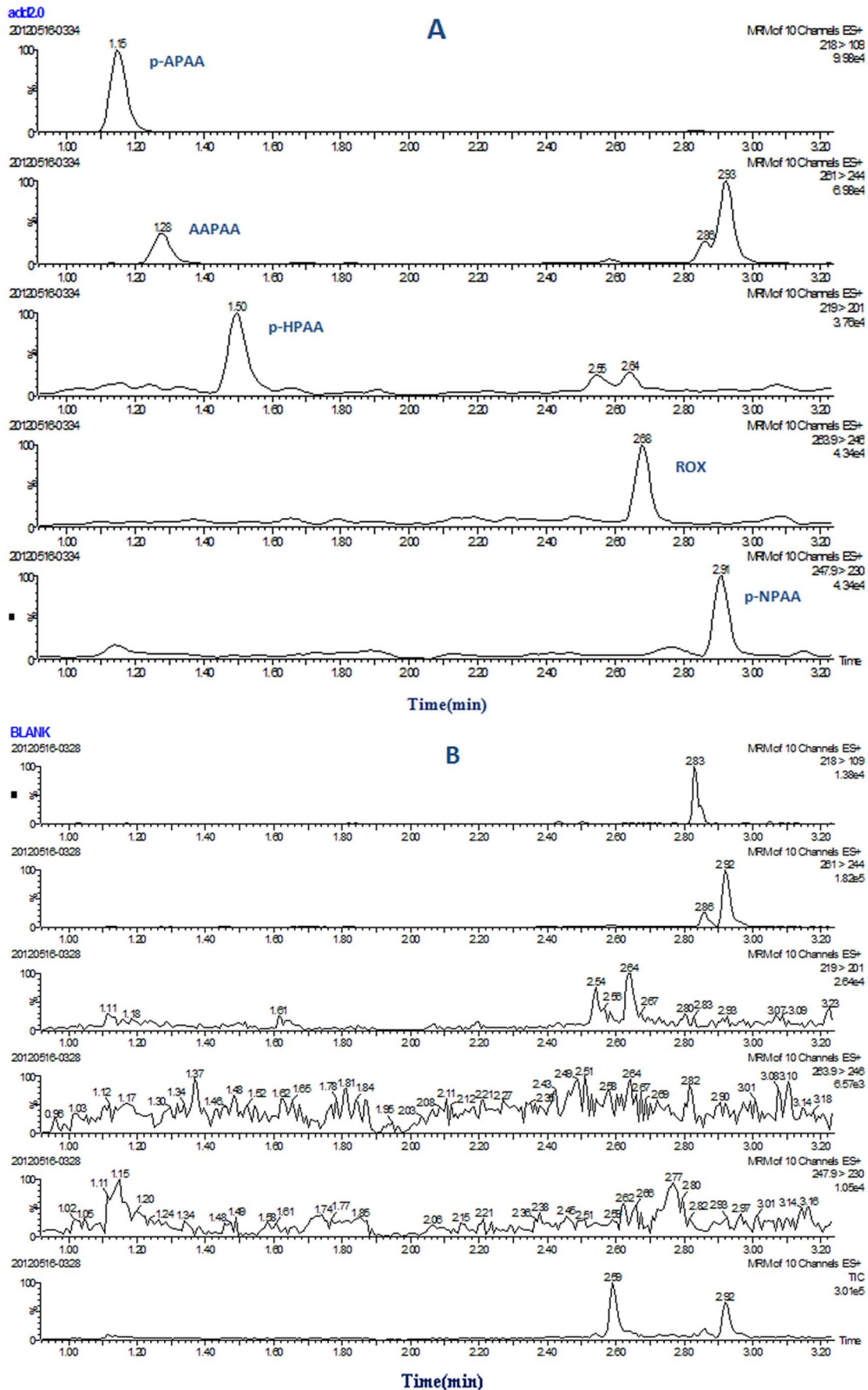
$\text{Sn}^{2+}$  at concentrations of 500, 5000, and 50000  $\mu\text{g/L}$  for each inorganic ions respectively, interference of inorganic ions on the adsorption of phenylarsonic acids was studied. No significant interferences were observed under the optimum conditions

described above in the presence of inorganic ions as high as 50000  $\mu\text{g/L}$ . The influence of four organic analogues including phenol, benzoic acid, p-hydroxybenzoic and m-hydroxybenzoic acid at concentrations of 50, 250 and 500  $\mu\text{g/L}$  for each organic

**Table 4. Analytical performance data with UPLC-MS/MS.**

Compounds	Linear range ( $\mu\text{g/L}$ )	Linear equation	R <sup>2</sup>	LOD ( $\mu\text{g/kg}$ )
p-APAA	0.2–10.0	$y = 7305.3x + 2211.7$	0.9952	0.05
AAPAA	0.2–10.0	$y = 2287.2x + 398.63$	1.0000	0.05
p-HPAA	0.2–10.0	$y = 5739.4x + 935.79$	0.9984	0.10
ROX	0.2–10.0	$y = 3249.2x - 144.89$	1.0000	0.10
p-NPAA	0.2–10.0	$y = 1712.3x - 87.287$	0.9988	0.11

x: the concentration of five phenylarsonic acids, y: peak area. LOD = 3 S/N of blank chicken tissues sample.  
doi:10.1371/journal.pone.0107147.t004



**Figure 9. MRM chromatogram of spiked chicken meat (2.0  $\mu$ g/kg) (A) and blank (B).**  
doi:10.1371/journal.pone.0107147.g009



**Table 5.** Recoveries of spiked arsenics in chicken liver and muscle sample (n = 5).

Compounds	Chicken liver			Chicken meat		
	Added ( $\mu\text{g}/\text{kg}$ )	Recovery (%)	RSD (%)	Added ( $\mu\text{g}/\text{kg}$ )	Recovery (%)	RSD (%)
p-APAA	0.2	80.7	5.0	0.2	87.3	8.0
	2.0	89.5	6.7	2.0	86.1	7.6
	20	87.7	4.2	20	101.8	5.2
p-HPAA	0.2	94.3	4.9	0.2	77.2	9.1
	2.0	97.9	6.6	2.0	95.1	5.1
	20	93.7	12.5	20	79.9	5.2
p-NPAA	0.2	102.2	4.7	0.2	91.8	7.6
	2.0	110.2	5.4	2.0	98.8	5.7
	20	99.0	10.8	20	102.8	6.3
AAPAA	0.2	82.4	4.4	0.2	83.7	12.1
	2.0	82.6	5.4	2.0	82.6	8.1
	20	78.3	2.0	20	79.6	8.5
ROX	0.2	93.4	2.9	0.2	96.9	10.1
	2.0	104.2	7.3	2.0	104.2	6.4
	20	106.1	4.8	20	108.7	5.2

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analogues respectively on the adsorption of target arsenic species was investigated. As shown in **Table 3**, the allowed concentrations of coexisting analogues were about 500  $\mu\text{g}/\text{L}$  or 250  $\mu\text{g}/\text{L}$  with a tolerance factor of less than 5%. However, p-NPAA was an exception. The tolerance concentration of p-hydroxybenzoic acid was only 50  $\mu\text{g}/\text{L}$ . Possible explanation is shown in **Fig. 7**. Despite other interactions, the hydrogen bonding between hydroxyl on the surface of CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> and amino groups of p-APAA and AAPAA was very strong but there was hardly any hydrogen bonding between the hydroxyl and nitro of p-NPAA. In any case, phenylarsonic acid could form inclusion complex with  $\alpha$ -CD and therefore the arsenate, hydroxyl or amino group were forced closer to the magnetic particles, leading to stronger interactions between the modified material and phenylarsonic acids. Both selectivity and adsorption efficiency were improved compared to that of Fe<sub>3</sub>O<sub>4</sub> nanoparticles without modification.

### Analytical performance

**Optimization of UPLC-MS-MS chromatograph separation condition.** A mixture of five arsenic compound standards, each at a concentration of 2.0  $\mu\text{g}/\text{L}$ , was successfully separated and analyzed by UPLC-MS/MS in less than 3 min on a C18 column. Under the optimal separation conditions, baseline separation was achieved for every arsenic compound. The multi-reaction monitoring (MRM) chromatograms of arsenic compounds are shown in **Fig. 8**.

**Optimization of sample pretreatment condition.** To extract phenylarsonic acids from chicken tissue samples, different extraction methods (including ultrasonic extraction, microwave extraction), extraction time (10, 20, 30, 40, 50 and 60 min) and extraction solvents (methanol, ethanol and toluene) were studied. The optimal recovery was above 75% with the ultrasonic extraction method and toluene as the extraction solvent. The proper extraction time was found to be 30 min and extraction should be conducted twice.

### Method evaluation

The developed method was validated by determining the linearity and LOD of arsenic species listed in **Table 4**. A linear response can be seen in the concentration range of 0.20–10  $\mu\text{g}/\text{L}$  (enrichment factor 2.5), with the  $R^2$  ranging from 0.9951 to 1.0000. The repeatability study was performed for each of the phenylarsonic acids under the optimal conditions. The LOD of each phenylarsonic acid was estimated by analyzing blank samples spiked at 0.2  $\mu\text{g}/\text{kg}$  of each target analytes and they were determined as the lowest concentrations of the analyte for which signal-to-noise ratios were 3 respectively. The resultant repeatabilities expressed as RSD varied from 0.85% to 4.49%. These results show that the method has a high sensitivity and good repeatability.

### Sample analysis

The phenylarsonic acid in tissue was stored with the prototype compound. So the sample preparation of real tissue samples is same as the spiked samples [17]. In order to validate the suitability of the developed method, the method was applied to analyze spiked chicken tissue samples. For comparison, the total arsenic in these samples was also determined.

In the samples of chicken meat and liver, 0.2, 2 and 20  $\mu\text{g}/\text{kg}$  of each phenylarsonic acids were spiked, respectively. The recoveries of five phenylarsonic acids in chicken tissue samples, as shown in **Table 5**, fell in the ranges of 77.2%–110.2%, with a RSD less than 12.5%. The chromatograms of spiked chicken meat sample (2.0  $\mu\text{g}/\text{kg}$ ) and blank chicken meat sample are shown in **Fig. 9**. To confirm the selectivity of CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub>, five phenylarsonic acids were spiked individually in each sample at 0.2  $\mu\text{g}/\text{kg}$  and determined by ICP-MS. Recoveries were in the range of 81.5%–119.2% which agreed with the UPLC-MS/MS results. This shows that the CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles have high selectivity for phenylarsonic acid and can be used for sample clean-up. Combining CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles clean-up procedure with UPLC-MS-MS technique would potentially result in practical application in the analysis of trace phenyl arsenic acids.

## Conclusions

In this work, CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles were synthesized to selectively extract and enrich phenylarsonic acids. CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles exhibited excellent selectivity and adsorption efficiency for five phenylarsonic acids because of the size selectivity of  $\alpha$ -CD and the affinity of Fe<sub>3</sub>O<sub>4</sub> to arsenic. In the sub ppb level of phenylarsonic acids, the enrichment factor was higher than 400 and the extraction efficiency higher than 70%. Coupled with UPLC-MS/MS, a fast, selective and convenient analytical method for the determination of phenylarsonic acid was developed. Comparing with published method [14], the developed method showed satisfactory sensitivity due to the selectively adsorption of CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> nanoparticles for phenylarsonic acids in complex sample matrix.

## References

1. Jaafar J (2001) Separation of phenylarsonic compounds by ion-pairing reversed phase-high performance liquid chromatography. *Jurnal Teknologi C UTM* 35: 71–83.
2. Wang PL, Zhao GL, Tian J, Su XO (2010) High-performance liquid chromatography-inductively coupled plasma mass spectrometry based method for the determination of organic arsenic feed additives and speciation of anionic arsenics in animal feed. *J. Agric. Food Chem* 58(9): 5263–70.
3. Chiou PW, Chen KL, Yu B (1997) Effects of roxarsone on performance, toxicity, tissue accumulation and residue of eggs and excreta in laying hens. *J. Sci. Food Agric* 74(2): 229–236.
4. Bartel M, Ebert F, Leffers L (2011) Toxicological characterization of the inorganic and organic arsenic metabolite thio-DMA(V) in cultured human lung cells. *J. Toxicol* 2011: 1–9.
5. FDA (2011) Study 275. 30 Arsenic speciation in broiler chickens. a) Summary Final Report. b) Amendments to final report. c) Analyst's report. d) Statistician's Report.
6. Thirunavukkarasu OS, Viraraghavan T, Subramanian KS, Tanjore S (2002) Organic arsenic removal from drinking water. *Urban Water* 4: 415–21.
7. Samanta G, Chowdhury TR, Mandal BK, Biswas BK, Chowdhury UK, et al. (1999) Flow Injection Hydride Generation Atomic Absorption Spectrometry for Determination of Arsenic in Water and Biological Samples from Arsenic-Affected Districts of West Bengal, India, and Bangladesh. *Microchemical J* 62(1): 174–91.
8. Ma MS, Le XC, Hu B, Jiang ZC (2000) High performance liquid chromatography with hydride generation atomic fluorescence detection for rapid urinary arsenic speciation. *J. Ana. Sci* 16: 89–96.
9. Liu JJ, Yu HX, Song HB, Qiu J, Sun FM, et al. (2008) Simultaneous determination of *p*-arsanilic acid and roxarsone in feed by liquid chromatography-hydride generation online coupled with atomic fluorescence spectrometry. *J. Environ. Monit* 10: 975–8.
10. Roerdink AR, Aldstadt JH (2004) Sensitive method for the determination of roxarsone using solid-phase microextraction with multi-detector gas chromatography. *J. Chromatogr. A* 1057 (1-2): 177–83.
11. Li P, Hu B (2011) Sensitive determination of phenylarsonic compounds based on a dual preconcentration method with capillary electrophoresis/UV detection. *J. Chromatogr. A* 1218 (29): 4779–87.
12. Rosal CG, Mompalaisir GM, Heithmar EM (2005) Roxarsone and transformation products in chicken manure: determination by capillary electrophoresis-inductively coupled plasma-mass spectrometry. *Electrophoresis* 26(7-8) 1606–14.
13. Bednar AJ, Garbarino JR, Burkhardt MR, Ranville JF, Wildeman TR (2004) Field and laboratory arsenic speciation methods and their application to natural-water analysis. *Water Res* 38 (2): 355–64.
14. Pergantis SA, Winnik W, Betowski D (1997) Determination of ten organoarsenic compounds using microbore high-performance liquid chromatography coupled with electrospray mass spectrometry-mass spectrometry. *J. Anal. At. Spectrom* 12: 531–6
15. Chen SL, Yeh SJ, Yang MH, Lin TH (1995) Trace element concentration and arsenic speciation in the well water of a Taiwan area with endemic Blackfoot disease. *Biol. Trace Elem. Res* 48 (3): 263–74.
16. Kuehnelt D, Goessler W (2003) Organometallic compounds in the environment, ed. P. J. Craig, John Wiley & Sons Ltd., Chichester, pp. 223–75.
17. Conklin SD, Shockey N, Kubachka K, Howard KD, Carson MC (2012) Development of an ion chromatography-inductively coupled plasma-mass spectrometry method to determine inorganic arsenic in liver from chickens treated with roxarsone. *J. Agric. Food Chem.* 60(37): 9394–404.

## Supporting Information

**Figure S1 FTIR spectra of Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles (a), CM- $\alpha$ -CD (b) and CM- $\alpha$ -CD-Fe<sub>3</sub>O<sub>4</sub> (c).** (TIF)

## Author Contributions

Conceived and designed the experiments: PW RZ. Performed the experiments: JJ. Analyzed the data: JW PW. Contributed reagents/materials/analysis tools: WZ PW. Contributed to the writing of the manuscript: PW RZ.

18. Chen WR, Huang CH (2012) Surface adsorption of organoarsenic roxarsone and arsenic acid on iron and aluminum oxides. *J. Hazard. Mater* 227–228: 378–85.
19. Mao XJ, Chen BB, Hu B (2011) Titania immobilized polypropylene hollow fiber as a disposable coating for stir bar sorptive extraction–high performance liquid chromatography–inductively coupled plasma mass spectrometry speciation of arsenic in chicken tissues. *J. Chromatogr. A* 1218 (1): 1–9.
20. Mayo JT, Yavuz C, Yean S, Cong L, Shipley H, et al. (2007) The effect of nanocrystalline magnetite size on arsenic removal. *Sci. Technol. Adv. Mater* 8(1-2): 71–5.
21. Akin I, Arslan G, Tor A, Ersoz M, Cengelglu Y (2012) Arsenic(V) removal from underground water by magnetic nanoparticles synthesized from waste red mud. *J. Hazard. Mater* 235–236: 62–8.
22. Li RX, Liu SM (2011) Preparation and characterization of cross-linked  $\beta$ -cyclodextrin polymer/Fe<sub>3</sub>O<sub>4</sub> composite nanoparticles with core-shell structures. *Chin. Chem. Lett* 22 (2): 217–20.
23. Kan XW, Zhao Q, Shao DL, Geng ZR, Wang ZL, et al. (2010) Preparation and Recognition Properties of Bovine Hemoglobin Magnetically Molecularly Imprinted Polymers. *J. Phys. Chem. B* 114 (11): 3999–4004.
24. Liu ZG, Zhang FS, Sasai R (2010) Arsenate removal from water using Fe<sub>3</sub>O<sub>4</sub>-loaded activated carbon prepared from waste biomass. *Chem. Eng. J.* 160 (1): 57–62.
25. Badruddoza AZM, Hidajat K, Uddin MS (2010) Synthesis and characterization of  $\beta$ -cyclodextrin-conjugated magnetic nanoparticles and their uses as solid-phase artificial chaperones in refolding of carbonic anhydrase bovine. *J. Colloid. Interface Sci* 346 (2): 337–46.
26. Zhang CC, Li X, Pang JX (2001) Synthesis and adsorption properties of magnetic resin microbeads with amine and mercaptan as chelating groups. *J. Appl. Polym. Sci* 82 (7) 1587–92.
27. Atia AA, Donia AM, El-Enein SA, Yousif AM (2007) Effect of Chain Length of Aliphatic Amines Immobilized on a Magnetic Glycidyl Methacrylate Resin towards the Uptake Behavior of Hg(II) from Aqueous Solutions. *Sep. Sci. Technol.* 42 (2): 403–20.
28. Atia AA, Donia AM, Yousif AM (2008) Removal of some hazardous heavy metals from aqueous solution using magnetic chelating resin with iminodiacetate functionality. *Sep. Purif. Technol* 61 (3): 348–57.
29. Mohan D, Pittman CU (2007) Arsenic removal from water/wastewater using adsorbents—A critical review. *J. Hazard. Mater* 142 (1-2): 1–53.
30. Badruddoza AZM, Hazel GSS, Hidajat K, Uddin MS (2010) Synthesis of carboxymethyl- $\beta$ -cyclodextrin conjugated magnetic nano-adsorbent for removal of methylene blue. *Colloids Surf. A* 367 (1-3): 85–95.
31. Badruddoza AZM, Tay ASH, Tan PY, Hidajat K, Uddin MS (2011) Carboxymethyl- $\beta$ -cyclodextrin conjugated magnetic nanoparticles as nano-adsorbents for removal of copper ions: Synthesis and adsorption studies. *J. Hazard. Mater.* 185 (2-3): 1177–86.
32. Zhi J, Tian XL, Zhao W, Shen JB, Tong B, et al. (2008) Self-assembled film based on carboxymethyl- $\beta$ -cyclodextrin and diazo resin and its binding properties for methylene blue. *J. Colloid. Interface Sci.* 319 (1): 270–6.
33. Wu W, He QG, Jiang CZ (2008) Magnetic Iron Oxide Nanoparticles: Synthesis and Surface Functionalization Strategies. *Nanoscale Res. Lett* 3(11): 397–415.
34. Krachler M, Radner H, Irgolic KJ (1996) Microwave digestion methods for the determination of trace elements in brain and liver samples by inductively coupled plasma mass spectrometry. *Fresenius' Journal of Analytical Chemistry* 355(2): 120–8.
35. Wu YP, Zuo F, Zheng ZH, Ding XB, Peng YX (2009) A Novel Approach to Molecular Recognition Surface of Magnetic Nanoparticles Based on Host-Guest Effect. *Nanoscale Res. Lett.* 4 (7): 738–47.