Report on Collaborative Trial

FoodStuffs - Determination of Inorganic Arsenic in Food of Marine and Plant Origin

CEN/TC 275/WG10 Elements and their chemical species

Work item: Inorganic arsenic in food of marine and plant origin (WI00275238)

DTU Food
National Food Institute

Report on Collaborative Trial

FoodStuffs - Determination of Inorganic Arsenic in Food of Marine and Plant Origin

National Food Institute
Research Group of Nano-Bio Science

FoodStuffs - Determination of Inorganic Arsenic in Food of Marine and Plant Origin

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Mandate: SA/CEN/EN/TR//422/2011-07 ”Heavy metals”
Project responsible: CEN TC275
Secretariat: DIN (organisational work on behalf of CEN TC275)
Work item: Inorganic arsenic in food of marine and plant origin (WI00275238)
Project leader: Dr. Jens J. Sloth, National Food Institute DTU, Denmark

This report is available at
www.food.dtu.dk

National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19
DK-2860 Søborg

Tel: +45 35 88 70 00
Fax: +45 35 88 70 01

Collaborative trial report

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Content

Collaborative trial report prepared by:
Dr. Jens J. Sloth, National Food Institute DTU, Denmark

Date: 06. February 2014

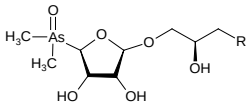
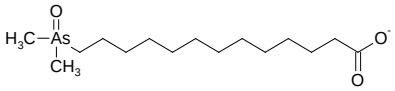
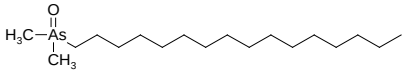
1. Introduction

Arsenic is a metalloid that occurs in different inorganic and organic forms, which are found in the environment from both natural and anthropogenic sources. Until now more than 50 different naturally occurring arsenic compounds have been identified, primarily in samples of marine origin (Francesconi 2010). Examples of arsenic compounds are given in Table 1. The inorganic forms of arsenic comprise the two oxyanions arsenite (As(III)) and arsenate (As(V)). In food samples, these analytes are likely to be bound to thiol groups of peptides and proteins and for quantitative determination liberation of the inorganic forms from the thiol groups is required (Styblo 1996, Muñoz, 1999). The inorganic forms of arsenic are more toxic as compared to the organic arsenic, but so far most occurrence data with regards to food control is reported as total arsenic. Recently, two toxicological evaluations were issued from EFSA (2009) and JECFA (2010), respectively; providing benchmark dose level values for intake of inorganic arsenic; $BMDL_{01}$ = 0.3-8 µg/kg bw/day (EFSA) and $BMDL_{0.5}$ = 3 µg/kg bw/day (JECFA) (EFSA 2009, JECFA 2010). Consequently, there is an evident need for development of robust, validated and standardized methods for specific determination of inorganic arsenic in food as emphasized in the two toxicological evaluations by EFSA and JECFA. Commodities of special focus are seafood, due to the high concentration of total arsenic often reported in these sample types (Francesconi 2010) and rice, which have higher arsenic concentrations compared to most other terrestrial plants (Heitkemper 2009).

2. Project background and time frame

In 2010 a tender for a project with the aim to develop a European standard method for the determination of inorganic arsenic in foodstuffs of marine and plant origin was set up by DIN on behalf of CEN TC275. Several proposed methods were discussed within the CEN TC 275/WG10 expert group, who gave advice to selection committee that finally selected the proposal from DTU Food and Dr. Jens J. Sloth was assigned as project leader. A service contract between DIN and DTU Food was signed in 2012 and the project official start date was 01-01-2012. The method was developed and validated at DTU Food during the period 2012-2013. The collaborative trial was conducted in 2013 with participants from 15 different laboratories from 10 different countries. The results from the collaborative trial were presented and discussed by the project leader at the CEN TC275/WG10 meeting in Paris 18. October 2013 and comments were received from the expert group members of WG10. The present report has been prepared in January-February 2014.

Table 1 Examples of arsenic compounds found in the marine environment. For simplicity the compounds are depicted in their fully deprotonated form. Names and acronyms as proposed by Francesconi and Kuehnelt (2004) and Sele et al (2012).

Acronym	Arsenic species	Formula
As^{III}	Arsenite	$As(O^-)_3$
As^{V}	Arsenate	$AsO(O^-)_3$
MA	Methylarsonate	$CH_3AsO(O^-)_2$
DMA	Dimethylarsinate	$(CH_3)_2AsO(O^-)$
AB	Arsenobetaine	$(CH_3)_3As^+CH_2COO^-$
TMAO	Trimethylarsine oxide	$(CH_3)_3AsO$
AC	Arsenocholine	$(CH_3)_3As^+CH_2CH_2OH$
TETRA	Tetramethylarsonium ion	$(CH_3)_4As^+$
TMAP	Trimethylarsoniopropionate	$(CH_3)_3As^+CH_2CH_2COO^-$
Arsenosugars		
Arsenosugar **1** (glycerol sugar)		R = OH
Arsenosugar **2** (phosphate sugar)		R = $OP(O)(OH)OCH_2CH(OH)CH_2OH$
Arsenosugar **3** (sulphonate sugar)		R = SO_3H
Arsenosugar **4** (sulphate sugar)		R = OSO_3H
Arsenolipids		
	Arsenic fatty acids	
	Arsenic hydrocarbons	

3. Scope

The aim of the present project is to develop a European standard method (EN) for the determination of inorganic arsenic in foodstuffs of marine and plant origin. The method should be based on HPLC-ICPMS. Furthermore a collaborative trial was conducted to evaluate the performance characteristics of the method.

4. Sample material – preparation and homogeneity testing

Several different sample materials were evaluated as candidate test materials for the collaborative trial. The samples included several (certified) reference materials and proficiency test materials, for which suitable homogeneity already has been verified by the supplier. For one of these material a certified value for inorganic arsenic has been established and this value was used to evaluate the accuracy of the present methodology. The following Table 2 provides an overview of the sample materials selected for the collaborative trial.

Table 2 Overview of sample material for the collaborative trial and their expected approximate concentrations.

Sample no	**Sample material**	**Producer**	**Approx conc (mg/kg)**
CEN iAs 1	Rice meal (white rice based)	Retail sample, Denmark (purchased in local supermarket)	0.05-0.1
CEN iAs 2	Rice meal (wholemeal based)	FAPAS T07151QC (Proficiency test material) (target value on iAs)	0.39
CEN iAs 3	Leach powder	National Food Agency, Denmark (NFA) (national reference material – not certified for iAs)	0.05-0.15
CEN iAs 4	Mussel powder	Seagarden (commercial seafood powder producer) (obtained from their production line)	0.2-0.4
CEN iAs 5	Fish muscle (DORM-4)	National Research Council Canada (NRCC) (certified for trace element – not iAs)	0.2-0.4
CEN iAs 6	Seaweed (Hijiki CRM 7405-A)	National Metrological Institute of Japan (NMIJ) (certified for total and inorganic arsenic)	10.1 +/- 0.5

Samples 2, 3, 5 and 6 are certified or reference samples and thus the homogeneity has already been verified. These samples were rebottled in small dark glass containers in order not to reveal the identity of the samples to the participants. For samples 1 and 4 homogeneity tests have been performed successfully ensuring sufficient homogeneous sample material (annex 10). Both samples were obtained as fine powders from the producers and the sample material was bottled in small dark glass containers. All bottles were clearly labelled and numbered consecutively according to bottling order.

5. Participant invitation and information

The method was tested in a collaborative trial with 15 participating laboratories from 10 different countries (Denmark, Sweden, Norway, Belgium, Germany, France, Spain, Switzerland, UK and USA). The invitation letter to participants can be found in Annex 1. A list of participants can be found in Annex 5.

The samples were dispatched from DTU Food on the 6th of May 2013 and the participants received the following information and documents:

1) Accompanying letter with information on the collaborative trial (Annex 2)
2) Method procedure
3) Results scheme (Annex 3)
4) Questionnaire (Annex 4)

The results from the participating laboratories were received in June and July 2013.

6. Method principle

Inorganic arsenic consists of arsenite, As(III) and arsenate, As(V), and the present method can be used for the determination of inorganic arsenic (=sum of As(III) and As(V)). Briefly, a representative test portion of the sample is treated with an extraction solution of dilute nitric acid and hydrogenperoxide in a waterbath at 90˚C for 60 min. Hereby the sample is solubilised and As(III) is oxidised to As(V). The inorganic arsenic is subsequently determined as As(V) by a method based on anion-exchange high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICPMS). Quantification is done by matrix-matched external calibration.

7. Results and statistical evaluation

7.1. Results

The reported results from the participating laboratories can be found in annex 8. An overview of the ICPMS instrumentation used as well as details regarding the chromatography (column type and dimensions, injection volume, mobile phase concentration and flow rate) can be found in annex 6. Various types of ICPMS instruments have been used both from Agilent, Thermo, Perkin Elmer and Varian. Three different anion-exchange columns have been used; IonPac AS7 (Dionex), ICSep Ion 120 (Transgenomics) and PRP X-100 (Hamilton). A wide range of different injection volumes are reported from 5 -100 µL as well as variation in mobile phase concentration (20 – 180 mM) and flow

rates 0,8 – 1,5 ml/min. The variation in these parameters indicate that the method is robust and various choices with regards to chromatographic set-up can be used successfully. In annex 11 examples of chromatograms for the six different sample types (sample 1-6) can be seen.

7.2. Laboratories compliance

Sixteen laboratories signed up to participate in the collaborative trial. One laboratory did not report any results. The laboratories also filled in a questionnaire regarding the analysis and some laboratories reported deviations from the method procedure and were judged as non-compliant. In Table 3 a list of non-compliant laboratories can be found and the rationale for this judgment.

Table 3 List of non-compliant laboratories

Non-compliant lab	**Reason**
L06	Used a different extraction solution (H2O/H2O2) than stated in the method procedure.
L11	Used a different extraction appraoch (enzymes and ultrasound) than stated in the method procedure. Used a different mobile phase (acetate buffer) solution than stated in the method procedure.
L12	Did not submit results.

The results from the non-compliant laboratories were excluded from the statistical analysis of the data from the collaborative trial. This decision was supported by CEN TC275/WG10 group at their meeting on 18/10-2013 in Paris.

7.3. Outlier identification

Following the initial identification of non-compliant laboratories, results from the remaining 13 laboratories were subjected to statistical analysis following international standard recommendations ISO5725-2 and ISO 13528. First step was to identify outliers (1% confidence level) and stragglers (5% confidence level) by the Cochran and Grubbs tests.

Table 4 provides an overview of the outlying results identified and the outlier/straggler type. Two Cochran outliers were identified (sample 2 and sample 3), two Cochran stragglers (sample 1 and sample 5) and one Grubbs straggler (sample 4). For sample 6 no outliers or stragglers were identified.

Table 4 Overview of outliers and stragglers identified by the Cochran and Grubbs tests.

No	Sample type	Outlier lab	Outlier/Straggler type
1	White rice	L01	Cochran straggler
2	Wholemeal rice	L13	Cochran outlier
3	Leach	L16	Cochran outlier
4	Blue mussel	L05	Grubbs straggler
5	Fish muscle	L02	Cochran straggler
6	Seaweed	-	-

In all cases the number of outliers is below the threshold recommended by the AOAC guideline, where a maximum outlier rate of 2/9 is established.

7.4. Statistical evaluation of the results

Following exclusion of outlying results the remaining measurements were used to evaluate relevant performance characteristics related to trueness and precision of the method under validation. The following method characteristics were calculated:

- The overall mean, Xobs (of all values after outlier elimination) and associated observed variability (expressed as one standard deviation, u_{obs})
- The standard deviation S_r and the relative standard deviation RSD_r obtained under repeatability conditions (within-laboratory observed variability),
- The standard deviation S_R and relative standard deviation RSD_R, obtained under reproducibility conditions (between-laboratory observed variability),
- The repeatability r_L (as 2.8 * S_r) and reproducibility limits R_L (as 2.8 * S_R) [10, 11],
- The percentage of identified and excluded outliers
- The Horwitz value was calculated in two different ways I) by the Horwitz equation $2*C^{-0,15}$ (Horwitz, 2006) and II) by the Thompsons modified Horwitz equation (Thompson, 2000)

$$\sigma = \begin{cases} 0.22c & \text{if } c < 1.2\times10^{-7} \\ 0.02c^{0.8495} & \text{if } 1.2\times10^{-7} \le c \le 0.138 \\ 0.01c^{0.5} & \text{if } c > 0.138 \end{cases}$$

- The HorRat value was calculated by dividing the RSD_R value with the calculated Horwitz values .

An overview of the method performance characteristics can be found in Table 5.
The relative standard deviation under repeatability conditions (within-laboratory), RSD_r was in the range from 1,9 – 6,3 % and the relative standard deviation under reproducibility conditions (between-laboratory), RSD_R was in the range 9,1 – 14,9 %. These values are very satisfactory and indicate that the method has a satisfactory precision. Sample 6 (Seaweed) is a certified reference material from NMIJ with a certified value for inorganic arsenic at 10,1 + /- 0,5 mg/kg (NMIJ, 2010). The mean value in the present study was calculated at 10.3 mg/kg and in good agreement with the certified value indicating satisfactory accuracy for the method. The method working range was established in the concentration range 0,073 mg/kg to 10,3 mg/kg. The RSD_r and RSD_R values for sample 1 (White rice) with mean value 0.073 mg/kg were 4,9% and 11.0% , respectively. These low RSD values may indicate that the method is suited for analysis at even lower concentration levels. The participants were asked to estimate the LOD of the method in the test solution and the values can be found in annex 6. The stated LODs range from 0,057 – 0,5 µg/L, which corresponds to a LOD in the samples in the range from 0,003 – 0,025 mg/kg (assuming 0,20 gram sample intake and extraction volume of 10 mL). HorRat values in the range of 0,47-1,05 were obtained,, which is very satisfactory and all below the guideline value of 2.

7.5. Participants comments

In annex 7 an overview of reported comments by the participants can be found. Some laboratories report that they have deviated slightly from the method procedure. This information can be used to evaluate the robustness of the method as the deviations did not deteriorate the performance of the laboratory and quality of the data:

- L03 kept the samples at room temperature for three days instead of keeping them cooled.
- L07 used extraction in a dry block system instead of waterbath.
- L08 centrifuged the sample extracts at 13000 rpm instead of filtering.
- L14 added the two reagents in the extraction solution one at a time and not as a prepared solution.
- L16 used different test portion sizes than prescribed.

7.6. Additional results

In annex 12 extra datasets from two laboratories L03 and L08 are given. In their second dataset the method was varied in two different ways: L03 use of conventional oven heating instead of waterbath heating during extraction and L08 use of hydride-generation (HG) coupled to ICPMS in

Table 5 Method performance characteristics from the collaborative trial.

Samples	White rice	Wholemeal rice	Leek	Blue mussel	Fish muscle	Seaweed
Number of reporting laboratories	13	13	13	13	13	13
Number of laboratories after elimination of outliers	13	12	12	13	13	13
Number of outlying laboratories	0	1	1	0	0	0
Mean value $\overline{x}$, mg/kg	0,073	0,47	0,086	0,33	0,27	10,3
Repeatability limit r, mg/kg	0,010	0,025	0,015	0,057	0,049	1,2
Repeatability standard deviation s(r), mg/kg	0,036	0,0090	0,0054	0,020	0,017	0,44
RSD(r), %	4,9	1,9	6,3	6,2	6,3	4,3
Reproducibility limit R, mg/kg	0,022	0,12	0,033	0,14	0,11	3,4
Reproducibility standard deviation s(R), mg/kg	0,008	0,043	0,012	0,049	0,038	1,2
RSD(R), %	11,0	9,1	13,8	14,9	13,8	11,8
Horwitz value according to Horwitz	23,54	17,79	22,95	18,79	19,29	11,20
HorRat value according to Horwitz	0,47	0,51	0,60	0,79	0,72	1,05
Horwitz value according to Thompson	22,00	17,92	22,00	18,93	19,43	11,26
HorRat value according to Thompson	0,50	0,51	0,63	0,79	0,71	1,04

the detection step. In both cases the obtained results were in good agreement with the results obtained from the same lab using the present methodology and also in good agreement with the mean value calculated from the collaborative trial.

8. Conclusion

A method for the determination of inorganic arsenic in foodstuffs of marine and plant origin was developed at DTU Food. The method principle is based on waterbath extraction with dilute nitric acid and hydrogen-peroxide followed by determination of inorganic arsenic by anion-exchange chromatography HPLC-ICPMS.

The method performance characteristics were assessed in a collaborative trial with 13 participating laboratories on six different food samples within the concentration range of 0.073 – 10.3 mg Kg^{-1}. Based on the statistical evaluation of the results from the collaborative trial it is concluded that the proposed method is suitable for the quantitative analysis of inorganic arsenic in foodstuffs of marine and plant origin.

9. Acknowledgements

Dr. Rie R. Rasmussen (DTU Food) has been an immense help with the practical work on the method development as well as evaluation of data. Mrs Birgitte Koch Herbst (DTU Food) has skilfully conducted most of the practical work with the characterisation of test materials as well as preparation and shipping of test materials to the participants. Finally and important all the participating laboratories are thanked for their voluntary participation in the collaborative trial, for their production of good results and their useful comments on the method. Thanks go also to the members of the CEN TC275/WG10 expert group for their constructive comments and encouragement during the project period.

10. References

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Annex 1. Invitation letter to collaborative trial

CEN / TC 275 `Foodstuffs:
Working group 10 'Elements and their species'

Invitation to participate in a collaborative trial.

Method: Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICPMS following waterbath extraction

Søborg, February 2013

Dear colleague,

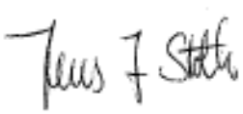
You are hereby invited to participate in a collaborative study of a method for the determination of inorganic arsenic in foodstuffs of marine and plant origin. The method principles are based on waterbath extraction followed by selective determination of inorganic arsenic by anion-exchange HPLC-ICPMS.

The participants will be supplied with the following:

- Method procedure (to be followed strictly)
- 5- 6 samples of marine or plant origin to be analysed (in duplicate on two separate days)
- Reporting scheme and questionnaire
- Report on the outcome of the collaborative trial (Lab ID will not be made public available)

I hope you will find it attractive to participate in the development of a future European CEN standard method for food control. Further information can be found in the following pages and if interested please fill in and send the registration form. Your efforts are very much appreciated thanks in advance.

Best regards,



Dr. Jens J. Sloth (senior scientist)

Some practical information regarding the collaborative trial

Introduction:

An international collaborative study will be conducted under the CEN leadership to evaluate a method for the determination of inorganic arsenic (iAs) in foodstuffs of marine and plant origin. The proposed method approach has been discussed and agreed upon in the CEN/TC275/WG10 group and The National Food Institute at the Technical University in Denmark has been appointed to organize this collaborative trial.

Principle of the method:

Extraction of inorganic As is done in a waterbath with dilute nitric acid and hydrogenperoxide for solubilisation and oxidation of AsIII to AsV. Determination of inorganic arsenic (as AsV) will be done by anion-exchange HPLC-ICPMS. A description of the method procedure to be followed will be sent to the participating laboratories.

Samples and sample analysis:

5-6 samples of marine or plant origin with unknown concentrations will be sent to the participating laboratories. The sample materials shall be analyzed in duplicate on two separate days and the results reported on dry matter basis. A reporting scheme and a questionnaire will furthermore be provided.

Requirements to the participating laboratories:

The following equipment should be available at the participating labs.

- Waterbath capable of maintaining a temperature of 90°C
- Strong anion exchange (SAX) column suitable for arsenic speciation analysis
- HPLC-ICPMS equipment

Time schedule:

- Registration for the collaborative trial – **15 March 2013**
- Estimated time for dispatch of samples – **beginning of April 2013**
- Deadline for submission of results: **17. May 2013.**
- Discussion of results will subsequently take place in CEN TC275/WG10 - **during 2013-2014**
- A report on the collaborative trial will be made and made public available – **during 2013**
- The individual results from the participating laboratories will be kept anonymous, but a list of participants will be given

Contact details:

Jens J. Sloth (project leader)
E-mail: jjsl@food.dtu.dk
Phone: +45 35887625
National Food Institute
Mørkhøj Bygade 19
DK-2860 Søborg
Denmark

Registration form - Collaborative study: Foodstuffs - Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICPMS following waterbath extraction	
Name of contact person	
E-Mail adress	
Organisation	
Shipping address	
City and postal zip code	
Country	

Please inform about which equipment will be used for the collaborative trial:

Strong Anion exchange (SAX) column used?	
HPLC-ICPMS system used?	
Waterbath available? (at 90°C)	
Previous experience with inorganic arsenic determination? (~years)	
Comments?	

Please send this registration form by mail to: **jjsl@food.dtu.dk**

Before Friday 15 March 2013

Annex 2 Accompanying letter to participants

To the participants of the
collaborative trial on inorganic As
in foodstuffs by HPLC-ICPMS

May 2013
/jjsl

CEN TC275/WG10 Collaborative trial on the determination of inorganic arsenic in foodstuffs of marine and plant origin by HPLC-ICPMS

Dear participant,

Thank you for participating in the collaborative trial on the determination of inorganic arsenic in foodstuffs. The aim of the project is to establish a European standard for the analysis of inorganic arsenic in foodstuffs. Your participation is a very important contribution and very much appreciated.

In this shipment you receive the sample materials to be analysed. Please read and follow the instructions in this letter carefully prior to starting with the analysis.

The deadline for submission of results is Friday 21/06/2013.

Contact details:
And if there are any questions don´t hesitate to contact:

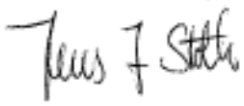
Jens J. Sloth: email: jjsl@food.dtu.dk or phone +45 3588 7625

or

Rie R. Rasmussen: email: riro@food.dtu.dk or phone +45 3588 7455

Your contribution is important for a successful outcome of this project and for a continued high level of food safety measures in Europe. Thank you for very much your collaboration,

Best regards,



Jens J. Sloth

INSTRUCTIONS

Please read and follow the instructions carefully. Any deviation from the instruction or method protocol must be reported.

A: Sample materials

Six different sample materials each in two bottles and one bottle with a standard solution are included in the study.

Sample ID	Sample type	Sample amount (g/bottle)	Test portion size for analysis (g)
CEN iAs – sample 1	Rice	3	0.5
CEN iAs – sample 2	Rice	1	0.2
CEN iAs – sample 3	Plant material	3	0.5
CEN iAs – sample 4	Shellfish	2	0.3
CEN iAs – sample 5	Fish powder	0.9	0.2
CEN iAs – sample 6	Algae	0.9	0.2
CEN iAs – sample 7	Aqueous standard	2 ml	Dilute 10 times before injection

NOTE: You should store the samples in a dark and cold place (at maximum 4 ºC) until analysis.

Please check whether the bottles containing the test material remained undamaged during transport, if not new sample material can be provided. Please confirm the receipt of the samples by email to jjsl@food.dtu.dk.

B: Analysis of samples

For the collaborative study please perform two independent measurements on the same day using one of the bottles and please remember to follow the draft method procedure carefully. It is crucial that the sample is wetted sufficiently prior to putting it in the waterbath (6.2), so please pay extra attention to this part in the procedure: Shake the tubes thoroughly and leave the sample and extractant solution in contact for an extended time period e.g. overnight prior to the waterbath extraction step.
Please also determine the drymatter content and use this to correct the results in order to report in mg As kg^{-1} dry matter as inorganic arsenic with at least 3 significant figures. Use approximately the test portion sizes indicated in the table above for the analysis.

For determination of dry matter content use oven drying of two portions of minimum 0.2 gram at 103 +/- 2°C until constant mass is attained.

THIS IS A STUDY OF THE METHOD NOT OF THE LABORATORY. THE METHOD MUST BE STRICTLY FOLLOWED AS DESCRIBED.
It is very important that you report any deviation from the method.

C: Standard solution (CENiAs – sample 7)

The standard solution (CENiAs – sample 7) contains a mixture of 3 arsenic species: arsenobetaine (AB); monomethylarsonous acid (MA) and arsenate (AsV) and addition of HCl. Analyse the mixture following a 10 fold dilution in the extraction solvent. Please provide a copy of the chromatogram of this solution and quantify the AsV content in the solution.

- AB is added as a marker of the void volume of the chromatographic system.
- MA is added to demonstrate satisfactory resolution between MA and AsV by the chromatographic system.
- HCl is added to demonstrate satisfactory resolution between AsV and Cl^-. (please monitor both m/z 75 and m/z 35)

NOTE: if you do not get a satisfactory resolution between MA and AsV as well as between AsV and Cl^-, the chromatographic conditions should be optimised, e.g. by changing the mobile phase concentration or the mobile phase flow rate.

D: Reporting of results

Report the values (at least 3 significant figures) in the results form and send it to the project coordinator (jjsl@food.dtu.dk). Please check your results carefully for any errors before submission.

Furthermore please fill in the questionnaire. This information is valuable for the subsequent evaluation of the results. Remember to note all deviations and if anything unexpected happens during analysis. Please also provide copies of the chromatogram obtained for each of the samples and for one of the standards.

E: Method procedure and other forms

You will per email receive the following:

- Draft method procedure description (MUST BE STRICTLY FOLLOWED)
- Reporting scheme (results with at least 3 significant figures)
- Questionnaire to be answered and returned together with the results

F: Summary

Please provide the following:

1. Confirmation upon receipt of samples to jjsl@food.dtu.dk
2. A reporting scheme with the results from the analysis of the samples following the method protocol

3. Copies of the chromatograms of each of the samples 1-7.
4. Fill in the questionnaire
5. Report any deviation and unexpected observations

G: Thanks for your contribution – highly appreciated

If you have questions – please contact:
Jens J. Sloth (jjsl@food.dtu).dk or Rie R. Rasmussen (riro@food.dtu.dk)

Results scheme

CEN TC275/WG10 Collaborative trial on inorganic arsenic in foodstuffs by HPLC-ICPMS

Laboratory: _____

Date of extraction: _____
Date of analysis: _____

Results

Sample	Bottle no	Dry matter (%)	Result replicate 1 (mg kg^{-1})	Result replicate 2 (mg kg^{-1})
CEN iAs Food – sample 1				
CEN iAs Food – sample 2				
CEN iAs Food – sample 3				
CEN iAs Food – sample 4				
CEN iAs Food – sample 5				
CEN iAs Food – sample 6				

All results shall be given in mg As kg^{-1} as inorganic arsenic with at least 3 significant figures.

CEN iAs – sample 7		-	µg/L	µg/L
Procedural blank	-	-	µg/L	µg/L

Please send copies of the chromatograms of each of the samples 1-7.

Deadline for submission of results: **Friday 21. June 2013**
Remember to fill in the questionnaire.

Please send to: jjsl@food.dtu.dk

Annex 4. Questionnaire

CEN TC275/WG10 Collaborative trial on the determination of inorganic arsenic in foodstuffs of marine and plant origin by HPLC-ICPMS

Please complete this questionnaire.

Laboratory: _____

1. Method related questions

1.1 Which equipment did you use?

HPLC: _____

ICPMS: _____

Column (type and dimensions): _____

Column temperature (°C): _____

1.2 Please provide the settings for HPLC:

Mobile phase concentration (mM) _____

Mobile phase flowrate (ml/min) _____

Injection volume (µL) _____

1.3 Which calibration working range have you used? Indicate lowest and highest standard (µg/l):

_____

1.4 Have you diluted any of the samples prior to measurement? If yes how much?

CEN-iAs-sample 1: _____

CEN-iAs-sample 2: _____

CEN-iAs-sample 3: _____

CEN-iAs-sample 4: _____

CEN-iAs-sample 5: _____

CEN-iAs-sample 6: _____

CEN-iAs-sample 7: _____

1.5 How did you ensure good wetting of the sample with the extractant solution prior to the waterbath treatment (section 6.2 in method protocol)? _____

_____

1.6 How did you store the sample extracts in the time period from extraction to analysis?_____

_____

1.7 Did you apply a recovery factor for correction of the results? If yes how (e.g. recovery from a reference material)?

_____

1.8 Have you identified any interference(s)? If yes, how did you correct?_____

_____

1.9 Did you control the instrument sensitivity during the analytical run (e.g. by analysing calibration standards throughout the run)? If yes, please elaborate._____

_____

1.10 What is the estimated limit of detection in solution (µg/L)? _____

2. **The method description should be followed strictly. However, if any deviation were made please report here.**

Please specify the modifications introduced **(VERY IMPORTANT !!)**:

_____
_____
_____
_____
_____

3. **Does your laboratory carry out HPLC-ICPMS analysis on a routine basis?**

O No O Yes

If yes, please estimate the number of samples:

a) **0-50 samples per year** _____
b) **50-200 samples per year** _____
c) **>200 samples per year** _____

4. **Does your laboratory have a quality system in place?**

O No O Yes

If yes, which:

a) **ISO17025** _____
b) **ISO 9000 series** _____
c) **Other, please specify:** _____

5. **Is your laboratory accredited for this kind of analysis?**

O No O Yes

If yes, which accreditation body: _____

6. **Do you have any comments or suggestions? Please let us know:**

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Please return questionnaire to jjsl@food.dtu.dk together with the results of the analysis.
Thanks for your time ☺

Annex 5. List of participating laboratories

Lab	Country	Contact person
FAVV-FLVVG	Belgium	Inge van Hauteghem
DTU Food	Denmark	Jens J Sloth
ANSES LSA CIME Unit	France	Thierry Guerin
Brooks Rand Labs	USA	Michelle Briscoe
Bayerische LGL	Germany	Peter Fecher
NIFES	Norway	Heidi Amlund
SLV - National Food Agency	Sweden	Barbro Kollander
University of Aberdeen	UK	Asta Petursdottir/Jörg Feldmann
NQAC Cergy Nestle France	France	Vincent Dufailly
NRC - QS department - Mineral Laboratory	Switzerland	Eric Poitevin
US FDA/CFSAN	USA	Sean Conklin
CODA-CERVA	Belgium	Ann Ruttens
FVST - Danish Food Administration	Denmark	Inge Rokkjær
BVL - Fed Off for Consumer Protection and Food Safety	Germany	Timo Kapp
Universitat de Barcelona - Departament de Quimica Analytica	Spain	Jose Fermin Lopez Sanchez

The laboratories are listed in random order and the order of appearance does not correspond to the lab numbers given in the results.

Annex 6. Overview of instruments, columns and analytical parameters

Lab	ICPMS	Column	Length (mm)	i.d. (mm)	particle size (µm)	Temp (°C)	Inj vol (µL)	Mobile phase (mM)	Flow rate (mL/min)	Calibration range (µg/L) Min	Max	LoD (µg/L)
1	Agilent 7500ce	IonPac AS7	250			30	5	40	1	0,5	20	0,08
2	Agilent 7500cx	ICSep Ion120	120	4,6	10	20	25	30	1	0,8	10	0,08
3	Agilent 7500i	ICSep Ion120	120	4,6	10	20	25	30	1	5	50	0,42
4	Agilent 7700x	IonPac AS7	250			30	25	50	1	0,05	10	0,05
5	Perkin Elmer DRCII	Hamilton PRP X100	250	4,1		30	20	50	1,5	0,1	20	0,1
6	Thermo X-series II	IonPac AS7	250	4		20	100	50	1,35	0	20	0,06
7	Thermo Element 2	IonPac AS7	250	4		room	20	50	0,8	1	100	0,1
8	Agilent 8800	Hamilton PRP X100	250	4.6		22	100	20	1	1	200	0,05
9	Perkin Elmer DRCe	Hamilton PRP X100	250	4.6		25	50	180	1	0,25	10	0,25
10	Agilent 7700	IonPac AS7	250	4		room	25	50	1	0,2	20	0,2
11	Agilent 7700x	IonPac AS7	250	4		room	25	gradient	1,3	0,2	10	0,2
12	-	did not report	-	-	-	-	-	-	-	-	-	-
13	Perkin Elmer DRCe	Hamilton PRP X100	150	4.6		35	60	50	1	0,5	25	0,5
14	Varian 820	Hamilton PRP X100	250	4.6	10	30	60	40	1	0	5	0,06
15	Agilent 7500ce	Hamilton PRP X100	250	4.1	10	24	50	20	1,5	0	10	0,057
16	Agilent 7500ce	Hamilton PRP X100	250	4.1	10	room	100	30	1	0,5	200	-

Annex 7. Overview of reported comments by the participants

Lab no	Comment
L03	The samples were by mistake kept at room temperature for 3 days upon receipt and then put on cooled storage until analysis.
L04	The shellfish sample (sample#4) required extensive washing of the column to avoid memory effects. A blank sample was run after the shellfish sample to wash the column and then no disturbance was observed. Possible contamination by As in some batches of H2O2 is suspected.
L05	The results for sample 6 were above the upper calibration range of the instrument. However, the reported results agree well with those obtained for the sample by an alternate method.
L06	Used extraction with H2O/H2O2 instead of HNO3/H2O2. Used gradient elution with A: 0,8 mMHNO3/MeOH (99/1) and B: 50 mM HNO3/MeOH (99/1).
L07	Extraction in dry block system (type QBD2, Grant instruments, Cambridge, UK) at 90°C. Nitric acid with density 1.38 g/ml was used and therefore 6.68 ml HNO3 was used for preparation of extraction solution (paragraph 4.4)
L08	Filters were not available and the samples were centrifuged at 13000rpm instead. HNO3 was of analytical grade and H2O2 was of laboratory reagent grade.
L10	Overestimation of results expected due to 115% recovery in in-house referencematerial.
L11	Used a different mobile phase (Acetate buffer pH4.65/HNO3 0.5mM/HNO3 50mM). Extraction was done by ultrasonic enzymatic extraction.
L14	The extraction solution was added to each tube as 9 mL HNO3 0.11 M + 1 mL H2O2 (30%) and not as a prepared solution. No methanol was added to the mobile phase. Mobile phase was 40 mM (NH4)2CO3 at pH 9.4.
L15	Used SRM1568a, NMIJ CRM 7503a and ERM BC211 rice reference materials to access accuracy. The results obtained were in agreement with the certified values.
L16	Suggested test portion sizes were not strictly followed.

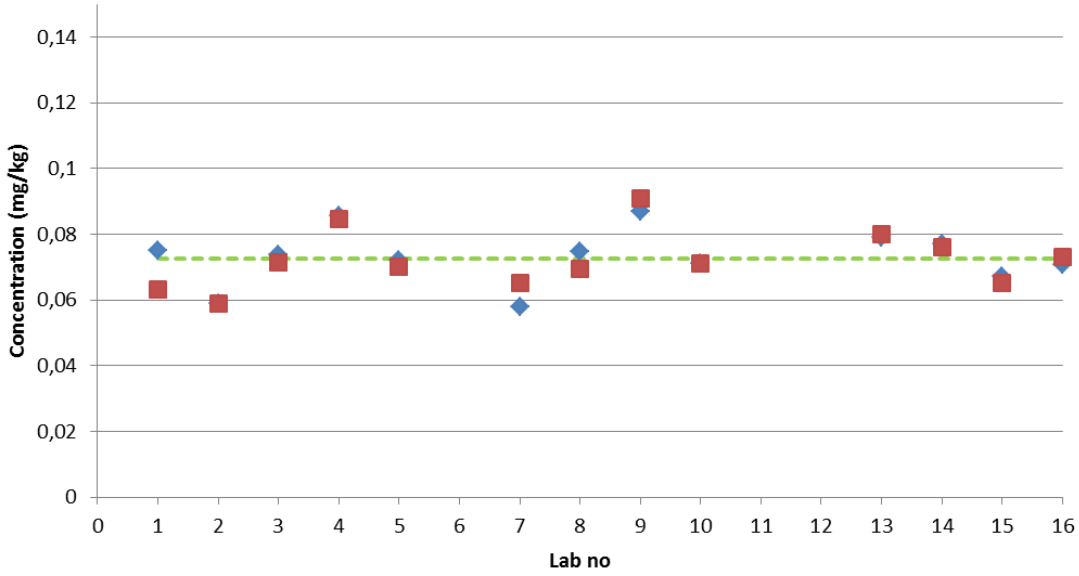
Annex 8 Results reported by the participating laboratories

Lab No	sample 1 White rice		sample 2 Wholemeal rice		sample 3 Leek		sample 4 Blue mussels		sample 5 Fish muscle		sample 6 Seaweed	
1	0,075	0,063	0,493	0,498	0,085	0,086	0,309	0,312	0,26	0,294	10,91	9,84
2	0,059	0,059	0,39	0,4	0,075	0,07	0,26	0,28	0,28	0,22	9,69	9,7
3	0,0736	0,0713	0,492	0,493	0,0919	0,0919	0,337	0,338	0,283	0,285	12	12,1
4	0,0856	0,0847	0,514	0,531	0,0968	0,0909	0,344	0,412	0,29	0,282	10,5	10,6
5	0,072	0,07	0,526	0,529	0,063	0,061	0,196	0,194	0,178	0,187	7,297	7,787
6	0,095	0,091	0,592	0,573	0,064	0,075	0,418	0,383	0,261	0,289	9,58	9,93
7	0,058	0,065	0,392	0,406	0,086	0,084	0,341	0,372	0,276	0,252	9,902	10,621
8	0,0746	0,0695	0,503	0,485	0,0758	0,0781	0,327	0,316	0,282	0,268	9,52	9,51
9	0,087	0,091	0,479	0,468	0,08	0,082	0,315	0,337	0,255	0,26	9,969	9,885
10	0,071	0,071	0,501	0,501	0,106	0,119	0,372	0,381	0,373	0,363	13,268	12,351
11	0,061	0,047	0,374	0,369	<0,025	<0,025	0,072	-	0,161	0,181	1,817	-
12*	-	-	-	-	-	-	-	-	-	-	-	-
13	0,079	0,08	0,438	0,483	0,084	0,095	0,391	0,407	0,308	0,296	10,208	10,77
14	0,077	0,076	0,505	0,515	0,100	0,101	0,318	0,335	0,268	0,269	11,6	11,4
15	0,067	0,065	0,431	0,443	0,087	0,077	0,317	0,313	0,288	0,280	9,315	10,158
16	0,0706	0,0730	0,401	0,402	0,125	0,0898	0,326	0,356	0,274	0,268	9,67	9,39

* L12 did not report results

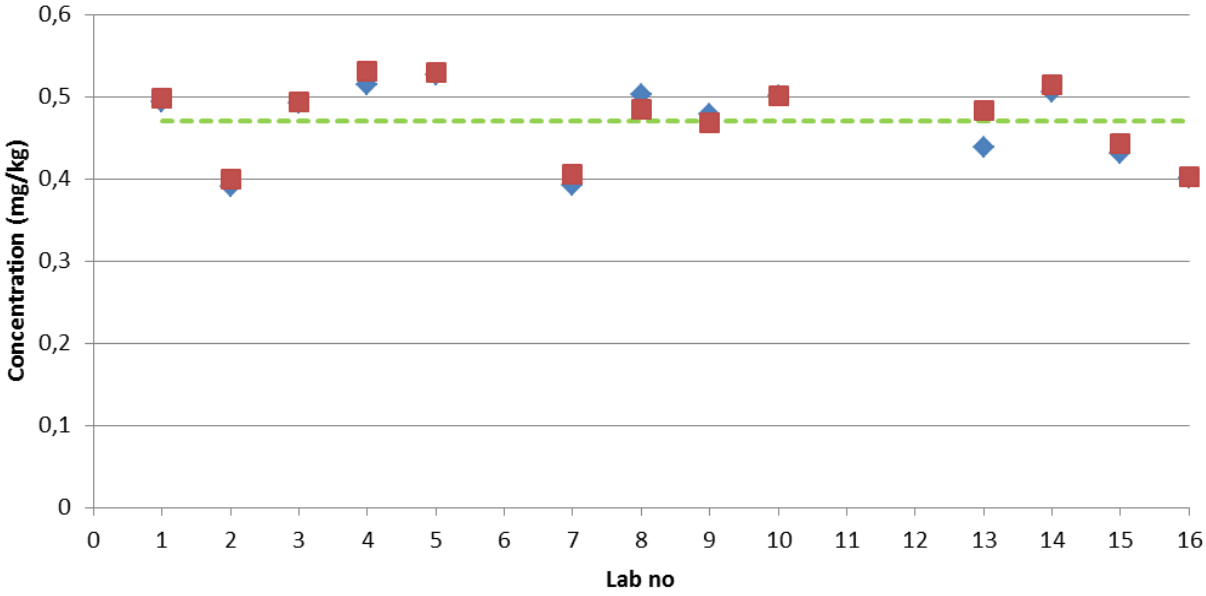
Annex 9 Plots of results from compliant laboratories

Sample 1 White rice (mean value +/- u_{obs} = 0.073 + /- 0.006 mg/kg)



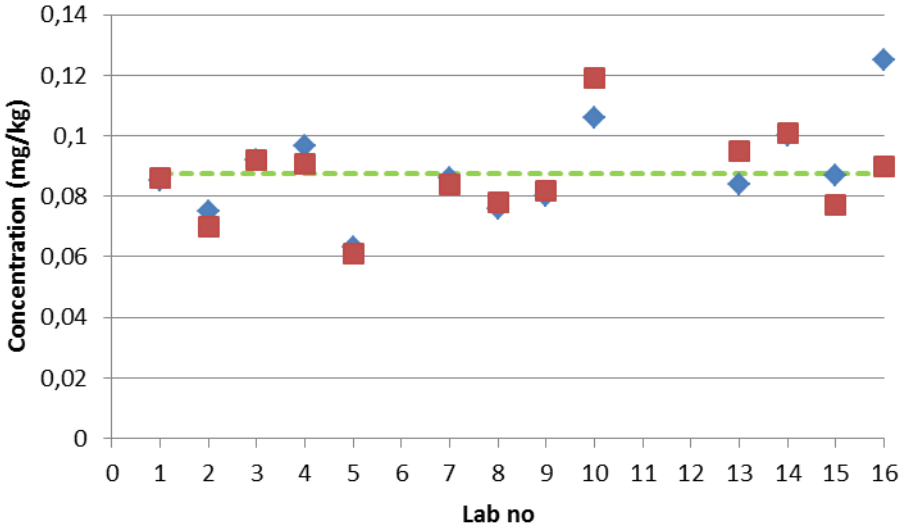
L01 is a Cochran straggler

Sample 2 Wholemeal rice (mean value +/- u_{obs} = 0.47 +/- 0.05 mg/kg)



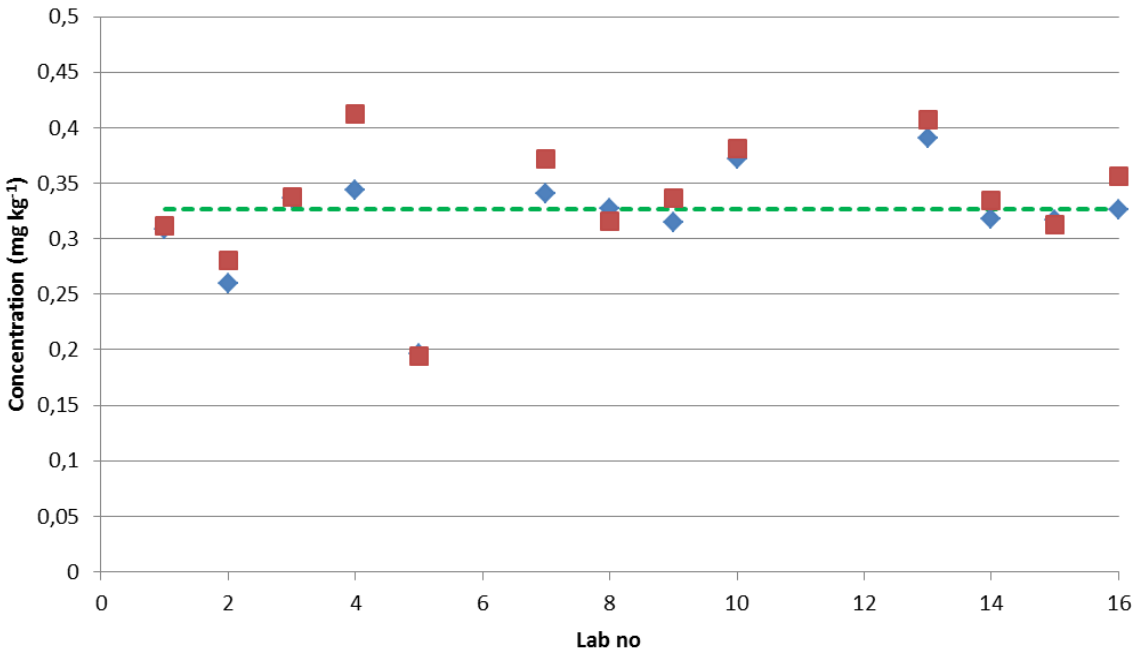
L13 is a Cochran outlier

Sample 3 Leek (mean value +/- u_{obs} = 0.086 +/- 0.013 mg/kg)



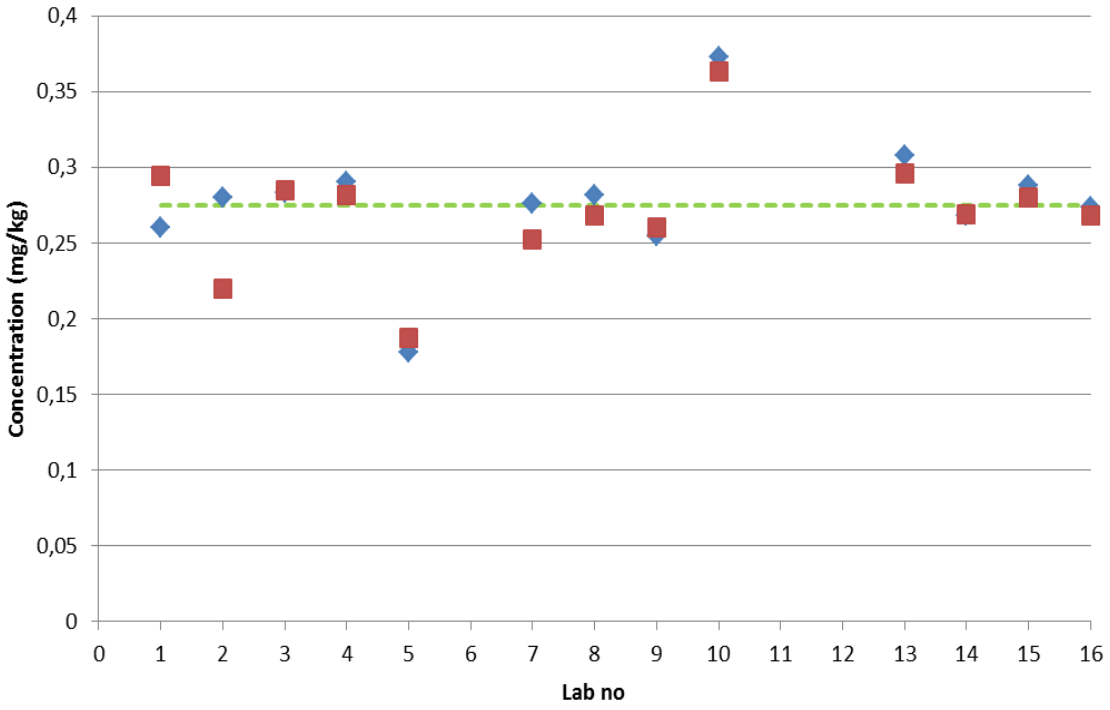
L16 is a Cochran outlier

Sample 4 Blue mussel (mean value +/- u_{obs} = 0.33 +/- 0.05 mg/kg)



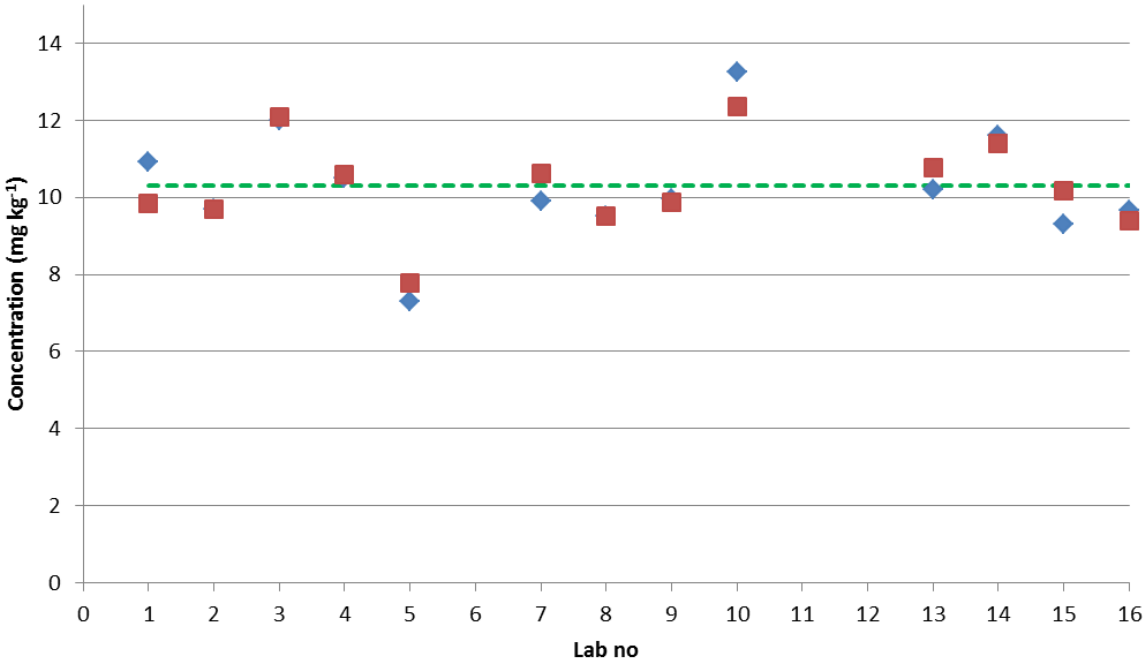
L05 is a Grubbs straggler

Sample 5 Fish muscle (mean value +/- u_{obs} = 0.27 +/- 0.04 mg/kg)



L02 is a Cochran straggler

Sample 6 Seaweed (mean value +/- u_{obs} = 10.3 +/- 1.3 mg/kg)



Annex 10 Results from the homogeneity testing of sample 1 and sample 4

Sample 1 – White rice

Results of the homogeneity studies

Sample	No1 white rice	
Bottle ID	Replicate 1	Replicate 2
2	0,069	0,068
4	0,069	0,067
12	0,070	0,071
13	0,070	0,070
42	0,071	0,070
61	0,072	0,070
70	0,078	0,070
9	0,066	0,064
35	0,071	0,068
65	0,071	0,070
Grand Mean	0,070	
Number of bottles, m	10	

S_{DD} Sum of Squares	Cochran Test	Cochran value
0,000	0,719	0,602

	Sum_i	$Difference_i$	D^2	$Vs = (Si-S^{-})^2/(m-1)$
	0,137	0,001	0,00000	0,00000
	0,136	0,002	0,00000	0,00000
	0,141	-0,001	0,00000	0,00000
	0,140	0,000	0,00000	0,00000
	0,141	0,001	0,00000	0,00000
	0,142	0,002	0,00000	0,00000
	0,148	0,008	0,00006	0,00001
	0,130	0,002	0,00000	0,00001
	0,139	0,003	0,00001	0,00000
	0,141	0,001	0,00000	0,00000
$\sum$	**1,395**	0,019	**0,00009**	**0,00002**
S^{-}	0,140			
$S_{an}^2 = \sum D^2/2m$	**0,000004**			
$S_{sam}^2 = (Vs/2-S_{an}^2)/2$	**0,0000032**			
F1	*1,88*			
F2	*1,01*			
σ-hat (%)	**15**			
$\sigma^2_{all} = (0,3\sigma_p)^2$	0,000010			
$c = F1\sigma^2_{all}+F2S_{an}^2$	0,000023			
$S_{sam}^2 < c$	passed			

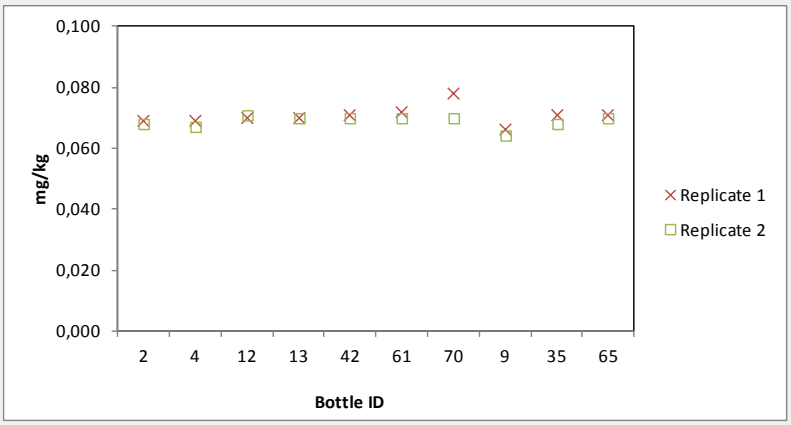
Cochran outlier test

Conclusion:
outliers!!

Homogeneity test

Conclusion:
material homogeneous

Visual appraisal of results:



Sample 4 – Blue mussels

Results of the homogeneity studies

Sample	sample 4 blue mussel	
Bottle ID	Replicate 1	Replicate 2
12	0,273	0,273
24	0,277	0,268
42	0,268	0,252
10	0,287	0,147
54	0,286	0,274
58	0,282	0,282
68	0,298	0,282
43	0,294	0,286
27	0,270	0,258
49	0,274	0,288
Grand Mean	0,271	
Number of bottles, m	10	

S_{DD} Sum of Squares	Cochran Test	Cochran value
0,021	0,945	0,602

	Sum_i	$Difference_i$	D^2	$Vs = (Si-\bar{S})^2/(m-1)$
	0,546	0,000	0,00000	0,00000
	0,545	0,009	0,00008	0,00000
	0,520	0,016	0,00026	0,00005
	0,434	0,140	0,01960	0,00129
	0,560	0,012	0,00014	0,00004
	0,564	0,000	0,00000	0,00005
	0,580	0,016	0,00026	0,00016
	0,580	0,008	0,00006	0,00016
	0,528	0,012	0,00014	0,00002
	0,562	-0,014	0,00020	0,00004
$\sum$	**5,419**	0,199	**0,02074**	**0,00183**
$\bar{S}$	0,542			
$S_{an}^2 = \sum D^2/2m$	**0,001037**			
$S_{sam}^2 = (Vs/2-S_{an}^2)/2$	**0,0000000**			
F1	*1,88*			
F2	*1,01*			
σ-hat (%)	**15**			
$\sigma^2_{all} = (0,3\sigma_p)^2$	0,000149			
$c = F1\sigma^2_{all}+F2S_{an}^2$	0,001327			
$S_{sam}^2 < c$	passed			

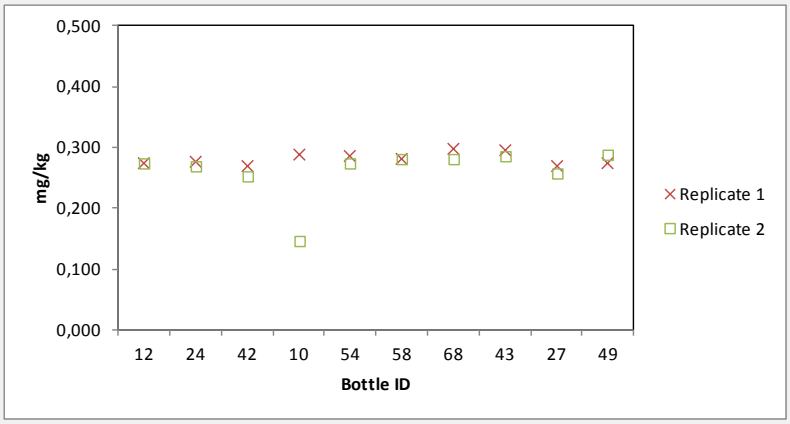
Cochran outlier test

Conclusion:
outliers!!

Homogeneity test

Conclusion:
material homogeneous

Visual appraisal of results:



Comment:
Bottle 10 was identified as an analytical outlier by the cochran test and excluded from the dataset.

Sample 4 – Blue mussels (after exclusion of bottle 10 as outlier)

Results of the homogeneity studies

Sample	sample 4 blue mussel	
Bottle ID	Replicate 1	Replicate 2
12	0,273	0,273
24	0,277	0,268
42	0,268	0,252
54	0,286	0,274
58	0,282	0,282
68	0,298	0,282
43	0,294	0,286
27	0,270	0,258
49	0,274	0,288
Grand Mean	0,277	
Number of bottles, m	9	

S_{DD} Sum of Squares	Cochran Test	Cochran value
0,001	0,224	0,602

	Sum_i	$Difference_i$	D^2	$Vs = (Si-\bar{S})^2/(m-1)$
	0,546	0,000	0,00000	0,00001
	0,545	0,009	0,00008	0,00001
	0,520	0,016	0,00026	0,00013
	0,560	0,012	0,00014	0,00000
	0,564	0,000	0,00000	0,00001
	0,580	0,016	0,00026	0,00008
	0,580	0,008	0,00006	0,00008
	0,528	0,012	0,00014	0,00007
	0,562	-0,014	0,00020	0,00001
$\sum$	**4,985**	0,059	**0,00114**	**0,00039**
$\bar{S}$	0,554			
$S_{an}^2 = \sum D^2/2m$	**0,000063**			
$S_{sam}^2 = (Vs/2-S_{an}^2)/2$	**0,0000663**			
F1	*1,94*			
F2	*1,11*			
σ-hat (%)	**15**			
$\sigma^2_{all} = (0,3\sigma_p)^2$	0,000155			
$c = F1\sigma^2_{all}+F2S_{an}^2$	0,000372			
$S_{sam}^2 < c$	passed			

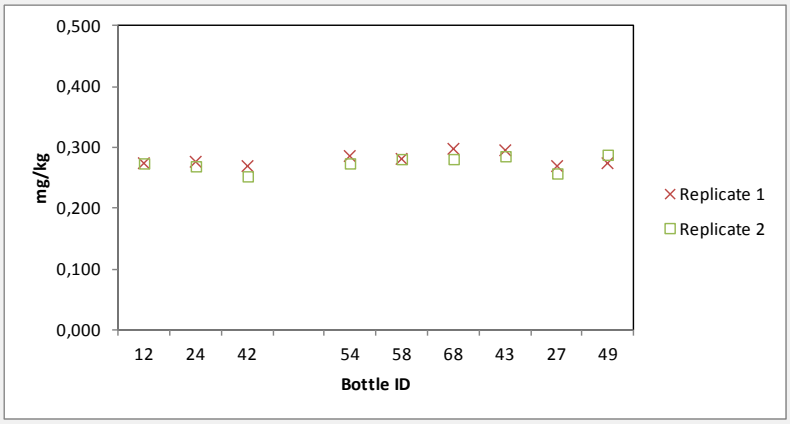
Cochran outlier test

Conclusion:
no analytical outliers

Homogeneity test

Conclusion:
material homogeneous

Visual appraisal of results:

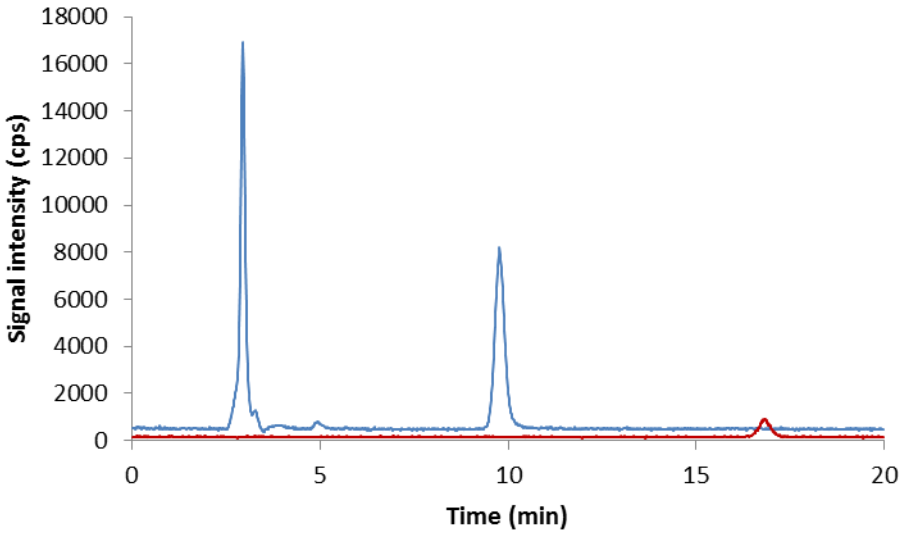


Comment:
Bottle 10 was identified as an analytical outlier by the cochran test and excluded from the dataset and the homogeneity re-assessed.

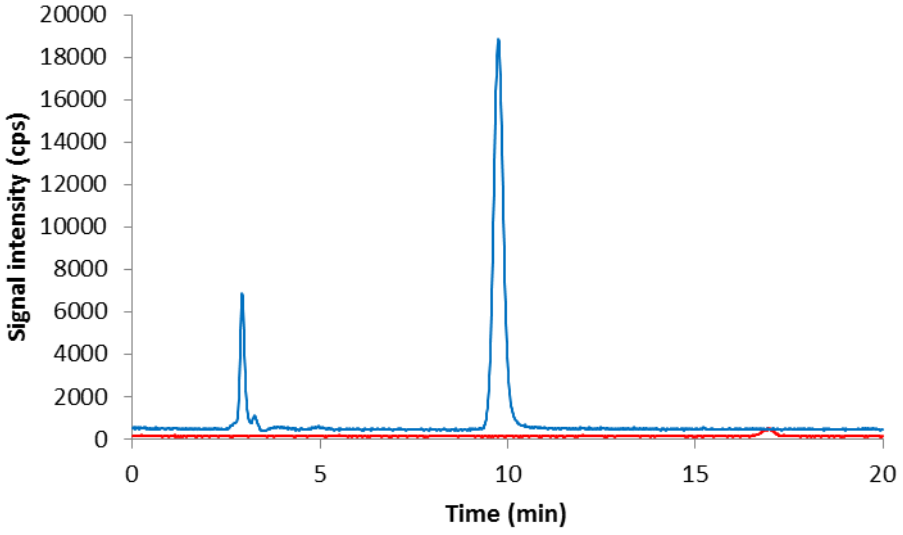
Annex 11 Examples of chromatograms

The following figures show typical chromatograms obtained from the analysis of the six sample materials included in the present study. The blue traces are for m/z 75 (As) and inorganic arsenic is eluting (as arsenate) at a retention time of approximately 9,5-10 min. The other peaks in the chromatograms represent organoarsenic compounds. The red traces are for m/z 35 (Cl) which have been recorded in order to illustrate that the chloride peak is well separated from iAs and potential interference from ArCl can be neglected.

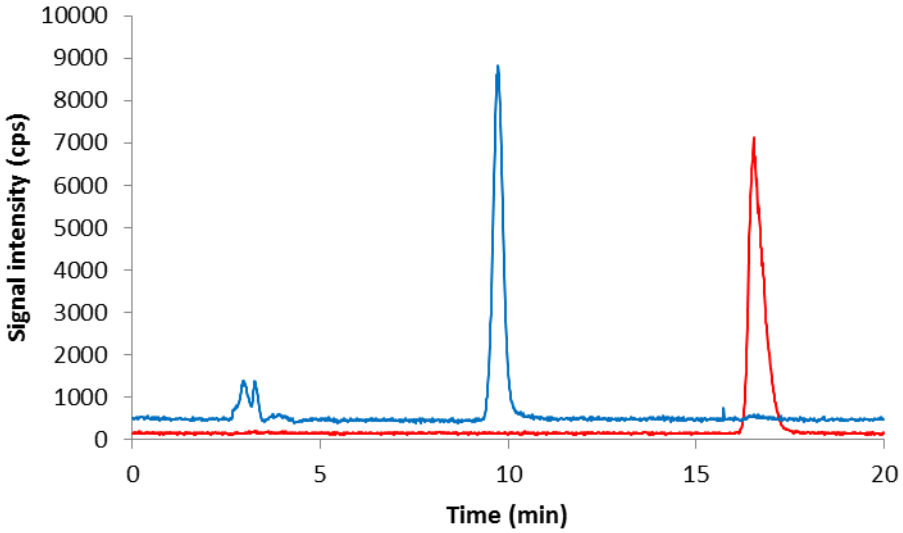
Sample 1 – White rice



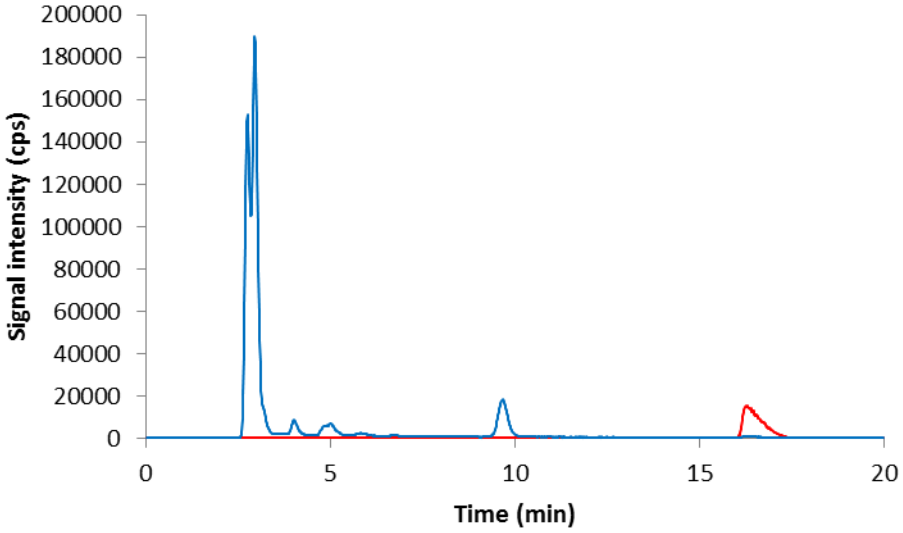
Sample 2 – Wholemeal rice



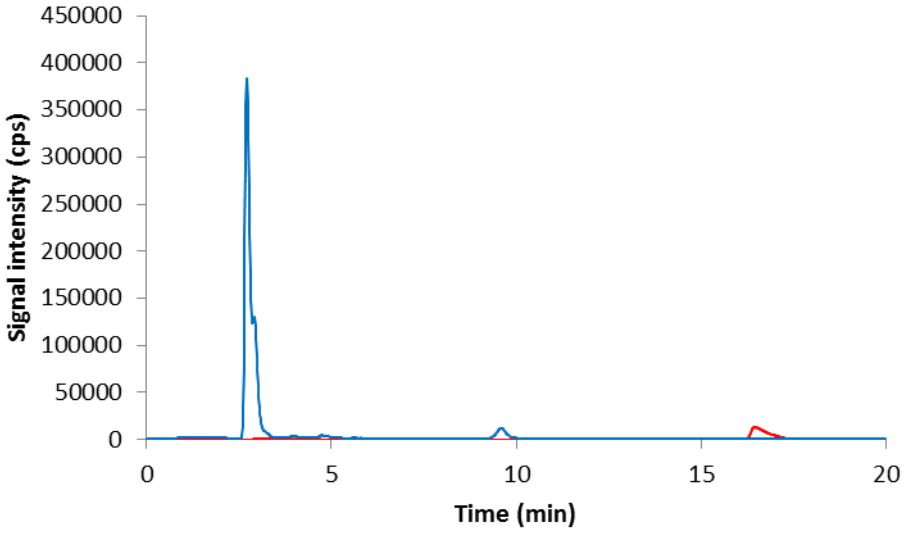
Sample 3 – Leek



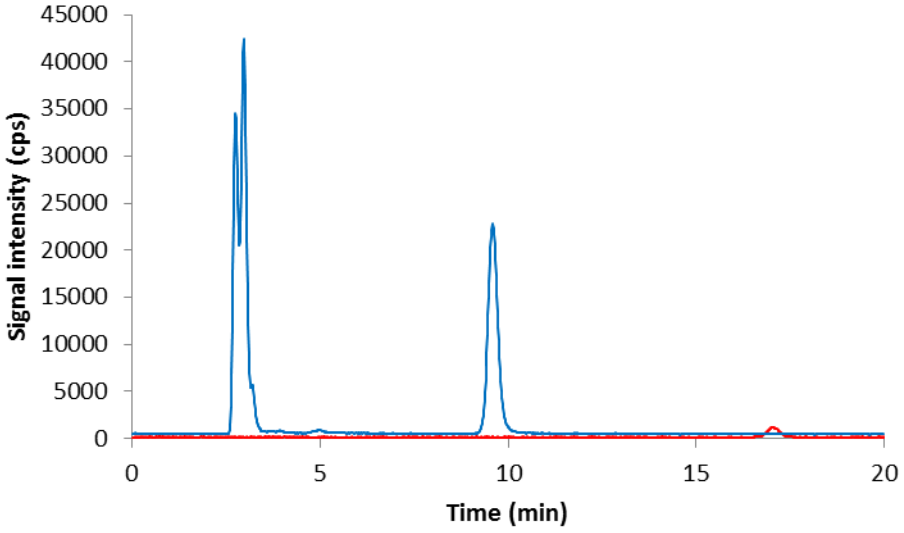
Sample 4 – Blue mussels



Sample 5 – Fish muscle



Sample 6 – Seaweed



Annex 12 Additional results

Two laboratories L03 and L08 send in two datasets. In their second dataset the method was varied in two different ways.

- L03 use of conventional oven heating instead of waterbath heating during extraction
- L08 use of hydride-generation (HG) coupled to ICPMS in the detection step

In both cases the obtained results were in fairly good agreement with the results obtained from the same lab using the present methodology and also in fairly good agreement with the mean value calculated from the collaborative trial.

L03a – use of conventional oven heating in the extraction step

	1	2	3	4	5	6
L03	0,074	0,492	0,092	0,337	0,283	12,0
	0,071	0,493	0,092	0,338	0,285	12,1
L03a	0,070	0,500	0,091	0,332	0,267	11,4
	0,070	0,498	0,093	0,331	0,267	11,6
Overall mean	0,073	0,470	0,086	0,327	0,275	10,3

L08a – use of HG-ICPMS in the detection step.

	1	2	3	4	5	6
L08	0,075	0,50	0,076	0,33	0,28	9,52
	0,070	0,49	0,078	0,32	0,27	9,51
L08a	0,073	0,40	0,082	0,40	0,38	9,85
	0,69	0,40	0,086	0,38	0,33	9,60
Overall mean	0,073	0,47	0,086	0,33	0,27	10,3

National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19
DK - 2860 Søborg

Tel. 35 88 70 00
Fax 35 88 70 01

www.food.dtu.dk

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