National Food Institute

Technical University of Denmark DTU

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
- Quistions?

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**.

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

CONtaminants in Food and Feed: Inexpensive DEtectioN for Control of Exposure (CONFIDENCE)

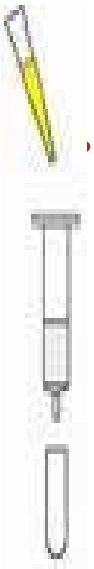
- **Improvement of consumer exposure assessments. The developed fast and cost-efficient methods will allow a higher sampling and analysis density in monitoring. Thus, a better understanding of contaminant levels in food and feed will be achieved.**

- **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**

CONFIDENCE……..

- 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

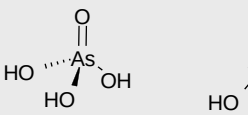
High concentrations of arsenic has been found in samples from the marine environment.

Seawater	1 - 2	µg/L
Marine fish	0,2 - >100	mg/kg
Marine invertebrates	0,2 - >100	mg/kg
Marine algae	0,02 - 40	mg/kg
Freshwater fish	<0,01 – 2	mg/kg
Terrestrial biota	<0,2	mg/kg

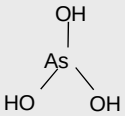
All results on wet weight basis

Marine organisms can bioaccumulate arsenic by a factor of up to **100.000** compared with seawater!!!

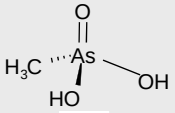
Arsenic compounds in the marine environment



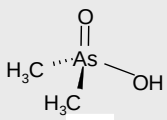
As(V)



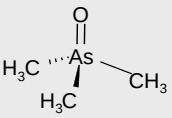
As(III)



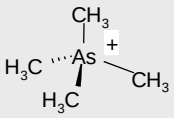
MA



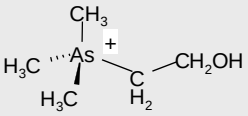
DMA



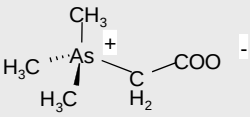
TMAO



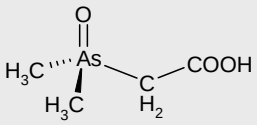
TETRA



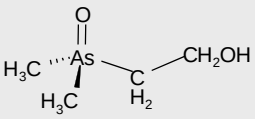
AC



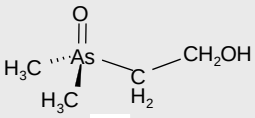
AB



DMAA

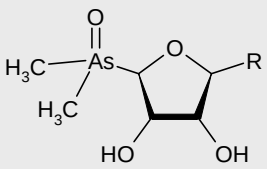


DMAE

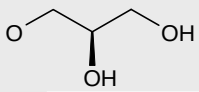


TMAP

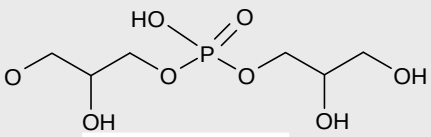
More than **40** different arsenic species have been found in the marine environment



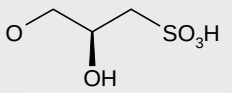
Dimethylarsinoylriboside



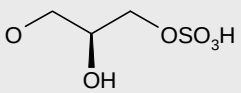
Dimethyl As-sugar 1



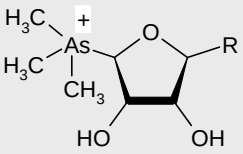
Dimethyl As-sugar 2



Dimethyl As-sugar 3



Dimethyl As-sugar 4

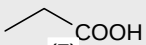


Trimethylarsonioriboside

R = 1,2,3,4,5

OMe
(5)

H
(6)



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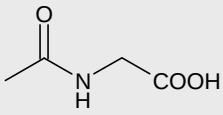

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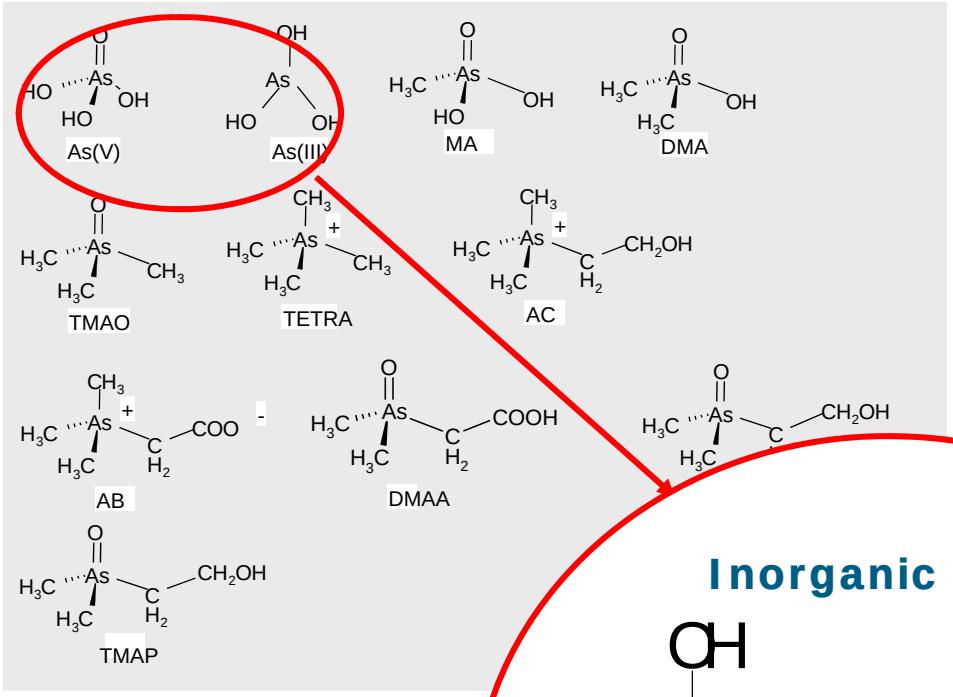
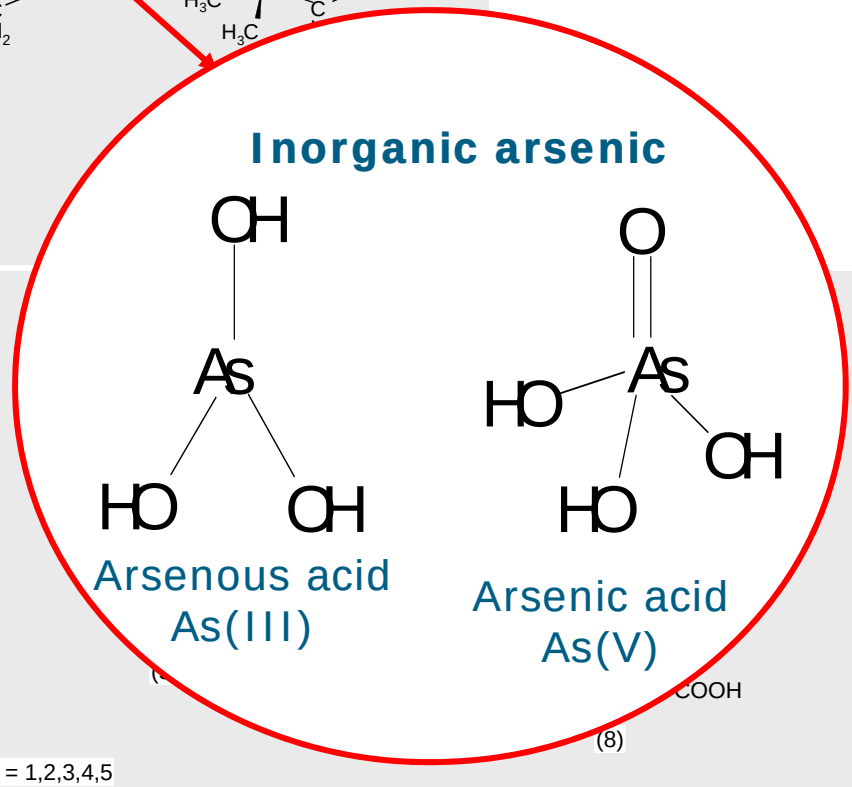
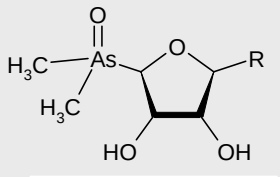
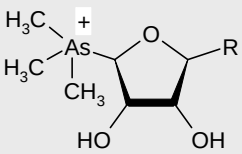
Arsenic compounds in the marine environment



More than 40 different arsenic species have been found in the marine environment







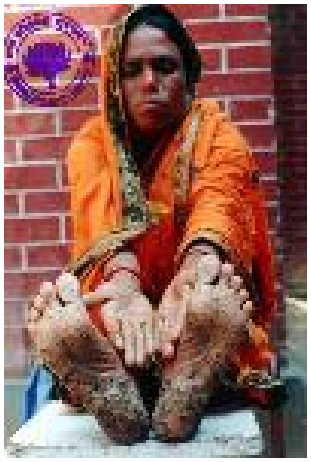
R = 1,2,3,4,5

(8)

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 $(mg\ kg^{-1}\ day^{-1})^{-1}$
  (US EPA 2005)

WHO PTWI for inorganic arsenic: 15 µg/kg bw/week
(**P**rovisional **T**olerable **W**eekly **I**ntake)
For a 70 kg person => 150 µg / day

Arsenic - toxicity

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

Inorganic arsenic

LD_{50} - values (mg/kg)	
As(III)	15-42
As(V)	20-800
TETRA	890
MA	700-1800
DMA	1200-2600
AC	6500
AB	>10000

Values for mice and rats

Kaise & Fukui (1992); Shiomi (1994); Donohue & Abernathy (1999)

Commission Directive 2003/100/EC on animal feed

Undesirable substances	Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12 %
(1)	(2)	(3)
'1. Arsenic (8)	Feed materials with the exception of:	2
	— meal made from grass, from dried lucerne and from dried clover, and dried sugar beet pulp and dried molasses sugar beet pulp	4
	— palm kernel expeller	4 (9)
	— phosphates and calcareous marine algae	10
	— calcium carbonate	15
	— magnesium oxide	20
	— feedingstuffs obtained from the processing of fish or other marine animals	15 (9)
	— seaweed meal and feed materials derived from seaweed	40 (9)
	Complete feedingstuffs with the exception of:	2
	— complete feedingstuffs for fish and complete feedingstuffs for fur animals	6 (9)
	Complementary feedingstuffs with the exception of:	4
	— mineral feedingstuffs	12

Max levels for total arsenic

Footnote in the Commission directive

(9) 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 *Hizikia fusiforme*.'

Arsenic and food/feed control – present status

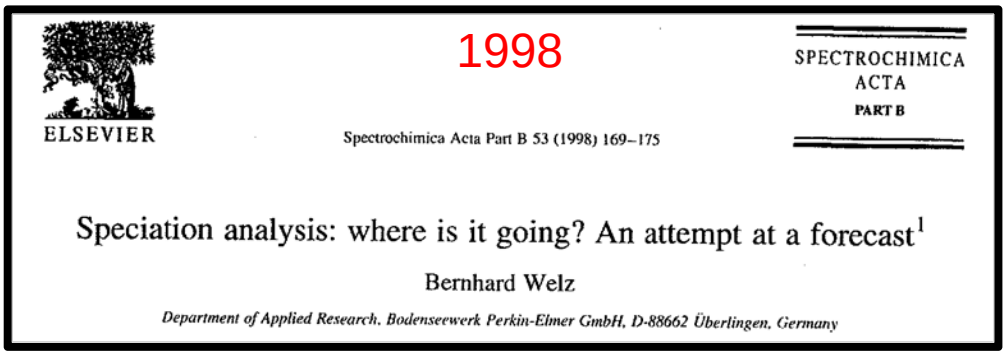
- Food – no maximum levels established
- Feed – maximum levels for total arsenic

- 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)

efsa
European Food Safety Authority

Speciation and regulation - some historical viewpoints



1998

ELSEVIER

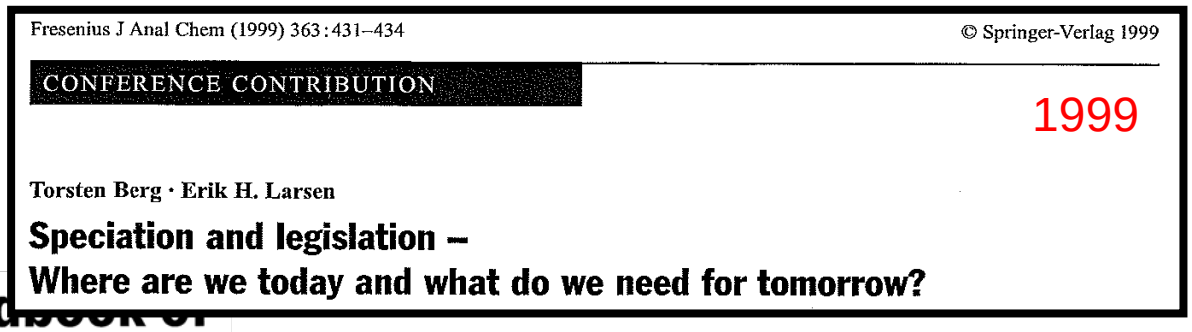
Spectrochimica Acta Part B 53 (1998) 169–175

SPECTROCHIMICA ACTA PART B

Speciation analysis: where is it going? An attempt at a forecast[1]

Bernhard Welz

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



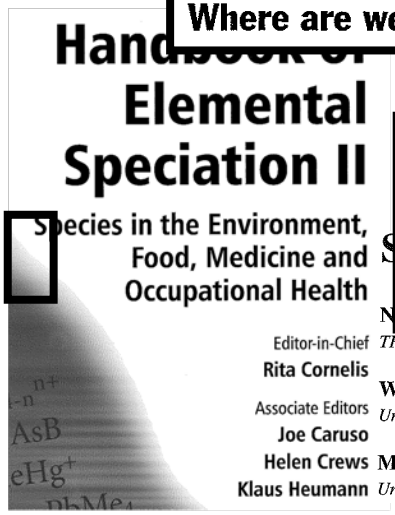
Fresenius J Anal Chem (1999) 363:431–434

CONFERENCE CONTRIBUTION

1999

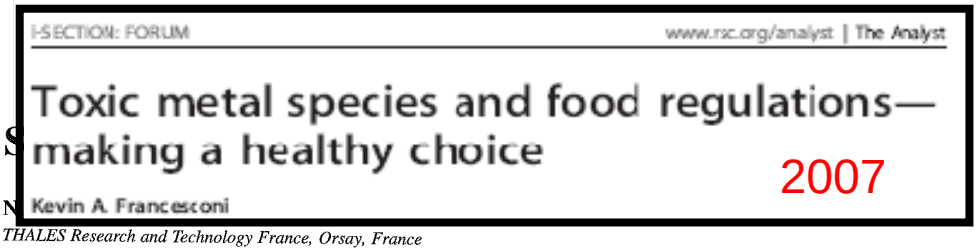
Torsten Berg · Erik H. Larsen

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



Handbook of Elemental Speciation II

Species in the Environment, Food, Medicine and Occupational Health



I-SECTION: FORUM www.rsc.org/analyst | The Analyst

Toxic metal species and food regulations—making a healthy choice

2007

Kevin A. Francesconi

THALES Research and Technology France, Orsay, France

Wiley 2005

National Food Institute

DTU

Sloth et al, J.Agri.Food Chem, 2005, 53, 6011-6018

Sample identification	Inorganic arsenic	Total arsenic
Salmon (*Salmo salar*)	< 0.0006	1.9 ± 0.2
Cod (*Gadus morhua*)	< 0.0006	17 ± 2
Cod (*Gadus morhua*)	< 0.0006	15 ± 2
Wolffish (*Anarhichas lupus*)	< 0.0006	4.1 ± 0.5
Wolffish (*Anarhichas lupus*)	< 0.0006	31 ± 4
Anglerfish (*Lophius piscatorius*)	< 0.0006	15 ± 2
Anglerfish (*Lophius piscatorius*)	< 0.0006	44 ± 6
Atlantic halibut (*Hippoglossus hippoglossus*)	< 0.0006	12 ± 1
Mackerel (*Scomber scombrus*)	< 0.0006	1.7 ± 0.2
Mackerel (*Scomber scombrus*)	< 0.0006	2.8 ± 0.4
Herring (*Clupea harengus*)	< 0.0006	1.5 ± 0.2
Herring (*Clupea harengus*)	< 0.0006	1.7 ± 0.2
Herring (*Clupea harengus*)	< 0.0006	1.7 ± 0.2
Tuna fish (Thunnus alalunga)	0.008 ± 0.001	0.9 ± 0.1
Lobster, tail meat (*Homarus gammarus*)	< 0.0006	14 ± 2
Lobster, head and thorax meat (*Homarus gammarus*)	0.030 ± 0.004	22 ± 3
Crab, white meat (*Cancer pagurus*)	0.016 ± 0.002	32 ± 4
Crab, head and thorax meat (*Cancer pagurus*)	0.060 ± 0.009	26 ± 3
King crab, white meat (*Paralithodes camschaticus*)	0.005 ± 0.001	26 ± 3
Norway lobster (*Nephrops norvegicus*)	0.020 ± 0.003	21 ± 3
Shrimp (*Pandalus borealis*)		3.8 ± 0.5
Shrimp (*Pandalus borealis*)		60 ± 8
Shrimp (*Pandalus borealis*)		67 ± 8
Horse mussel (*Modilous modiolus*)		3.4 ± 0.4
Scallop muscle (*Pecten maximus*)	0.008 ± 0.001	3.1 ± 0.3
Oyster (*Ostrea edulis*)	0.014 ± 0.002	1.8 ± 0.2
Mink whale (*Balaenoptera Acutorostrata*)	< 0.0006	0.61 ± 0.08
Harp seal (*Pagophilus groenlandicus*)	< 0.0006	0.9 ± 0.1
Hooded seal (*Cystophora cristata*)	< 0.0006	0.22 ±0.03

Fish muscle

Crustaceans & bivalves

For all samples inorganic arsenic constitutes less than 1% of total arsenic

PK_a for different Arsenic compounds

Species	pK_a - values	pH? 1 2 3 4 5 6 7 8 9 10 11 12 13
As(III)	9.2	H_3AsO_3 | $H_2AsO_3^-$
As(V)	2.3/6.7/11.6	H_3AsO_4 | $H_2AsO_4^-$ | $HAsO_4^{2-}$ | AsO_4^{3-}
MA	3.6/8.2	$CH_3AsO(OH)_2$ | $CH_3AsO_2(OH)^-$ | $CH_3AsO_3^-$
DMA	1.3/6.3	$(CH_3)_2As^+(OH)_2$ | $(CH_3)_2AsO(OH)$ | $(CH_3)_2AsO_2^-$
DMAA	?	$(CH_3)_2AsOCH_2COOH_2^+$? $(CH_3)_2AsOCH_2COOH$? $(CH_3)_2AsOCH_2COO^-$
AB	2.2	$(CH_3)_3As^+CH_2CO_2^-$ | $(CH_3)_3As^+CH_2CO_2^-$
TMAO	3.6	$(CH_3)_3AsOH^+$ | $(CH_3)_3AsO$
DMAE	?	$(CH_3)_2AsOCH_2CH_2OH_2^+$? $(CH_3)_2AsOCH_2CH_2OH$
AC	none	$(CH_3)_3As^+CH_2CH_2OH$
TETRA	none	$(CH_3)_4As^+$

The charge of the arsenic compound depends on pH and inorganic arsenic should be separated by ainionic 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 / H_2O_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 H_2O_2
III: Time / temperature may be reduced

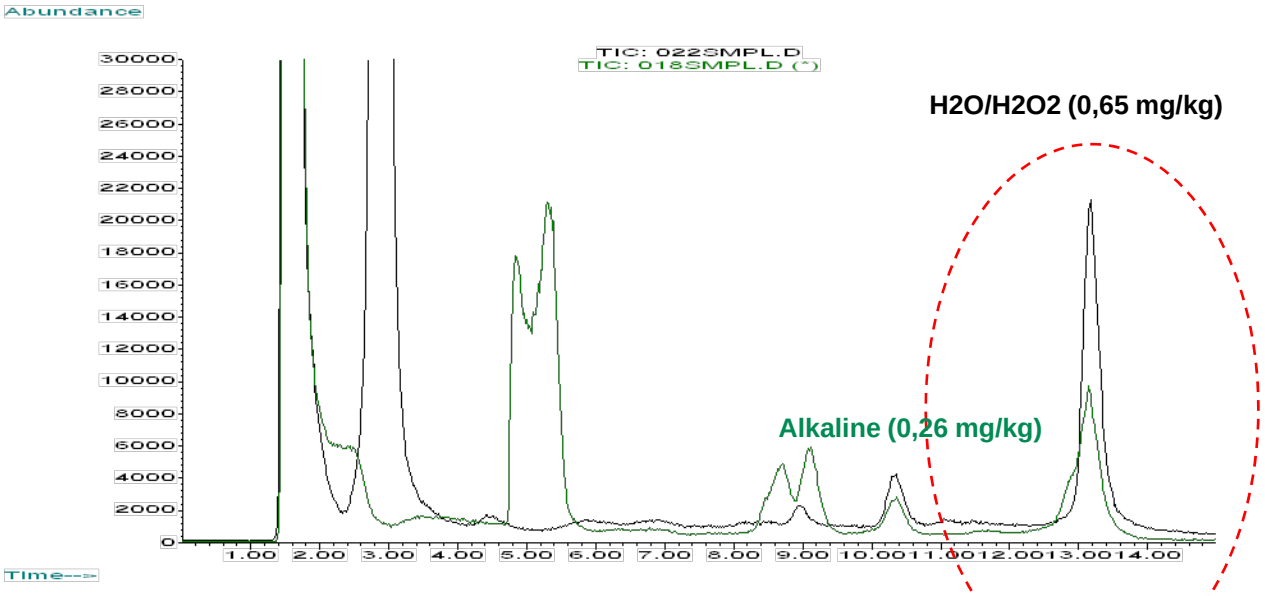
↓

Ajust 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 % H_2O_2

- extraction and oxidation of As(III) to As(V) (=total iAs)
- more compatible with SPE

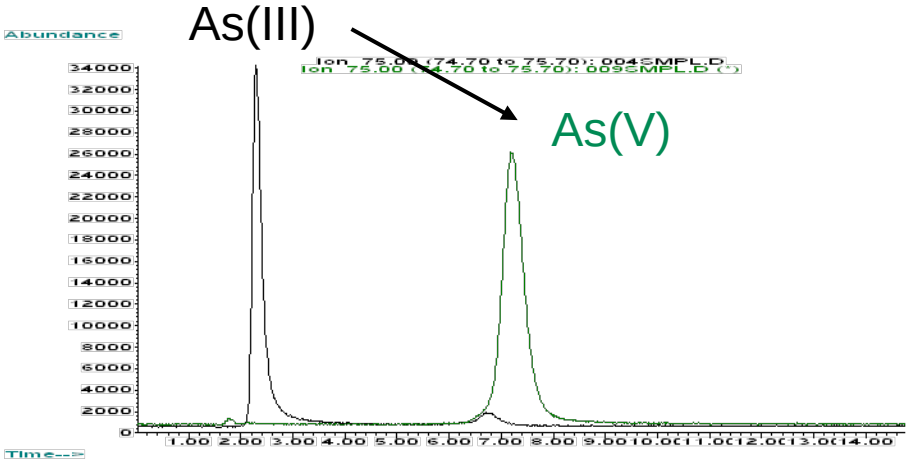
FREEZE drying a necessary step not wet sample when operating with water

Solvent to extraction of inorganic arsenic

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

Recovery of spiked As(III)

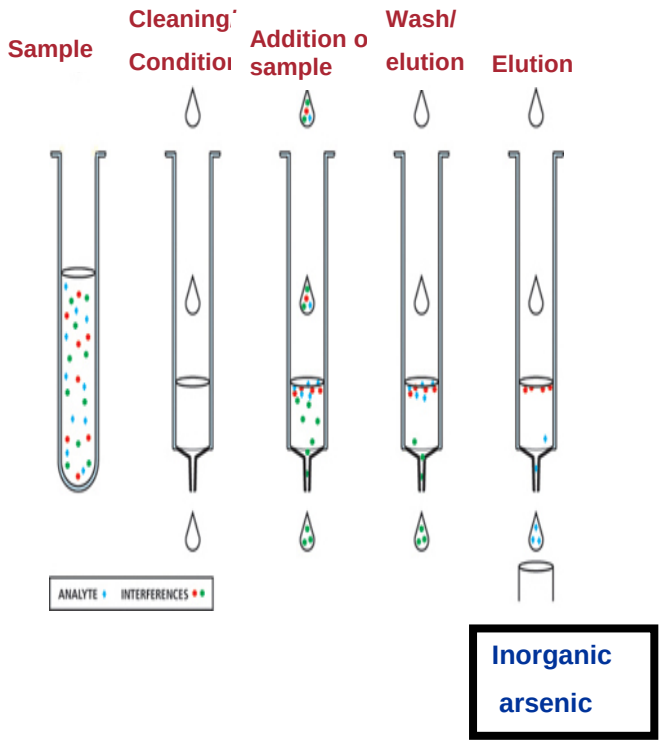
Quantitative conversion of As(III) to As(V) by H_2O_2



SOLID PHASE EXTRACTION (SPE)

SAX (strong anionic exchange)

Inorganic Arsenic negatively charged



-**Cleaning with 2 mL methanol**

-**Conditioning with 2 mL solvent**

-**4 mL sample** (diluted 1:1)

-**3 mL elution with 1 M Acetic acid**

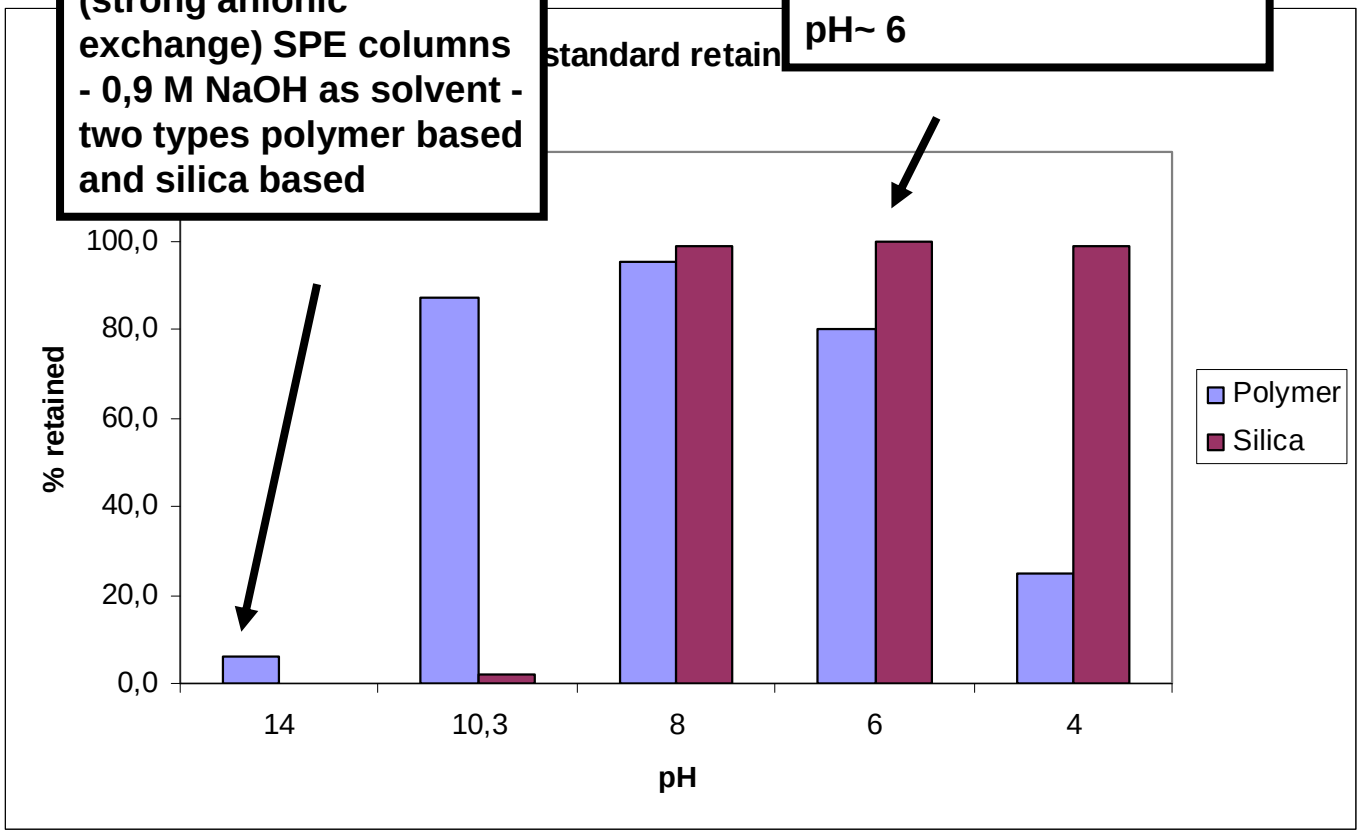
-**1 mL elution with 1 M HCl**

-**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

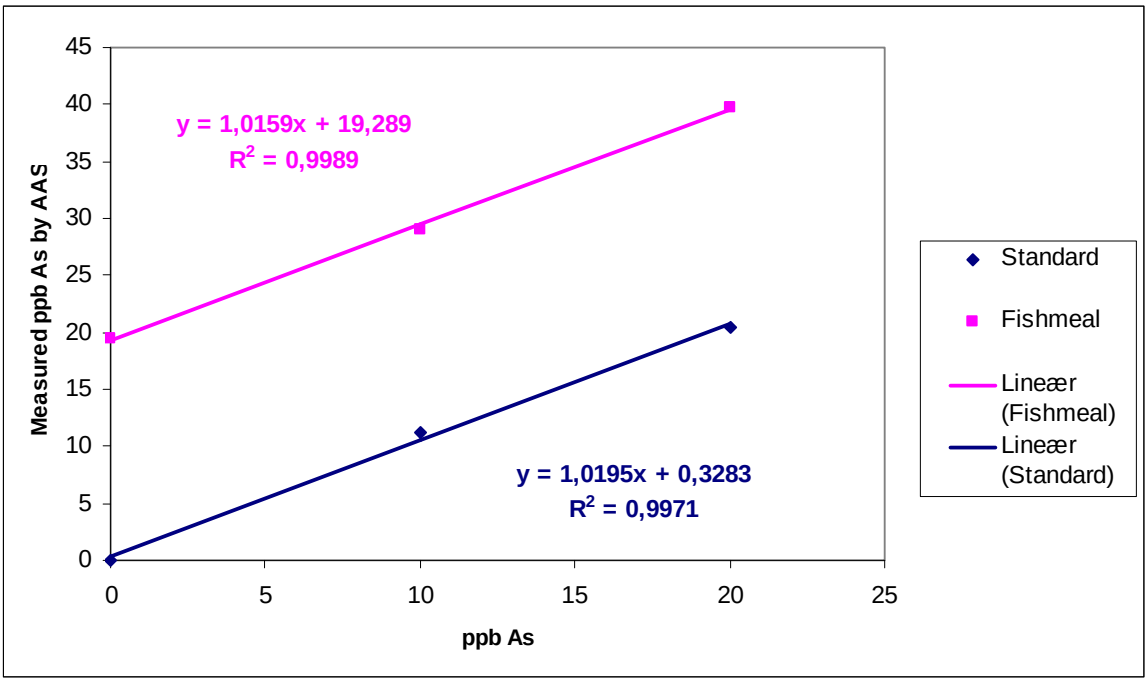
Pre-reduction of samples

- Samples diluted 1:5 with 10 % HCL contaning 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)



AAS compared to ICP-MS

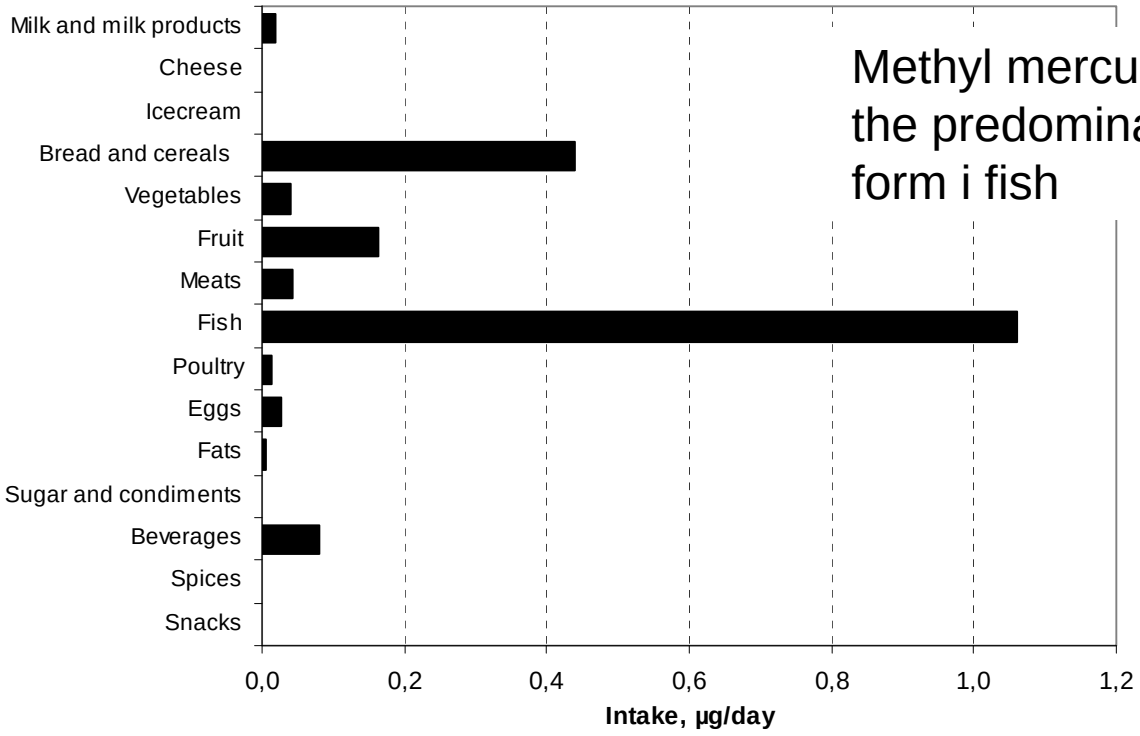
	AAS (ppm)	ICP (ppm)
Tort-2 (Lobster hepatopancreas)	**0,94**	**0,95**
Blue mussel	**0,38**	**0,37**
Ris	**1,07**	**1,29**
Reje m skald	**0,22**	**0,20**

-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 i fish

A MAE-SPE-HG-AAS method to detection of methyl mercury is to be developed

Some balanced views on seafood consumption:

Denmark: Danish Food Administration (Fødevarestyrelsen):

Helhedssyn på fisk og fiskevarer (2003)

Free download from www.fvst.dk

Thanks for your attention!!!

"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."