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A microwave-assisted sequential extraction of water and dilute acid soluble arsenic species from marine plant and animal tissues

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Abstract

This paper describes the use of dilute nitric acid for the extraction and quantification of arsenic species. A number of extractants (e.g. water, 1.5 M orthophosphoric acid, methanol–water and dilute nitric acid) were tested for the extraction of arsenic from marine biological samples, such as plants that have proved difficult to quantitatively extract. Dilute 2% (v/v) nitric acid was found to give the highest recoveries of arsenic overall and was chosen for further optimisation. The optimal extraction conditions for arsenic were 2% (v/v) HNO_3, 6 min^{-1}, 90 °C. Arsenic species were found to be stable under the optimised conditions with the exception of the arsenoriboses which degraded to a product eluting at the same retention time as glycerol arsenoribose. Good agreement was found between the 2% (v/v) HNO_3 extraction and the methanol–water extraction for the certified reference material DORM-2 (AB 17.1 and 16.2 $\mu g\,g^{-1}$, respectively, and TETRA 0.27 and 0.25 $\mu g\,g^{-1}$, respectively), which were in close agreement with the certified concentrations of AB 16.4 ± 1.1 $\mu g\,g^{-1}$ and TETRA 0.248 ± 0.054 $\mu g\,g^{-1}$.

To preserve the integrity of arsenic species, a sequential extraction technique was developed where the previously methanol–water extracted pellet was further extracted with 2% (v/v) HNO_3 under the optimised conditions. Increases in arsenic recoveries between 13% and 36% were found and speciation of this faction revealed that only inorganic and simple methylated species were extracted.

Keywords: Arsenic extraction; Arsenic speciation; Dilute nitric acid; Biological tissues

1. Introduction

Over 30 arsenic species are found in marine plants and animals [1] with thirteen that are relatively common (Fig. 1). The measurement of arsenic species in environmental samples requires three main steps, extraction, separation and quantification. Separation systems such as HPLC, coupled to element-specific detectors (i.e. ICP-MS) provides a mean to separate and quantify individual arsenic species, although care must be taken to use appropriate columns and eluants [2,3]. However, an extraction step that quantitatively extracts arsenic species without altering their chemical form is required. The most commonly used solvents to extract arsenic species are water, methanol and methanol–water mixtures of varying concentrations [4,5]. Other extractants such as orthophosphoric acid, [4,6] trifluoroacetic acid [7] and sodium hydroxide [8] have been used, mainly for the extraction of plant tissues. To date, few extraction methods have been able to quantitatively extract arsenic species from marine plant tissue [9] and non-muscle marine animal tissues [5,10]. Current methods for the extraction of arsenic from biological samples are limited to water-soluble species [11–13]. The majority of known arsenic species are polar and hence, methanol–water soluble, but inorganic arsenic is normally poorly extracted [4]. Many of the investigations into the composition of arsenic species in plants have shown that the majority of arsenic is inorganic [12,14–18], hence extraction of arsenic from plants will require solvents able to solubilise inorganic arsenic. One of the reasons for the apparent insolubility of arsenic in plants is that As(V) is reduced to As(III) and sequestered in vacuoles by phytochelatins ($[\gamma$-glutamate-cysteine$]_n$-glycine) [19,20].

Methanol–water mixtures have been shown to give low extraction efficiencies for plant material [6,14,18]. Bohari et al. [6] used methanol–water (1:9, 1:1, 9:1, v/v), among other extractants, for the determination of arsenic species in terrestrial plant and soil samples with the aid of sonication, agitation and

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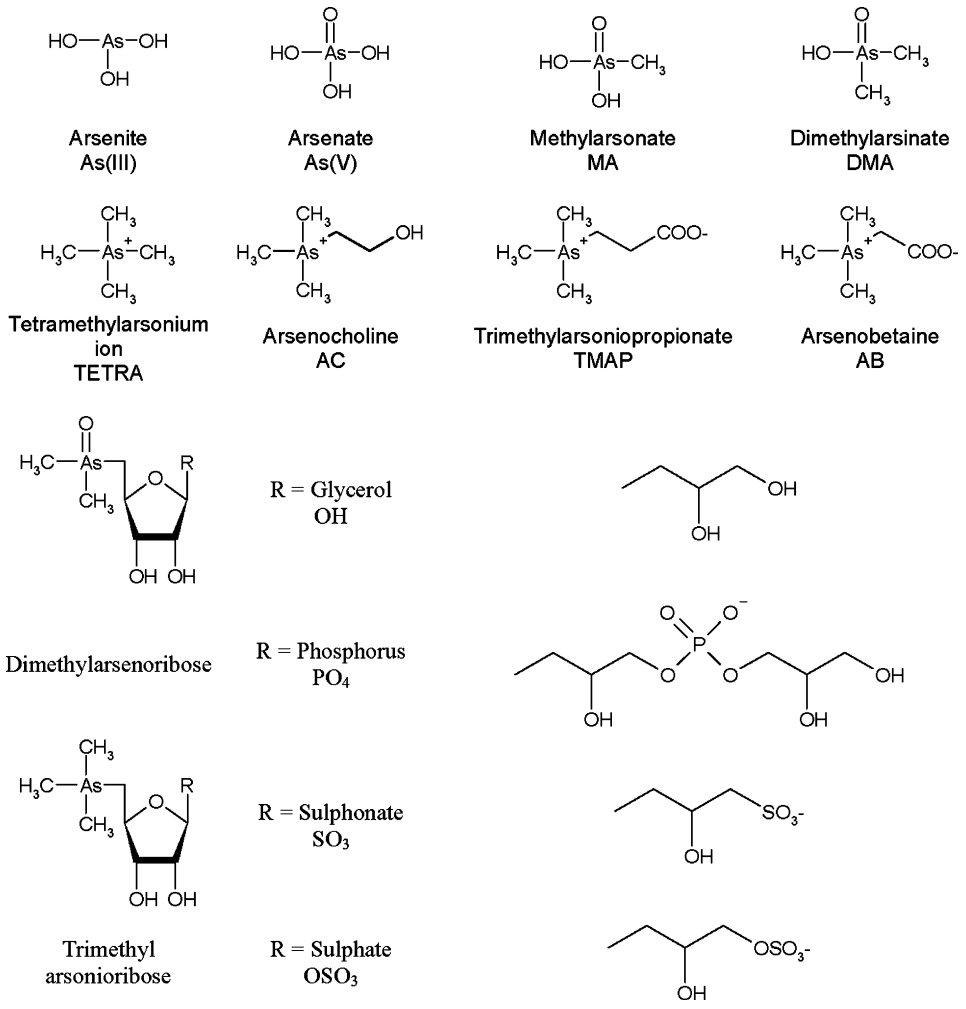

Fig. 1. Common arsenic species found in marine animal and plant tissues.

microwave irradiation. They found that the extraction of arsenic decreased with increasing methanol content. Microwave irradiation using methanol–water (1:9, v/v) was found to give the best recoveries for arsenic (31%) from plant tissue. Kuehnelt et al. [18] used mechanical agitation and methanol–water (9:1, v/v) for the extraction of arsenic species from plant and lichens, and obtained lower recoveries for arsenic (4–22%) than those for the same material extracted with water (7–71%). Zheng et al. [14] used mechanical agitation and methanol–water (9:1, v/v) for the extraction of arsenic from two species of submerged and terrestrial plants. Recovery of arsenic from these materials was low (6–6%) [14]. Heitkemper et al. [7] extracted rice using methanol, water and/or mixtures of methanol–water in varying concentrations with the assistance of accelerated solvent extraction at room temperature and found for the SRM 1568a (rice flour) that all the extractants used gave similarly good results (76–105%), while real world samples only yielded between 24% and 36%.

Plants are not the only tissues that have been consistently difficult to extract arsenic. Extraction of arsenic from marine animal tissues, such as liver and digestive tissues, has also been found to be difficult [21,22]. Goessler et al. [22] extracted arsenic from the livers of several species of whales and seals using mechanical agitation and methanol–water (9:1, v/v) with arsenic extraction yields of 44–77%. Maher et al. [21] extracted arsenic from tissues of *Mugil cephalus* (mullet) using mechanical agitation and methanol–water (9:1, v/v) with arsenic recoveries for the intestine, stomach and liver of 21%, 19% and 19%, respectively.

Other extractants have been tried with varying success. Bohari et al. [6] found that the use of 0.3 mol dm^{-3} orthophosphoric acid with microwave heating extracted reasonable amounts of arsenic from CRM GBW-08501 Peach leaves (79%) and CRM BCR 279 *Ulva lactuca* (67%). The use of orthophosphoric acid (1.5 mol dm^{-1}) was also investigated by Kuehnelt et al. [4] who found arsenic recoveries of 94% for DORM-2 and 76% for *Hijiki fuziforme*. Heitkemper et al. [7] used 2 M trifluoroacetic acid with heating for 6 h at 100 °C for the extraction of the NIST SRM 1568a (rice flour) and rice samples and obtained good recoveries for both the SRM (112%) and rice samples (92%). However, 2 M trifluoroacetic acid was found to reduce As(V) in the samples to As(III) resulting in an apparent increase in As(III) and subsequent loss of the spiked As(V) [7]. Krachler and Emons [8] extracted freeze dried plant material with several solvents (0.2 M acetic acid, 1.25 M EDTA pH 4.7, 0.66 M sodium hydroxide, 9:1 (v/v) methanol–water and 10 mM