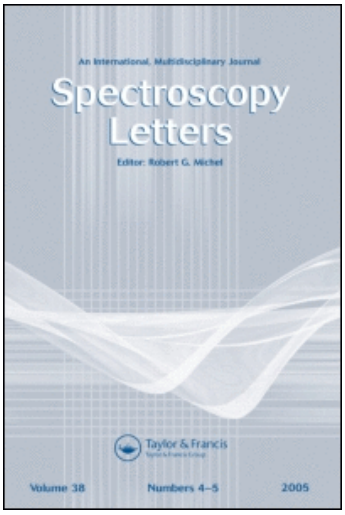

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Arsenic Speciation in Chinese Herb *Ligusticum chuanxiong* Hort by Hydride Generation Atomic Fluorescence Spectrometry

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Abstract: Trace arsenic in *Ligusticum chuanxiong* Hort in the forms arsenite, As(III), arsenate, As(V), and organic arsenic were determined by hydride generation atomic fluorescence spectrometry (HG-AFS). It was observed that As(V) has significant atomic fluorescence that interferes with the signals of As(III) in the 10% HCl matrix. Interferences from heavy metals such as Pb(II) and Cu(II) can cause severe increase of the signals as compared to the insignificant effects caused by Zn(II), Mn(II), Cd(II), and Fe(III). The masking agent 8-hydroxyquinoline was found to be an efficient agent to eliminate interference of transition metals and to remove entirely the atomic fluorescence of As(V) in a mixed As(III)/As(V) solution.

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A sensitive and interference-free procedure was developed for the arsenic speciation of the Chinese herb samples by use of HG-AFS.

Keywords: Arsenic speciation, HG-AFS, *Ligusticum chuanxiong* Hort

INTRODUCTION

The great beneficial effects of traditional Chinese herbs on people's health have increasingly been noticed.[1] Unfortunately, contamination of soil with heavy metals has occurred on a global scale. Those herbs grown while exposed to toxic heavy metals may cause adverse effects on human health. Many arsenic compounds are widely present in the natural environment, and the toxicity and bioavailability of arsenic compounds depend on their chemical forms.[2] Inorganic arsenic compounds such as arsenite, As(III), and arsenate, As(V), are more toxic than organic arsenic compounds, and arsenite has been recognized as a human carcinogen mainly, in lung and skin cancer.[3] The trace arsenic existing in Chinese herbs has a pharmacological effect as well as a curative effect.[1] Because of the variable toxicity levels of arsenic species found in Chinese herbs, total arsenic determinations alone do not provide adequate assessment information, and thus the determination of individual arsenic species is necessary.[4,5]

Publications on arsenic speciation of traditional Chinese herbs are very rare other than on natural waters,[6] soils,[7–10] and foods.[11] The purpose of this study was to establish a rapid and sensitive method for arsenic speciation and analysis in order to understand the behavior of arsenic on solubility, mobility, and transport in the Chinese herb *Ligusticum chuanxiong* Hort.

EXPERIMENTAL

Equipment

An AFS-2202 instrument equipped with a continuous flow injection hydride generation system and a boosted discharge hollow cathode lamp, both from Haiguang (Beijing Haiguang Instrument Company, Beijing, China), was used for the atomic fluorescence determination for all the experiments reported in this paper. Hydride compounds were atomized in an argon–hydrogen flame produced during the hydride generation process. The instrument was operated under the following conditions: hollow-cathode lamp current, 55 mA; negative photomultiplier voltage, 270 V; carrier gas (Ar), 400 mL/min; shield gas (Ar), 1000 mL/min; temperature of furnace, 200°C; carrier solution, 10% (v/v) HCl; sample flow rate, 9 mL/min; flow rate of $NaBH_4$, 4.5 mL/min; delay time,

4 s; analysis time, 16 s; signal type, peak area. The limit of detection (3σ) of the instrument was 0.01 μg/L, and the linear range was up to 100.0 μg/L for As(III).

Materials and Reagents

Materials

The herbaceous plant samples used in this study were collected from five planting bases of good agriculture practice (GAP) for the traditional Chinese herb *Ligusticum chuanxiong* Hort, located in the low-altitude places of Dujiangyan City (Xudu countryside, altitude 623 ± 5 m; Minxing countryside, altitude 630 ± 5 m), Penzhou City (Aoping countryside, altitude 588 ± 5 m), and the high-altitude places of Wenchuan County (Shimo countryside, altitude 1210 ± 5 m) and Shifang City (Bajiao countryside, altitude 1120 ± 5 m), respectively, in Sichuan Province, China. After harvest, the whole plants were washed with double-deionized water in the lab and then separated into three different groups for samples collected as rhizomes, stems (upper and lower stems), and frondages. The samples were freeze-dried, ground to fine powder using a ceramic mortar and pestle, and stored in 20-mL plastic bags until use.

Reagents

Stock solutions of 1000 mg/L As(III) and As(V) were prepared from commercial salts: sodium arsenite ($NaAsO_2$, Chengdu Sima Co., China), sodium hydrogenarsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, Chengdu Sima Co., China) in 10% (v/v) HCl (A.R., Chengu Chemicals Factory, China).

A 2.5% sodium tetrahydroborate (99%, Chengdu Sima Co., China) solution stabilized in 0.4% (w/v) NaOH (A.R., Chengu Chemicals Factory, China) was prepared. The solution was filtered through a 0.45 μm membrane. A stock solution of 50% (w/v) potassium iodide (A.R., Shanghai Chemicals Factory, China) containing 10% w/v L-ascorbic acid (A.R., Chengu Chemicals Factory, China) was prepared.

To prepare a 0.1 % (w/v) 8-hydroxyquinoline stock solution, 0.10 g of 8-hydroxyquinoline (A.R., Chengdu Sima Co., China) was dissolved in 1.0 mL of methanol (99.8%, A.R., Chengdu Chemicals Factory, China) to expedite its solubility, then diluted with double-deionized water to 100.0 mL in 10% (v/v) HCl. Stock solutions of 1000 mg/L Cd(II), Zn(II), Cu(II), Mn(II), Fe(III), and Pb(II) dissolved in 1% HNO_3 (A.R. for all agents from either Chengdu or Shanghai Chemicals Factory, China) were used for interference study.

For all solutions, hydrochloric acid (A.R., Chengu Chemicals Factory, China) and double-deionized water were used. All stock solutions were stored at 4°C in the dark and used for daily preparation of dilute standard solutions.

Analytical Procedures

Sample Extraction Procedure for Total Inorganic Arsenic

A precise 0.50 g of the herbal powder (0.074 mm) was weighed into a 25-mL Teflon digestion bomb separately. After the addition of 10.0 mL of 1% (v/v) HCl, the bomb cap was tightened and the sample was extracted at room temperature overnight.[12,13] Then, the dissolved phase and the residue were filtered through a 0.22 μm acid-resistant cellulose nitrate membrane and made up to 25.0 mL with double-deionized water.

Analytical Procedures for As(III)

In an aliquot of 5.0 mL of the filtered solution, 1.25 mL of 0.1% 8-hydroxyquinoline solution and 2.5 mL HCl (conc.) were added to obtain 25.0 mL of final solution in 0.005% 8-hydroxyquinoline and 10% HCl matrix. The concentration of As(III) in the sample was determined directly by HG-AFS.

Determination of Total Inorganic Arsenic As(V) + As(III)

In another 5.0 mL of the filtered solution, 1.0 mL of 50% KI solution, 1.25 mL of 0.1% 8-hydroxyquinoline solution, and 2.5 mL of HCl (conc.) were added to obtain a 25.0 mL final solution in 2.0% KI, 0.005% 8-hydroxyquinoline, and 10% HCl. The concentration of As(V) was obtained by subtracting As (III) from the total inorganic As, As(V) + As(III).

Total Arsenic Analytical Procedure, As(V) + As(III) + Organic As

Another 0.50 g aliquot of the herbal powder (0.074 mm) was weighed precisely in a 25-mL Teflon digestion bomb. Then, 15.0 mL of HNO_3 (conc.) and 2.0 mL of H_2O_2 were added, and the cap was tightened. The sample was subjected to microwave oven digestion at 720 W for 5 min. After digestion, the sample was allowed to cool at room temperature and filtered into a clean graduated cylinder. Then, 1.0 mL of 50% KI, 1.25 mL of 0.1% 8-hydroxyquinoline, and 2.5 mL of HCl (conc.) were added. A 25.0 mL final solution was made up with double-deionized water and left for 20 min at room temperature, then determined by HG-AFS. The concentration of organic As was obtained by subtracting the sum of As(V) + As(III) from the total As concentration.

RESULTS AND DISCUSSION

KI and the Optimal Acidity in Prereduction

An appropriate masking agent in speciation analysis should not cause any undesired redox reactions, otherwise the speciation of As would not be possible. Because thiourea can be used as both a masking agent and a reductant,[14] a 2.0% thiourea solution can efficiently reduce As(V) to As(III) in 0.5% HCl. However, for arsenic speciation analysis, thiourea can only be used for total inorganic arsenic analysis of As(III) + As(V). It cannot allow for the accurate determination of As(III) only in samples because As(V) is reduced to As(III). For this reason, thiourea was not used as a reductant in this work.

The 2.0% KI solution has a negligible blank value. It was found that both 1.0% and 2.0 % KI solutions could efficiently reduce As(V) to As(III). A 2.0% KI solution was chosen in later studies. The effect of HCl concentration for As(V) prereduction with KI was studied. It was found that HCl concentration does not affect the As(III) signal and does not significantly affect the prereduction efficiency of As(V) to As(III). The recovery was best when the concentration of HCl was 10%.

A comparison of standard calibration plots of As(III) and As(V) was done under the optimal conditions. For As(III), the calibration curve is described by $y = 19.8\ (\pm 0.2), x + 2.9\ (\pm 0.3)$, $r^2 = 0.9999$, and $n = 4$, where y represents the peak area signal, x the As concentration expressed in $\mu g/L$, r is the correlation coefficient, and n is the number of measurements. For As(V) in 2.0% KI and 10% HCl, the calibration curve is given by $y = 20.5\ (\pm 0.1)$, $x + 0.67\ (\pm 0.01)$, $r^2 = 0.9998$, and $n = 4$. The standard deviations of the slopes are not significantly different. Under such conditions, As(V) could be completely reduced to As (III) and quantitatively measured.

The Atomic Fluorescence Emission of As(V) and Its Elimination During the Measurement of As(III)

Like Sb(V),[15] As(V) has a significant atomic fluorescence emission in an HCl matrix. It was found in the current study that the peak area signal varied from 10% to 40% with HG-AFS when compared to that produced by the As(III) species (Table 1). This suggests that a positive error could be introduced due to the emission of As(V) when As(III) is measured in a sample also containing As(V). An ideal situation would be to eliminate entirely the emission of As(V) during the measurement of As(III).

The masking agent 8-hydroxyquinoline was tested in this study. It was found that the presence of 8-hydroxyquinoline could prevent the atomic fluorescence emission of As(V) in a mixed As(III) and As(V) solution. In a mixed standard

Table 1. Elimination of As(V) atomic fluorescence emission by 8-hydroxyqu.

Conditions	Samples μg/L	Added As(III) μg/L	Added As(V) μg/L	Determined μg/L	Recovery of As(III) %
0.005% 8-hydroxyqu.	Herb 1	0.0	0.0	2.01	/
+10% HCl (n = 5)		2.5	0.0	4.77	106
		5.0	2.5	6.86	97.9
0.005%	Herb 2	0.0	0.0	6.82	/
8-hydroxyqu. + 2%		2.5	0.0	9.14	97.4
KI + 10% HCl		2.5	2.5	12.06	104
(n = 5)					

solution containing As(III) and As(V) in 0.005% 8-hydroxyquinoline and 10% HCl, no As(V) emission was observed over a period of 8 hr during which 5 measurements were performed. This is probably due to the formation of a chelate between As(V) and 8-hydroxyquinoline. Another solution with the same combination of As(III) and As(V) standards and 0.005% 8-hydroxquinoline and 2.0% KI in 10% HCl was set for 8 hr, then analyzed. A good recovery of total As was obtained (Table 2). Table 2 also indicates that the presence of 8-hydroxyquinoline did not induce any redox modification of the As species.

Interference Study

Because this procedure was designed to be used for As speciation in Chinese herbs, which were contaminated by transition metals, special care was taken in

Table 2. Recovery of As from herb samples by standard addition

Conditions	Added As(III) μg/L	Added As(V) μg/L	Amount recovered μg/L	Recovery of As(III) %
10% HCl	40.0	0.0	41.00	103
	10.0	10.0	13.91	139
	40.0	10.0	45.53	114
0.005% 8-hydroxyqu.	10.0	0.0	9.38	93.8
+10% HCl	20.0	0.0	19.63	98.2
	10.0	10.0	10.22	102
	40.0	10.0	39.62	99.1
0.005% 8-hydroxyqu.	10.0	10.0	19.82	99.1
+2% KI + 10% HCl	40.0	10.0	50.22	100

the interference study. In this series of tests, the 20.0 μg/L As(III) in 10% (v/v) HCl was used in all experiments.

It has also been observed that the presence of some transition metals can interfere with the determination of arsenic.[14] Figure 1 shows the results of the study done with Zn^{2+}, Fe^{3+}, Cd^{2+}, Mn^{2+}, and Pb^{2+}. It indicates that Cd^{2+}, Mn^{2+}, and Fe^{3+} have no interference up to 50.0 mg/L, respectively. Zn^{2+} has no significant signal depression when its concentrations were 50 mg/L, but the signal gradually dropped to about 93% when its concentration was decreased to 10 mg/L. Generally, this group of elements can be considered as noninterfering elements.

Besides some controversies on interferences from copper, most studies have often been done with relatively high concentrations of arsenic (normally higher than 100 μg/L), and the reports lack detailed information.[14,15] The case of Cu^{2+} and Pb^{2+} presents a severe positive interference on the arsenic signal with As(III) at 20.0 μg/L in 10% HCl (Fig. 1). Cu^{2+} started showing a serious increase of signal at a concentration of 10.0 mg/L, whereas the signal increased by 40% at a Cu^{2+} concentration of 50.0 mg/L. This group of elements is usually present in relatively high concentrations in the herb samples, particularly those with high levels of metal contamination.

The Study of Masking Agents

To select an appropriate masking agent, two important factors should be considered:[15] (1) it should not cause any unwanted redox reactions; (2) it

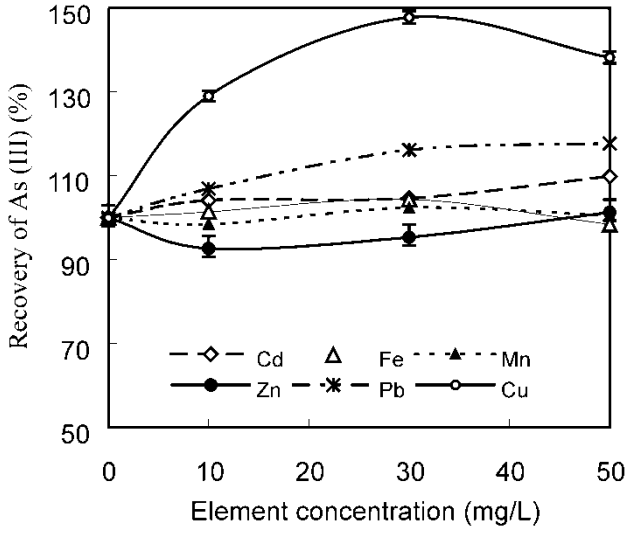

Figure 1. The interference study done with As(III) at 20.0 μg/L in 10% HCl.

should be stable at low concentrations for at least a working day. The masking agent 8-hydroxyquinoline was tested in this study.

The reagent 8-hydroxyquinoline was shown to be an efficient masking agent for Cu^{2+} and Pb^{2+}. Also, the stability of 8-hydroxyquinoline in solution was tested with the mixed 10 μg/L of As(III) and 10 μg/L of As(V) in the interference mixtures. Under these two conditions, the recoveries were close to 100% and the solution was stable for at least 8 hr (Fig. 2).

Determination of As Species in the Chinese Herb *Ligusticum chuanxiong* Hort

By the proposed procedures for the determination of As species associated with samples of the Chinese herb Ligusticum chuanxiong Hort, which samples are potted plants in our lab, the recovery for arsenic added into samples are given in Table 2. The good recovery proved that the method is feasible and varied from 97.4% to 106%.

According to the speciation procedures established in the section "Analytical Procedures", we conducted some work with five planting sites

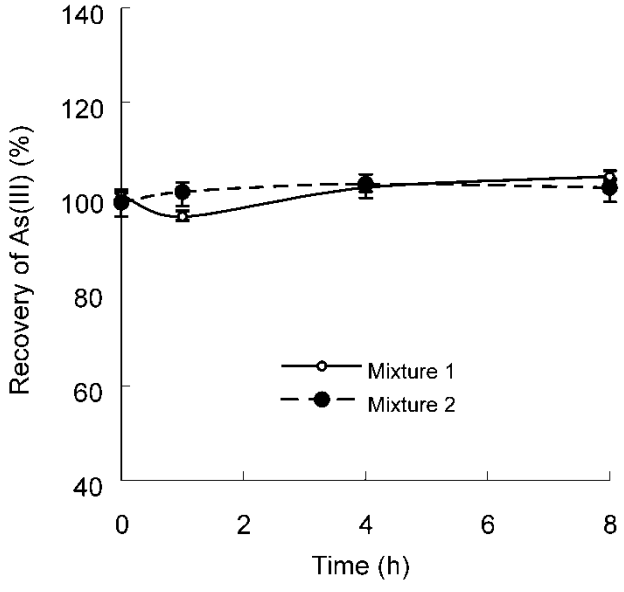

Figure 2. The stability of 0.005% 8-hydroxyquinoline in 10.0 μg/L of As(III) and 10.0 μg/L of As(V) standard solutions containing 2% KI, 10% HCl, and different concentrations of mixed interfering elements. Mixture 1 (mg/L): $Pb^{2+} = 30.0$, $Cu^{2+} = 30.0$. Mixture 2 (mg/L): $Zn^{2+} = 30.0$, $Fe^{3+} = 30.0$, $Cd^{2+} = 30.0$, $Mn^{2+} = 30.0$, $Pb^{2+} = 30.0$, $Cu^{2+} = 30.0$.

of *Ligusticum chuanxiong* Hort. The results are given in Table 3. The relative standard deviations for the measurements were usually less than 8%. Table 3 shows that (1) concentrations of As(III) in the planting areas of Dujiangyan City (Xudu, Minxing) and Penzhou City (Aoping) were the highest in the frondages, whereas concentrations of As(III) and As(V) in the tissues of stems and rhizomes were relatively lower, even not detectable; (2) concentrations of As(III), As(V), and As associated with natural organic compounds in the stem tissues from the places of Wenchuan County (Shuimo) and Shifang City (Bajiao) represent a high percentage of the total As in the plants, especially in the upper stems. On the other hand, the concentration of inorganic arsenic accumulated in the frondage of *Ligusticum chuanxiong* Hort is much higher than the concentrations accumulated in the stem and rhizome. It is fortunate that only the underground parts of the rhizome of *Ligusticum chuanxiong* Hort can be used as a traditional Chinese medicine. Based on the arsenic speciation concentrations presented in Table 3, the five areas could be divided into two types according to the

Table 3. Determined arsenic concentrations (mg/kg dry mass) of *Ligusticum chuanxiong* Hort tissues

Sites and tissues		As (III)	As (V)	As-org	Total As
Xudu	Frondage	1.49	0.22	ND	1.71
	Stem	0.030	0.56	2.52	3.11
	Rhizome	N.D.[a]	N.D.	0.80	0.80
Minxing	Frondage (n = 3)	0.64 (3.1)[b]	0.44 (4.4)	0.030 (7.6)	1.11 (4.9)
	Stem	0.080	0.40	0.61	1.09
	Rhizome	0.45	0.60	0.040	1.09
Aoping	Frondage (n = 4)	0.90 (4.3)	0.090 (6.8)	0.10 (6.1)	1.09 (5.4)
	Stem	N.D.	N.D.	1.19	1.19
	Rhizome	N.D.	N.D.	1.63	1.63
Shuimo	Frondage	0.98	1.38	0.57	2.93
	Upper stem	4.60	1.70	1.75	8.05
	Lower stem	0.62	0.35	0.20	1.17
	Rhizome	0.17	0.32	1.14	1.63
Bajiao	Frondage	0.32	0.070	ND	0.39
	Upper stem	8.32	4.59	6.38	19.29
	Lower stem	0.11	0.070	0.20	0.38
	Rhizome	0.050	0.050	0.38	0.48

[a]Not detectable.

[b]Values in parentheses are relative standard deviations (RSD, %).

altitudes. Generally, the places with lower altitude are the most suitable places for GAP bases selected, especially Penzhou City (Aoping).

CONCLUSIONS

It was found that KI is an efficient prereducing reagent for the determination of total As(V) when the As(V) concentration is low. The optimal concentration of hydrochloric acid is 10% for the determination of As(III) and As(V) by HG-AFS. Iron(III), Mn(II), Cd(II), and Zn(II) do not cause any significant interference in As determination, whereas Cu(II) and Pb(II) interfere seriously with As determinations at concentrations as low as 10 mg/L. The reagent 8-hydroxyquinoline in acidic solutions can efficiently eliminate the interferences. This masking agent is stable, has a low blank value, and is easy to use. Apart from all the mentioned advantages, the presence of 8-hydroxyquinoline also efficiently eliminates the unwanted As(V) atomic fluorescence emission during the determination of As(III), which greatly simplifies the process and improves the quality of As speciation. The established speciation procedures are robust, sensitive, reproducible, and accurate.

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