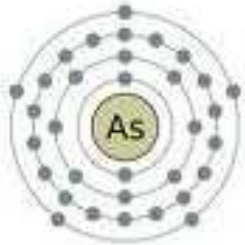
INORGANIC ARSENIC DETERMINED BY SPE SEPARATION AND AAS DETECTION – A NOVEL SPECIATION APPROACH

Rasmussen RR, Hedegaard RV and Sloth JJ

RAFA 3rd November 2011



DTU Food
National Food Institute

Arsenic - occurrence

total As

High concentrations of arsenic in samples from the marine environment:

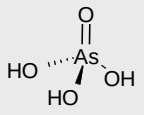
Seawater	1	-	2	µg/L
Marine fish	0,2	-	**>100**	mg/kg
Marine invertebrates	0,2	-	**>100**	mg/kg
Marine algae	0,02	-	40	mg/kg
Freshwater fish	<0,01	-	2	mg/kg
Terrestrial biota	<0,2			mg/kg

All results on wet weight basis

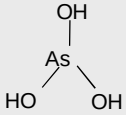
Marine organisms can bioaccumulate arsenic by a factor of up to 100.000 compared with seawater!!!

Arsenic species

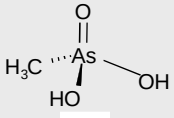
in the marine environment



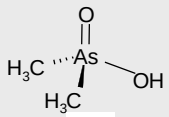
As(V)



As(III)



MA



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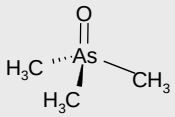

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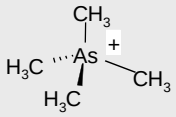

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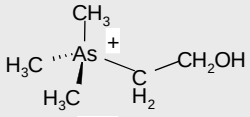

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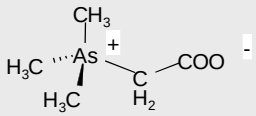

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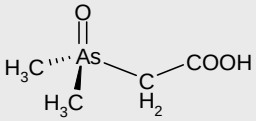

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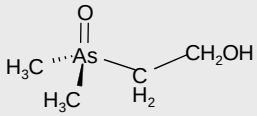

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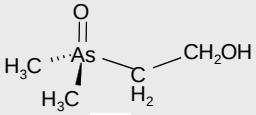

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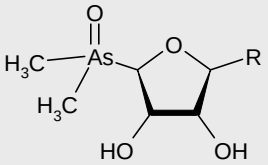
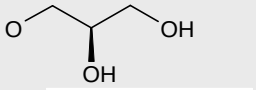
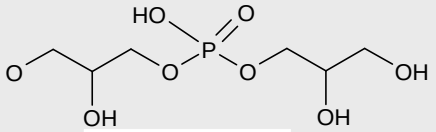


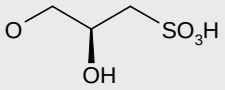
Dimethylarsinoylriboside



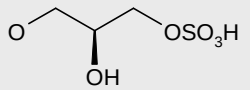
Dimethyl As-sugar 1



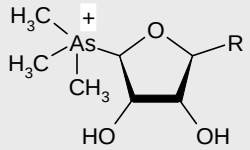
Dimethyl As-sugar 2



Dimethyl As-sugar 3



Dimethyl As-sugar 4

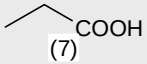


Trimethylarsonioriboside

R = 1,2,3,4,5

OMe (5)

H (6)



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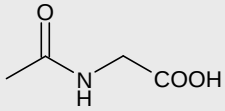


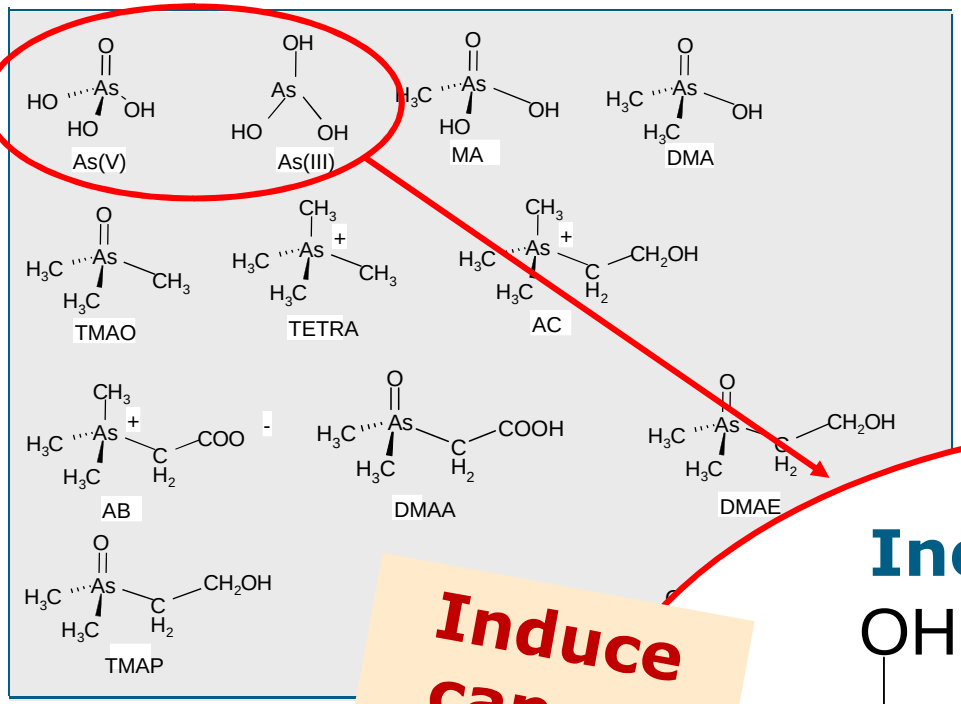
(8)

More than **50** different **arsenic species**:

- **inorganic arsenic**
- **organic arsenic**
  - methylated species
  - arsenosugars
  - arsenolipids

Arsenic species

in the marine environment



More than **50** different **arsenic species**

- **in** **nic**
- **o** **ic**

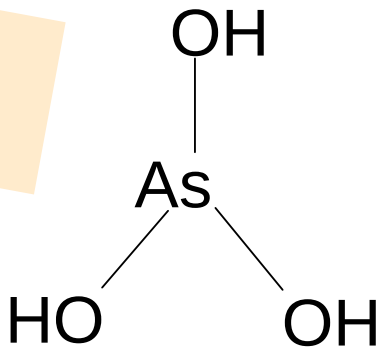
species

rs

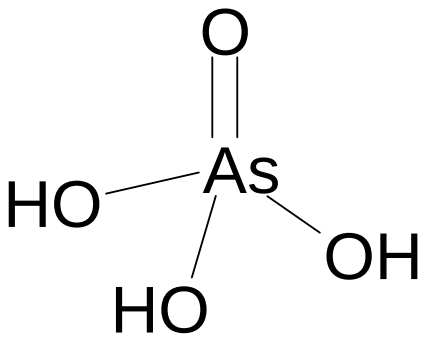
Most toxic form of arsenic!!

Induce cancer

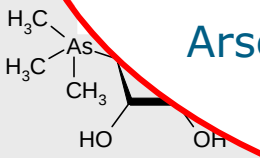
Inorganic arsenic



Arsenous acid
As(III)

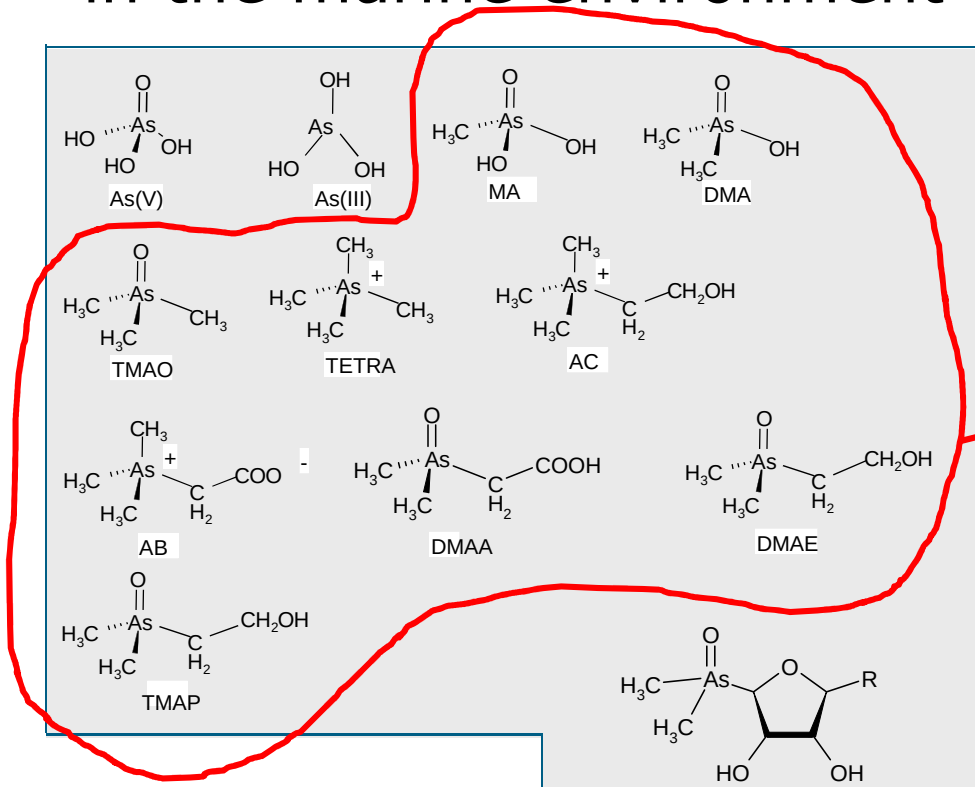


Arsenic acid
As(V)



Arsenic species

in the marine environment



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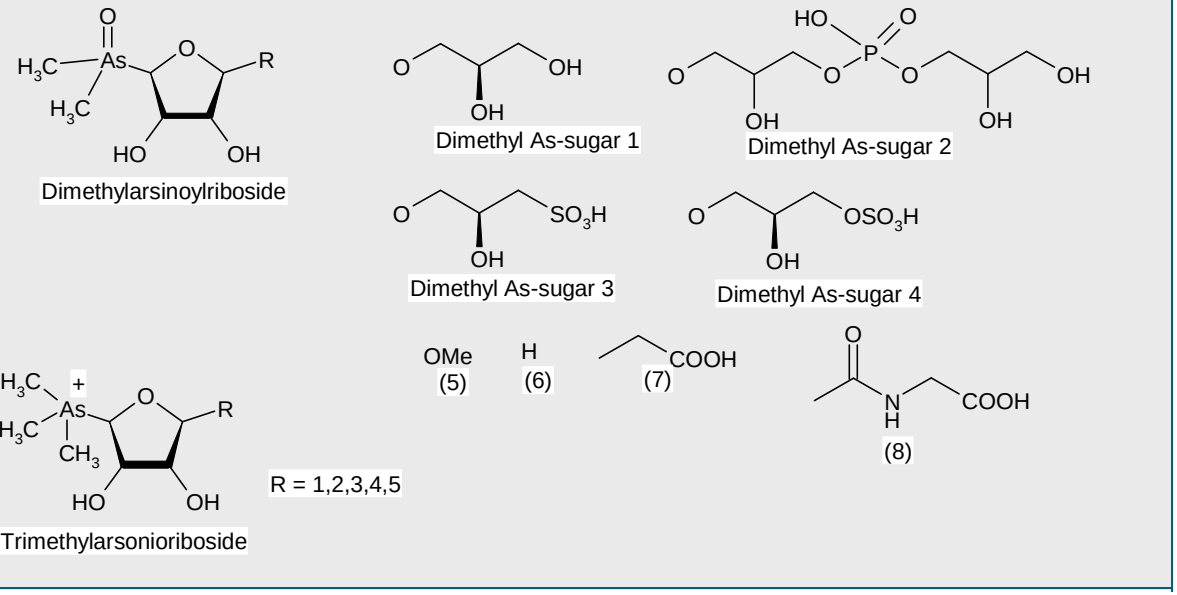
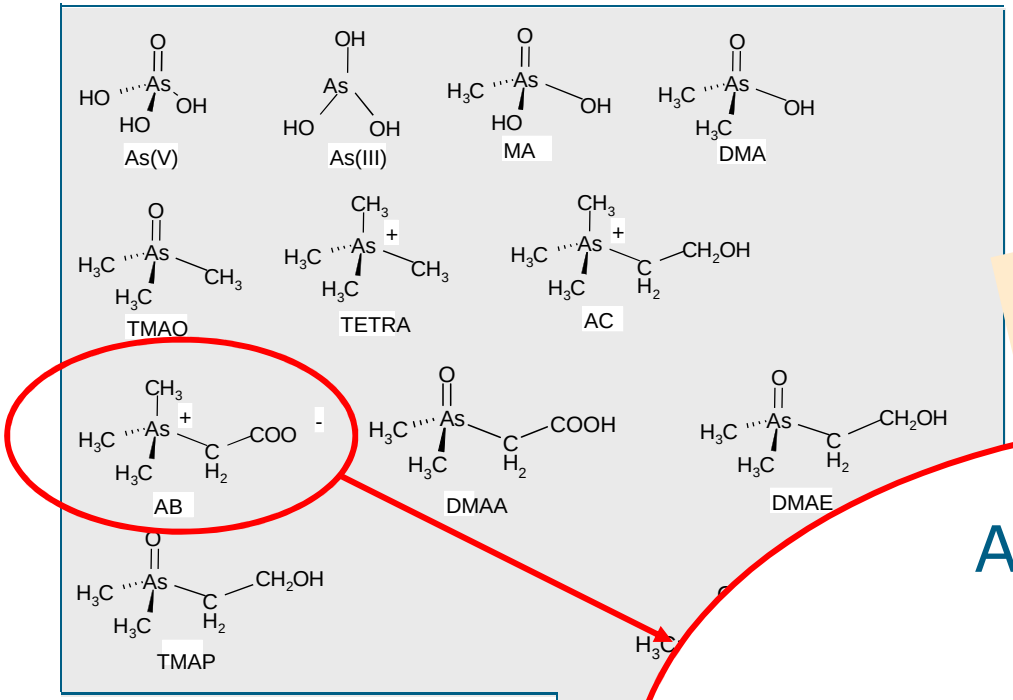


Arsenic species

in the marine environment



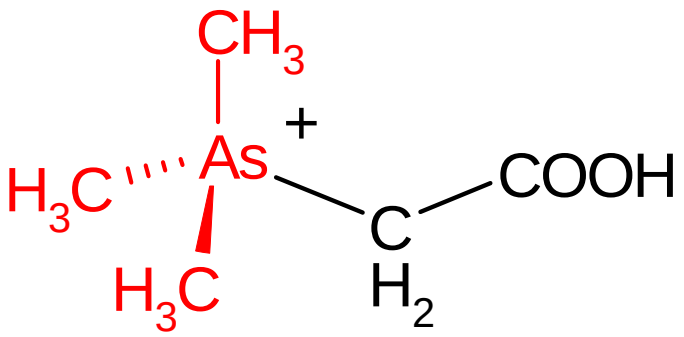
More than **50** different **arsenic species**:

- **in**[illegible]**nic**
- **c**
- [illegible]pecies
- [illegible]ars

Predominant form of arsenic in most seafood

Arsenobetaine

AB



Non toxic!!

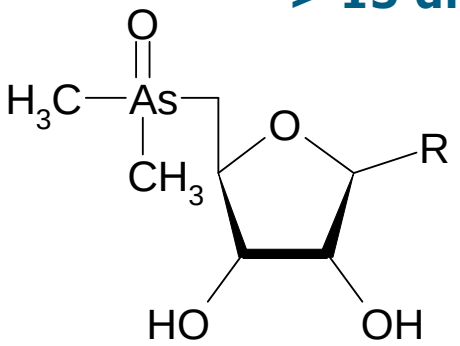
Arsenic species

in the marine environment

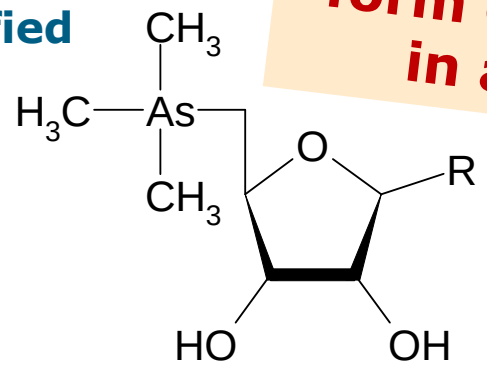
Arsenosugars

> 15 different identified

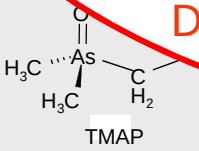
Predominant form of arsenic in algae!!



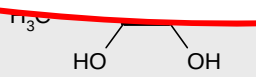
Dimethylarsinoylribosides



Trimethylarsonioribosides



TMAP



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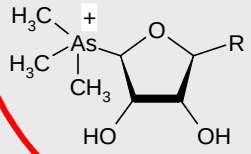
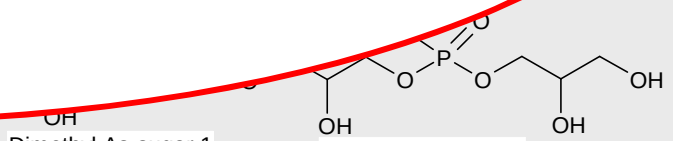


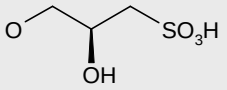
Trimethylarsonioriboside

R = 1,2,3,4,5

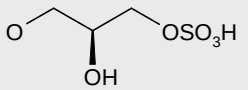


Dimethyl As-sugar 1

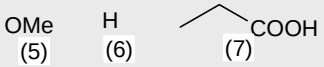
Dimethyl As-sugar 2

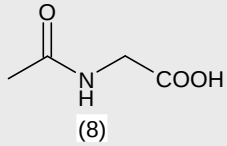


Dimethyl As-sugar 3



Dimethyl As-sugar 4



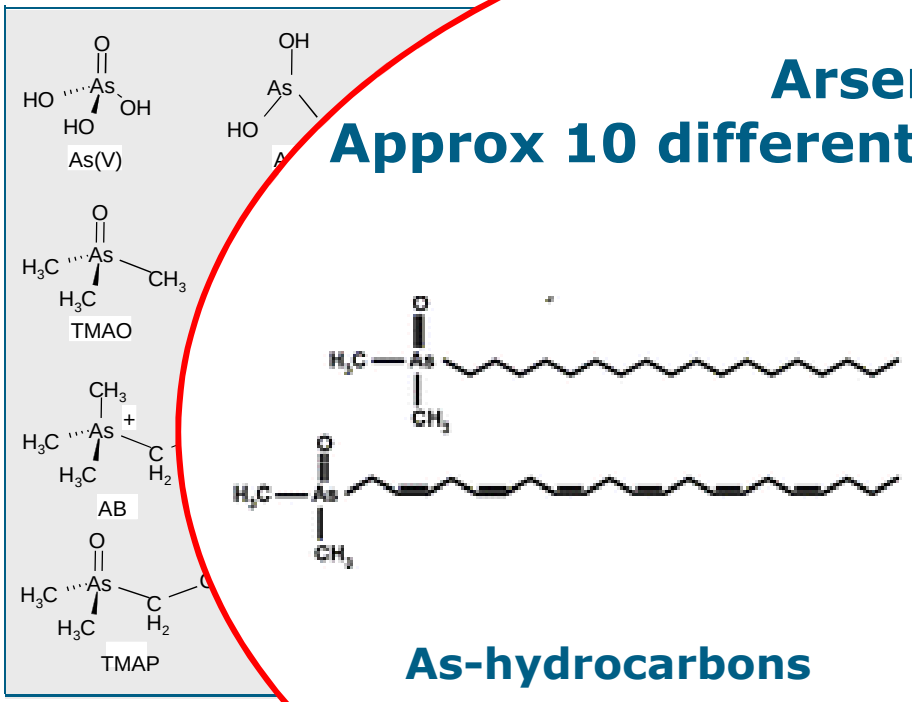


Arsenic species

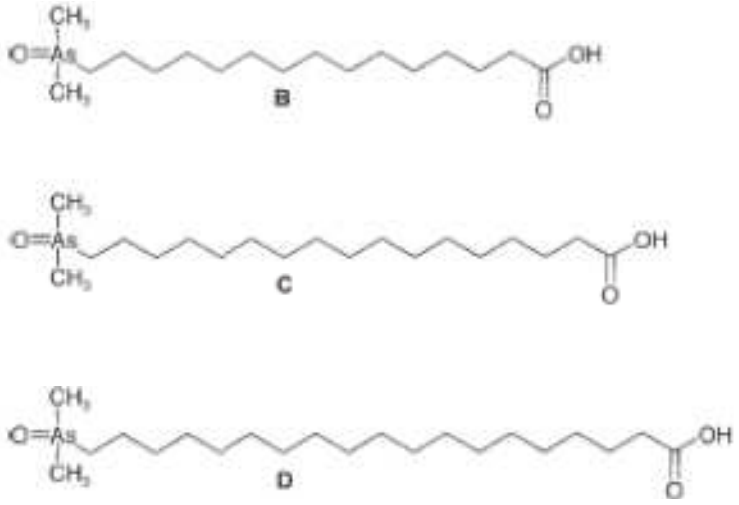
in the marine envir

Lipid soluble arsenic compounds

Arsenolipids
Approx 10 different compounds identified



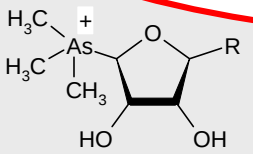
As-hydrocarbons



As-fatty acids

Present in fish oils and fatty fish

Arsenosugars and arsenolipids toxicity not available (EFSA, 2009)



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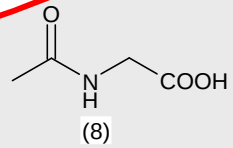


Laws, expert opinions

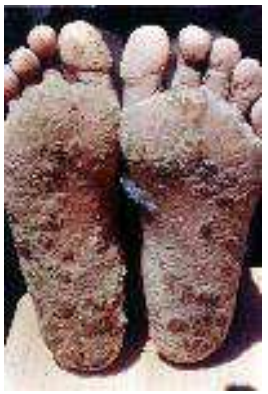
EFSA (2009), JECFA (2010)

Legislation, maximum levels (ML)

- Food — No EU legislation on arsenic
  AU and NZ →1-2 mg/kg iAs in certain seafood
- Feed — EU → 2-40 mg/kg total As
  EU → < 2 mg/kg iAs - demonstrated upon request

Experts

- inorganic As (iAs) is the most toxic form of As
- iAs causes skin, lung and bladder cancer + skin lesions:
- $BMDL_{01}$ = 0.3 – 8 µg/kg bw per day for iAs (EFSA)
- **Risk to some consumers cannot be excluded**
- Lack of specific data on iAs
- Lack of validated, standardised methods



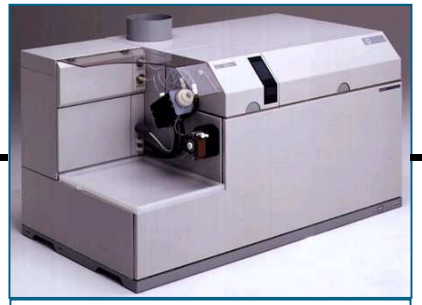
Keratosis

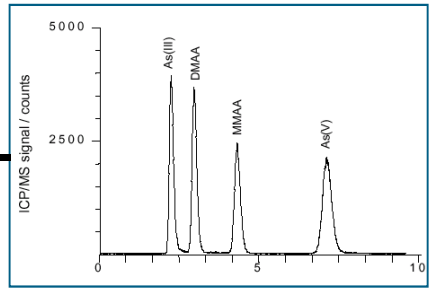
This presentation:

iAs analysis of marine feed and food samples

Arsenic speciation analysis

Workhorse: **HPLC-ICPMS**





HPLC

Sample introduction

Column

Separation

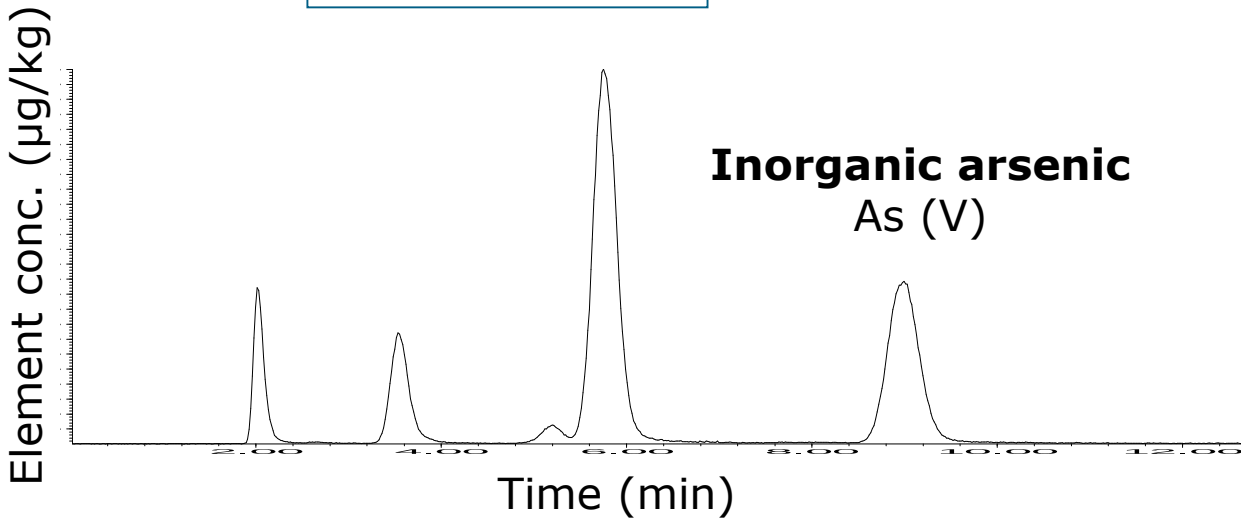
ICPMS

Element specific detection

Result

Chromatogram

Output:
Arsenic specific chromatogram



Arsenic speciation analysis

Workhorse: **HPLC-ICPMS**

HPLC

Sample introduction

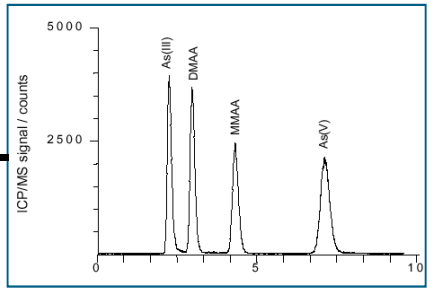
Column

Separation

ICPMS

☹

high costs

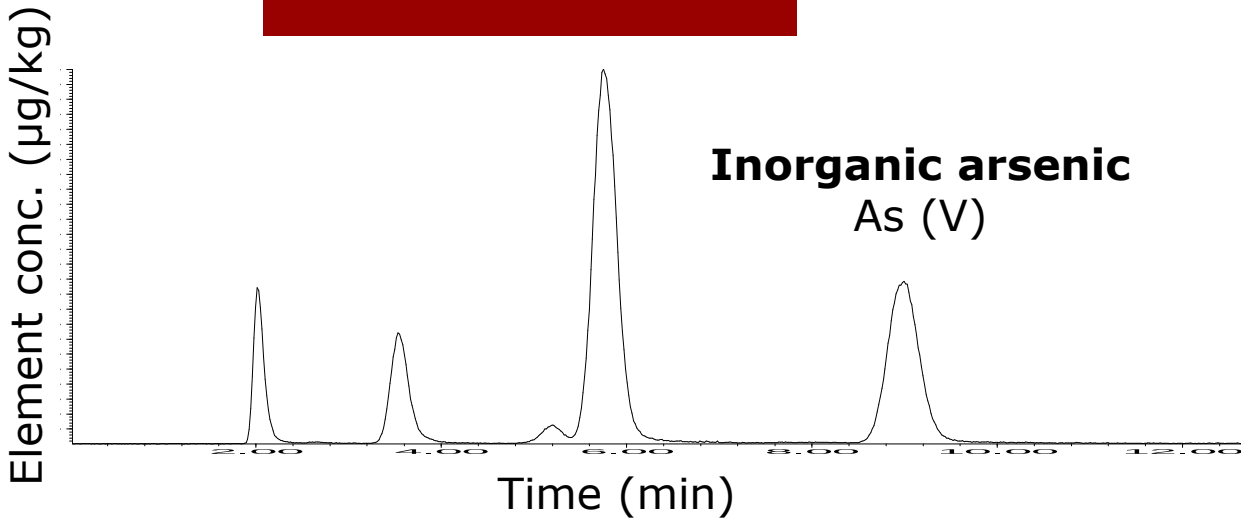


Result

Chromatogram

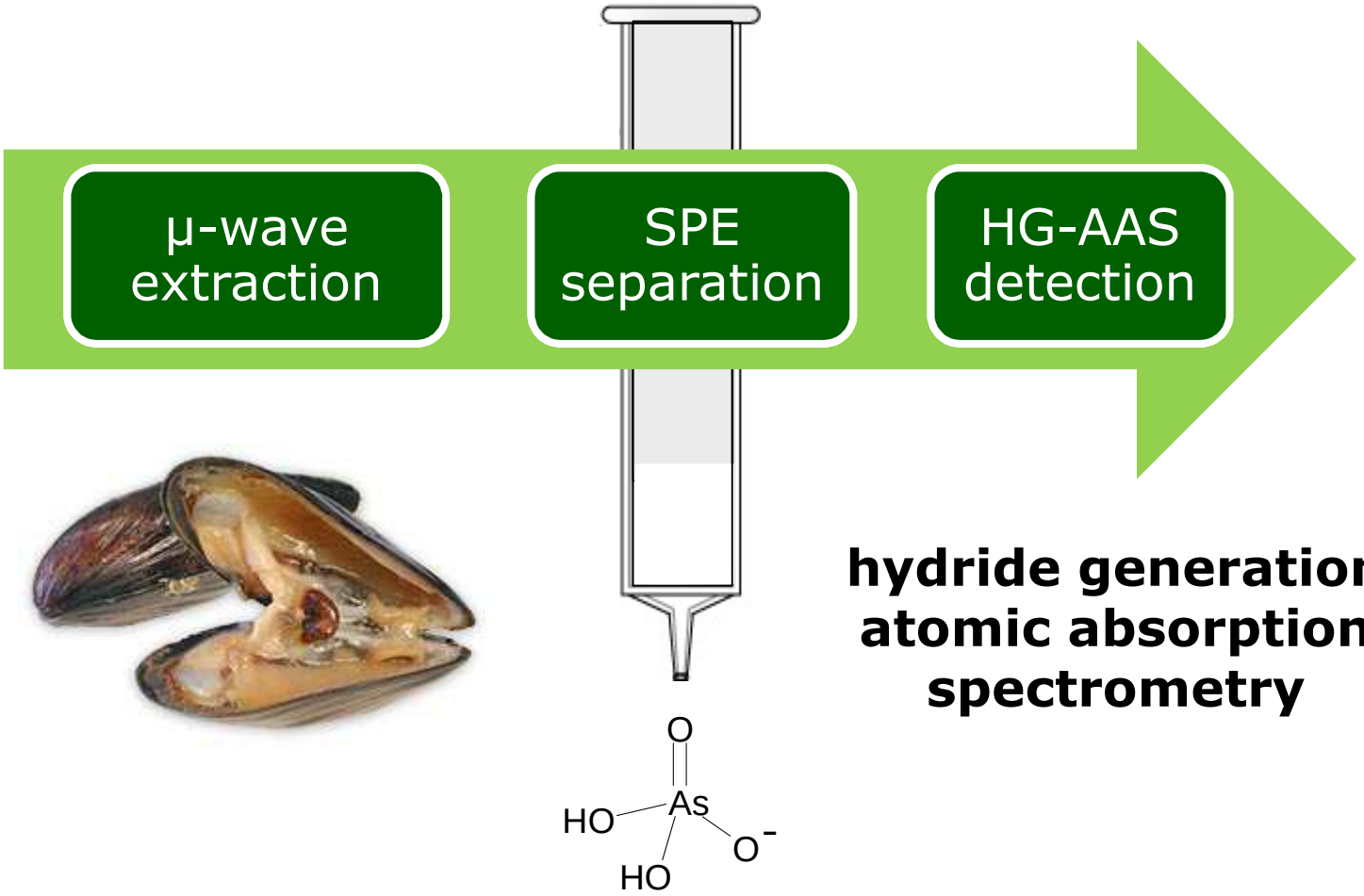
Output:

Arsenic specific chromatogram



Arsenic speciation analysis

speciation alternative: **SPE, HG-AAS**



hydride generation atomic absorption spectrometry

inorganic arsenic

Arsenic speciation analysis

speciation alternative: **SPE, HG-AAS**

low costs

- **Successfully in-house validated**
- **Tested in a collaborative trail**
  Method technically approved →
  CEN T327/WG4 final vote stage

µ-wave extraction

oxidation of As(III) to As(V)

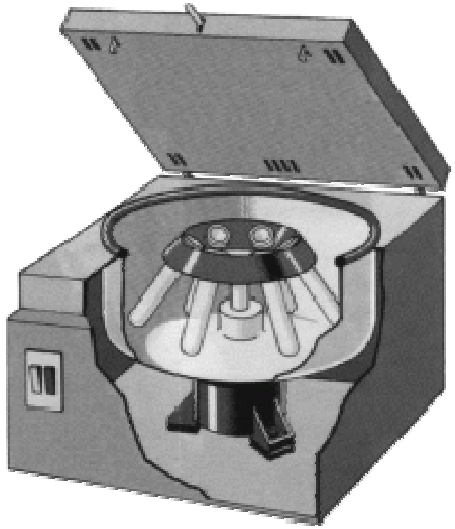
0.2 g sample
+ 10 mL extractant
(0.06 M HCl, 3% H_2O_2)

Glas vessel

25 minutes at 90°C

µ-wave oven

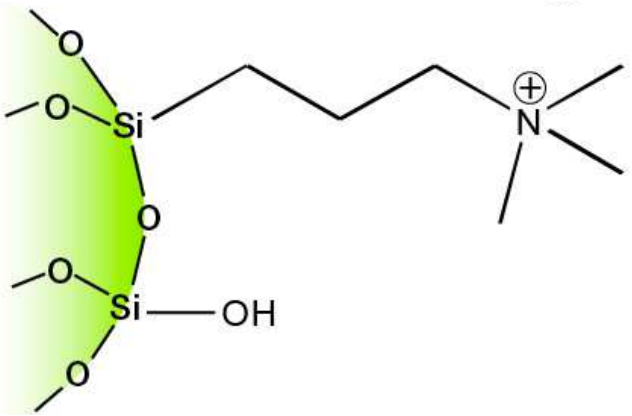
Centrifugation
10 min 2100 x *g*

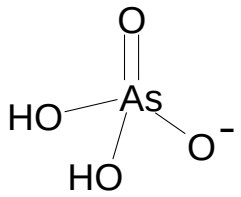


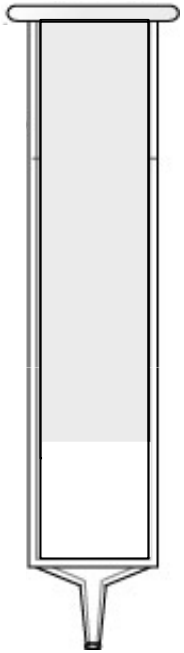
centrifuge

SPE protocol

Separation of As species







Strong anion exchange SPE column
silica based
Strata SAX
500 mg/6 mL, Phenomenex

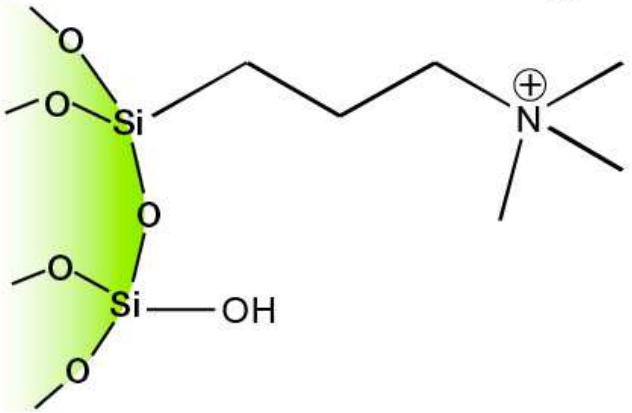
The **charge** of the arsenic species depends on pH

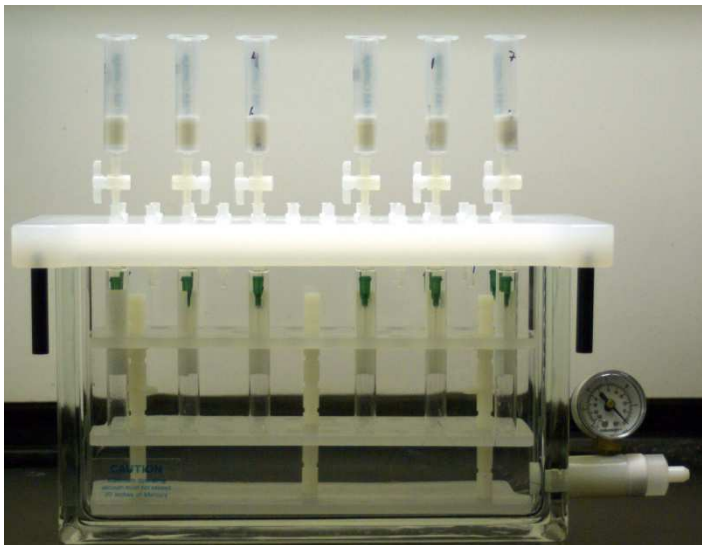
@ pH = 6 **iAs(V)** is **negatively charged**

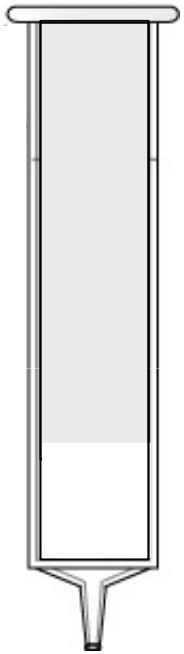
Sequential elution
Separation of inorganic As from organo As species by SPE

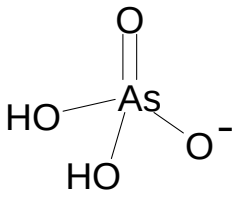
SPE protocol

Separation of As species









Condition
100 % MeOH

Equilibrate
Buffer: 20mM $(NH4)_2CO_3$, 0.03 M HCl and 1.5% H_2O_2

Load
Buffered sample: pH 5.0-7.5

Wash 0.5 M CH_3COOH

Elute 0.5 M HCl

Illustrations: Crawfordscientific, Phenomenex and Jeff Dahl.

Arsenic in SPE eluates

load, wash and sample fraction

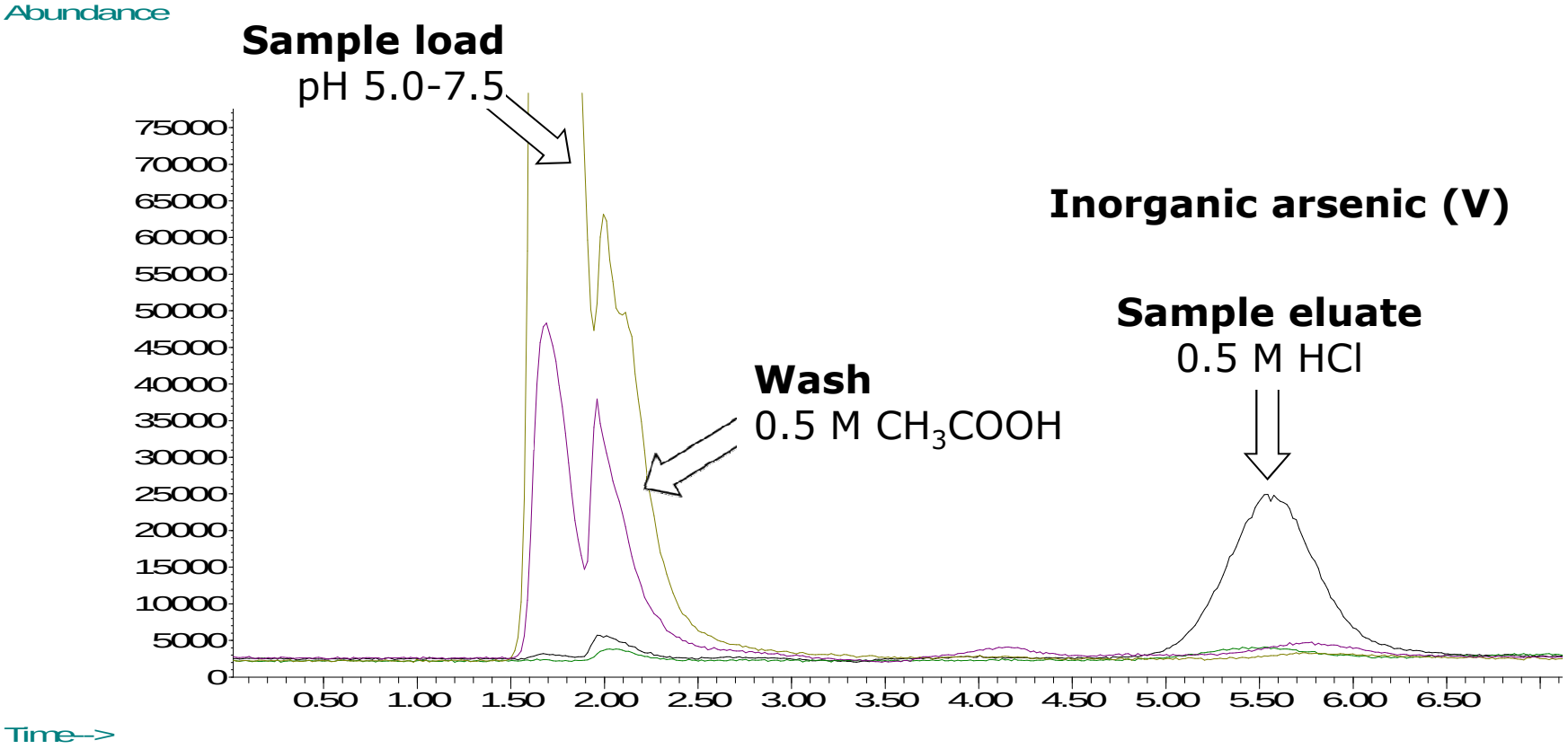


Figure. HPLC-ICP-MS chromatogram of fish protein (TORT-2) 3 SPE fractions separated on an anion exchange column (ION-120 part nr. ANX-00-6550, 120x4.6 mm), 40 mM carbonate pH 10.3.

HG-AAS detection

arsenic in sample eluate

Hydride generation atomic absorption spectrometry

Thermo Scientific

HG-AAS
total arsenic
in eluate

Gaseous arsenic hydrides is transported by argon gas to the cell

→ Atomisation reaction
→ Atomic absorption of arsenic

HG-AAS detection

arsenic in sample eluate

Pre-reduction: As(V) → As(III)

• Mix sample eluate with KI and ascorbic acid, 3 M HCl
60 min incubation

• Add more 3 M HCl
Another 60 min incubation

Hydride generation reagents

HCl (4.7 M)
$NaBH_4$ / NaOH (0.5 % w/v)

Instrument settings:

Electrical heated cell (900° C)
Element specific lamp for As
Wave length (193.7 nm)
Slit width (0.5 nm)

Hydride generation atomic absorption spectrometry

Thermo Scientific

HG-AAS
total arsenic
in eluate

In-house validation

SPE, HG-AAS

Setup

Spiked samples → Trout, oyster, fish feed
Natural incurred samples → TORT-2, DORM-3
Analysed in triplicates on 3 different days
2 technicians

Method performance

	Spike low	Spike medium	Spike high	TORT-2	DORM-3
iAs level (mg/kg)	0.5	1	1.5	0.9*	0.2*
Observations (N)	9	9	9	6	6
Mean recovery (%)	101	103	104	100	90
Repeatability RSD_r (%)	4	8	5	3	7
Reproducibility RSD_{IR} (%)	5	9	6	9	13
Horwitz Rel. Std. (%)	18	16	15	16	20

**Reference value determined by HPLC-ICP-MS.*

In-house validation

SPE, HG-AAS

0.08 mg/kg limit of detection (LOD)
3-8% repeatability
5-13% reproducibility
90-104% recovery

Method performance

	Spike low	Spike medium	Spike high	TORT-2	DORM-3
iAs level (mg/kg)	0.5	1	1.5	0.9*	0.2*
Observations (N)	9	9	9	6	6
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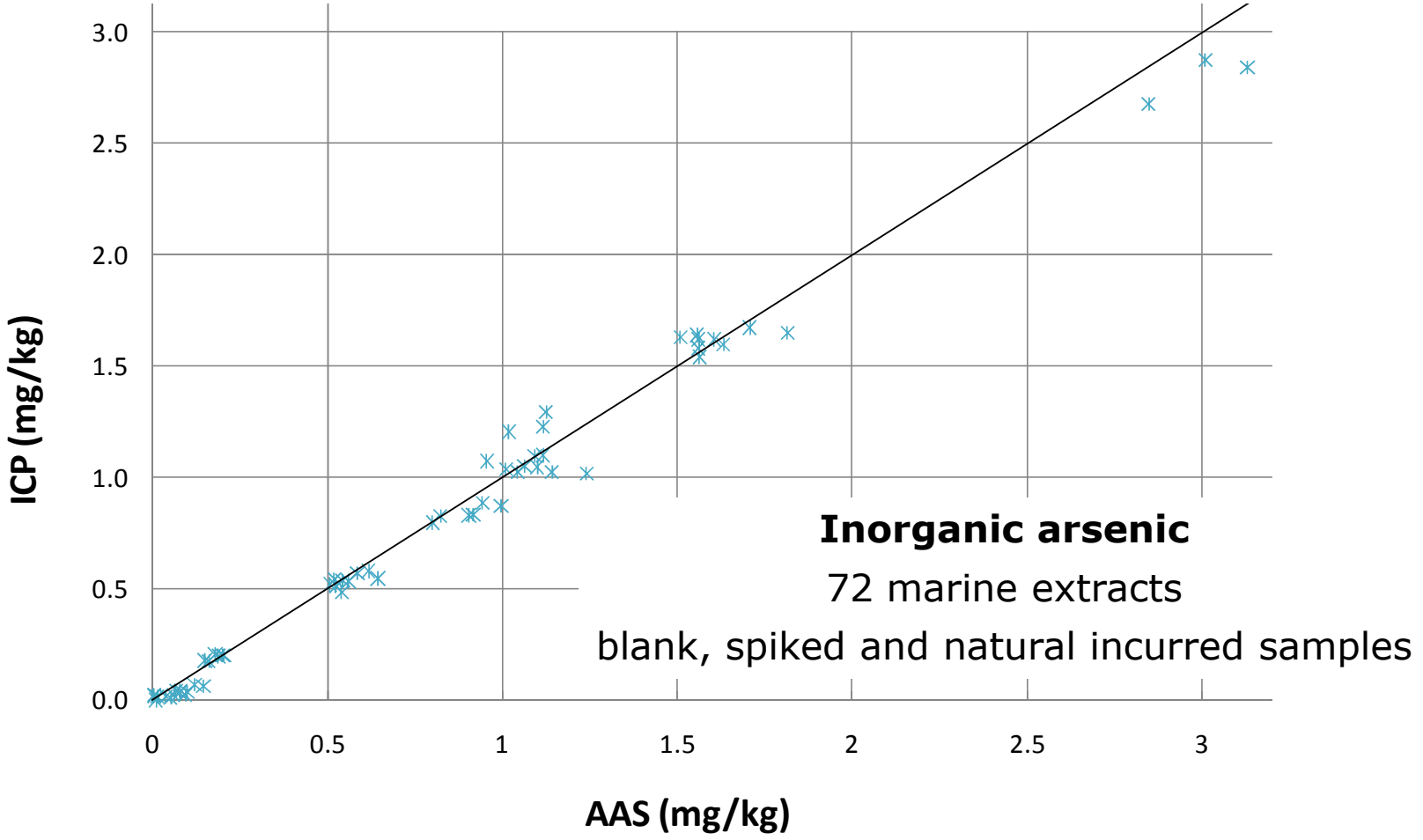
**Reference value determined by HPLC-ICP-MS.*

Methodology

SPE, HG-AAS **versus** HPLC-ICP-MS



The detection methods were not significantly different (*t* Test, 95% confidence)

Conclusion

- Marine organisms can bioaccumulate arsenic (As)
- As toxicity is species specific, iAs is most toxic
- iAs causes cancer & skin lesion
- As speciation analysis and monitoring data needed
- Low cost iAs method developed for marine feed and food samples:

SPE, HG-AAS

In-house validated &

tested in a collaborative trail, successfully.

Open Day CONffIDENCE

CONffIDENCE: Safer food through rapid and cost-efficient tests for chemical contaminants in the food chain

Open Day at RAFA 2011

3 November 2011

Stella Hall: 13:00 – 16:00

Posters (23)

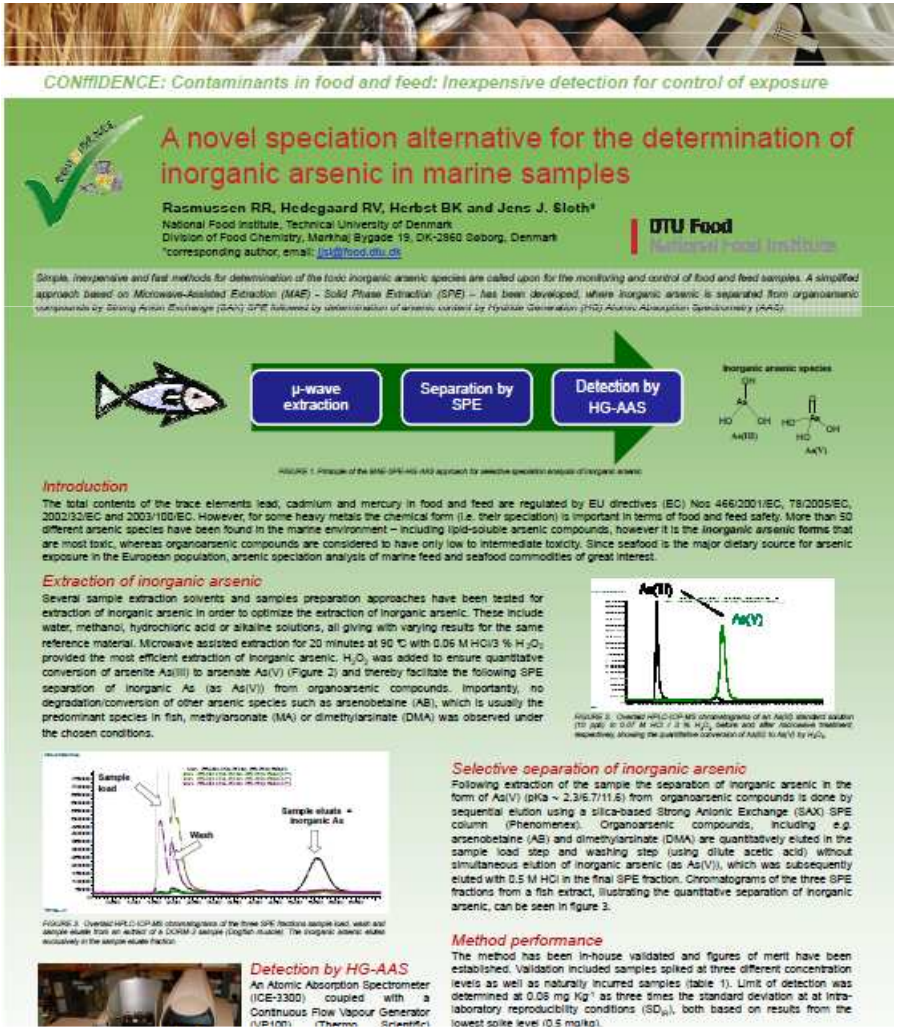
Demonstrations (8)

RIKILT WAGENINGEN UR

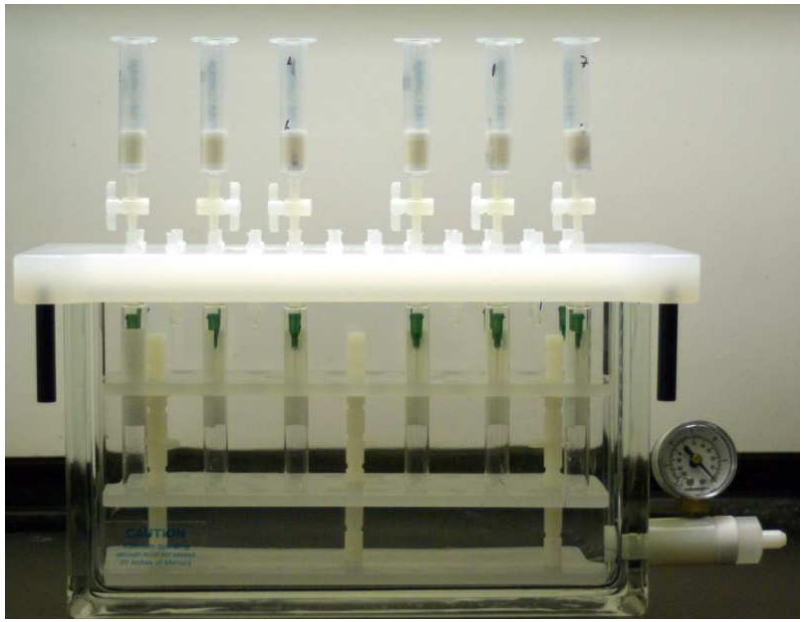
Metal speciation

posters and demonstration

Inorganic arsenic



Demo: Small tricks



Metal speciation

posters and demonstration

Inorganic arsenic

CONFIDENCE: 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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*corresponding author, email: jjs@food.dtu.dk

DTU Food
National Food Institute

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).

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.

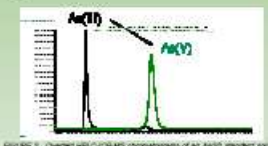

FIGURE 2. Overlaid HPLC-ICP-MS chromatograms of an As(III) standard solution (10 µg/l in 0.07 M HCl / 3 % H_2O_2 before and after microwave treatment) respectively, showing the quantitative conversion of As(III) to As(V) by H_2O_2.

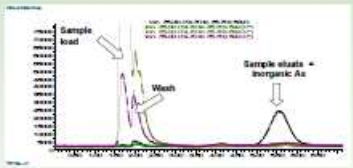

FIGURE 3. Overlaid HPLC-ICP-MS chromatograms of the three SPE fractions sample load, wash and sample eluate from an extract of a DORM-2 sample (Dogfish muscle). The inorganic arsenic elutes exclusively in the sample eluate fraction.

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 0.5 M 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 0.05 mg Kg^{-1} as three times the standard deviation at at intra-laboratory reproducibility conditions (SD_{IP}), both based on results from the lowest spike level (0.5 mg/kg).

Detection by HG-AAS

An Atomic Absorption Spectrometer (ICE-3300) coupled with a Continuous Flow Vapour Generator (VP100) (Thermo Scientific)

Methylmercury

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

Mercury speciation analysis in marine samples by HPLC-ICPMS

DTU Food
National Food Institute

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A simple method for determination of mercury species in marine origin has been developed and in-house validated. It is based on acid extraction by sonification, HPLC separation of inorganic mercury and methylmercury by cation-exchange column using isocratic elution and detection by ICPMS.

Introduction

Mercury (Hg) exists as elemental mercury (metallic), inorganic mercury and organic mercury (primarily methylmercury). Methylmercury is probably by far the most toxic form of mercury in food. It is highly toxic, particularly to the nervous system, and the developing brain is thought to be the most sensitive target organ for methylmercury toxicity. It bioaccumulates and biomagnifies along the food chain and is the most common mercury species in fish and seafood. Human exposure to methylmercury is mainly from fish and other seafood consumption. Inorganic mercury is probably the predominant form of mercury in foods other than fish and seafood. The methylmercury species in feed and food is currently not regulated by the European Union. Only for total mercury maximum levels have been laid down. There is a current need for fully standardized methods for determination of methylmercury.

Extraction of methylmercury

The applied method was inspired by Valient et al (2007). Samples were extracted twice with 5 M hydrochloric acid by sonication. Hereby the protein-bound mercury species are released. Before re-extraction the samples were centrifuged (10 min at 3170 x g) and the supernatant decanted. The combined sample extracts were added 10 M sodium hydroxide to increase pH, diluted in the mobile phase and filtered.

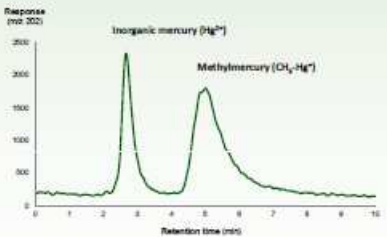
Detection of methylmercury

Analysis of methylmercury were performed using HPLC-ICPMS equipped with a MicroMist nebuliser. Typical plasma conditions were 1500 W RF power, 15 l/min plasma, 0.97 l/min carrier and 0.17 l/min makeup gas. Analysis was performed in the time resolved analysis mode monitoring the ^{202}Hg, ^{199}Hg, ^{35}Cl (m/z) with 1 s (Hg) and 0.01 s (Cl) integration time per data point.

Separation of mercury species

Since the ICP-MS detector is not selective towards specific mercury species, the HPLC separation is crucial for selective determination. Separation of inorganic mercury and methylmercury was obtained on a polymer-based cation-exchange column (Hamilton PRP-X200,150 x 2.1 mm Id, 10 µm) using isocratic elution (0.2 ml/min at 40° C). The mobile fase (pH- 3) consisted of L-cysteine (0.5% w/w), pyridine (50 mmol/L), methanol (5% v/w) and formic acid (0.8% v/w) dissolved in water. Separation of the inorganic mercury and methyl-mercury can be seen in Figure 1.

Methylmercury was quantified using peak height (m/z 202) and external calibration standards (0-10 µg/l) which were run before and after the samples.



Method performance

The method has been in-house validated using three different certified reference materials (DORM-2, TORT-2 and DORM-3) and four other fish and feed samples of marine origin (Table 1). All samples were analysed in triplicate and repeated on three different days. Limit of detection (LOD) and quantification (LOQ) were calculated as three and six times the standard deviation at intra laboratory reproducibility conditions (SD_{IP}) divided by average recovery for certified reference material (R_{CRM}), respectively. LOD was 0.027 mg/kg and LOQ 0.054 mg/kg.

The in-house reproducibility standard deviations (RSD_{IP}) were less than ≤12% for samples containing 0.15 to 0.47 mg/kg methylmercury and less than ≤ 20% for samples with 0.06 mg/kg.

Table 1. Performance of the HPLC-ICPMS method for determination of methylmercury.

	DORM-2	TORT-2	DORM-3	Fish feed spiked	Codfish	Salmon	Fish feed
Ref. level (mg/kg)	4.47	0.15	0.38	0.21	0.17	0.06	0.06
Observations (N)	9	18	9	9	9	9	9
Mean recovery (%)	94	102	96	-	-	-	-
Repeatability RSD_r (%)	3	4	3	11	5	13	13
Reproducibility RSD_R (%)	8	12	8	11	12	20	15

Fish feed

The method was applied to fish feed in use in Denmark. In total 23 samples (4 complete fish feed products, 5 fish silage samples and 14 fish meal samples) were collected by the Danish Plant Directorate. All samples were kept at -18°C. Samples (except fish silage) were homogenised before analysis. Certified reference material was used for quality control. The results can be seen in Table 2. For all samples the concentrations were below the EU maximum levels for total mercury in fish feed at 0.2 mg/kg and marine feed materials at 0.5 mg/kg (EU dir 2010/6/EC).

Table 2. Analysis of feed samples.

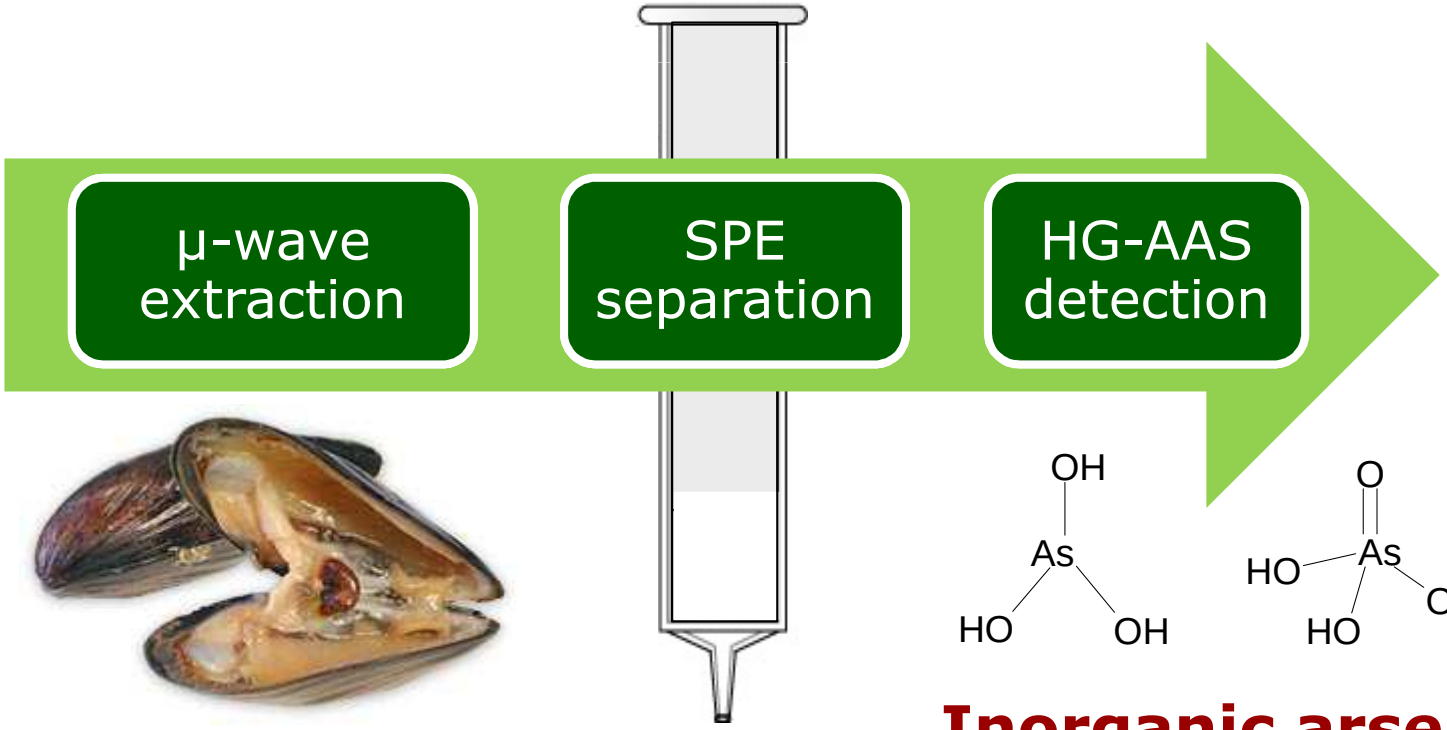
Feed sample type	CH_3-Hg^+ (µg/kg)	Feed sample type	CH_3-Hg^+ (µg/kg)
Fish Silage	<0.027	Fish meal	<0.027
	<0.027		<0.027
	<0.027		<0.027
	<0.027		<0.027
	<0.027		<0.027
Complete feed	<0.027		<0.027
	<0.027		0.030
	<0.027		0.032
	0.032		0.045

Thank you for your attention!

and funding from...

European Community's Seventh Framework Programme

European Committee for standardisation (CEN)



Inorganic arsenic