National Reference Laboratory for Heavy Metals in Food
Metals Unit, Division of Environmental Health
Croatian Institute of Public Health

DETERMINATION OF INORGANIC ARSENIC SPECIES IN FOOD BY *IN SITU* IRRIDIUM TRAPPING ETAAS METHOD

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Arsenic species

Predominant species: arsenite, arsenate, methylarsonate, methylarsenite, arsenobetaine, arsenocholine

Toxicity →
- **As^{3+} is 60 to 80 times more toxic than As^{5+};**
- **organic As < inorganic As, arsenocholine and arsenobetaine are not toxic**
- **As^{3+} binds thiol groups inhibiting oxidative phosphrilation**
- **As^{5+} interferes the synthesis of ATP**

Metabolism →
- **Absorption** → **$As^{5+} > As^{3+}$ (not trough the skin);**
  Inorg. As > Org. As
- **Transported in the blood and accumulated in liver, kidney and lungs**
- **Excreted via urine $As^{5+} > As^{3+}$; organic As > inorg. As**

Detoxification → **Methylation of As^{5+} via reduction to As^{3+}**

EFSA (2009) and JECFA (2010) opinions on arsenic in food

- **Conclusion of opinions**: PTWI (15 mg kg^{-1}) body weight value (WHO, 1988) was withdrawn
- **NEW!:** $BMDL_{01}$ = 0.3 – 8 µg/kg b.w. per day for inorganic arsenic

=> EU dietary exposures within this range

=> Risk to some consumers cannot be excluded

- **NEW!** $BMDL_{0.5}$ = 3 µg/kg b.w. per day for inorganic arsenic

=> 0.5% increased incidence of lung cancer for 12 y exposure

- *"…there is a need to produce speciation data for different food commodities to support dietary exposure assessment…"*
- *"…more accurate information on the inorganic arsenic content of foods is needed to improve assessments of dietary exposures to inorganic arsenic"*
- *"…need for validated methods for selective determination of inorganic arsenic in food matrices"*


Commentary
Exposure to inorganic ar
Yong-Guan Zhu
Food
Elemental Analysis Manual: Section 4.10: High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometric Determination of Four Arsenic Species in Fruit Juice
Version Draft 0.82 (August 2010)
Author: Sean D. Conklin
Arsenic and Paddy Rice: A Neglected Cancer Risk?
11 JULY 2008 VOL 321 SCIENCE www.sciencemag.org
RICHARD STONE
Environmental Pollution 152 (2008) 746–749
Rapid communication
Inorganic arsenic levels in baby rice are of concern
Andrew A. Meharg, Guoxin Sun, Paul N. Williams, Eureka Adomako, Claire Deacon, Yong-Guan Zhu, Joerg Feldmann, Andrea Raab
Median consumption of organic arsenic levels for UK babies from baby rice is above threshold considered safe.
Analytical Methods
An improved HPLC–ICP
Application to rice, wheat and
in food:
Georg Raber, Natascha Stock, Pia Hanel, Manue
n A. Francesconi
Institute of Chemistry – Analytical Chemistry, Karl-Franzens University Graz, 8010 Graz, Austria
Faculty of Chemistry, Brno University of Technology, Institute of Food Science and Biotechnology, 61200 Brno,
FOOD CHEMISTRY

Current situation in EU legislation

Inorganic Arsenic in food

COMMISSION REGULATION (EU) 2015/1006

Commission Regulation (EU) 2015/1006 – amending Regulation (EC) No 1881/2006 - sets new MLs for inorganic arsenic in various rice products to range from 0.10 to 0.30 mg kg^{-1}.

According to Commission Recommendation (EU) 2015/1381, Member States should monitor in 2016-2018 the presence of arsenic in a wide variety of foodstuffs, by determining the content of inorganic and total arsenic and, if possible, other relevant arsenic species.

METHODS FOR iAs QUANTIFICATION (1)

Standardised methods

EN 15517(2008): iAs in seaweed by HG-AAS after acid extraction

"Nearly selective method":
(DMA and MMA may interfere)

EN 16278(2012): iAs by HG-AAS after microwave extraction and SPE for feed. Validated in the range 0.190-2.7 mg kg^{-1}

GB/T5009.11-2003: Determination of total arsenic and abioarsenic in foods, issued by Ministry of Health, the Standardisation Administration of China

BS EN 16802:2016:Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS

METHODS FOR iAs QUANTIFICATION (2)

Analysis of inorganic arsenic in seafood

- Inorganic arsenic affinity to thiol groups in proteins
- Hydrolysis of the As-S bond is required to liberate inorganic As to solution
- ”Classical” As extraction using aqueous MeOH not sufficient

Only 31 % of spiked As^{3+} was recovered using MeOH/water solvent

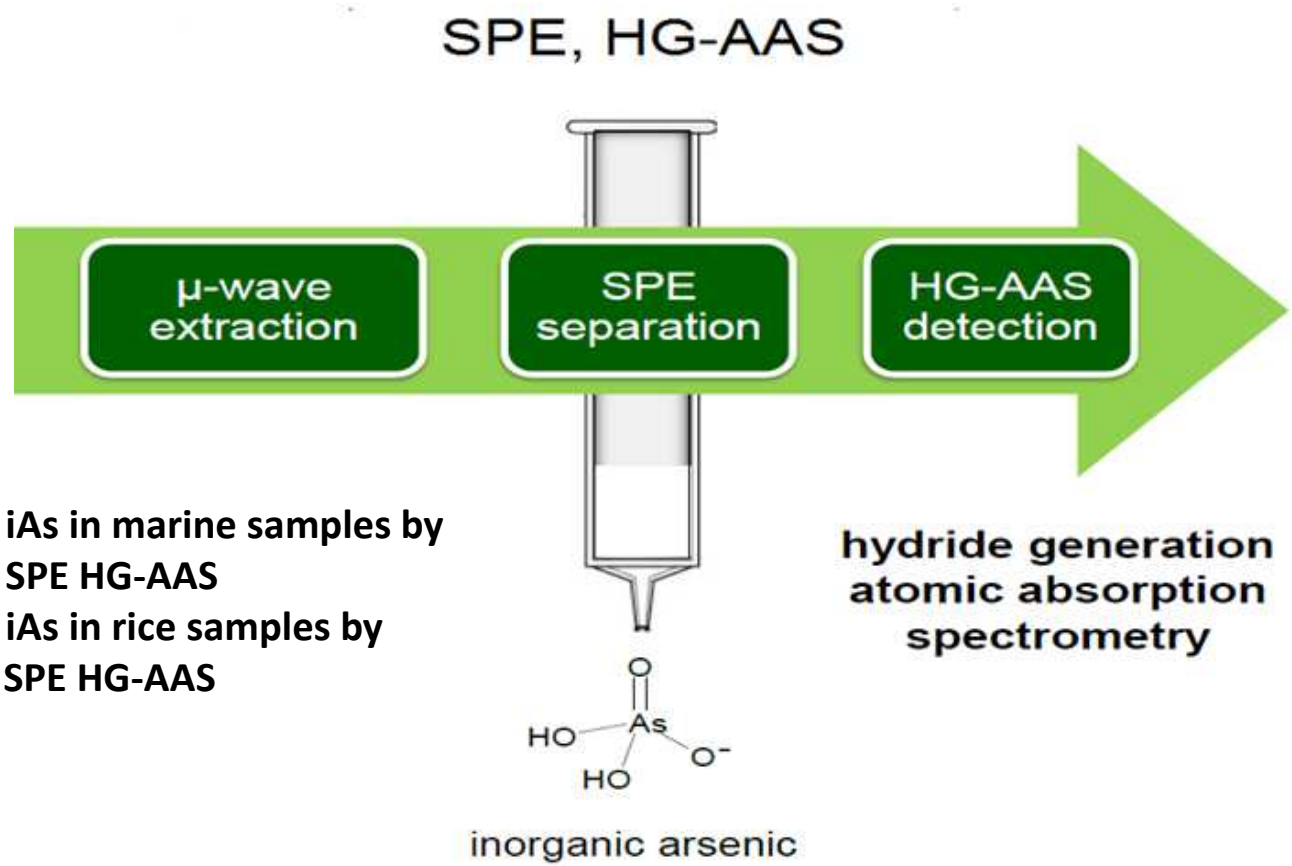
Typical approach:

- Digestion by strong HCl => $AsCl_3$
- Extraction by organic solvents (CCl_4) or destillation
- Co-extraction of MA and DMA possible => overestimation
- Determination by HG-AAS

Muñoz et al, Analyst, 1999, 124, 601-7
Oygard et al, J. AOAC, 1999, 82, 1217-1223

METHODS FOR iAs QUANTIFICATION (3)

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



- **iAs in marine samples by SPE HG-AAS**
- **iAs in rice samples by SPE HG-AAS**

METHODS FOR iAs QUANTIFICATION (4)

Extraction from matrix

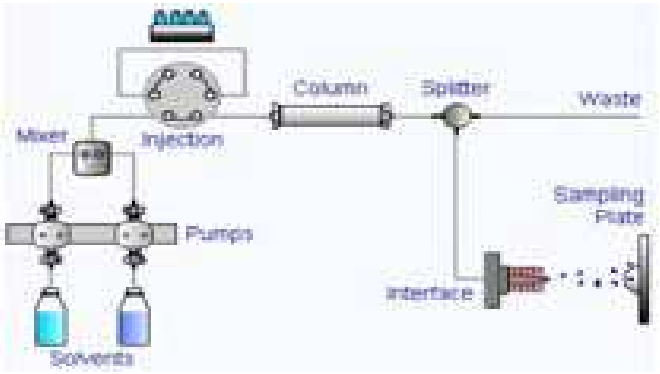
0.2-1 % HNO3 or
0.07 M HCl or
0.02 M TFA

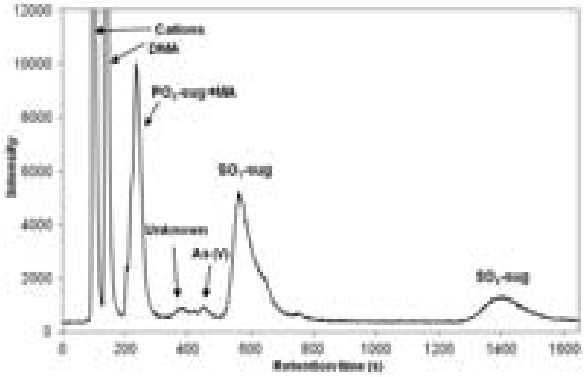
+

1 -30 % H_2O_2

Microwave

90-95 C
20-60 min





Determination of iAs in different food commodities

- rice and rice products (N=8)


- cereals and cereal-based food (N=7)




- potatoes, carrots, cabbage, lettuce/vegetables (N=25)

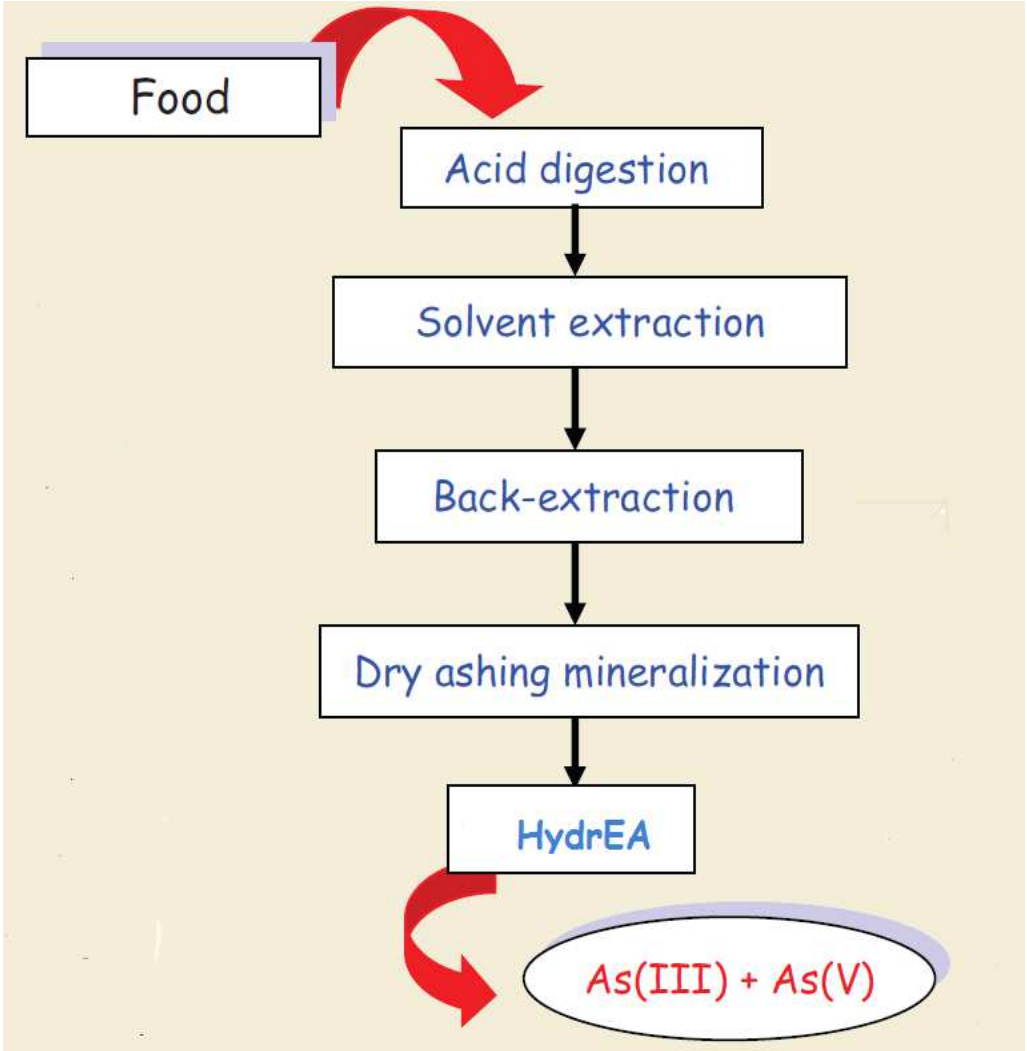

- barley, corn/Grains (N=4)

- sardines, tuna and mackerel/Sea fish (N=30)

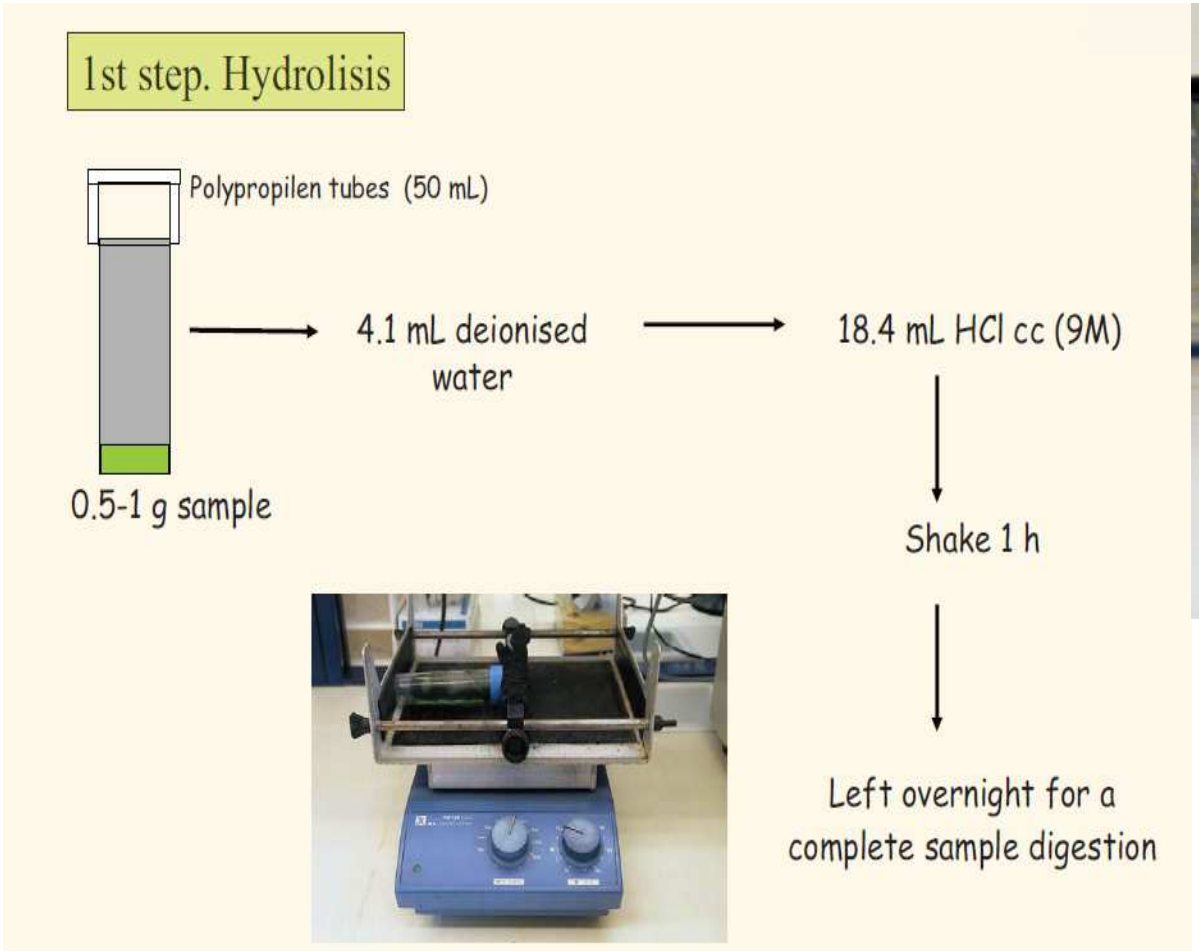


METHODS FOR iAs QUANTIFICATION (4)

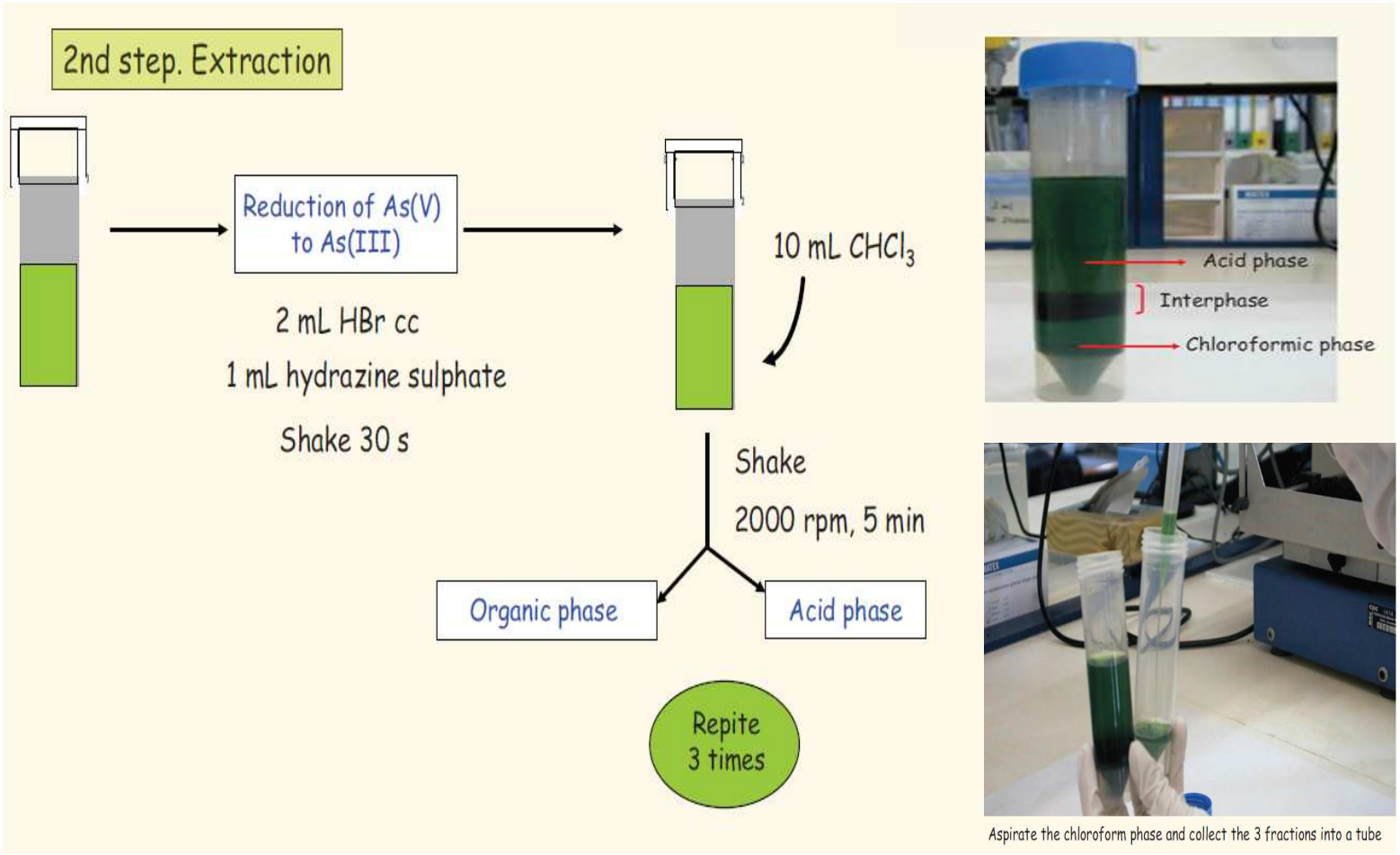
BY *IN SITU* IRRIDIUM TRAPPING ETAAS METHOD



Sample preparation for detrmination inorganic arsenic (1)



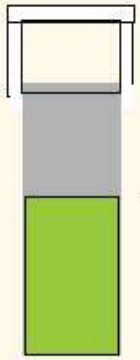
Sample preparation for detrmination inorganic arsenic (2)



Sample preparation for detrmination inorganic arsenic (3)

3rd step. Clean-up

Pool of chlroformic phases

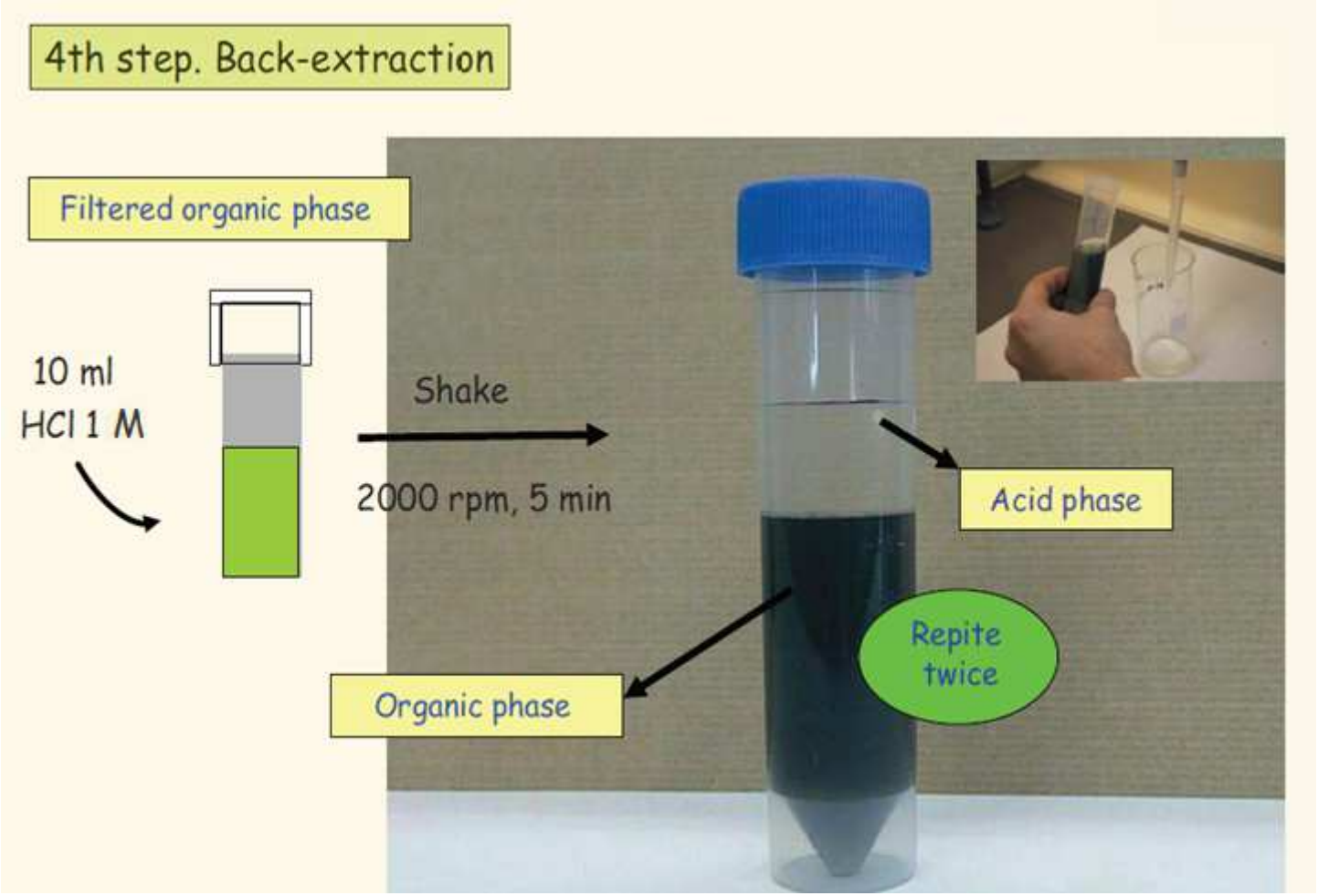


Centrifugation

Remove acid phase residues by pipetting

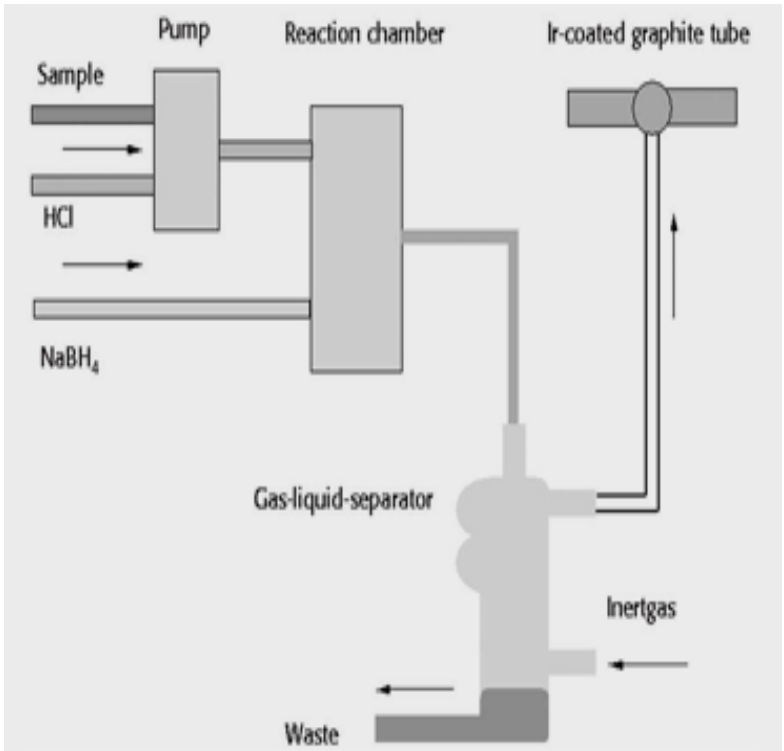
Filtration through a Whatman GD/X filter with PTFE membrane

Sample preparation for detrmination inorganic arsenic (6)



DETERMINATION OF INORGANIC ARSENIC SPECIES IN FOOD BY *IN SITU* IRRIDIUM TRAPPING ETAAS METHOD

5th step. Quantification → **Dry mineralisation and quantification by HydrEA/continuous technique**



Limit of detection: 0.005 – 0.01 mg kg^{-1}

Precision: 3 %

Recovery: As^{3+}= 91-114 %

The measurements were carried out using an AAS ZEEnit 700 and a hydride system HS 51

Instrumental parameters

Element	Wavelength [nm]	Slit [nm]	Lamp current [mA]
As	193.7	0.8	HCL/ 5.0

Element	Graphite tube	TPyr. [°C]	TAtom. [°C]	Ramp [°Cs^{-1}]
As	Standard tube Ir coated	310	2100	1200

Hydride generation parameters

Operation mode	Continous flow	Sample load time	16 s
Pump speed	3	Reasction time	30 s
Enrichment cycles	1	Rinse time	15 s
Gas flow	6NL/h	Carrier solution	3 % HCl
Cleaning	15 s with acid	Reduction agent	0.3 % $NaBH_4$ in 0.1 % NaOH

Calibration function

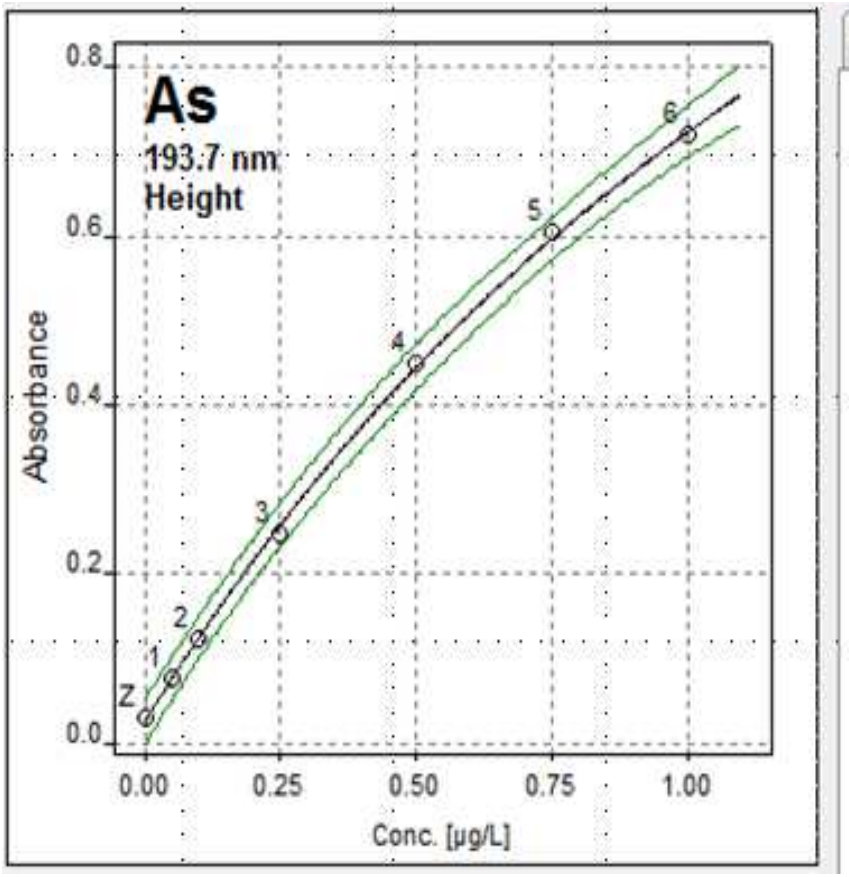


Table | Parameters | Residuals | LOD / LOQ

Calibration data

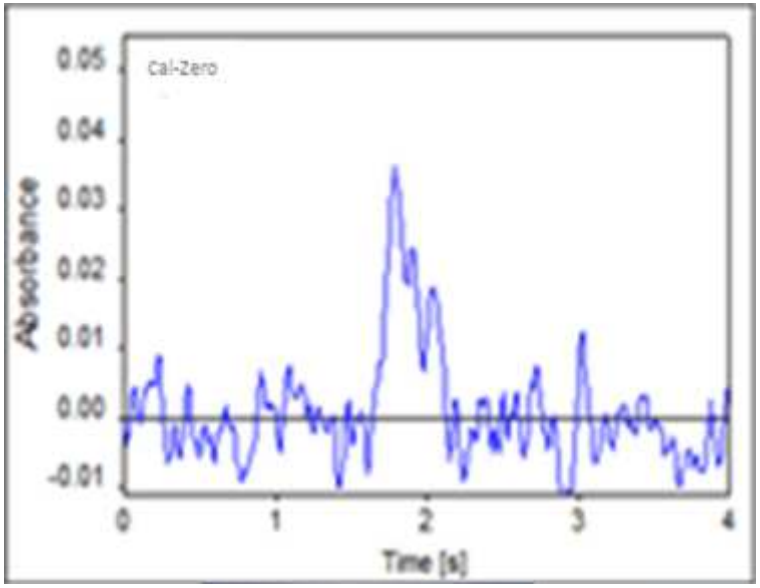
R:	0.999723983
Slope:	1.05265 Abs./µg/L
Method SD:	0.01056 µg/L
Char.conc.:	0.00414 µg/L/1%A

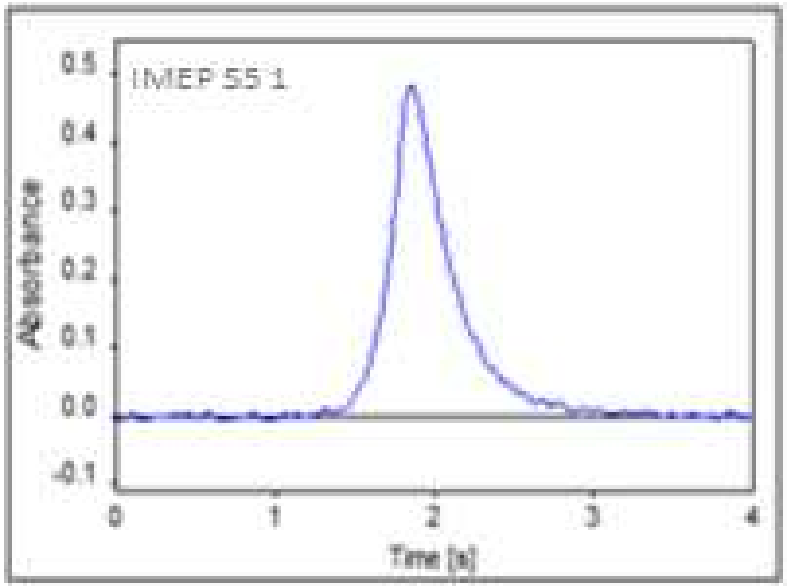
y=(a+bx)/(1+cx)

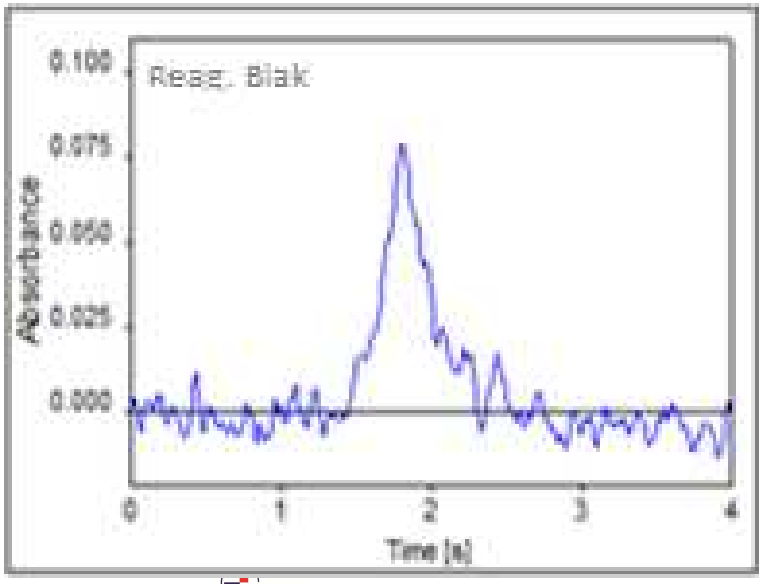
a=0.0250665 b=1.0653891 c=0.5082431

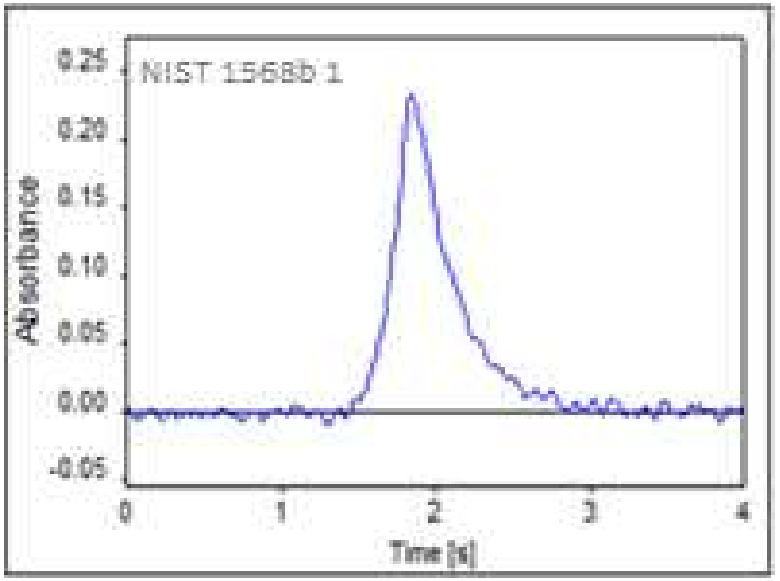
Calibration shall be performed by means of external calibration. The calibration curve ranges from 0.05 to 1.0 µg L^{-1} of As^{3+}. All As^{3+} calibration standard solutions shall be freshly prepared before each calibration.

Signals of the As^{3+} - Determination by HydrEA









Measured Inorganic arsenic concentration in rice and rice product

Sample	Country/region of origin	mg kg^{-1}	
		Inorganic arsenic (As^{3+}+As^{5+})	ML
Long grain white rice	Cambodia	0.068±0.010	0.20
Long grain, parboiled rice	Italy	0.125±0.018	0.25
Active Rice-snack classic (rice crackers with sea salt)	Germany	0.196±0.030	0.30
Brown rice flakes	Italy	0.075±0.012	0.20
Sesam bio-reis snack	Germany	0.139±0.020	0.30
Rice medium grain	Italy	0.065±0.010	0.20
Lang grain rice	Italy	0.060±0.009	0.20
Long grain white rice	Italy	0.063±0.009	0.20

results are given as mean ± U (k=2; p=95 %); (n = 2)
U' = The expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 % (U = 2u).

Measured Inorganic arsenic concentration in Rice destined for the production of food for infants and young children

Sample	Country/ region of origin	mg kg^{-1}	
		Inorganic arsenic (As^{3+} + As^{5+})	ML
Fruit yogurt duet with apple and strawberry (under 10 months)	Hungary	0.017±0.002)	0.10
Processed cereal-based foods for infants	Germany	0.119±0.017	0.10
Cereal with rice for infants and young children	Croatia	0.061±0.009	0.10
Organic Eco, cereal-based foods from organic farming, rice flakes	Croatia	0.071±0.010	0.10
3 cereals (rice, millet, buckwheat) with an initial milk	Croatia	0.030±0.004	0.10
Instant cereal porridge of rice and corn with transitional milk of infant formulas	Spain	0.035±0.005	0.10
Cereal-based foods, rice flakes - BIO	Poland	0.107±0.016	0.10

results are given as mean ± U (k=2; p=95 %); (n = 2)
U' = The expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 % (U = 2u).

Measured Inorganic arsenic concentration in fish, grains and vegetables

Food product	n	Concentration range (mg kg^{-1})
Fresh tuna	15	0.015 (0.008 - 0.021)
Canned tuna	12	0.024 (0.013 - 0.048)
Canned mackerel	3	0.036 (0.030 - 0.053)
Canned sardine	1	0.07
Grains (barley, corn)	4	0.010 - 0.016
Vegetables (potatoes, carrots, cabbage, lettuce)	25	0.010 - 0.013

Trueness (%) of inorganic arsenic determination in certified reference materials

Certified Reference Materials	Inorganic arsenic (As^{3+} + As^{5+}) (mg kg^{-1})		
	Measured value	Certified values	Recovery (%)
NIST 1568b Rice Flour	0.085±0.007 (4)	0.092±0.01	93 (90 - 99)
IMEP-112 Wheat	0.161±0.006 (2)	0.165 ±0.021	98 (95 - 100)
IAEA 359 Cabbage	0.084±0.029 (5)	0.091±0.016	92 (88 – 101)
IMEP 107 Rice Flour	0.098±0.002 (2)	0.108±0.011	91 (89 - 93)
ERM-CE278k Mussels	0.098±0.004 (4)	0.0863±0.008	114(109 – 119)

CONCLUSIONS

- The method does not imply the use of sophisticated /expensive instrumentation and can be implemented, even in challenging matrices;
- Pre-treatment uses basic analytical chemistry;
- The methodology provides a very good performance for the analysis of a wide range of food commodities with low detection levels, good precision, accuracy and recoveries;
- Fish matrices were particularly challenging. The filtration step is complicated for some samples (with lipids);
- The main drawback of the method is that it implies the use of such an organic solvent as chloroform.

THANK YOU FOR YOUR ATTENTION !!!!!!!!!!!

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