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Speciation of arsenic — A review of phenylarsenicals and related arsenic metabolites

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ABSTRACT

Environmental and health studies of arsenic require the determination of arsenic species of diverse properties and toxicities. This review summarizes recent analytical advances in speciation of phenylarsenicals. Arsenic speciation techniques have taken advantage of high performance liquid chromatography (HPLC) separation with inductively coupled plasma mass spectrometry (ICPMS) and electrospray ionization tandem mass spectrometry (ESI-MS/MS) detection. These techniques have enabled the identification and quantitation of new arsenic species. Characterization of phenylarsenical metabolites has contributed to better understanding of the fate of phenylarsenicals and the potential risk of human exposure. Binding of trivalent phenylarsenicals to proteins has facilitated the development of analytical techniques for the capture and identification of arsenic-binding proteins.

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1. Introduction

Arsenic has attracted worldwide attention due to its environmental impact and human health concerns. Chronic exposure to inorganic arsenic (iAs) is strongly associated with the prevalence of lung, bladder, and skin cancers, and increased risk of many other diseases, such as diabetes, heart disease, and neurodevelopmental effects [1]. The toxicity of arsenic varies greatly and is dependent upon its chemical composition, whether it is inorganic or organic, and its oxidation state [2–6]. The bioavailability, environmental fate, and remediation efficiencies of arsenicals also depend on the specific species of arsenic [5–7].

Regulatory efforts have been put in place to primarily reduce human exposure to iAs from drinking water, with the current guideline for arsenic in drinking water established by the World Health Organization (WHO) as 10 μg/L [8]. Another main route of arsenic exposure occurs through the ingestion of food containing arsenic, although arsenic levels in food are less strictly regulated compared to drinking water [8,9]. Exposure to iAs via food can exceed exposure via drinking water, and total arsenic concentrations consumed through food can be as much as ~40 μg/day, with the majority consisting of organoarsenicals [9,10].

While organoarsenicals in seafood have been extensively studied, characterization of arsenic species in other food items have only recently received attention. For example, several phenylarsenic compounds, such as 4-hydroxy-3-nitrophenylarsonic acid or Roxarsone (Rox), have been used as feed additives in poultry and swine farms. These benzene-containing arsenicals offer growth-promoting and disease-controlling effects, but can also form toxic metabolites in animals raised for human consumption [11–13].

Despite the discontinuation of phenylarsenical feed additives in Europe and the United States in 1988 and 2013, respectively, potential exposure and the associated health risks of phenylarsenicals remains an important research topic. Roxarsone is still manufactured and widely distributed in other parts of the world [14], and decisions to regulate phenylarsenical feed additives in other countries are not yet in place. The public can also be exposed to phenylarsenicals through other sources such as environmental pollution from remnants of warfare compounds [15] and anticancer drugs [16,17].

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2. Phenylarsenicals

Phenylarsenicals are synthetic compounds and do not have a natural source. They contribute to contamination in the environment and in biological systems, resulting mainly from the usage of feed supplements and drugs, and from the degradation of warfare chemicals that have leaked from storage. However, the fate, biotransformation, and toxicity of phenylarsenicals have not been comprehensively studied. The phenylarsenicals and other relevant arsenic compounds discussed in this paper are summarized in Table 1.

Phenylarsenicals have been utilized for well over 70 years [18]. Four phenylarsenicals were commonly used as feed additives since the 1940s. Rox and 4-aminophenylarsonic acid (*p*-arsanilic acid, ASA) were used for reducing hemorrhage and mortality in farm animals suffering from parasitic infections, while 4-nitrophenylarsonic acid (4-NPAA, Nitarsone) and *p*-ureidophenylarsonic acid (pUPAA, Carbarsone) were used for controlling blackhead disease in turkeys [19]. The routine use of arsenic-based feed additives has been phased out in Europe since 1988 [13]. The United States Food and Drug Administration (FDA) withdrew Rox from the U.S. market in 2013 [20,21]. Soon after, the other three phenylarsenicals, ASA, 4NPAA, and pUPAA, were also withdrawn from the list of approved animal feed supplements in the U.S. [22]. However, the phenylarsenicals are still used as feed additives for poultry and swine in many other countries.

3. Speciation of phenylarsenicals in biological and environmental samples

3.1. Extraction and enrichment/pre-concentration

A wide variety of extraction techniques have been developed in an attempt to maintain the integrity of arsenic species, maximize extraction efficiency, reduce interference from matrix, and pre-concentrate the arsenic species of interest. These techniques included extraction with water and methanol under mild conditions, liquid extraction (LE), accelerated solvent extraction (ASE), liquid-liquid microextraction (LLME), and solid phase microextraction (SPME). Sonication, microwave digestion, and enzymes have been used to further improve the efficiency (Table 2).

Initial steps for preparing solid samples often involve drying or freeze drying followed by or preceded by homogenization, grinding, powdering, or chopping. The purpose of these steps is to achieve a homogeneous sample to which the chosen extraction technique can be applied. They also increase the surface area of the sample to optimize the efficiency of the extraction. The most common method for extraction involves a methanol/water mixture at a 1:1 volume ratio. Although this method may not provide the same efficiency as complete digestion methods, the use of water and methanol under mild conditions reduces the possibility of species conversion [23]. Monasterio et al. [24] used a 1:1 methanol/water mixture to extract Rox, 4NPAA, ASA, dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) from arugula, a plant matrix. Similarly, Geng et al. [25] carried out methanol/water extraction for arsenic species analysis in rice.

Several studies used only water to extract arsenic species from dog food [24], soil [26], and rice plants [27,28]. Kutschera et al. [26] pre-dried soil and extracted arsenic species with 25 mL of deionized water assisted by sonication for 10 min, and centrifuged at 5000 rpm for 5 min. The extraction yield from dry soil ranged from 6 to 32%. Arroyo-Abad et al. [27] used a similar method, in which the soil was first dried at 100 °C, homogenized, soaked with deionized water, and shaken to extract the arsenic species. Yao et al. [28] used water to extract arsenic species from rice straw, grain, and hull where the harvested rice was first air dried, dehulled, lyophilized, and powdered. Ultrapure water was added to the samples, left overnight, then sonicated at 55 °C for 20 min, and centrifuged for 10 min at 4000 rpm. The residue was extracted two more times. The overall extraction efficiencies were (61 ± 1)% for the grain, (92 ± 1)% for the hull, and (89 ± 2)% for the straw [28].

Extraction with buffer solutions, plus ultrasonication and mechanical shaking, has shown excellent extraction efficiencies. Schmidt et al. [29] used sodium hydrogen phosphate buffer at pH 7.7 to extract arsenic species from the leaves, roots, and stems of *T. majus*. With ultrasonication and 24-h total extraction time, this method achieved an extraction efficiency of 90%. The detected species included *p*-arsanilic and *o*-aminophenylarsonic acid (ASA, *o*-APAA), PAO, phenylarsonic acid (PAA), and Rox. Nachman et al. [13] used 20 mM malonic acid and 1% of 30% (by volume) hydrogen peroxide to extract arsenic species from chicken breast meat. With mechanical shaking and controlled temperature at 50 °C, this method resulted in a near quantitative extraction.

Accelerated solvent extraction was used by Cui et al. [30] to extract ASA, 4NPAA, and Rox from porcine and chicken liver samples. A mixture of methanol/water at a volume ratio of 3–7 was used as the extraction solvent. An elevated temperature and pressure were set at 80 °C and 1500 psi, respectively, to accelerate the extraction process. Extraction was performed for 3 cycles, each lasting for only 4 min.

Enzymatic hydrolysis of biological tissue samples has increasingly been employed to enhance the extraction efficiency of the traditional water-methanol extraction systems. Under the mild temperature and pH conditions typical of enzymatic reactions, there is a minimum risk of conversion of the arsenic species, while the breakage of biological tissue and cells exposes the arsenic species for efficient extraction. The combined use of enzymatic hydrolysis and ultrasonication is an appealing method to extract phenylarsenicals from the solid biological samples, such as meat and plant matter [31]. For example, Liu et al. [32] used ultrasonication-assisted papain hydrolysis to extract arsenic species from chicken muscle. This method achieved better extraction efficiency (80%) than traditional 1:1 water/methanol extraction (28%). Other proteolytic enzymes, such as pepsin and trypsin, have also been utilized, with good success [12,33].

Microwave assisted extraction (MAE) has also been used for speciation analysis. Hu et al. [11] reported microwave-assisted extraction of Rox, ASA, AsB, iAs, DMA, and MMA from 100 mg of chicken meat, using 5 mL of 22% (v/v) methanol, 90 mM $(NH_4)_2HPO_4$, and 0.07% (v/v) trifluoroacetic acid (TFA) at pH 10.0. Recovery of arsenic species from the spiked samples was (104 ± 14)%. Nachman et al. [34] used MAE to extract Rox, iAs, arsenobetaine (AsB), MMA, and DMA from chicken feathers, using 5 mL of 0.1 M TFA and 50 μL of hydrogen peroxide (30% v/v). The mixture was sonicated for 10 min, left overnight and microwave-assisted extraction was performed at an argon pressure of 4×10^6 Pa and a temperature of 95 °C for 1 h. The median extraction efficiency for this method was 80%.

Roerdink and Aldstadt [35] used solid phase microextraction (SPME) to extract ASA and Rox in surface water from a hog farm, a turkey farm, and the Milwaukee River. The sample was acidified to pH 2, converting the phenylarsenicals to their protonated neutral forms, to increase the hydrophobicity of the analytes. The sample was then heated to 70 °C and derivatized with 1,3-propanedithiol. A 65 μm polydimethylsiloxane-divinylbenzene SPME fiber was allowed to equilibrate with the sample for 15 min. The average recovery for Rox was (103 ± 11)% from several fortified samples. In terms of the derivatization of phenylarsenic compounds, Roerdink

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