Determination of inorganic arsenic in food and feed by MAE-SPE-HG-AAS – a simple, inexpensive and fast speciation alternative

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National Food Institute (DTU Food)
Technical University of Denmark
Department of Food Chemistry at DTU Food

Research, consultancy to authorities, teaching

Analysis in food for:

- Pesticides
- Veterinary drug residues
- Migration from Food Contact Materials
- Biotoxins
- Organic pollutants (POPs)
- Metals and minerals
- Nutrients and vitamins
- Food additives
Todays agenda

- Speciation of arsenic, WHY?
  - EU-projects
    - CEN Standard
    - Confidence
- Background
- Speciation af arsenic
- Development of AAS-method
- Results
- Questions?

Is seafood safe to eat?
- a consumers dilemma
CEN/TC 327/WG 4
“Heavy metals, trace elements and minerals”
Work item: Inorganic arsenic

• “Animal feeding stuffs – Determination of inorganic arsenic”
  Project leader: Jens J. Sloth

• **Scope:**
  - The aim is to develop a European standard method for the determination of inorganic arsenic in marine-based feedingstuffs for animals.
CONtaminants in Food and Feed: Inexpensive DEtection for Control of Exposure (CONFIDENCE)

• Improvement of consumer exposure assessments. The developed fast and cost-efficient methods will allow a higher sampling and analysis density in monitoring. Thus, a better understanding of contaminant levels in food and feed will be achieved.

• Large EU-project, Several other work packages measuring: Persistent Organic Pollutants (POPs), Perfluorinated compounds (PFCs): Pesticides, Veterinary drugs, Heavy metals, Biotoxins.

• The main task is to develop a SPE-AAS based method for quantification of inorganic As in both food of marine origin and feed
Additionally develop a SPE-AAS based method for quantification of methyl mercury in feed and food of marine origin.

A already developed HPLC or GC-ICPMS method for detection of either inorganic arsenic or methylmercury will be used as support for quantification of both inorganic arsenic and methylmercury.
**Arsenic - occurrence**

High concentrations of arsenic has been found in samples from the marine environment.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater</td>
<td>1 - 2</td>
<td>µg/L</td>
</tr>
<tr>
<td>Marine fish</td>
<td>0,2 - &gt;100</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Marine invertebrates</td>
<td>0,2 - &gt;100</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Marine algae</td>
<td>0,02 - 40</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Freshwater fish</td>
<td>&lt;0,01 – 2</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Terrestrial biota</td>
<td>&lt;0,2</td>
<td>mg/kg</td>
</tr>
</tbody>
</table>

*All results on wet weight basis*

Marine organisms can bioaccumulate arsenic by a factor of up to **100,000** compared with seawater!!!
Arsenic compounds in the marine environment

More than 40 different arsenic species have been found in the marine environment.
More than 40 different arsenic species have been found in the marine environment.
Arsenic – chronic toxicity

Long term exposure => skin diseases
• Keratosis, gangrene, melatosis
• Skin cancer

… and also
• lung, kidney, liver, bladder cancers
• Cancer slope factor: $1.5 \text{ (mg kg}^{-1} \text{ day}^{-1})^{-1}$
  (US EPA 2005)

WHO PTWI for inorganic arsenic: 15 µg/kg bw/week (Provisional Tolerable Weekly Intake)
For a 70 kg person => 150 µg / day
Arsenic - toxicity

Toxicity: As(III) > As(V) > TETRA > MA > DMA > AC/AB

Inorganic arsenic

<table>
<thead>
<tr>
<th>Compound</th>
<th>LD₅₀ values (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As(III)</td>
<td>15-42</td>
</tr>
<tr>
<td>As(V)</td>
<td>20-800</td>
</tr>
<tr>
<td>TETRA</td>
<td>890</td>
</tr>
<tr>
<td>MA</td>
<td>700-1800</td>
</tr>
<tr>
<td>DMA</td>
<td>1200-2600</td>
</tr>
<tr>
<td>AC</td>
<td>6500</td>
</tr>
<tr>
<td>AB</td>
<td>&gt;10000</td>
</tr>
</tbody>
</table>

Values for mice and rats

Kaise & Fukui (1992); Shiomi (1994); Donohue & Abernathy (1999)
### Commission Directive 2003/100/EC on animal feed

<table>
<thead>
<tr>
<th>Undesirable substances</th>
<th>Products intended for animal feed</th>
<th>Maximum content in mg/kg (ppm) relative to a feedingsstuff with a moisture content of 12%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
</tr>
</tbody>
</table>

| 1. Arsenic (%)          | Feed materials with the exception of: |  |
|                        | — meal made from grass, from dried lucerne and from dried clover, sunflower and beet pulp and dried molasses sugar beet pulp | 2 |
|                        | — corn kernel expeller              | 4 (*)                                                                                     |
|                        | — phosphates and calcareous marine algae | 10                                                                                       |
|                        | — calcium carbonate                | 15                                                                                       |
|                        | — magnesium oxide                  | 20                                                                                       |
|                        | — feedingsstuffs obtained from the processing of fish or other marine animals | 15 (*)                                                                                   |
|                        | — seaweed meal and feed materials derived from seaweed | 40 (*)                                                                                   |

Complete feedingsstuffs with the exception of:
- complete feedingsstuffs for fish and complete feedingsstuffs for fur animals
- complementary feedingsstuffs with the exception of:
  - mineral feedingsstuffs

*(Upon request of the competent authorities, the responsible operator must perform an analysis to demonstrate that the content of inorganic arsenic is lower than 2 ppm. This analysis is of particular importance for the seaweed species *Hizika fusiforme*.)
Arsenic and food/feed control – present status

- Food – no maximum levels established
- Feed – maximum levels for total arsenic
- EFSA opinion on arsenic in food – expected in 2009
- CEN (European Standardization Organization)
  - TC327 WG4 Feedingstuffs (Heavy metals and minerals)
  - TC275 WG10 Foodstuffs (Trace elements)
Speciation and regulation - some historical viewpoints

1998

Speciation analysis: where is it going? An attempt at a forecast

Bernhard Welz

Department of Applied Research, Rudolphsweck Perkin-Elmer GmbH, D-88662 Überlingen, Germany

1999

Conference Contribution

Torsten Berg · Erik H. Larsen

Speciation and legislation – Where are we today and what do we need for tomorrow?

1999

2007

Handbook of Elemental Speciation II

Species in the Environment, Food, Medicine and Occupational Health

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Joe Caruso

Helen Crewe

Michael Sperling

Klaus Heumann

University of Münster, Münster, Germany

Wiley 2005
<table>
<thead>
<tr>
<th>Sample identification</th>
<th>Inorganic arsenic</th>
<th>Total arsenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon (Salmo salar)</td>
<td>&lt; 0.0006</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Cod (Gadus morhua)</td>
<td>&lt; 0.0006</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Cod (Gadus morhua)</td>
<td>&lt; 0.0006</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Wolffish (Anarhichas lupus)</td>
<td>&lt; 0.0006</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>Wolffish (Anarhichas lupus)</td>
<td>&lt; 0.0006</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>Anglerfish (Lophius piscatorius)</td>
<td>&lt; 0.0006</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Anglerfish (Lophius piscatorius)</td>
<td>&lt; 0.0006</td>
<td>44 ± 6</td>
</tr>
<tr>
<td>Atlantic halibut (Hippoglossus hippoglossus)</td>
<td>&lt; 0.0006</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Mackerel (Scomber scombrus)</td>
<td>&lt; 0.0006</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Mackerel (Scomber scombrus)</td>
<td>&lt; 0.0006</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>Herring (Clupea harengus)</td>
<td>&lt; 0.0006</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Herring (Clupea harengus)</td>
<td>&lt; 0.0006</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Tuna fish (Thunnus alalunga)</td>
<td>&lt; 0.0006</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Lobster, tail meat (Homarus gammarus)</td>
<td>&lt; 0.0006</td>
<td>3.8 ± 2</td>
</tr>
<tr>
<td>Lobster, head and thorax meat (Homarus gammarus)</td>
<td>0.016 ± 0.002</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Crab, white meat (Cancer pagurus)</td>
<td>0.060 ± 0.009</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Crab, head and thorax meat (Cancer pagurus)</td>
<td>0.005 ± 0.001</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>King crab, white meat (Paralithodes camtschaticus)</td>
<td>0.020 ± 0.003</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Norway lobster (Nephrops norvegicus)</td>
<td>0.008 ± 0.001</td>
<td>3.1 ± 0.3</td>
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<tr>
<td>Shrimp (Pandalus borealis)</td>
<td>3.8 ± 0.5</td>
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<tr>
<td>Shrimp (Pandalus borealis)</td>
<td>60 ± 8</td>
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<tr>
<td>Shrimp (Pandalus borealis)</td>
<td>67 ± 8</td>
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<tr>
<td>Horse mussel (Modiolus modiolus)</td>
<td>3.4 ± 0.4</td>
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<tr>
<td>Scallop muscle (Pecten maximus)</td>
<td>0.014 ± 0.002</td>
<td>1.8 ± 0.2</td>
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<tr>
<td>Oyster (Ostrea edulis)</td>
<td>&lt; 0.0006</td>
<td>0.61 ± 0.08</td>
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<tr>
<td>Mink whale (Balaenoptera Acutorostrata)</td>
<td>&lt; 0.0006</td>
<td>0.9 ± 0.1</td>
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<tr>
<td>Harp seal (Pagophilus groenlandicus)</td>
<td>&lt; 0.0006</td>
<td>0.22 ±0.03</td>
</tr>
</tbody>
</table>
**PKₐ for different Arsenic compounds**

<table>
<thead>
<tr>
<th>Species</th>
<th>pKₐ - values</th>
<th>pH?</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
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<tr>
<td>As(III)</td>
<td>9.2</td>
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<td>As(V)</td>
<td>2.3/6.7/11.6</td>
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<tr>
<td>MA</td>
<td>3.0/8.2</td>
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<td>DMA</td>
<td>1.3/6.3</td>
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<td>TMAO</td>
<td>3.6</td>
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<tr>
<td>AC</td>
<td>none</td>
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<tr>
<td>TETRA</td>
<td>none</td>
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</tbody>
</table>

The charge of the arsenic compound depends on pH and inorganic arsenic should be separated by anionic chromatography.
Reference method HPLC-ICPMS
Jens et al. 2005

0,2 g dry (or 1,0 g wet) sample has to be weighted into akvarts/teflon bomb

Add 10 mL 0,9 M NaOH in 50 % ethanol (Base solution)

CENTRIFUGATION: the samples will be transferred to 15 mL polypropylen centrifugation containers and centrifuge for 10 minutes at 4000 rpm, transfer the supernatant to 10 mL polyethylen containers

FILTRATION: The samples must be filtered by a 0,45 um membrane filter

ANALYSE:: LC separation on a Agilent 1100 series polymer strong Anion changer HPLC colomn ION-120 (4,6 x 120 mm, 10 um partikler) Mobilefase: 30 mM (NH4)2CO3 in water (MilliQ) with 3 % methanol, pH 10,3 isocratisk.

Detection: Agilent ICPMS 7500cs (Yokogawa Analytical Systems Inc. Tokyo, Japan)
Determination of AS by SPE-HGAAS

Microwave assisted acidic/$\text{H}_2\text{O}_2$ hydrolysis:

- **Freeze drying of sample** (Addition of solvent)
- **Microwave treatment 20 min, 90°C**
  
  *I: Solubilisation of sample matrix*
  *II: Conversion of As(III) to As(V) by $\text{H}_2\text{O}_2$*
  *III: Time/temperature may be reduced*

- **Adjust pH of sample to pH 6 in order to obtain maximum retention on SPE column**
Extraction of inorganic arsenic

Inorganic arsenic by anion exchange HPLC-ICPMS

First approach: Alkaline extraction and µ-waves
- not compatible with SPE!!!!!!!!!!
- and apparently not the most efficient !!!

New approach – DIFFERENT SOLVENT 0,07 M HCl/10 % H₂O₂
- extraction and oxidation of As(III) to As(V) (=total iAs)
- more compatible with SPE

FREEZE drying a necessary step not wet sample when operating with water
Solvent to extraction of inorganic arsenic

Ensure: no conversion of other organic Arsenic compounds is converted to iAs

Recovery of spiked As(III)

Quantitative conversion of As(III) to As(V) by $H_2O_2$
SOLID PHASE EXTRACTION (SPE)

SAX (strong anionic exchange)

- Cleaning with 2 mL methanol
- Conditioning with 2 mL solvent
- 4 mL sample (diluted 1:1)
- 3 mL elution with 1 M Acetic acid
- 1 mL elution with 1 M HCl
- Matrix matched standard curves
Solid phase extraction (SPE): Silica versus polymer

- Started out with a SAX (strong anionic exchange) SPE columns - 0.9 M NaOH as solvent - two types polymer based and silica based

100 % retention with SAX Silica columns at pH~ 6

Polymer column only about 80 % retention
Measurement on a ICE 3300 from Thermo Scientific
Pre-reduction of samples

• Samples diluted 1:5 with 10 % HCL containing 0.5 % KI and 0.5 % Ascorbic acid
• After mixing left for 1 hour
• Diluted up to 1:10 with 10 % HCl
• After mixing left for one hour before measurement
• (total dilution of 1:10) of the sample and sample matrix)
Anti-foaming agent

- Foaming in samples
- Other method in the literature diluted 1:25
- Further dilution not a possible due to sensitivity of the method
- Silicone anti-foaming agent added to the samples solved the problem
- 0.05 % in the samples
STANDARD CURVES
Fishmeal (spiked)

\[ y = 1.0159x + 19.289 \]
\[ R^2 = 0.9989 \]

\[ y = 1.0195x + 0.3283 \]
\[ R^2 = 0.9971 \]
# AAS compared to ICP-MS

<table>
<thead>
<tr>
<th></th>
<th>AAS (ppm)</th>
<th>ICP (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tort-2 (Lobster hepatopancreas)</td>
<td>0,94</td>
<td>0,95</td>
</tr>
<tr>
<td>Blue mussel</td>
<td>0,38</td>
<td>0,37</td>
</tr>
<tr>
<td>Ris</td>
<td>1,07</td>
<td>1,29</td>
</tr>
<tr>
<td>Rejm skald</td>
<td>0,22</td>
<td>0,20</td>
</tr>
</tbody>
</table>

- test of several different marine matrices
- in-house validated this Fall
- collaborative trial with 5-6 laboratories participation early 2010
Intake of mercury from various food

Methyl mercury is the predominant form in fish

A MAE-SPE-HG-AAS method to detection of methyl mercury is to be developed
I had a fatty fish the other day, so I think I’ll go for a lean fish this time!!

Some balanced views on seafood consumption:
Denmark: Danish Food Administration (Fødevarestyrelsen):
Helhedssyn på fisk og fiskevarer (2003)
Free download from www.fvst.dk
"Here's a dish I used to cook for my late husband. If you want to try it just follow the recipe, but ignore the part where it mentions a pinch of arsenic."

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