Microchemical Journal 141 (2018) 176–180

Contents lists available at ScienceDirect

Microchemical Journal

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

Trace arsenic speciation analysis of bones by high performance liquid chromatography-inductively coupled plasma mass spectrometry

Huimin Yu[a], Huan Du[a], Li Wu[b], Ruolin Li[c], Qun Sun[c], Xiandeng Hou[a,b,*]

[a] Key Laboratory of Green Chemistry & Technology, Ministry of Education, College of Chemistry, Sichuan University, Chengdu, Sichuan 610064, China
[b] Analytical & Testing Center, Sichuan University, Chengdu, Sichuan 610064, China
[c] College of Life Sciences, Sichuan University, Chengdu, Sichuan 610064, China

ARTICLE INFO

Keywords:
Arsenic
Panda bone
Pig bone
Microwave-assisted extraction
HPLC
ICP-MS

ABSTRACT

The speciation analysis of arsenic (As) has always received great attention, in which the analysis of complex matrix samples remains challenging, mainly manifested in selection of suitable sample pretreatment methods and sensitive analytical spectrometers. In this work, microwave-assisted extraction of total As and various As species in bones, with their respective determination by ICP-MS and HPLC-ICP-MS, was studied, and was successfully utilized to determine As species in panda and pig bones. The results reveal the content of As ranging from 28 to 75 μg g^{-1} in the panda bone samples and 0.27 μg g^{-1} in pig bone, in which > 90% is inorganic As^{V} except for little As^{III} and organic As. The method could be applicable to the analysis of diverse bone samples.

1. Introduction

From natural and anthropogenic sources, As pollution is ubiquitous in water, soil and air. > 30 kinds of inorganic and organic As species are subsistent in various environmental and biological samples, with diverse toxicological, biochemical and environmental effects [1]. Inorganic As (iAs) is classified as a human carcinogen by the International Agency for Research on Cancer (IARC) [2], while organoarsenic compounds are usually less toxic or nontoxic; and the toxicity is also dependent on the specific oxidation state [3,4]. Therefore, the determination of trace As species has always been receiving worldwide attention.

Arsenic can also be accumulated in animals and cause teratogenic, carcinogenic, and mutagenic effects. The giant panda, as one of the world-known vulnerable mammals according to the International Union for Conservation of Nature, is under the influence of agriculture, industry and other human activities and even natural disasters, thus exposed to the hazard of heavy metals. Studies have shown that, by microwave digestion-hydride generation method, the content of As in the bamboo of Qinling giant panda habitat can be up to 0.99 μg g^{-1} [5]. Furthermore, the content of As in fecal samples from captive giant pandas was about 0.45 μg g^{-1}, nearly three times higher than that in wild ones in Qinling areas [6]. Therefore, the accurate evaluation of As content in giant panda-related samples and other animals is necessary.

The commonly-used strategy for As speciation analysis is the combination of a separation system with an atomic spectrometer [7], among which HPLC-ICP-MS with a simple interface is one of the most widely-used instrumental configurations. HPLC has a wide applicability in separating various As compounds by appropriate choice of the stationary and mobile phases, while ICP-MS offers excellent sensitivity and a wide linear range without any post-column treatment. Accordingly, Le et al. achieved the determination of 11 As species [8], wherein boric acid was used to selectively modify the separation of arsenosugars. Other species like arsenolipids [9,10] and phenylarsenicals [11] have also been determined by taking advantage of HPLC separation with ICP-MS detection. In consideration of the specific content level, a few As species, arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB) and arsenocholine (AsC) are generally involved in practical sample analysis [12,13].

Elemental analysis of bones was generally related to archaeology [14,15] in which the samples of bones and teeth were superior to hairs and nails, because the latter with sulphur-rich amino acids could diagenetic uptake exogenous As [16]. In their works, however, only total As was quantified as some certain As species had already transformed over time. However, for properly preserved bones or fresh bones, the speciation analysis is necessary for better understanding of the individual's situation on As exposure. Giant panda is a kind of precious and rare species, and there are few related works on its elemental analysis [17]. Thus, an investigation about the As species of panda bone is significative and that of pig bone is also conducted to benefit the accurate acquisition of elemental information.

* Corresponding author at: Key Laboratory of Green Chemistry & Technology, Ministry of Education, College of Chemistry, Sichuan University, Chengdu, Sichuan 610064, China.
E-mail address: houxd@scu.edu.cn (X. Hou).

https://doi.org/10.1016/j.microc.2018.05.013
Received 9 May 2018; Accepted 11 May 2018

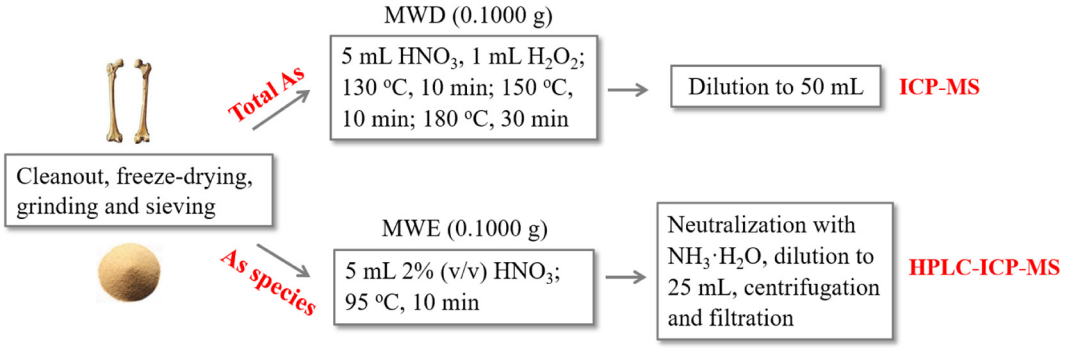

Fig. 1. Schematic illustrating the processes of sample preparation, analytes extraction and detection.

Sample pretreatment is the most important step in As determination, especially in As speciation analysis [18,19]. The matrix of bone or skeleton samples is relatively complex because of their high contents of Si and P, which increase the difficulty of thorough digestion for determination of total As. Some solutions have been adopted including the addition of aqua regia [20] or hydrofluoric acid HF [15,21], dry ashing [22] and long digestion time [23]. Microwave-assisted wet digestion [14,24] has been proved to be one of the most efficient ways for less consumption of time and corrosive agents. Moreover, to maintain the intrinsic As species of various samples in extraction process, CH_3OH [25], H_3PO_4 [26] and dilute HNO_3, etc. are usually used as the extraction reagents, assisted by microwave radiation, sonication [27], vortexing or heating [12]. Foster et al. [28] has tested a number of extractants for extraction of As from marine biological samples and found dilute HNO_3 under microwave radiation achieved the highest recoveries of As within 10 min. However, the extraction and detection of various As species in bones have not been reported yet. In this work, therefore, microwave-assisted digestion (concentrated HNO_3 and H_2O_2) and extraction (2% (*v*/v) HNO_3) have been used in the pretreatment of bone samples for the acquisition of total As and As species, prior to determination by ICP-MS and HPLC-ICP-MS, respectively.

2. Experimental section

2.1. Instrumentation

Most of the instrumental measurements were conducted with an inductively coupled plasma mass spectrometer (iCAP Q, Thermo Fisher Scientific Inc., Germany), which was coupled with a high performance liquid chromatographer (U3000, Thermo Fisher Scientific Inc., Germany) to determine the As species. A double-channel hydride generation atomic fluorescence spectrometer (HG-AFS, AFS-9600, Beijing Haiguang Instrument Co., China) was used to quantify the total As content with the result compared to that of ICP-MS. The extractions of multi-elements and As species from panda and pig bones were achieved with the assistance of a Sineo Master 40 (Shanghai, China) microwave digestion system.

2.2. Reagents and samples

18.2 MΩ cm deionized water produced from a water purification system (Chengdu Ultrapure Technology Co., Ltd., Chengdu, China) was used in the whole work. High purity argon and helium were purchased from Qiaoyuan Gas Company (Chengdu, China). Chemicals used in this work were of at least analytical grade, in which guaranteed purity HCl, HNO_3, H_2O_2, HF and $HClO_4$ (Kelong Reagent Factory, Chengdu, China) were used for the extraction of multi-elements from bone samples, and chromatographical purity CH_3OH and $(NH_4)_2CO_3$ (Aladdin Reagent Co., Shanghai, China) were used to prepare the mobile phase. In addition, KOH, KBH_4, ascorbic acid, $NH_3{\cdot}H_2O$ and acetone were purchased also from Kelong Chemical Factory. Stock standard solutions purchased from the National Research Center for Standard Materials (NRCSM) of China included six kinds of As species consisted of As^{III}, As^{V}, MMA, DMA, AsB and AsC, and 1000 mg L^{-1} Cr, Mn, Cu, As, Se, Cd, Hg, Pb and In. The panda ribs were donated by the Chengdu Research Base of Giant Panda Breeding (CRBGPB, Chengdu, China) while the pig rib was bought from a local supermarket (Chengdu, China).

2.3. Sample handling

One pig and three panda bone samples were scraped off the adherent muscular tissue if any, immersed in acetone with 24 h to remove the superficial lipid, and then rinsed three times with deionized water. After being dried with 12 h in 50 °C, the tough bone was roughly broken with a hammer, followed by a vacuum freeze dry process for 24 h. Subsequently, smash the sample with a pulverizer until it became powder prior to dry preservation for further use.

2.4. Determination of total arsenic and other elements

A microwave assisted digestion (MWD) method was employed to decompose the bone samples for the analysis as described in Fig. 1. All the teflon digestion vessels used in this work were soaked in concentrated HNO_3 and rinsed with deionized water in advance. The powder sample was precisely weighed with 0.1000 g into a teflon vessel, followed by addition of 5 mL HNO_3 and 1 mL H_2O_2. The microwave digestion was operated as shown in Fig. 1, and the clear digestion solution was accurately diluted to 50 mL in a volumetric flask prior to determination of multi-elements by ICP-MS. The instrument was operated with parameters listed in Table 1, in which the internal standard was online added into the test solution. In order to verify the reliability of above digestion method, the mixing acid (6 mL HNO_3, 2 mL HCl and 2 mL HF) used for the digestion of complex matrix samples like soil was applied to decompose panda bone-3 sample with an operation procedure as follows: 15 min at 120 °C; 10 min at 160 °C; 20 min at 180 °C; and 30 min at 200 °C. After evaporating excess acid, the digestion solution was diluted to 50 mL and then determined by ICP-MS. The same panda bone-3 sample was also determined by HG-AFS to confirm the accuracy of total As by ICP-MS in which $^{75}As^{+}$ is easily affected by $^{40}Ar^{35}Cl^{+}$, and HG can effectively avoid this interference and alleviate matrix interference.

2.5. Arsenic speciation analysis

Because inorganic As^{III} and some certain organic As species are easy to be oxidized, especially in a strong acidic medium, the extraction method of As species needs to be carefully selected. In this work, the entire microwave assisted extraction (MWE) procedure is shown in Fig. 1 referring to the literature by Foster [28]. 0.1000 g sample dispersed in 5 mL 2% (*v*/v) HNO_3 was microwave-radiated in 95 °C with