Sample preparation for food chemistry analysis

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Sample preparation for food chemistry analysis

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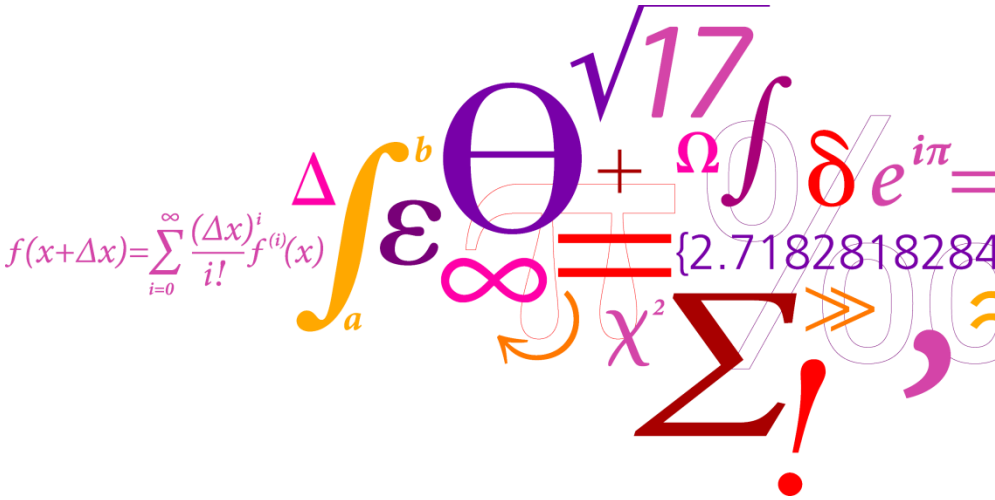


DTU Food
National Food Institute

National Food Institute

Focus on public health:

- human nutrition - food safety - food technology - environment and health -

Food products throughout the entire food chain:

- primary agricultural production
- industrial and home processing
- evaluating the impact on human health

Scientifically based risk assessments and advices

authorities and industry

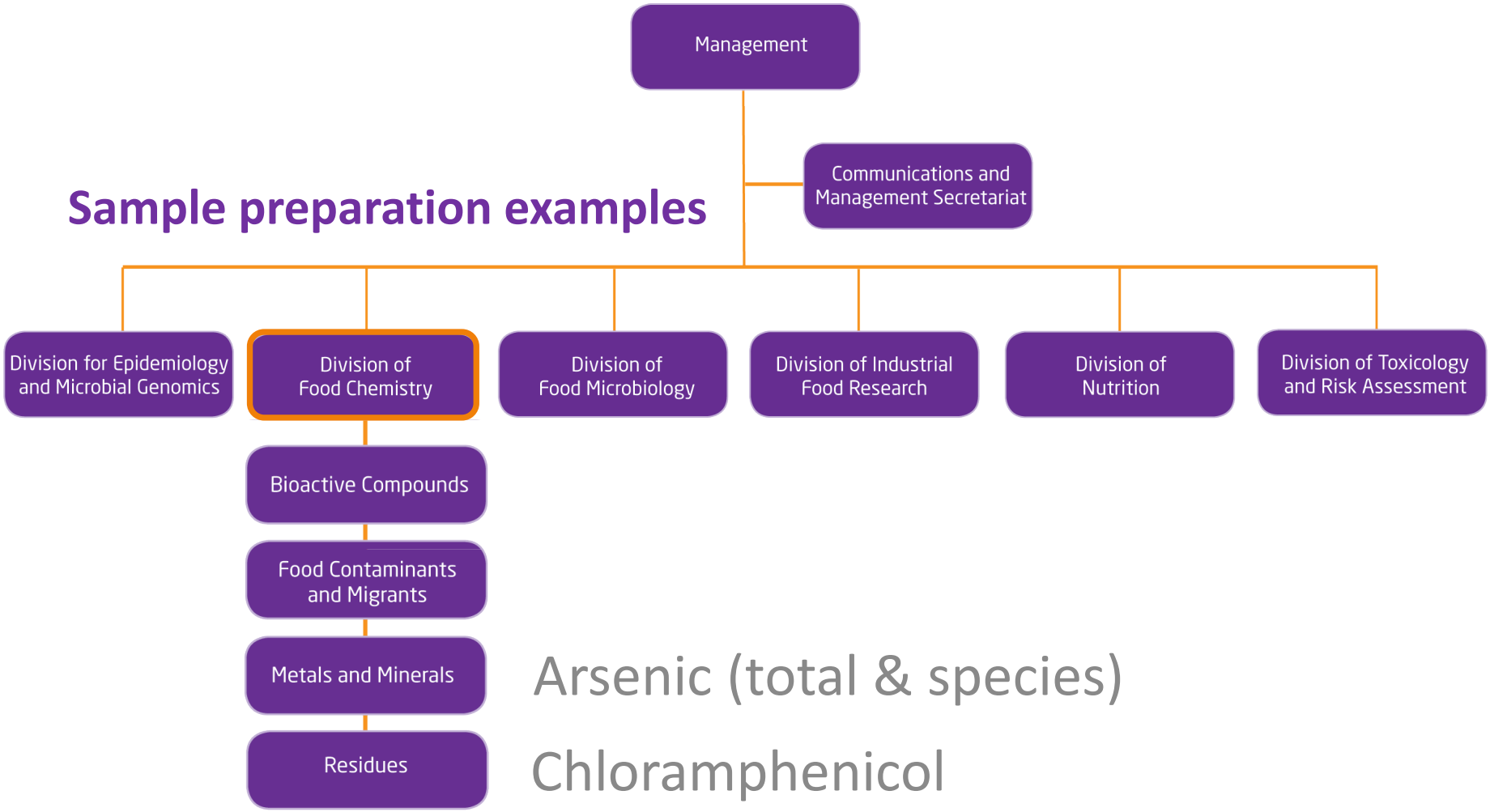
International and national reference laboratory

chemical and microbial food safety

Part of the national food safety contingency plan

National Food Institute

Approx. 400 employees in six divisions:

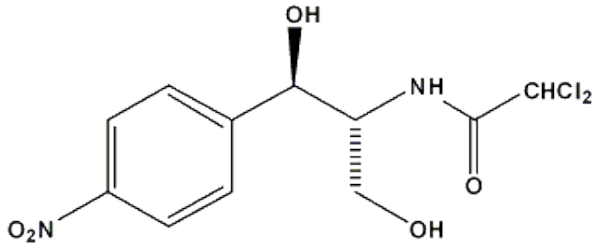


Sample preparation examples

Arsenic (total & species)

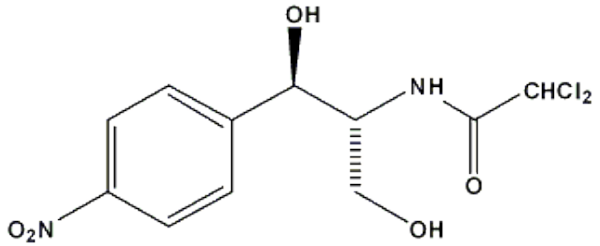
Chloramphenicol

Chloramphenicol



- Broad spectrum antibiotic
- Pharmaceutical prescribed for e.g., eye inflammation
- Severely toxic to bone marrow and the blood stem cells
- Banned in agricultural production in EU
- Analytical challenges; Chlorampenicol in honey
  - High sugar content
  - EU regulated at a low level (0.3 ng/g MRPL)
  - Verification method
    - GC-MS (4 ions required)
    - LC-MS-MS (1 precursor and 2 daughter ions required)

Chloramphenicol in honey



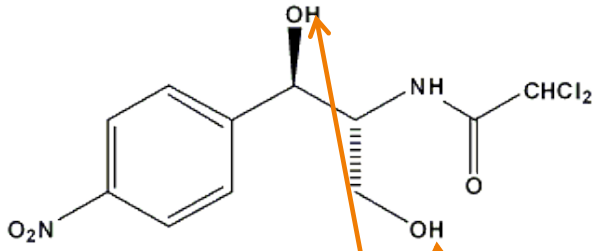
Sample preparation for GC-MS

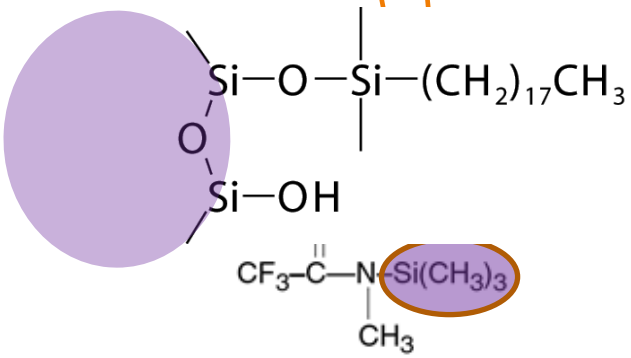
- Dissolve 1.5 g honey in 3 ml water
- Add internal standard (deuterium-labeled chloramphenicol)
- Load onto Extrelut column® (Merck)
- Elute with ethyl acetate
- Evaporate the ethyl acetate phase to dryness

Chloramphenicol in honey

Sample preparation for GC-MS

- Dissolve 1.5 g honey in 3 ml water
- Add internal standard (deuterium-labeled chloramphenicol)
- Load onto Extrelut column® (Merck)
- Elute with ethyl acetate
- Evaporate the ethyl acetate phase to dryness
- Re-dissolve with 0.3 ml methanol and 10 ml water
- Load onto a C18 cartridge
  (conditioned with methanol and water)
- Wash
  - water
  - water/methanol (90/10)
- Elute: 5 ml methanol/water (55/45)
- Evaporate the eluate to dryness
- Derivate with MSTFA (1 hour at 60 °C)
- Evaporate to dryness and re-dissolve in 50 µl heptane





MSTFA
replaces labile H with
-Si(CH$_3$)$_3$

Chloramphenicol in honey

GC-MS

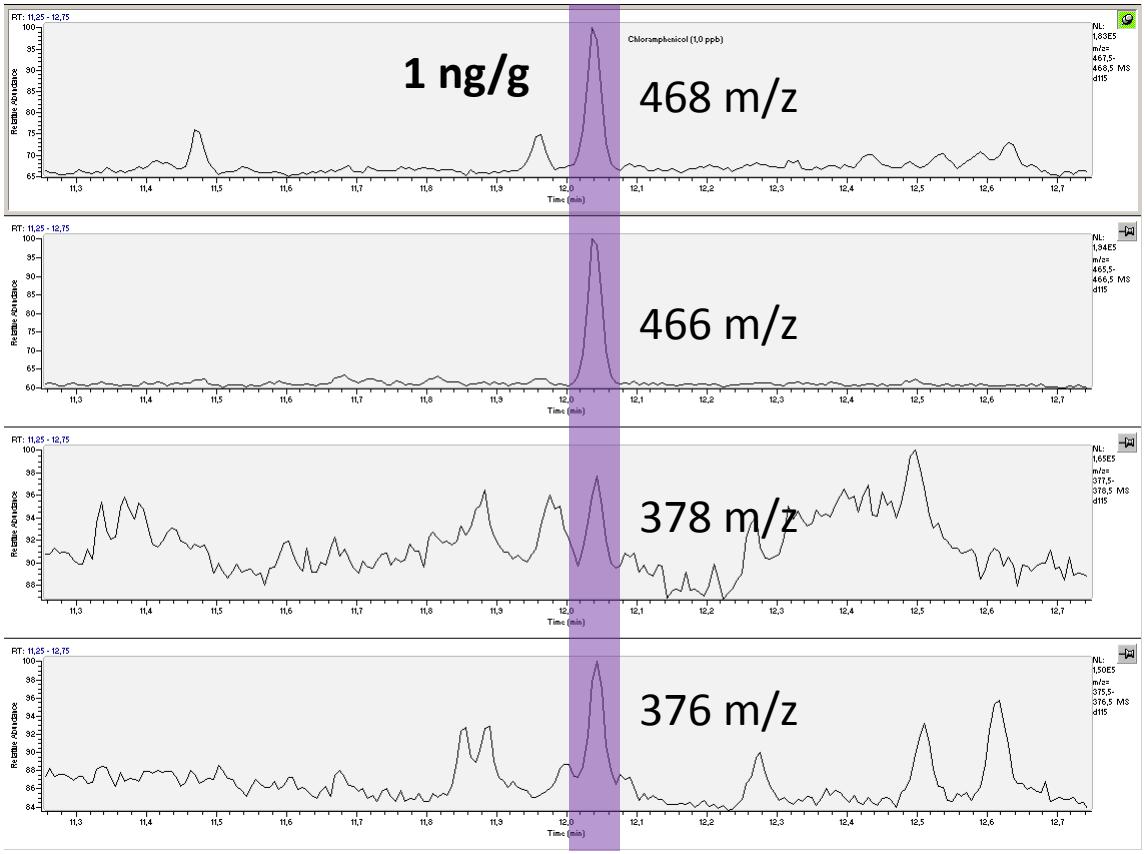
Temperature ramped separation on a 5% diphenyl column, negative chemical ionisation

Required identification

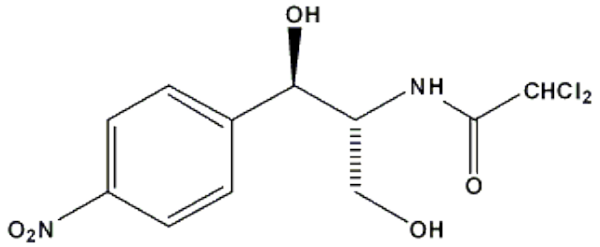
RT & 2 ion-ratios

not obtained

→ LC-MS/MS



Chloramphenicol in honey



Sample preparation for LC-MS/MS

- Dissolve 1.0 g honey in 1 ml water
- Add internal standard (deuterium-labeled chloramphenicol)
- Load onto a molecular imprinted polymers (MIP) cartridge (conditioned first with methanol and then with water)

- **Extensive wash**
  - 5% acetonitrile in 0.5% acetic acid
  - 1% ammonia
  - 20% acetonitrile in 1% ammonia
  - Water
  - 5% acetonitrile in 0.5% acetic acid
- Dry column with vacuum
- Elute with methanol
- Evaporate the eluate to dryness
- Re-dissolve in 500 µl 10% methanol

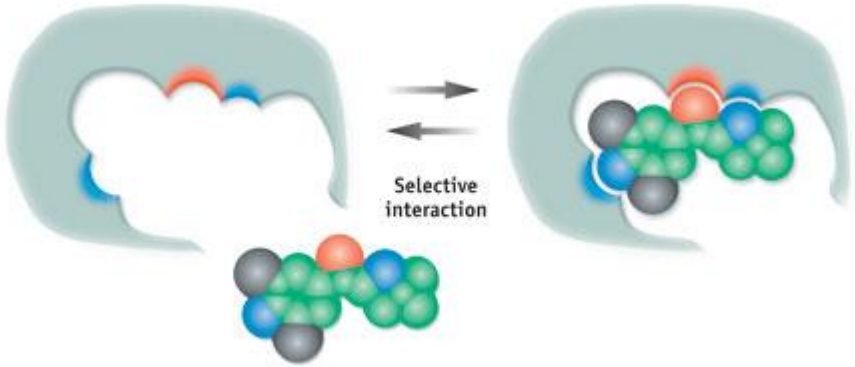


Illustration: www.sigmaaldrich.com

Chloramphenicol in honey

LC-MS/MS

Kolonne: C-18 Inertsil ODS2 (2 x 150 mm, 3 µm)
Gradient elution:
A: 10 % methanol / 90 % 0.001 M acetic acid
B: 100 % methanol

1 precursor and 2 daughter ions:

320.90 > 152.15 primary
320.90 > 257.15 secondary
325.93 > 157.18 internal standard

Detection capability = 10 times less than the required 0.3 ng/g

Arsenic in seafood

Arsenic is a metalloid present in soils, groundwater, surface water, air and **foods**

hydrogen 1 H 1.0079																	helium 2 He 4.0026
lithium 3 Li 6.941	beryllium 4 Be 9.0122											boron 5 B 10.811	carbon 6 C 12.011	nitrogen 7 N 14.007	oxygen 8 O 15.999	fluorine 9 F 18.998	neon 10 Ne 20.180
sodium 11 Na 22.990	magnesium 12 Mg 24.305											aluminium 13 Al 26.982	silicon 14 Si 28.086	phosphorus 15 P 30.974	sulfur 16 S 32.065	chlorine 17 Cl 35.453	argon 18 Ar 39.948
potassium 19 K 39.098	calcium 20 Ca 40.078		scandium 21 Sc 44.956	titanium 22 Ti 47.867	vanadium 23 V 50.942	chromium 24 Cr 51.996	manganese 25 Mn 54.938	iron 26 Fe 55.845	cobalt 27 Co 58.933	nickel 28 Ni 58.693	copper 29 Cu 63.546	zinc 30 Zn 65.39	gallium 31 Ga 69.723	germanium 32 Ge 72.61	arsenic 33 As 74.922	selenium 34 Se 78.96	bromine 35 Br 79.904	krypton 36 Kr 83.80
rubidium 37 Rb 85.468	strontium 38 Sr 87.62		yttrium 39 Y 88.906	zirconium 40 Zr 91.224	niobium 41 Nb 92.906	molybdenum 42 Mo 95.94	technetium 43 Tc [98]	ruthenium 44 Ru 101.07	rhodium 45 Rh 102.91	palladium 46 Pd 106.42	silver 47 Ag 107.87	cadmium 48 Cd 112.41	indium 49 In 114.82	tin 50 Sn 118.71	antimony 51 Sb 121.76	tellurium 52 Te 127.60	iodine 53 I 126.90	xenon 54 Xe 131.29
caesium 55 Cs 132.91	barium 56 Ba 137.33	57-70 *	lutetium 71 Lu 174.97	hafnium 72 Hf 178.49	tantalum 73 Ta 180.95	tungsten 74 W 183.84	rhenium 75 Re 186.21	osmium 76 Os 190.23	iridium 77 Ir 192.22	platinum 78 Pt 195.08	gold 79 Au 196.97	mercury 80 Hg 200.59	thallium 81 Tl 204.38	lead 82 Pb 207.2	bismuth 83 Bi 208.98	polonium 84 Po [209]	astatine 85 At [210]	radon 86 Rn [222]
francium 87 Fr [223]	radium 88 Ra [226]	89-102 **	lawrencium 103 Lr [262]	rutherfordium 104 Rf [261]	dubnium 105 Db [262]	seaborgium 106 Sg [266]	bohrium 107 Bh [264]	hassium 108 Hs [269]	meitnerium 109 Mt [268]	ununnilium 110 Uun [271]	unununium 111 Uuu [272]	ununbium 112 Uub [277]		ununquadium 114 Uuq [289]				

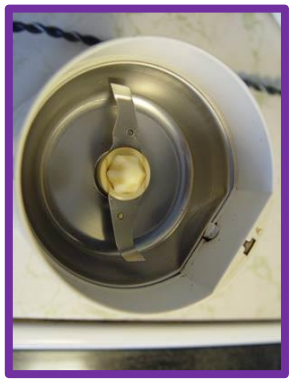
*Lanthanide series	lanthanum 57 La 138.91	cerium 58 Ce 140.12	praseodymium 59 Pr 140.91	neodymium 60 Nd 144.24	promethium 61 Pm [145]	samarium 62 Sm 150.36	europium 63 Eu 151.96	gadolinium 64 Gd 157.25	terbium 65 Tb 158.93	dysprosium 66 Dy 162.50	holmium 67 Ho 164.93	erbium 68 Er 167.26	thulium 69 Tm 168.93	ytterbium 70 Yb 173.04
**Actinide series	actinium 89 Ac [227]	thorium 90 Th 232.04	protactinium 91 Pa 231.04	uranium 92 U 238.03	neptunium 93 Np [237]	plutonium 94 Pu [244]	americium 95 Am [243]	curium 96 Cm [247]	berkelium 97 Bk [247]	californium 98 Cf [251]	einsteinium 99 Es [252]	fermium 100 Fm [257]	mendelevium 101 Md [258]	nobelium 102 No [259]

Total arsenic

- Sample homogenisation
  - Ultra turrax
  - Kitchen mill

Contamination:

Dust and steel contain some trace elements. Apply titanium knives and a LAF bench.



Total arsenic

- Sample homogenisation
- Microwave assisted extration
  - Strong acid (65 % v/v HNO_3)
  - High pressure and temperature

Only small samples size (organic material)

Glass can release arsenic.
Apply high quality quartz vessels.
Store samples and solutions in plastic containers

Total arsenic

Inductively **C**oupled **P**lasma **M**ass **S**pectrometry

→ Dilute conc. extract with water (to ~2 % v/v HNO_3)

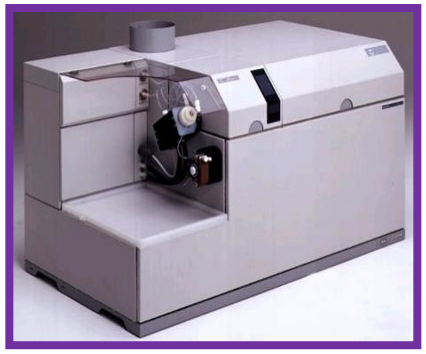
- Add internal standard mix (corrects for sensitivity drift)

☺ High sensitivity

☹ High purchase and running costs

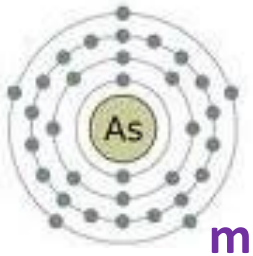
Inductively coupled (argon) plasma is 6000-8000 ^{o}K

- **Desolvation**
- **Vaporisation**
- **Atomisation**
- **Ionisation**



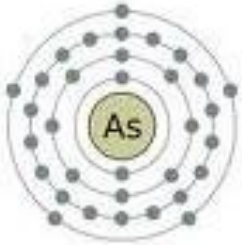
ICP-MS

Element specific detection



m/z 75

Total arsenic



Hydride generation atomic absorption spectrometry

☺ **Low purchase and running costs**

- Continuous Flow Vapor Generation System
- Atomic Absorption Spectrometer

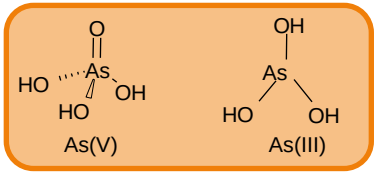
Instrument settings

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

Gaseous arsenic hydrides is transported by argon gas to the cell
→ Atomisation reaction
→ Atomic absorption of arsenic

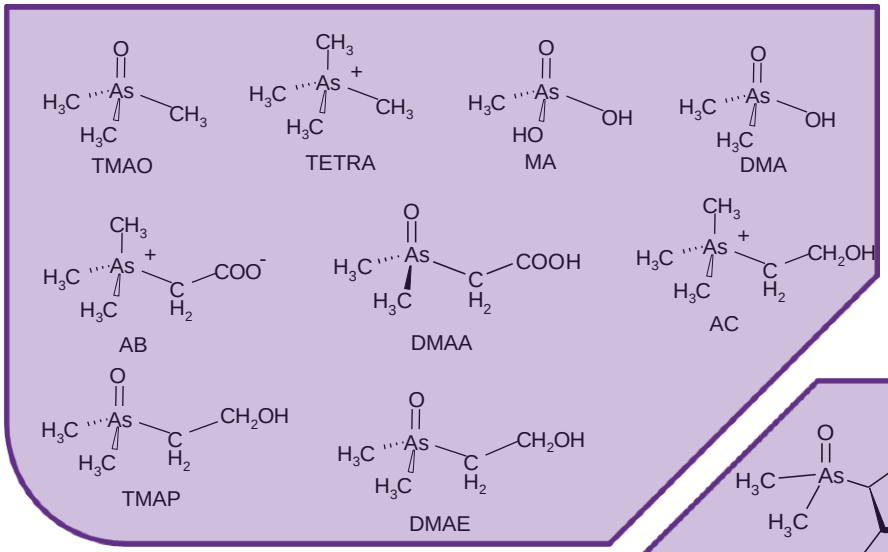
HG-AAS
total arsenic

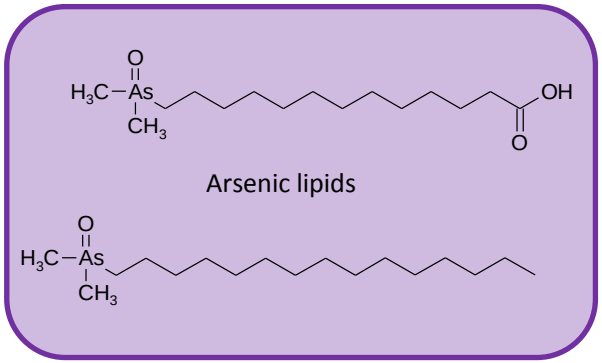
Arsenic species

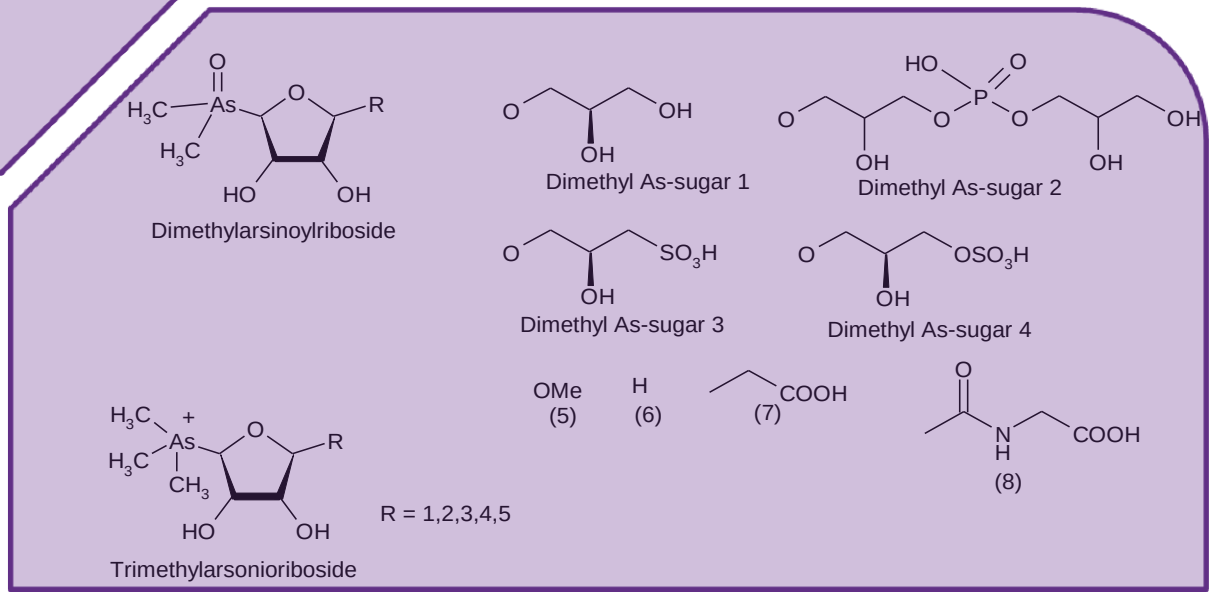


More than **50** different marine **arsenic forms**:

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







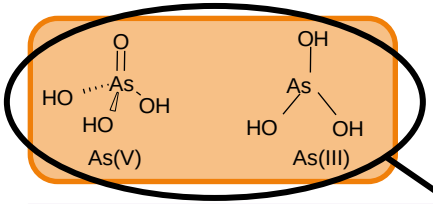
Arsenic species

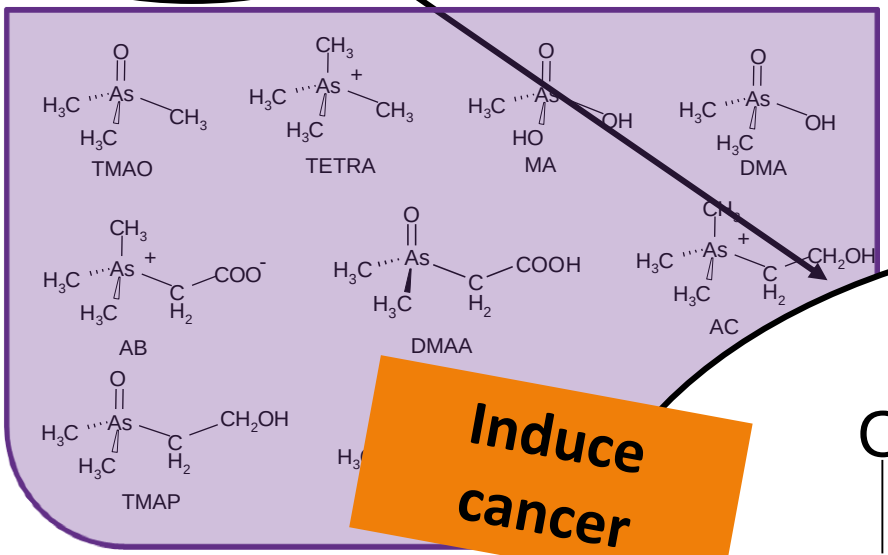
More than **50** different marine **arsenic forms**:

- inc
- or

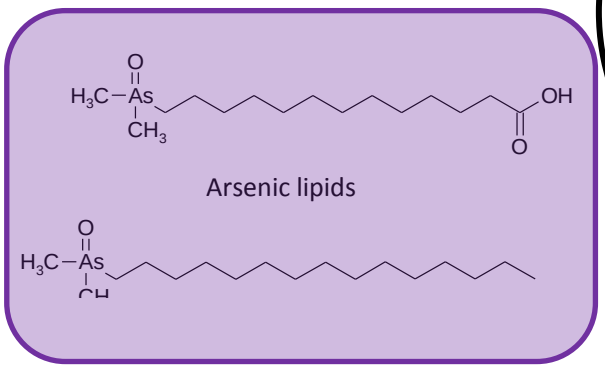
ecies

Most toxic form of arsenic!!

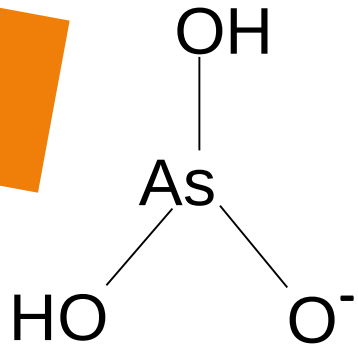




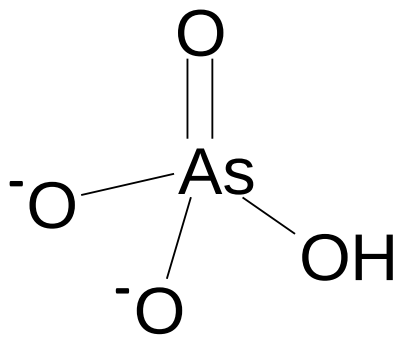
Induce cancer



Inorganic arsenic



Arsenous acid
As(III)



Arsenic acid
As(V)

Arsenic species

- Marine organisms can bioaccumulate arsenic (As)
- Major part of arsenic exists as organic arsenic in seafood
- As toxicity is species specific, inorganic arsenic (iAs) is most toxic
- iAs causes cancer & skin lesion
- Comprehensive As speciation data for food items missing
- Inorganic As and not total As in food should be determined for better risk assessment

- **Sample extraction aim**

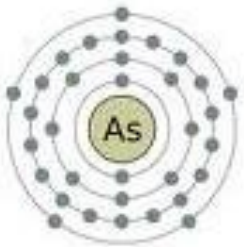
High extraction efficiency, no interconversions between inorganic As and organo As species

Arsenic species

HPLC-ICP-MS (anion exchange column)

☺ Species have different properties (pKa values)

☹ ICP-MS → high purchase and running costs



m/z 75

HPLC

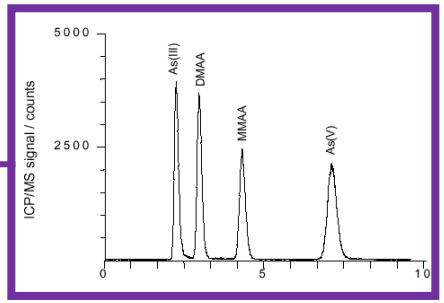
Sample introduction

Column

Separation

ICP-MS

Element specific detection



Result

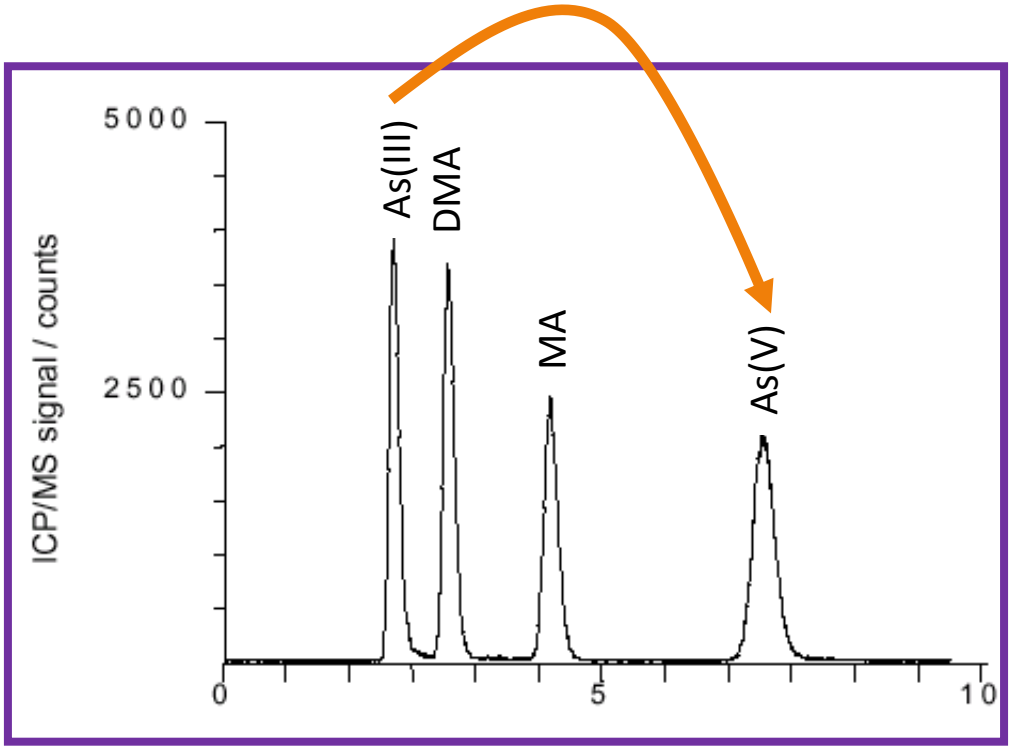
Chromatogram

Arsenic species

- **Sample preparation aim**

Isolate inorganic arsenic in one fraction

→ Measure arsenic with the cheap HG-AAS detector



HPLC (anion exchange) -ICP-MS

Trick

Convert As^{III} to As^{V} for higher anion properties by an oxidant

SPE separation

by anion exchange

Arsenic speciation analysis

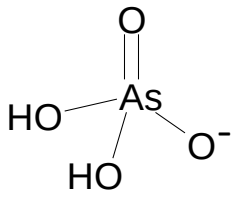
speciation alternative: SPE, HG-AAS

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

μ-wave extraction → SPE separation → HG-AAS detection

25 minutes at 90° C

Centrifugation
10 min 2100 x *g*



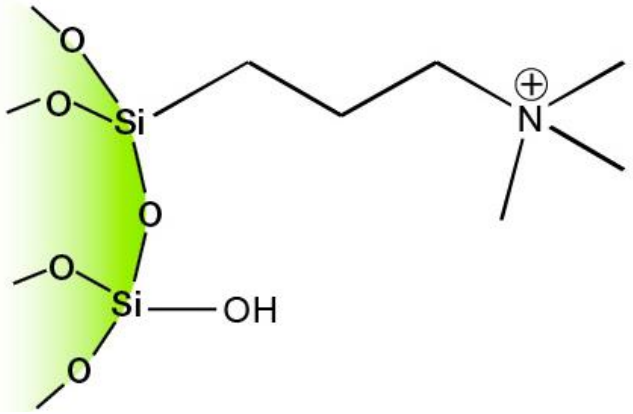
inorganic arsenic

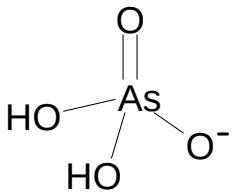
hydride generation atomic absorption spectrometry

☺ low costs

SPE protocol

Separation of As species





Strong anion exchange SPE column
silica based

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

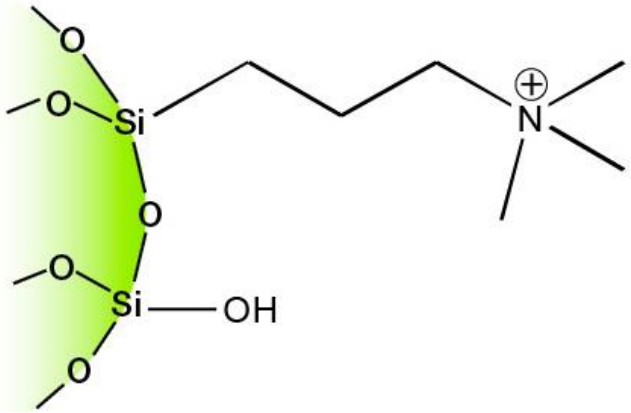
@ pH = 6 **iAs(V)** is **negatively charged**

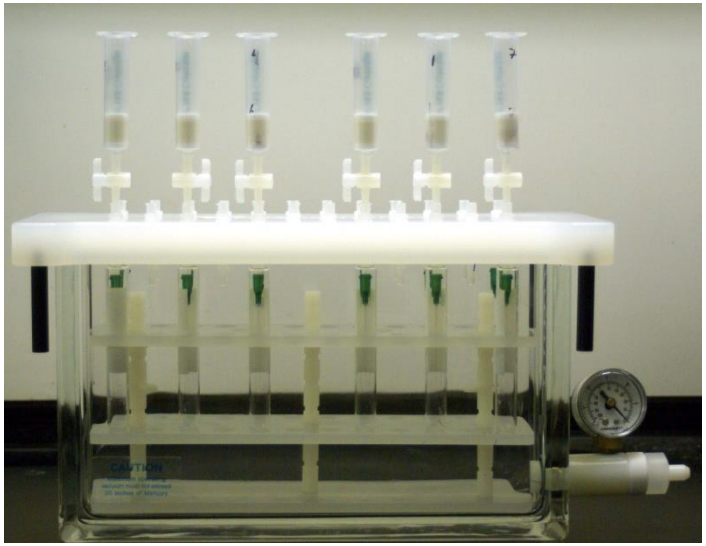
Load of pH buffered sample

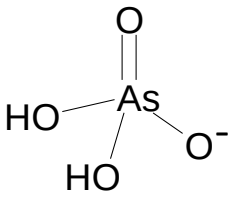
Sequential elution
Separation of inorganic As from organo As species by SPE

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

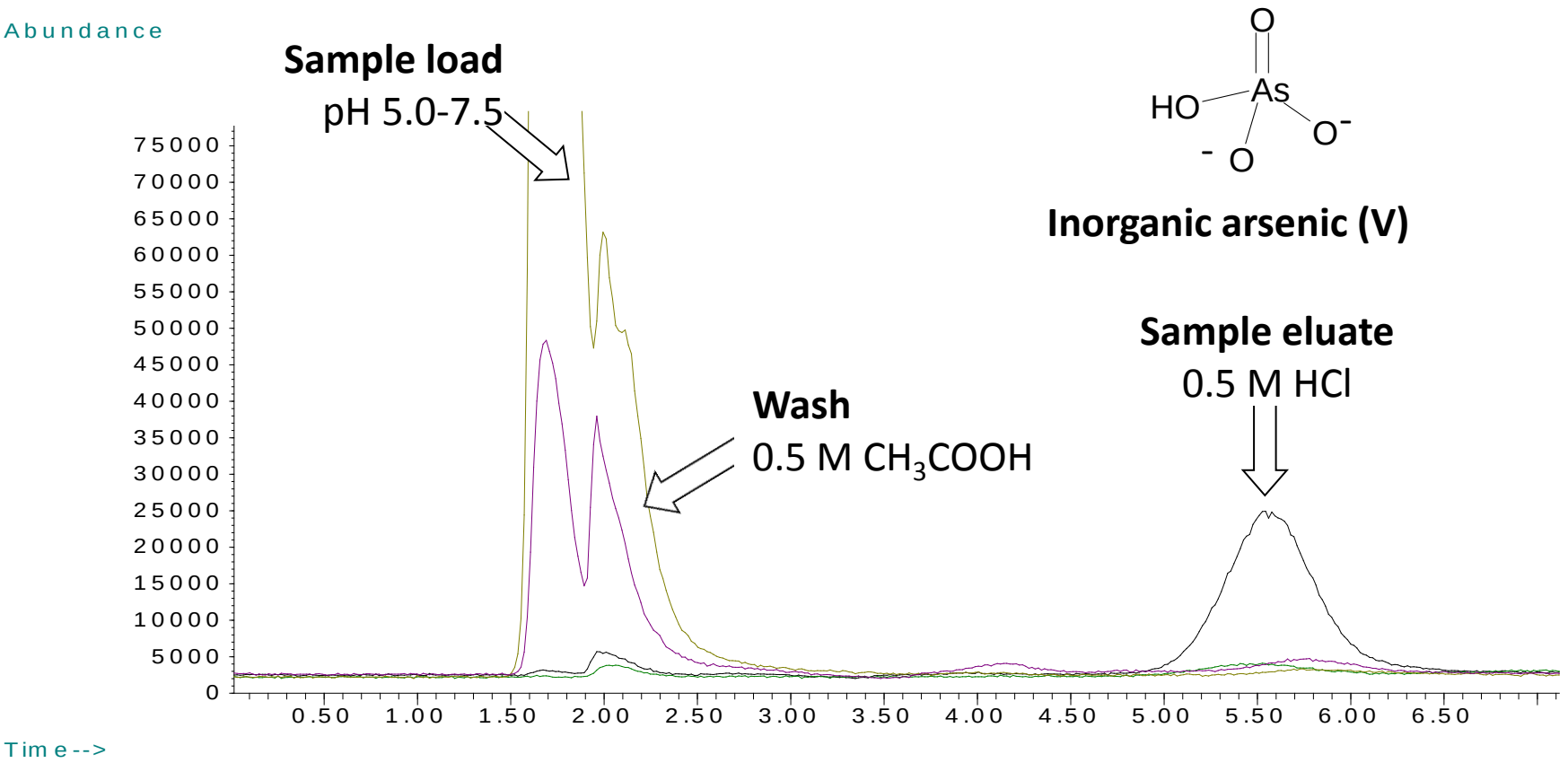


Figure. HPLC-ICP-MS chromatogram of fish protein (TORT-2) 3 SPE fractions separated on an anion exchange column (ION-120 part nr. ANX-00-6550, 120x4.6 mm), 40 mM carbonate pH 10.3.

HG-AAS detection

arsenic in sample eluate

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)

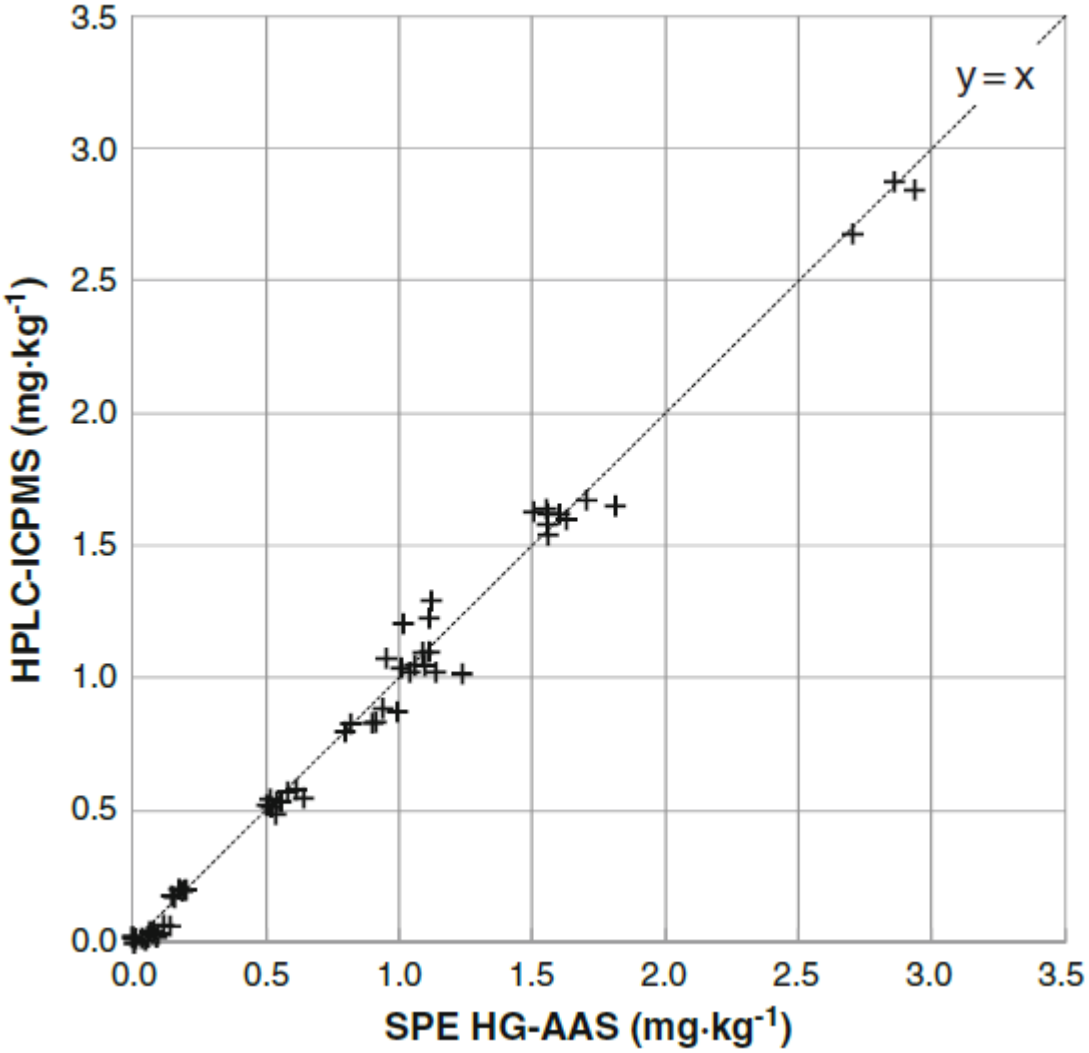
Hydride generation atomic absorption spectrometry

Thermo Scientific

HG-AAS

total arsenic in eluate

Arsenic speciation analysis



Inorganic arsenic (As^V) by two different methods:

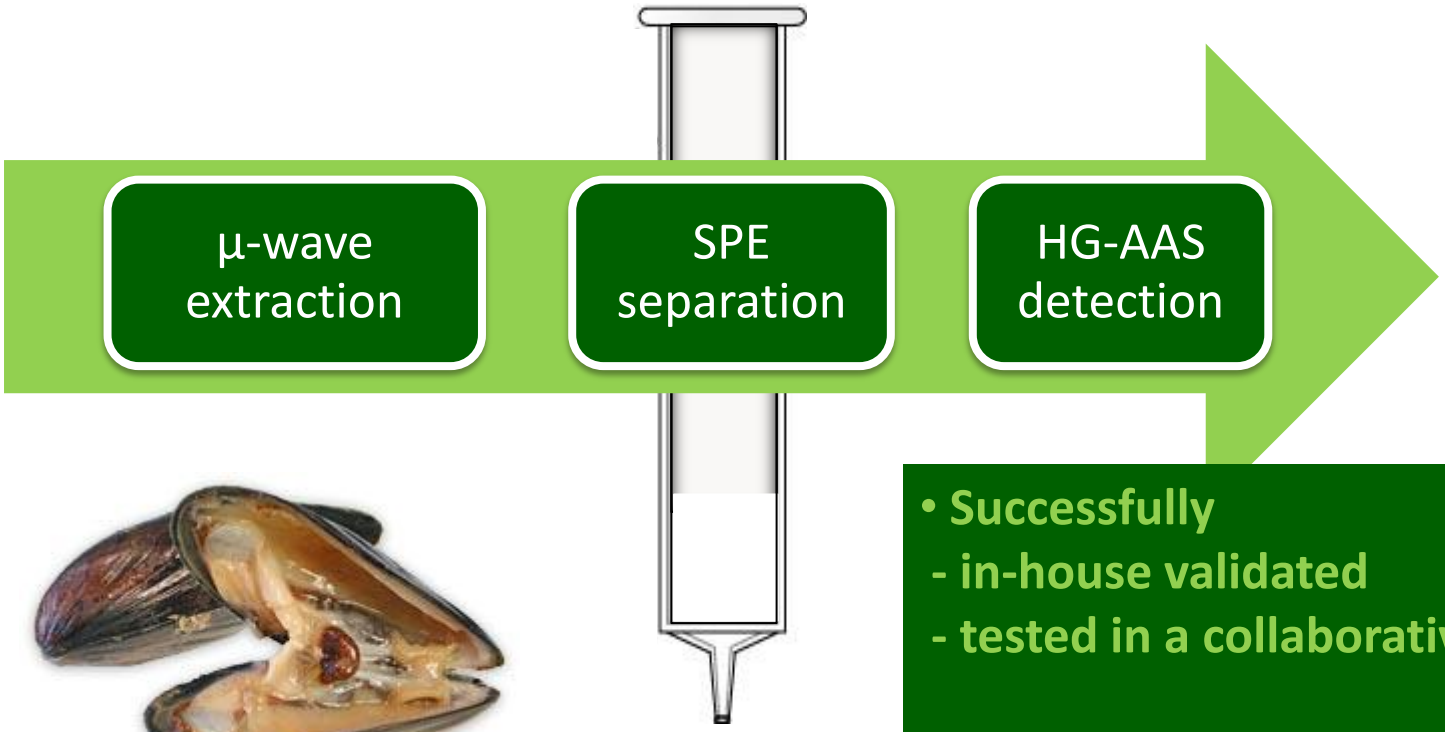
- HPLC-ICP-MS
- SPE HG-AAS

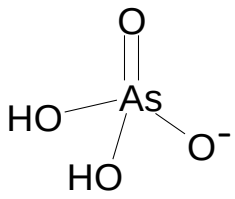
In total 72 blank, spiked and natural incurred marine samples were analysed.

No significant difference (P<5%)

Arsenic speciation analysis

speciation alternative: SPE, HG-AAS





inorganic arsenic

- **Successfully**
  - **in-house validated**
  - **tested in a collaborative trail**

→ **CEN standard (EN 16278:2012)**
Animal feeding stuffs – Determination of inorganic arsenic

Sample preparation @ DTU Food

- Cartridges examples

  – **Extrelut**

  Replacement for traditional liquid-liquid extraction, removed also water

  – **C18**

  Retains apolar compounds. Removed only part of interfering compounds (chloramphenicol in honey by GC-MS)

  – **Molecular imprinted polymers** (MIP)

  Compound specific, extensive wash possible, low detection capability obtained (chloramphenicol in honey by LC-MS/MS)

  – **Strong anion exchange**

  Retain negatively charged compounds, sequential elution, inorganic arsenic isolated in one fraction

Thank you for your attention!