Analytica Chimica Acta 818 (2014) 23–31

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Speciation analysis of arsenic in prenatal and children's dietary supplements using microwave-enhanced extraction and ion chromatography–inductively coupled plasma mass spectrometry

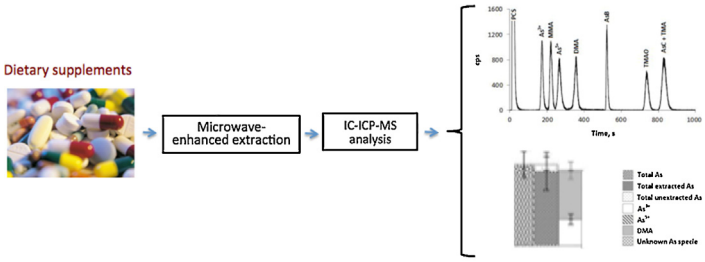
Mesay M. Wolle[a,*], G.M. Mizanur Rahman[b], H.M. 'Skip' Kingston[a], Matt Pamuku[b]

[a] Department of Chemistry and Biochemistry, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282, United States
[b] Applied Isotope Technologies, 2403 Sidney Street, Suite 280, Pittsburgh, PA 15203, United States

HIGHLIGHTS

- Microwave-enhanced extraction of arsenic from plant-based dietary supplements.
- Ion chromatography inductively plasma mass spectrometry method for arsenic.
- Optimal use of collision cell to eliminate molecular ions interfering with ^{75}As.
- Analysis of widely consumed prenatal and children's dietary supplements.
- Validation of analytical data using mass balance.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 September 2013
Received in revised form 23 January 2014
Accepted 30 January 2014
Available online 10 February 2014

Keywords:
Arsenic
Speciation analysis
Microwave-enhanced extraction
Ion chromatography
Inductively coupled plasma mass spectrometry
Dietary supplement

ABSTRACT

A study was conducted to develop a microwave-enhanced extraction method for the determination of arsenic species in prenatal and children's dietary supplements prepared from plant materials. The method was optimized by evaluating the efficiency of various solutions previously used to extract arsenic from the types of plant materials used in the dietary supplement formulations. A multivitamin standard reference material (NIST SRM 3280) and a prenatal supplement sample were analyzed in the method optimization. The identified optimum conditions were 0.25 g of sample, 5 mL of 0.3 mol L^{-1} orthophosphoric acid (H_3PO_4) and microwave heating at 90 °C for 30 min. The extracted arsenic was speciated by cation exchange ion chromatography–inductively coupled plasma mass spectrometry (IC–ICP-MS). The method detection limit (MDL) for the arsenic species was in the range 2–8 ng g^{-1}. Ten widely consumed prenatal and children's dietary supplements were analyzed using the optimized protocol. The supplements were found to have total arsenic in the concentration range 59–531 ng g^{-1}. The extraction procedure recovered 61–92% of the arsenic from the supplements. All the supplementary products were found to contain arsenite (As^{3+}) and dimethylarsinic acid (DMA). Arsenate (As^{5+}) was found in two of the supplements, and an unknown specie of arsenic was detected in one product. The results of the analysis were validated using mass balance by comparing the sum of the extracted and non-extracted arsenic with the total concentration of the element in the corresponding samples.

1. Introduction

The human diet provides a diverse blend of nutrients needed for growth, maintenance and overall health. For some people, however,

* Corresponding author. Tel.: +1 412 396 4106; fax: +1 412 396 4013.
E-mail addresses: wollem@duq.edu, mesmul@yahoo.com (M.M. Wolle).

http://dx.doi.org/10.1016/j.aca.2014.01.060
0003-2670/

food alone may not supply adequate amounts of the required nutrients. Furthermore, nutritional needs change with age, pregnancy, specific metabolism and lactation, or due to acute and chronic diseases and other medical conditions. Pregnant women take dietary supplements that contain macro- and micronutrients to decrease the risk of several complications including congenital malformations, maternal anemia and preeclampsia, thereby ensure safe pregnancy and healthy babies [1]. Supplements are also given to children who are at risk of nutrient deficiency due to lack of appetite or highly selective diet. The demand for such supplementary products has increased significantly especially in the industrialized areas including the US, Canada and Europe [2].

Dietary supplements are prepared through laboratory synthesis or from natural products such as plants and fish oil that are characterized by high contents of vitamins, minerals and other essential nutrients [3]. The products are often considered to be exclusively beneficial to health and free from toxic side effects. However, studies found high levels of toxic and xenobiotic elements [4–10], pesticides [11] and bacteria [5] in some types of supplements. Exposure of a population, especially pregnant women and children, to such substances is a major concern. This concern would be even more troubling if the exposure occurs through dietary supplements; a source unexpected by the public.

Arsenic is among the elements of primary concern due to the toxicity of some of its species. Contamination of dietary supplements by arsenic can result mainly through the plants that are used as product ingredients. Arsenic is released into the environment through natural processes such as weathering of minerals, volcanic activity and soil erosion [12], as well as anthropogenic activities including mining and ore smelting, coal combustion, waste incineration, and use of pesticides [13]. Plants take up arsenic from soil and water, and accumulate it in their edible parts mainly as arsenate, which can cross the plasma membrane as a phosphate analogue [14]. In addition to the raw materials, the manufacturing steps which include extraction, formulation, etc. may also contribute to the contamination of the supplement products.

Several regulatory bodies have emphasized the need to focus on dietary arsenic exposure. The United Nations' Food and Agricultural Organization (FAO), and World Health Organization (WHO) set benchmark dose levels of 0.3–8 μg per kg of body weight per day for inorganic arsenic, i.e. arsenite (As^{3+}) and arsenate (As^{+5}), associated with risks for several diseases [15]. Arsenic is also one of the toxic substances listed by the US Environmental Protection Agency (EPA) [16] and in the State of California Proposition 65 [17] with a limit of 10 μg per day. The Chinese regulation has established a tolerance limit of 0.3 mg kg^{-1} for arsenic in dietary supplements [18]. Although the European Union has recently set maximum levels for several toxic elements in relation to contamination of food supplements, no value has been put for arsenic [19].

So far, a limited number of studies evaluated the level of arsenic in dietary supplements [5–7,9,10,20,21] mostly based on the determination of the total concentration of the element in the products [5–7,20,21]. Such studies, however, should be conducted based on the determination of the individual species of the element because the toxicity of arsenic depends on its chemical forms. For example, long-term exposure to inorganic arsenic is associated with a range of adverse effects on humans, including skin lesions, cancer, developmental toxicity, neurotoxicity, cardiovascular diseases, abnormal glucose metabolism and diabetes [22]. The methylated forms of arsenic such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) have been labeled as cancer promoters [23,24]. Arsenobetaine (AsB) and arsenocholine (AsC) are believed to be virtually nontoxic, however, this belief is still up for debate due to recent findings [25]. To the authors' best knowledge, no work has been reported yet on the comprehensive speciation analysis of arsenic in dietary supplements, except for two studies which aimed only at the inorganic species of the element with [9] or without [10] their differential determination.

Table 1
Optimum conditions of the chromatographic method.

Parameter	Optimum condition
Column	PRP-X200 (cation exchange), 250 mm long, 4.1 mm i.d., 10 μm particle size, working pH 1–9
Mobile phases	(A) 1 mmol L^{-1} HNO_3 and 1% methanol in water, pH 2.5, and (B) 2 mmol L^{-1} HNO_3, 20 mmol L^{-1} ammonium nitrate and 1% methanol in water, pH 2.5
Elution mode	Gradient: 0.0–3.0 min (95% A and 5% B), 3.1–16.0 min (5% A and 95% B), 16.1–18.0 min (95% A and 5% B)
Flow rate	0.9 mL min^{-1}
Column temperature	Ambient
Injection volume (sample and post-column standard)	100 μL

The study presented in this paper aimed to develop an extraction method for the comprehensive speciation analysis of arsenic in prenatal and children's dietary supplements prepared from plant materials such as fruits, vegetables, grains and herbs. Numerous methods have been reported for extracting arsenic species from environmental, nutritional, botanical, biological and other samples [26,27], but none exists for samples of the type considered in the present study. Hence, the novelty of this study resides in the development of the extraction protocol and its application in supplement materials produced for vulnerable population subgroups, i.e. children and pregnant women. The method was developed based on a microwave-enhanced protocol. Several solutions previously used to extract arsenic species from the types of plant materials used in the dietary supplement formulations were evaluated by analyzing a multivitamin standard reference material and a prenatal supplement sample. The extracted arsenic was speciated by cation exchange ion chromatography–inductively coupled plasma mass spectrometry (IC–ICP-MS). Several widely consumed prenatal and children's supplements were analyzed using the proposed method. The analytical results were validated using mass balance by comparing the sum of the concentrations of the extracted and non-extracted arsenic species with the total arsenic found in the corresponding samples.

2. Experimental

2.1. Instrumentation and software

Extraction and sample decomposition were performed using an Ethos 1 laboratory microwave system (Milestone). The instrument was equipped with temperature and pressure feedback control and magnetic stirring capability. The device accurately senses within ±2.0 °C of the set temperature, and automatically adjusts the microwave field output power.

A SAVANT SPD1010 SpeedVac concentrator (Thermo Scientific) was used solvent for solvent evaporation.

Electrochemical potential (E_h) measurement was made with a Keithley 169 multimeter using a Pt-electrode (Metrohm AG) against a saturated Calomel electrode (Accument).

The ion chromatographic system was Metrohm 850 Professional IC (Metrohm). The system's hardware was made from PEEK, and it consisted of an auto-sampler (858 professional sample processor), a six-port sample injector, two pumps, a column thermostat, an eluent degasser, and an automated post-column injection unit (800 Dosino). A PRP X-200 cation exchange column (Hamilton) was used. Table 1 provides the optimum chromatographic conditions.