CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

A novel speciation alternative for the determination of inorganic arsenic in marine samples

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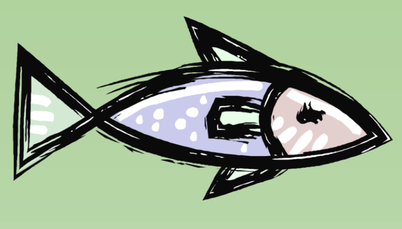
Simple, inexpensive and fast methods for determination of the toxic inorganic arsenic species are called upon for the monitoring and control of food and feed samples. A simplified approach based on Microwave-Assisted Extraction (MAE) - Solid Phase Extraction (SPE) – has been developed, where inorganic arsenic is separated from organoarsenic compounds by Strong Anion Exchange (SAX) SPE followed by determination of arsenic content by Hydride Generation (HG) Atomic Absorption Spectrometry (AAS).



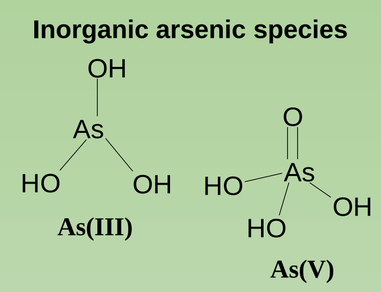


FIGURE 1. Principle of the MAE-SPE-HG-AAS approach for selective speciation analysis of inorganic arsenic.

Introduction

The total contents of the trace elements lead, cadmium and mercury in food and feed are regulated by EU directives (EC) Nos 466/2001/EC, 78/2005/EC, 2002/32/EC and 2003/100/EC. However, for some heavy metals the chemical form (i.e. their speciation) is important in terms of food and feed safety. More than 50 different arsenic species have been found in the marine environment – including lipid-soluble arsenic compounds, however it is the ***inorganic arsenic*** **forms** that are most toxic, whereas organoarsenic compounds are considered to have only low to intermediate toxicity. Since seafood is the major dietary source for arsenic exposure in the European population, arsenic speciation analysis of marine feed and seafood commodities of great interest.

Extraction of inorganic arsenic

Several sample extraction solvents and samples preparation approaches have been tested for extraction of inorganic arsenic in order to optimize the extraction of inorganic arsenic. These include water, methanol, hydrochloric acid or alkaline solutions, all giving with varying results for the same reference material. Microwave assisted extraction for 20 minutes at 90 °C with 0.06 M HCl/3 % H_2O_2 provided the most efficient extraction of inorganic arsenic. H_2O_2 was added to ensure quantitative conversion of arsenite As(III) to arsenate As(V) (Figure 2) and thereby facilitate the following SPE separation of inorganic As (as As(V)) from organoarsenic compounds. Importantly, no degradation/conversion of other arsenic species such as arsenobetaine (AB), which is usually the predominant species in fish, methylarsonate (MA) or dimethylarsinate (DMA) was observed under the chosen conditions.

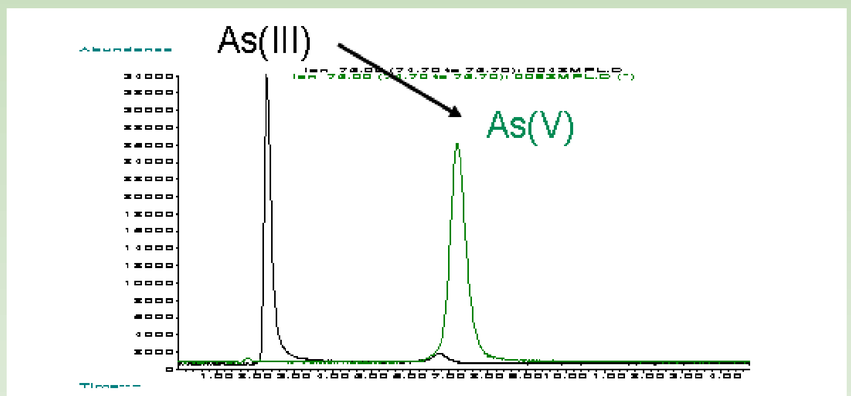


FIGURE 2. Overlaid HPLC-ICP-MS chromatograms of an As(III) standard solution (10 ppb) in 0.07 M HCl / 3 % H_2O_2 before and after microwave treatment, respectively, showing the quantitative conversion of As(III) to As(V) by H_2O_2.

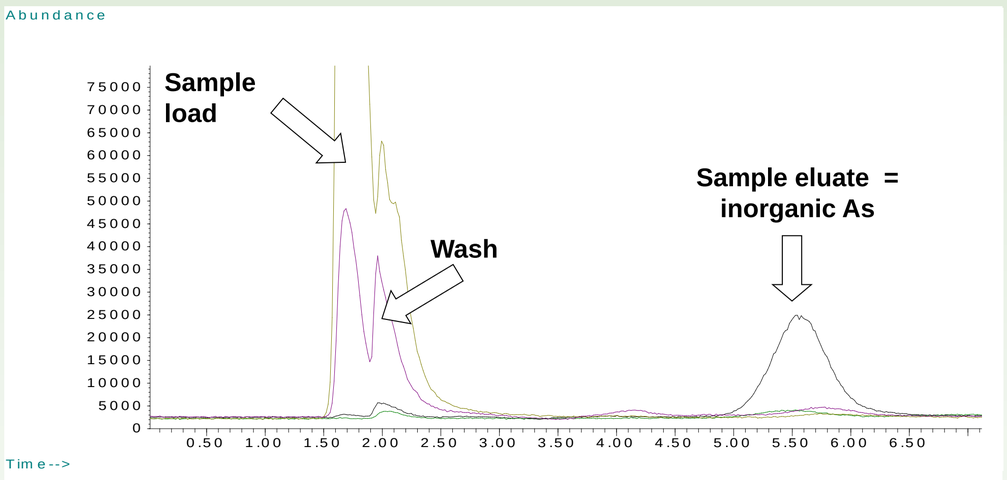


FIGURE 3. Overlaid HPLC-ICP-MS chromatograms of the three SPE fractions sample load, wash and sample eluate from an extract of a DORM-3 sample (Dogfish muscle). The inorganic arsenic elutes exclusively in the sample eluate fraction.

Selective separation of inorganic arsenic

Following extraction of the sample the separation of inorganic arsenic in the form of As(V) (pKa ~ 2.3/6.7/11.6) from organoarsenic compounds is done by sequential elution using a silica-based Strong Anionic Exchange (SAX) SPE column (Phenomenex). Organoarsenic compounds, including *e.g.* arsenobetaine (AB) and dimethylarsinate (DMA) are quantitatively eluted in the sample load step and washing step (using dilute acetic acid) without simultaneous elution of inorganic arsenic (as As(V)), which was subsequently eluted with 1M HCl in the final SPE fraction. Chromatograms of the three SPE fractions from a fish extract, illustrating the quantitative separation of inorganic arsenic, can be seen in figure 3.

Method performance

The method has been in-house validated and figures of merit have been established. Validation included samples spiked at three different concentration levels as well as naturally incurred samples (table 1). Limit of detection was determined at 0.08 mgKg-1 as three times the standard deviation at at intra-laboratory reproducibility conditions (SD_{IR}), both based on results from the lowest spike level (0.5 mg/kg).

TABLE 1. Figures of merit obtained in the validation study.

	Low	Medium	High	Total	TORT-2	DORM-3
Spike level (mg/kg)	0.5	1.0	1.5	0.5-1.5	0.90*	0.20*
Observations (N)	9	9	9	27	6	6
Mean recovery (%)	101	103	104	102	100	90
Repeatability RSD_r (%)	4	8	5	6	3	7
Reproducibility RSD_{IR} (%)	5	9	6	7	9	13

** Target level established by measurements with reference method HPLC-ICPMS.*

PICTURE 1. ICE 3300 AAS from Thermo Scientific with a VP100 for hydride generation system and an electrically heated cell.

Detection by HG-AAS

An Atomic Absorption Spectrometer (ICE-3300) coupled with a Continuous Flow Vapour Generator (VP100) (Thermo Scientific) (picture 1) is used for the determination of the arsenic content in the eluate from the SPE separation. The samples are prereduced with potassium iodide and ascorbic acid reducing As(V) to As(III) prior to analysis, due to the better hydride generating capacity of As(III).

Future use and impact

The method present a novel alternative allowing speciation analysis of inorganic arsenic with inexpensive equipment and can be used for control of inorganic arsenic concentrations in food and feed of marine origin. This will enable a more correct risk assessment compared to classical chemical analysis on the total arsenic content.

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