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Selective Determination of Inorganic Arsenic in Food and Feed by Microwave-assisted Extraction and Solid Phase Extraction

– A simple, inexpensive and fast speciation alternative

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ABSTRACT

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 pre-separated from organoarsenic compounds by Strong Anion Extraction (SAX) SPE followed by determination with ICP/MS. The method principle using SPE separation of arsenate As(V) from organoarsenic species will be presented together with extraction data from marine reference materials using an HPLC-ICPMS method.

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. Since seafood is the major dietary source for arsenic exposure in the European population, marine feed and seafood are of interest.

EXTRACTION OF INORGANIC ARSENIC

TABLE 1. Content of Inorganic arsenic in 2 marine reference materials TORT-2 and DORM-3 extracted with 3 different solvents in a microwaveoven for 20 min at 90 °C measured by anion exchange HPLC-ICP/MS.

EXTRACTION SOLVENT	CONTENT OF INORGANIC ARSENIC (mg/kg as As) Mean (RSD%)	
	TORT-2* N= 5	DORM-3** N=4
0.9 M NaOH/50 % EtOH	0.26 (17.5)	0.13 (5.6)
H2O/10 % H_2O_2	0.71 (14.9)	0.22 (8.0)
0.07 M HCl/10 % H_2O_2	0.78 (16.1)	0.24 (7.1)

Several sample extraction solvents and samples preparation approaches have been applied for analysis of inorganic arsenic. Water, methanol, hydrochloric acid or alkaline solutions have been used as extraction solvents with different results for the same reference material. Different extraction solvents was tested in order to optimize the extraction of inorganic arsenic from 2 marine reference materials TORT-2 and DORM-3 with a known totals content of arsenic (TABLE 1), since there does not exist reference materials for inorganic arsenic. Microwave assisted digestion for 20 minutes at 90 °C with 0,07 M HCl/10 % H_2O_2 were most efficient. H_2O_2 was added to ensure quantitative conversion of arsenite As(III) to arsenate As(V) (FIGURE 1) and thereby facilitate the following SPE separation by measuring total inorganic arsenic as As(V). 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 condition.

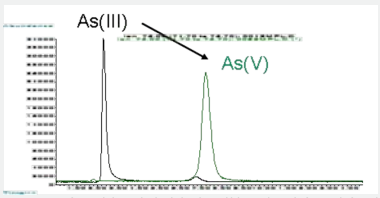

FIGURE 1. Chromatograms of an As(III) standard solution (10 ppb) in H_2O/H_2O_2 before and after microwave treatment, respectively, showing the quantitative conversion of As(III) to As(V) by H_2O_2.

METHOD

For the analysis of the total arsenic content in the SPE experiments (mass balance) an Agilent quadrupole ICPMS 7500ce instrument (Yokogawa Analytical Systems Inc., Tokyo, Japan),, was run in the standard mode. The sample introduction system consisted of an ASX-500 Auto sampler (CETAC Technologies, Omaha, NE, USA), a peristaltic pump, a concentric nebuliser (CPI International, Amsterdam, The Netherlands) and a water-cooled (15 °C) spray chamber. For the arsenic speciation analysis the ICPMS was coupled to an Agilent 1100 series quaternary HPLC pump, degasser and autosampler (Agilent Technologies, Waldbronn, Germany). Polypropylene autosampler vials were used. A strong anion exchange HPLC column, ICSep ION-120 (Transgenomics, San Jose, CA, USA) was used.

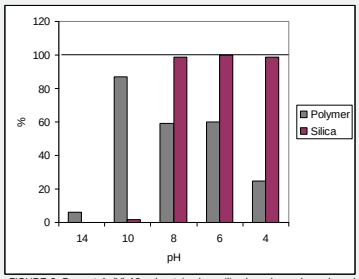

FIGURE 2. Percent As(V) 10 ppb retained on silica-based or polymer-based SAX SPE columns in the pH range 4 to 14. The pH of the samples were obtained by dissolving them in either 0.9 M NaOH, or 5 mM $(NH_4)_2CO_3$ buffer adjusted to the right pH with CH_3COOH or NH_3. At pH 14 the silica-based columns were dissolved and not possible to use.

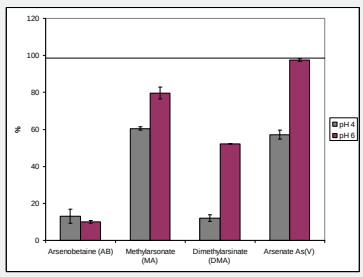

FIGURE 3. Retention of As(V) 10 ppb, Methylarsonate (MA) 10 ppb, Dimethylarsinate (DMA) 10 ppb and Arsenobetaine (AB) 10 ppb standards on a silica based SAX SPE column.

SPE SPECIATION

The retention of inorganic arsenic in the form of As(V) (pK_a ~ 2.3/6.7/11.6) on Strong Anionic Extraction (SAX) SPE columns were examined in the pH range 4-14 on both silica-based and polymer-based SPE columns from different producers. The columns were preconditioned with methanol and the solvent of the sample prior to loading the sample containing 10 ppb As(V). The retained arsenic was afterwards eluted with 1 M HNO_3. As(V) was retained 100 % on the SAX silica based SPE column (from Phenomenex or ThermoFisher) at pH 6, whereas the maximum retention on the polymer based SPE column (from Thermo Fisher) were 87 % at pH 10.3 (Figure 2). However at pH 6 other arsenic compounds commonly found in marine products such as MA (pK_a ~3.6/8.2), DMA (pK_a ~ 1.3/6.3)) and AB (pK_a 2.2) standards (10 ppb) were also retained on the SAX SPE column, by approximately 80, 52 and 10 %, respectively (Figure 3). However, it was possible to selectively elute MA, DMA and AB with 1 M CH_3COOH prior to elution with HNO_3 without eluting As(V) and thereby enable separation of As(V) from the organoarsenic compounds by SPE. Future studies will introduce the use of HG-AAS instead of ICP/MS instrumentation and thus provide a simple and inexpensive speciation alternative to HPLC-ICP/MS (FIGURE 4).

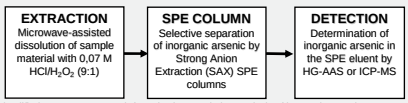

FIGURE 4. Principle of the simplified SPE AAS approach for selective speciation analysis of inorganic arsenic.

CONCLUSION

Extraction of As(V) with 0.07 M HCl/10 % H_2O_2 followed by selective separation of total inorganic arsenic in the form of As(V) from organoarsenic compounds by silica-based SAX SPE columns provides a simple and inexpensive speciation alternative to HPLC.

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