National Food Institute
Technical University of Denmark

Report on collaborative trial

Animal Feedingstuffs - Determination of inorganic arsenic in animal feed by anion-exchange HPLC-ICPMS

CEN/TC 327/WG4 Elements and their chemical species

Jens Jørgen Sloth
July 2018

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National Food Institute

Technical University of Denmark

Kemitorvet

Building 202

DK-2800 Kgs.Lyngby

Tel: +45 35 88 70 00

Collaborative trial report

Animal feedingstuffs – Determination of inorganic arsenic in animal feed anion-exchange HPLC-ICPMS
Mandate: 523
Project responsible: CEN TC327
Secretariat: NEN (organisational work on behalf of CEN TC327)
Work item: Inorganic arsenic in animal feedingstuffs
Project leader: Dr. Jens J. Sloth, National Food Institute DTU, Denmark

Content

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

1. Introduction

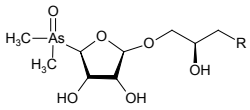
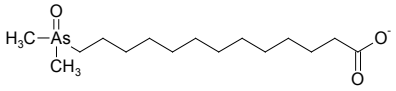
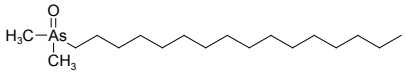
Arsenic is a metalloid that occurs in different inorganic and organic forms, which are found in the environment from both natural and anthropogenic sources. Until now more than 100 different naturally occurring arsenic compounds have been identified, primarily in samples of marine origin (Petursdottir, 2014). 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 animal feed 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 considered to be more toxic as compared to most of the organic arsenic compounds. Two toxicological evaluations related to dietary exposure to inorganic arsenic 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). In 2016 EU established maximum levels for inorganic arsenic in rice and rice-based products (EU Comm, 2016) and an analytical method for the specific determination of inorganic arsenic in food of marine and plant origin was published (EN16802-2016). In the feed legislation no maximum levels have currently been established for inorganic arsenic (EU Comm, 2002). However, in a footnote it is stated that "*Upon request of the competent authorities, the responsible operator must perform an analysis to demonstrate that the content of inorganic arsenic is lower than 2 ppm*". The method, which has been validated in the present study would support this.

2. Project background and time frame

In 2013 a tender for a project with the aim to develop a European standard method for the determination of inorganic arsenic in animal feed NEN on behalf of CEN TC327 was opened. Of the proposed projects, DTU Food (with Dr Jens J. Sloth as project leader) was selected. A service contract between NEN and DTU Food was signed in 2016 and the project official start date was 01-01-2016. The method was developed and validated at DTU Food during the period 2016-2018 and the method principle was based on the same analytical principle and procedure as EN16802:2016 as discussed and agreed upon in the CEN TC327/WG4 Elements and their chemical species. A collaborative trial was conducted in 2018 with participants from 12 different laboratories from 10 different countries. The results from the collaborative trial were presented and discussed by the project leader at the CEN TC327/WG4 meeting in Berlin 29. May 2018 and comments were

received from the expert group members of WG4. Following the meeting, the project leader prepared this report taken into account their comments.

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 and aim

The aim of the present project was to develop and validate a new European standard method (EN) for the determination of inorganic arsenic in animal feedingstuffs. The method should be based on HPLC-ICPMS and follow the same analytical principles as EN16802:2016. For validation a full collaborative trial should be conducted to evaluate the performance characteristics of the method.

4. Sample materials – preparation and homogeneity testing

Several sample materials were evaluated as candidate test materials for the collaborative trial. The samples included several (certified) reference materials and inter-laboratory comparison test materials from previous studies, for which suitable homogeneity already had been verified by the supplier/former project(s) (s2 (NMIJ), s3 (AGES), s4 and s6 (IMEP32), and s7(FAPAS)). For three of these materials (s2, s4 and s6) target values for inorganic arsenic were already established and these values were used to evaluate the accuracy of the present methodology. Furthermore, a synthetic solution containing the arsenic compounds AsV, MA and AB in dilute HCl aqueous solution was prepared in-house and included in the study. Table 2 provides an overview of the sample materials selected for the collaborative trial with the target concentration ranges for iAs and the approximate total arsenic concentration.

Samples s2, s3, s4 and s6 were rebottled in small white plastic containers in order not to reveal the identity of the samples to the participants. Sample s8 consisted on a liquid transparent solution and was transferred to 15 mL plastic tubes. For samples s1, s5 and s7 homogeneity tests have been performed (annex 10). For sample s5 the homogeneity test was approved in the statistical test although two clear outliers can be visually identified (bottles 12 and 14) and may have been caused by contamination in the analytical procedure. Since the results from the collaborative trial were acceptable the material was accepted for the collaborative trial. Samples s1 and s7 were obtained as fine powders and s5 as fine cut grass from the producers and the sample materials were bottled in small white plastic containers. All bottles were clearly labelled and numbered chronologically according to the bottling order.

Table 2 Overview of sample material for the collaborative trial, their expected iAs and total As concentration.

Sample ID	**Sample material**	**Producer/origin**	**Total iAs (mg/kg)**	**Total As (mg/kg)**
CENFEED-iAs-S1	Rice Meal *(Wholemeal Based)*	Retail sample purchased in local supermarked	0.1-0.5	0.18[I]
CENFEED-iAs-S2	Hijiki Seaweed *(NMIJ CRM 7405A)*	National Metrological Institute of Japan (NMIJ)	10.1 +/- 0.5 (certified value)	35.8+/-0.9[II]
CENFEED-iAs-S3	Mineral feed *(Mineral feed material)*	ALVA 16/1 Mineralfutter (Provided by AGES, Austria)	0.5-2	1.41+/-0.107[II]
CENFEED-iAs-S4	Fish meal *(marine-based ingredient)*	IMEP32-7 fish meal	0.432 +/- 0,066 (target value from ILC)	3.03[I]
CENFEED-iAs-S5	Grass meal *(Plant-based ingredient)*	Sächsisches Staatsministerium für Umwelt und Landwirtschaft (SMUL), Germany	1-5	2.85[I]
CENFEED-iAs-S6	Complete Feed 1 *(Marine based)*	IMEP32-2 fish feed	0.713 +/- 0,117 (target value from ILC)	5.93[I]
CENFEED-iAs-S7	Complete Feed 2 *(Cereal based)*	FAPAS T07299QC (Proficiency test material)	0.05-0.2	0.303[II]
CENFEED-iAs-S8	Aqueous Standard *(in-house prepared)*	Mix of AB, MA and AsV – in dilute HCl solution	0.05 µg/L *(target value based on production of solution)*	0.17 µg/L [I]

I: Data from analysis at DTU Food

II: Reference value provided by sample producer (+/- sd)

5. Participant invitation and information

The method was tested in a collaborative trial with 12 participating laboratories from 10 different countries (Belgium, Denmark, Germany, The Netherlands, Poland, Norway, United Kingdom, Iceland, Austria, and Czech Republic,). 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 21th of March 2018 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 April-May 2018.

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) as reported by the participants can be found in annex 6. Various types of ICPMS instruments have been used from various instrument companies, including Agilent, Thermo, Perkin Elmer and Varian. Three different anion-exchange columns have been used: IonPac AS7 (Dionex/Thermo-Fischer), PRP X-

100 (Hamilton) and column for As speciation in drinking water (Agilent). A wide range of different injection volumes are reported from 10 -100 µL as well as variation in mobile phase concentration (5.2 – 180 mM) and flow rates 0.1 – 1.2 ml/min. The variations in these parameters indicate that the method is robust and various choices with regards to chromatographic set-up can be used successfully depending on the choice of column (incl column dimensions) and mobile phase (incl concentration and flow rate). In annex 11 examples of chromatograms for the eight different sample types (sample s1-s8) can be found.

7.2. Laboratories compliance

Thirteen laboratories signed up to participate in the collaborative trial. One laboratory did not report results due to instrumental problems (reported by the laboratory). The laboratories also filled in a questionnaire regarding the analysis and some laboratories reported deviations from the method procedure. In Table 3 a list of non-compliant laboratories can be found and the rationale for this judgement.

Table 3 List of non-compliant laboratories

Non-compliant lab	**Reason**
L13	Did not submit results.

The results from the non-compliant laboratory were excluded from the statistical analysis of the data from the collaborative trial.

7.3. Outlier identification

Following the initial identification of non-compliant laboratories, results from the 12 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 (samples s4 and s8), three Cochran stragglers (samples s1, s2 and s7) and one Grubbs straggler (sample s8). For samples s3, s5 and s6 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
s1	Rice Meal	L02	Cochran straggler
s2	Seaweed Meal	L02	Cochran straggler
s3	Mineral Feed	-	No outliers
s4	Fish Meal	L12	Cochran outlier
s5	Plant Based	-	No outliers
s6	Complete Feed 1 (Marine)	-	No outliers
s7	Complete Feed 2 (Vegetable)	L08	Cochran straggler
s8	Aqueous standard	L12	Cochran outlier
		L12	Grubbs straggler

In all cases the number of outliers was below the threshold recommended by the AOAC guideline, where a maximum outlier rate of 2/9 is established (AOAC, 1995).

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 percentage of identified and excluded outliers
- The overall mean, X_{obs} (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) (ISO3534-1;AOAC 1995)
- The Horwitz value was calculated 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 6.

The relative standard deviation under repeatability conditions (within-laboratory), RSD_r was in the range from 3.7 – 11.9 % and the relative standard deviation under reproducibility conditions (between-laboratory), RSD_R was in the range 10.7 – 24.3 %. These values are very satisfactory and indicate that the method has a satisfactory precision.

For evaluation of the accuracy of the method, the overall means of samples s2, s4, s6 and s8 were compared with target values (Table 5). The obtained results were in good agreement with target values and recoveries from 96-112% were achieved.

Table 5 Accuracy evaluation. Overall mean values compared with target values.

No	**Type**	**Target value (mg/kg)**	**This ILC (mg/kg)**	**Recovery (%)**
s2	Seaweed meal	10.1 +/- 0.5 *(certified)*	9.69	96
s4	Fish meal	0.432 +/- 0.066 *(ILC mean value)*	0.45	104
s6	Fish feed	0.713 +/- 0.117 *(ILC mean value)*	0.80	112
s8	Standard solution	50 µg/L *(target value)*	50.1 µg/L	100

The method working range was established in the concentration range 0.149 mg/kg to 9.69 mg/kg. The RSD_r and RSD_R values for sample 1 (Rice meal) with the lowest mean value 0.149 mg/kg were 3.7 % and 13.4 %, 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 ranged from 0.04 – 1.0 µg/L. which corresponds to LODs in the samples in the range from 0.002 – 0.050 mg/kg (assuming 0.20 gram sample intake and extraction volume of 10 mL). HorRat values in the range of 0.49-1.56 were obtained. which is very satisfactory and all below the guideline value of 2.

Table 6 Method performance characteristics from the collaborative trial.

		CENFEEDiAs–S1	CENFEEDiAs–S2	CENFEEDiAs–S3	CENFEEDiAs–S4	CENFEEDiAs–S5	CENFEEDiAs–S6	CENFEEDiAs–S7	CENFEEDiAs–S8
		Rice meal	Seaweed meal	Mineral Feed	Fish Meal	Grass meal	Comp feed marine	Comp feed veg	Standard mix
No of labs		12	12	12	12	12	12	12	12
No of Cochran stragglers		1	1	0	0	0	0	0	0
No of Cochran outliers		0	0	0	1	0	0	0	1
No of Grubbs stragglers		0	0	0	0	0	0	1	1
No of Grubbs outliers		0	0	0	0	0	0	0	0
No of valid labs		12	12	12	11	12	12	12	11
Outlier percentage	%	0	0	0	9,1	0	0	0	9,1
Overall mean	mg kg^{-1}	0,149	9,69	0,975	0,450	1,76	0,802	0,312	50,1*
u_{obs}	mg kg^{-1}	0,020	1,56	0,232	0,107	0,40	0,119	0,048	5,3*
Sr	mg kg^{-1}	0,006	0,403	0,052	0,012	0,209	0,024	0,012	1,81*
RSDr	%	3,7	4,2	5,3	2,7	11,9	2,9	3,9	3,6
rL	mg kg^{-1}	0,015	1,13	0,145	0,034	0,586	0,066	0,034	5,08*
SR	mg kg^{-1}	0,020	1,60	0,237	0,109	0,403	0,122	0,049	5,37*
RSDR	%	13,4	16,5	24,3	24,3	22,9	15,2	15,5	10,7
RL	mg kg^{-1}	0,056	4,47	0,664	0,306	1,127	0,341	0,136	15,04*
Horwitz value (Thompson)		21,3	11,4	16,1	18,0	14,7	16,5	19,1	22,0
HorRat value (Thompson)		0,63	1,45	1,52	1,35	1,56	0,92	0,82	0,49

* *concentration is in µg/L*

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:

7.6. Comments related to sample 3 mineral mix

Sample 3 is a mineral feed material. The results obtained from the study indicate that the extraction procedure used does not extract iAs quantitatively from the sample matrix. The overall mean value corresponds approximately to 69% of the total arsenic content. The chromatograms obtained (example provided in appendix 11) show only one peak, indicating that all arsenic is present as inorganic arsenic and no organoarsenic compounds are present, which intuitively also should be the case in a mineral feed material, which is all inorganic in composition.

Consequently, it was decided to exclude mineral feed materials from the scope of the method. This decision has been supported by the members of CEN TC327WG4 and at the TC327 plenary meeting in June 2017.

It is recommended to perform total arsenic analysis in these sample types to achieve a correct result. The total arsenic result is equal to the total inorganic concentration in such sample types.

7.7. Additional results

In annex 12 three datasets, obtained by the same laboratory (L03) on the same instrument (8900 ICP-QQQ-MS from Agilent technologies), but operated in three different instrumental modes, are provided. The instrument used was an run in:

- Mass shift mode (O_2 as cell gas)
- No gas mode (no cell gas)
- He mode (He as cell gas)

In all 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, indicating.

8. Conclusion

A method for the determination of inorganic arsenic in animal feedingstuffs was developed at DTU Food and validated in a collaborative trial. 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 12 participating laboratories on seven different food samples within the concentration range of 0.149 – 9.69 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 animal feed samples with the exception of mineral feed materials.

9. Acknowledgements

Dr. Rie R. Rasmussen (DTU Food) has been an immense help with the practical work on the method development. Mrs Birgitte Koch Herbst and Mrs Annette Landin (both DTU Food) have 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. Mr Ralf Klose from SMUL in Germany is thanked for provision of test material s5 (grass meal). Very important contributors are all the participating laboratories, who are thanked for their voluntary participation in the collaborative trial for their production of good results and their useful comments on the method procedure. Finally big thanks go also to the convenor, secretary and expert members of the CEN TC327/WG4 expert group for their constructive comments and encouragement during the project period.

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Annex 1. Invitation letter to collaborative trial

CEN / TC 327 `Animal Feedingstuffs:
Working group 4 'Elements and their chemical species'

Invitation to participate in a collaborative trial

Method: Determination of inorganic arsenic in animal feed by anion-exchange HPLC-ICPMS following waterbath extraction

Søborg. March 2018

Dear colleague.

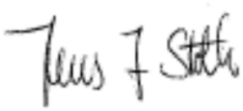
You are hereby invited to participate in a collaborative study of a method for the determination of inorganic arsenic in animal feed. The method principles are based on waterbath extraction followed by selective determination of inorganic arsenic by anion-exchange HPLC-ICPMS.

The participants will be supplied with the following:

- Method procedure (to be followed strictly)
- 7 feed samples to be analysed (in duplicate))
- 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 feed control. Further information can be found in the following pages. Please fill in and send the registration form. Your efforts are very much appreciated thanks in advance.

Best regards.



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 animal feed. The proposed method approach has been discussed and agreed upon in the CEN/TC327/WG4 group and The National Food Institute at the Technical University in Denmark has been appointed to organize this collaborative trial. The method principles are the same as for EN16802:2016 – Determination of inorganic arsenic in foodstuffs of marine and plant origin.

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:

Seven feed samples and one solution with unknown concentrations will be sent to the participating laboratories. The sample materials shall be analyzed in duplicate and the results reported to the organizer of the collaborative trial. 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 – **16. March 2018**
- Estimated time for dispatch of samples – **week 12 (i.e. 19-23/3-2018)**
- Deadline for submission of results and questionnaire: **4. May 2018.**
- Discussion of results will subsequently take place in CEN TC327/WG4 - **during 2018-19**
- A report on the collaborative trial will be made and made public available – **during 2018**
- Publication of CEN method – **expected in 2019**
- 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 93518857
National Food Institute
Kemitorvet B201. 128
DK-2800 KGS Lyngby
Denmark

Registration form - Collaborative study:

Animal feedingstuffs - Determination of inorganic arsenic in animal feed by anion-exchange HPLC-ICPMS following waterbath extraction

Name of contact person	
E-Mail adress	
Organisation	
Shipping address	
City and postal zip code	
Country	
Comments?:	

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

Deadline 16. March 2018

Annex 2 Accompanying letter to participants

To the participants of the
collaborative trial on inorganic arsenic
in animal feed by ICPMS

March 2018
/jjsl

CEN TC327/WG4 Collaborative trial on the determination of inorganic arsenic in animal feed by ICPMS

Dear participant.

Thank you for participating in the collaborative trial on the determination of inorganic arsenic in animal feed. The aim of the project is to establish a European standard for the analysis of inorganic arsenic in animal feedingstuffs. 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 on page 2 in this letter carefully prior to starting with the analysis.

The deadline for submission of results is Friday 04/05/2018

If there are any questions don´t hesitate to contact:

Jens J. Sloth: email: jjsl@food.dtu.dk or phone +45 93518857

or

Manuel Correia: email: manco@food.dtu.dk or phone +45 35887614

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

Best regards.

Jens J. Sloth

INSTRUCTIONS – Collaborative trial on inorganic arsenic in feed by ICPMS

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

A: Sample materials

Seven different sample materials and one bottle with a standard solution are included in the study. The recommended test portion sizes to be used for the analysis are also provided in the table as well as the expected concentration level of iAs in the samples.

Sample ID	Sample type	Sample amount (g/bottle)	Test portion size for analysis (g)	Conc. Range (mg/kg)
CENFEED-iAs – S1	Rice	5	0.3	0.1-0.5
CENFEED-iAs – S2	Seaweed	1.5	0.2	> 3
CENFEED-iAs – S3	Mineral feed	3	0.3	0.5-2
CENFEED-iAs – S4	Fish meal	3	0.3	0.1-0.5
CENFEED-iAs – S5	Plantbased	3	0.3	1-5
CENFEED-iAs – S6	Complete feed (marine)	3	0.3	0.5-1
CENFEED-iAs – S7	Complete feed (vegetable)	2	0.5	0.05-0.02
CENFEED-iAs – S8	Aqueous standard	2 ml/tube	Dilute 10 times with extraction solvent (4.4) prior to analysis	N/A

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 and manco@food.dtu.dk.**

B: Analysis of samples

For the collaborative study please perform two independent measurements of each sample on the same day using one of the bottles and remember to follow the method procedure provided carefully.
NOTE: 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.

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 (CENFEED-iAs – S8)

The standard solution (CENFEED-iAs – S8) 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 and manco@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 (S1-S7) and for one of the diluted standard solution (S8).

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 and manco@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 1-8.
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 **Manuel Correia (manco@food.dtu.dk)**

Results scheme

CEN TC327/WG4 Collaborative trial on determination of inorganic arsenic in animal feed by ICPMS

Laboratory:

_____

Date of extraction: _____
Date of analysis: _____

Results

Sample	Bottle no	Sample intake (g)	Result replicate 1 (mg kg^{-1})	Result replicate 2 (mg kg^{-1})
CENFEED-iAs – S1				
CENFEED-iAs – S2				
CENFEED-iAs – S3				
CENFEED-iAs – S4				
CENFEED-iAs – S5				
CENFEED-iAs – S6				
CENFEED-iAs – S7				

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

CENFEED-iAs – S8			µg/L	µg/L
Procedural blank			µg/L	µg/L

Please send copies of the chromatograms of each of the samples S1-S8.

Deadline for submission of results: **Friday 04/05/2018**
Remember to fill in the questionnaire.

Please send to: jjsl@food.dtu.dk and manco@food.dtu.dk

Annex 4. Questionnaire

CEN TC327/WG4 Collaborative trial on the determination of inorganic arsenic in animal feed by ICPMS

Please complete this questionnaire.

Laboratory name: _____

1. Method related questions

1.1 Which equipment did you use?

HPLC:

_____

ICPMS:

_____

Column type:

_____

Column characteristics (length. i.d.. particle size):

_____

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?

CENFEED-iAs-S1:

_____

CENFEED-iAs-S2:

_____

CENFEED-iAs-S3:

_____

CENFEED-iAs-S4:

_____

CENFEED-iAs-S5:

_____

CENFEED-iAs-S6:

_____

CENFEED-iAs-S7:

_____

CENFEED-iAs-S8:

_____

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 !!)**:
Please also report any other relevant observations here:

_____

_____

_____

_____

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?**

For feed: O No O Yes
For food: 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 and manco@food.dtu.dk together with the results of the analysis.
Thanks for your time ☺

Annex 5. List of participating laboratories

	Lab	Country	Contact person
1	FAVV-FLVVG	Belgium	Inge van Hauteghem
2	DTU Food	Denmark	Jens J Sloth
3	BVL - Fed Off for Consumer Protection and Food Safety	Germany	Timo Kapp
4	RIKILT	The Netherlands	Hanneke Brust
5	National Veterinary Research Institute	Poland	Agnieszka Nawrocka
6	Institute of Marine Research	Norway	Heidi Amlund
7	The State Laboratory	Ireland	Niamh Fitzgerald
8	University of Aberdeen	UK	Andrea Raab/Jörg Feldmann
9	Mátis	Iceland	Asta Petursdottir
10	AGES	Austria	Gerhard Liftinger
11	NRL – National Reference Laboratory	Czech Republic	Eva Cizmarova
12	Sciensano	Belgium	Ann Ruttens
13	FVST - Danish Food Administration	Denmark	Inge Rokkjær

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	HPLC	ICPMS	Column	Length (mm)	i.d. (mm)	particle size (µm)	Temp (°C)	Inj vol (µL)	Mobile phase conc (mM)	Flow rate (mL/min)	Calibration range (µg/L)		LoD (µg/L)
											Min	Max	
1	Agilent 1260 Infinity II	Agilent 7900	IonPac AS7	250	2.0		room	10	50	0.3	1	50	0.04
2	Agilent 1200	Agilent 7500ce	Hamilton PRP X100	250	4.6	10	20	100	30	1	0.6	60	0.05
3	Agilent 1260 Infinity	Agilent 8800	Hamilton PRP X100	150	4.1	10	40	20	25	1.1	0.5	5	0.20
4	Agilent 1200	Agilent 7500ce	Agilent column for As speciation in drinking water	150	4.6	0	25	25	8	1	0	100	0.3-0.4
5	Perkin Elmer Flexar HPLC	Perkin Elmer NexION 300D	Hamilton PRP X100	250	4.1	10	room	100	30	1.2	0.5	50	0.30
6	Agilent 1200	Agilent 7500i	IonPac As7	250	2.0	0	20	10	50	0.16	0	50	1.00
7	Agilent 1100	Agilent 7500ce	IonPac As7	250	0.0	0	room	10	50	0.1	0.2	30	0.08
8	Flexar PerkinElmer	DRCe PerkinElmer	Hamilton PRP X100	150	4.6	5	35	60	50	1	0.5	14	0.25
9	Varian Prostar	Varian 820 MS	Hamilton PRP X100	250	4.6	10	30	60	60	1	2	50	0.30
10	Perkin Elmer Flexar	PerkinElmer DRCe	Hamilton PRP X100	250	4.6	10	25	50	5.2-1117 (gradient)	1	0.5	20	0.08
11	Agilent 1220	Agilent 7700x	Hamilton PRP X100	250	4.6	10	room	50	50	0.9	0.5	100	0.50
12	Agilent 1290 Infinity	Agilent 8800	Supelco PRP X100	250	4.1	10	20	40	33.3	1	0.1	100	0.015 (from total arsenic in extract sol.)

Annex 7. Overview of reported comments by the participants

Lab no	Comment
L01	1) It seems we have misinterpreted this information "…perform two independent measurements of each sample on the same day …". We have weighed in and analysed two parallels on each sample. The weights are included in the results sheet (replicate 1/replicate2). 2) For sample S7 approx. 0.3g, and not 0.5 g sample were weighed in.
L02	Samples were homogenised in the vials they came in by thoroughly shaking and mixing with single use spatula – except for S3. The particle size was larger, the sample was fully transferred to an acid clean mortar and crushed with pestle, however, since no visible difference was a subsample (from the thoroughly mixed sample in the mortar) was placed in an analytical grinder to test if the particle size could be smaller. Since there was no difference the sample was transferred back from the mortar to the original vial (the sample from the grinder was discarded, as the procedure is to always throw away the first portion of sample being ground to minimise risk of contamination) and the samples weighed from the original vial in the original sample size (not a fine powder). Vials were closed when put in the water bath. There was pressure build-up in the vials, where s3 vials popped open after approx. 10 minutes. All vials were loosened a bit at that time point to prevent pressure build-up. After the extraction s3 had lost a significant amount of sample solution during the extraction and was prepared again (all others had similar weights before and after extraction).
L02	The samples were first measured on the 11th of April, but there were problems with the standards, hence the samples were measured again on the 16th. On the 11th of April S4 showed a single peak. On the 16th of April the peak indicated a co-elution of two compounds, this difference was probably due to small difference in the pH and/or concentration of the mobile phase. A spiking experiment was done (with AsV), which indicated that the peak in front was the iAs (but they were heavily co-eluting, hence some of the spike showed up in the latter peak as well). A similar trend was seen in Petursdottir et al 2012 (HPLC-HG-ICP-MS: a sensitive and selective method for inorganic arsenic in seafood) also for fish meal where the peak in front was the iAs (similar column conditions). Here we report the value of the first peak, the peak shape is not good where the iAs appears to be a shoulder on a nicer peak. The quantification may not be as accurate as it could be. If the shoulder and peak are considered the same compound then the concentration would be: 0.445 and 0.445 mg / kg for S4.
L04	Concerning the extraction procedure, we have used the shaking waterbath. In the draft standard was not clearly mentioned_whether the samples should be shaked or not during the 1 hour of extraction. We understand the meaning "to be extracted" while shaking (mixing) the samples in the waterbath because this is the way how we normally extract samples using waterbath.
L05	The mobile phase was 30 mM ammonium carbonate in 2% methanol at pH 9.2. This is the standard mobile phase we use for arsenic speciation. I did try the mobile phase as described in the method description, but the separation of the different compounds was not sufficient. Centrifugation was not performed at 4000 rpm, but at 3500 rpm, as that is the maximum rpm for our centrifuge.
L09	We did not add methanol in the mobile phase
L11	To prevent the plasma fluktuations we used 1% MetOH in the mobile phase

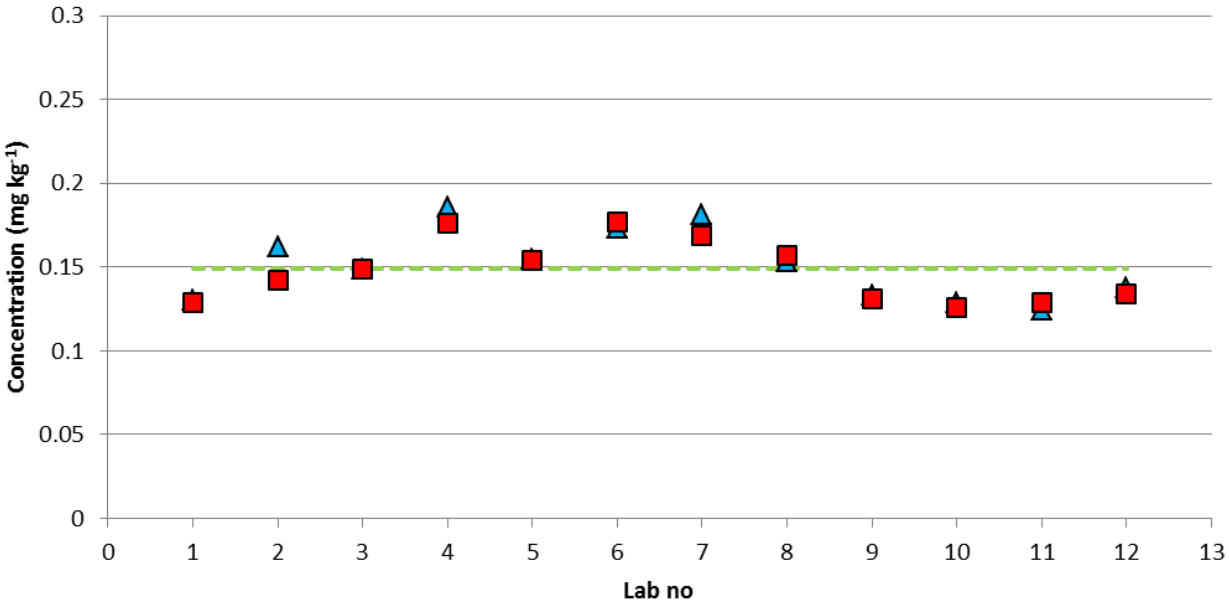
Annex 8 Results reported by the participating laboratories

Lab	S1		S2		S3		S4		S5		S6		S7		S8			
No	Rice Meal (mg kg^{-1})		Seaweed Meal (mg kg^{-1})		Mineral Feed (mg kg^{-1})		Fish Meal (mg kg^{-1})		Plant Based (mg kg^{-1})		Comp. Feed 1 (Marine) (mg kg^{-1})		Comp. Feed 2 (Vegetable) (mg kg^{-1})		Aqueous Standard (µg L^{-1})		Procedural Blank (µg L^{-1})	
1	0.130	0.129	8.73	8.69	0.606	0.615	0.397	0.403	1.25	1.35	0.691	0.7	0.304	0.304	49.4	49.8	0.1	0.1
2	0.162	0.142	10.16	11.68	0.81	0.938	0.237	0.201	1.41	1.43	0.789	0.752	0.343	0.347	36.5	37.5	0.1	0.1
3	0.149	0.149	9.439	9.360	1.069	1.069	0.484	0.491	1.419	1.684	0.764	0.834	0.331	0.325	51.2	49.5	0.0	0.0
4	0.186	0.176	7.396	7.422	1.106	1.092	0.416	0.402	1.282	1.534	0.825	0.760	0.238	0.218	47.3	48.2	6.9	6.9
5	0.16	0.15	10.70	11.00	1.06	1.03	0.58	0.56	2.23	1.88	0.85	0.84	0.34	0.35	50.4	49.5	0.1	0.0
6	0.173	0.177	10.300	10.200	0.805	0.898	0.486	0.515	1.630	1.820	0.765	0.804	0.353	0.324	49.8	50.0	1.1	1.4
7	0.181	0.169	9.789	9.875	0.891	0.851	0.517	0.514	1.559	1.533	0.870	0.867	0.349	0.351	59.9	55.1	0.1	-0.1
8	0.153	0.157	9.626	10.111	1.196	1.329	0.583	0.593	2.886	2.148	0.953	0.962	0.404	0.359	54.2	47.8	0.5	0.1
9	0.133	0.131	9.901	10.020	1.055	1.060	0.476	0.472	1.877	1.656	0.789	0.802	0.313	0.320	48.4	49.2	< 0.3	< 0.3
10	0.129	0.126	9.713	9.708	1.063	1.106	0.47	0.459	1.609	1.564	0.742	0.762	0.297	0.307	53.9	54.8	-0.13	-0.13
11	0.124	0.129	6.739	6.683	0.553	0.55	0.32	0.312	1.973	1.823	0.549	0.53	0.224	0.224	55.1	53.9	< 0.5	< 0.5
12	0.138	0.134	12.1	13.2	1.394	1.265	0.56	0.479	2.12	2.48	1.01	1.02	0.281	0.291	69.6	86.6	0.019	0.036
13*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*L13 did not report results

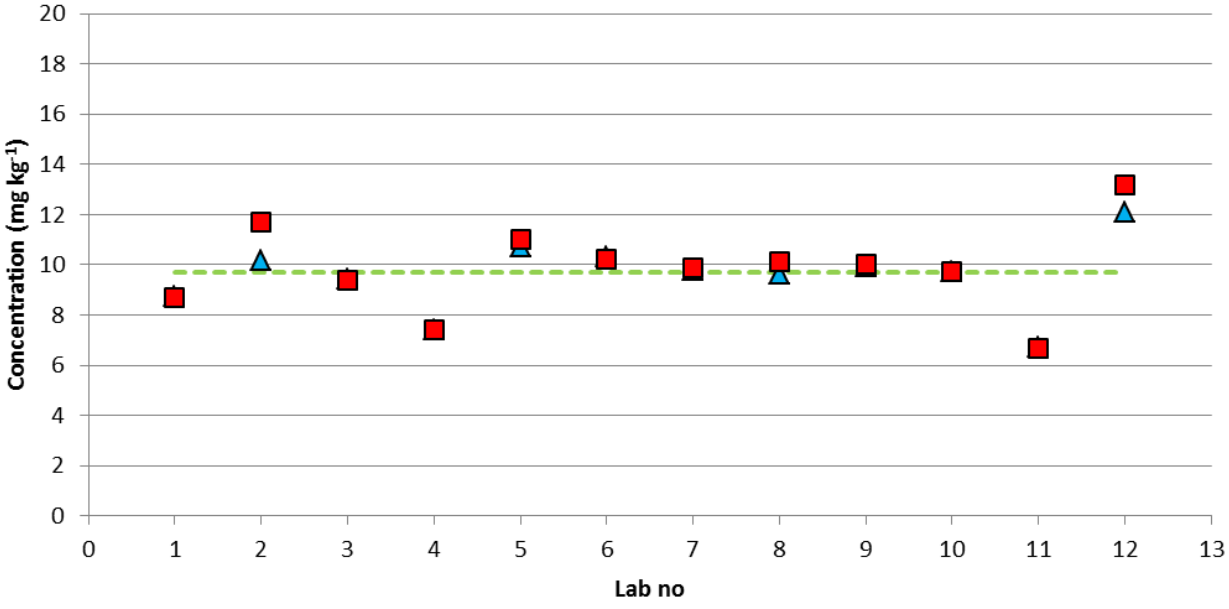
Annex 9 Plots of results from compliant laboratories

Sample s1 Rice Meal (mean value +/- u_{obs} = 0.149 + /- 0.02 mg/kg)



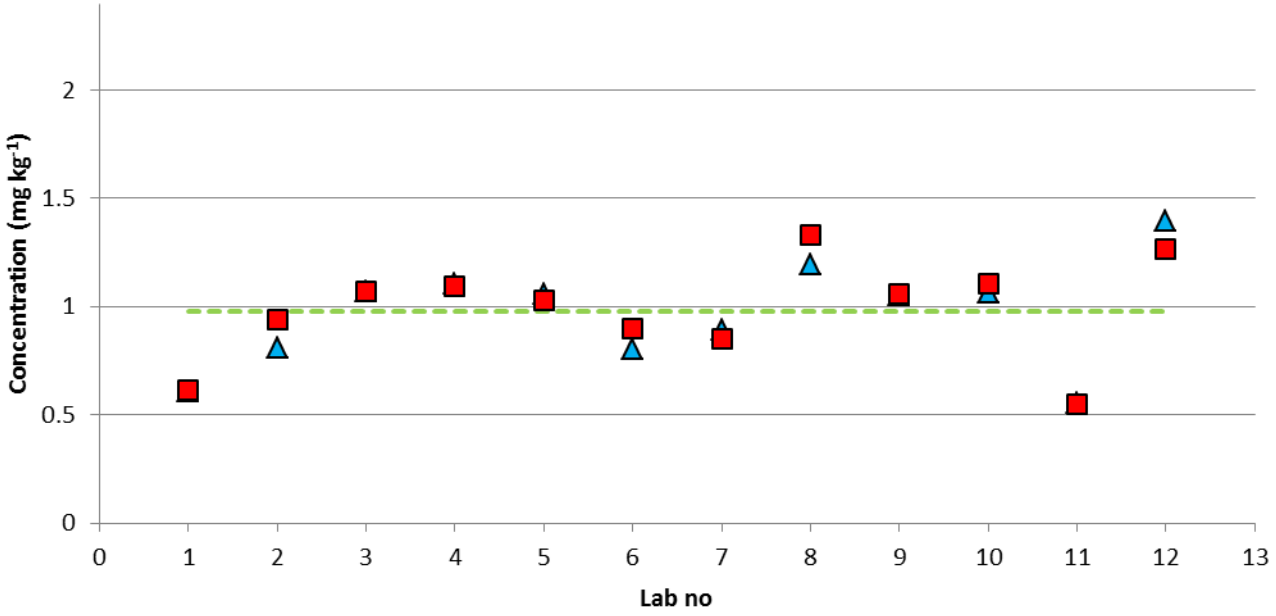
L02 is a Cochran straggler

Sample s2 Seaweed Meal (mean value +/- u_{obs} = 9.69 +/- 1.57 mg/kg)



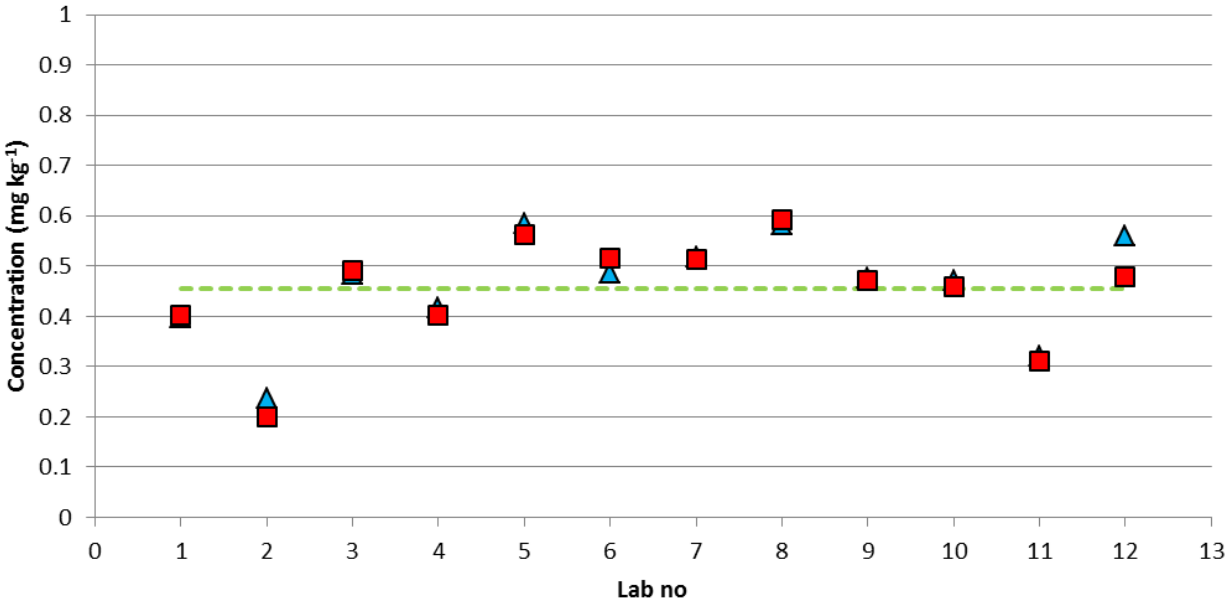
L02 is a Cochran straggler

Sample s3 Mineral Feed (mean value +/- u_{obs} = 0.975 +/- 0.232 mg/kg)



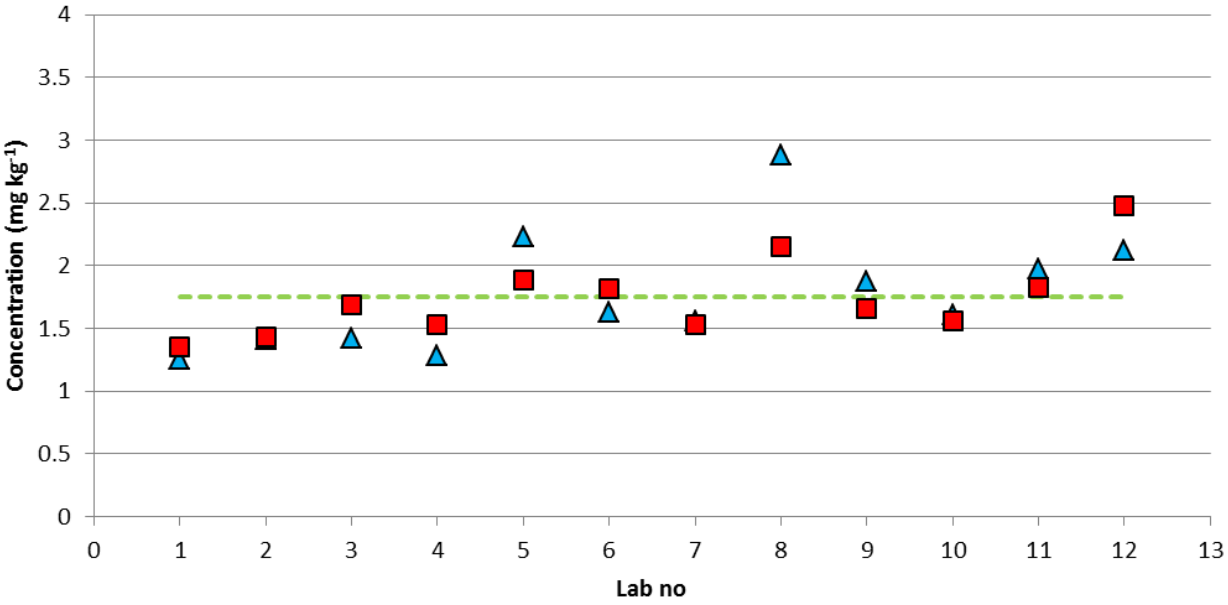
No outliers/stragglers

Sample s4 Fish Meal (mean value +/- u_{obs} = 0.456 +/- 0.105 mg/kg)



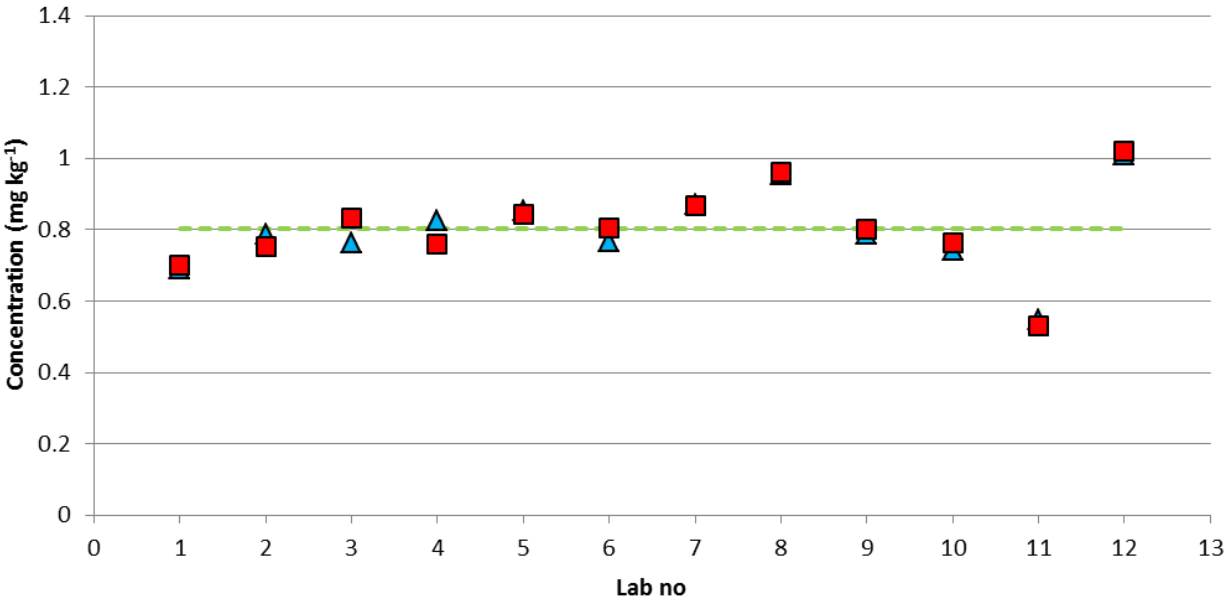
L12 is a Cochran outlier

Sample s5 Plant Based (mean value +/- u_{obs} = 1.756 +/- 0.396 mg/kg)



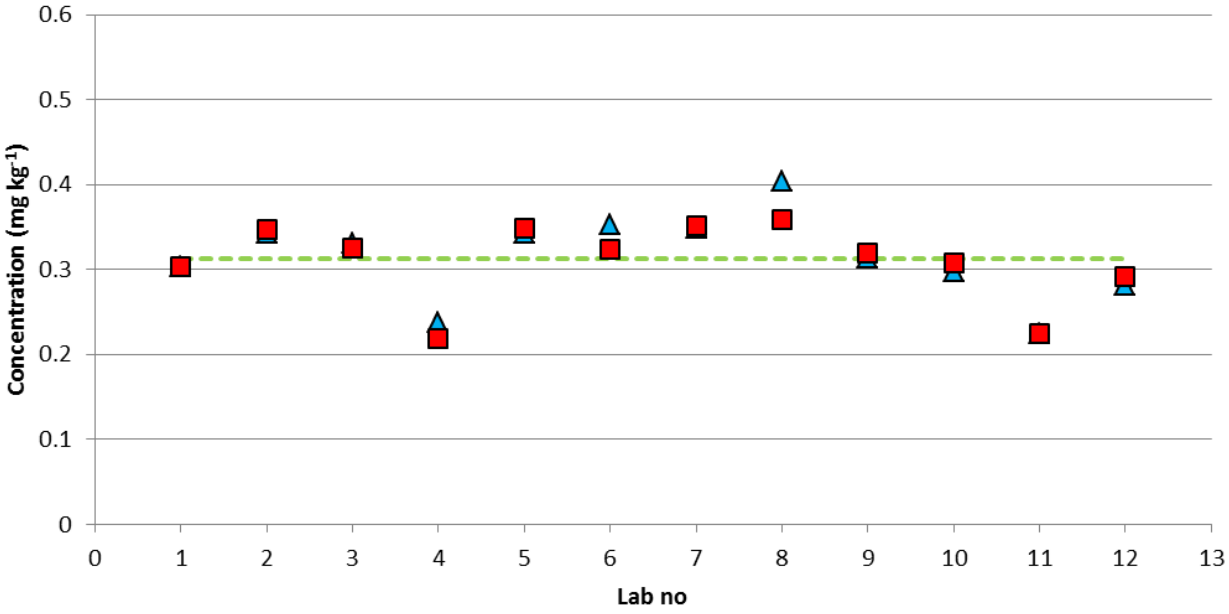
No outliers/stragglers

Sample s6 Complete Feed 1 (Marine) (mean value +/- u_{obs} = 0.802 +/- 0.119 mg/kg)



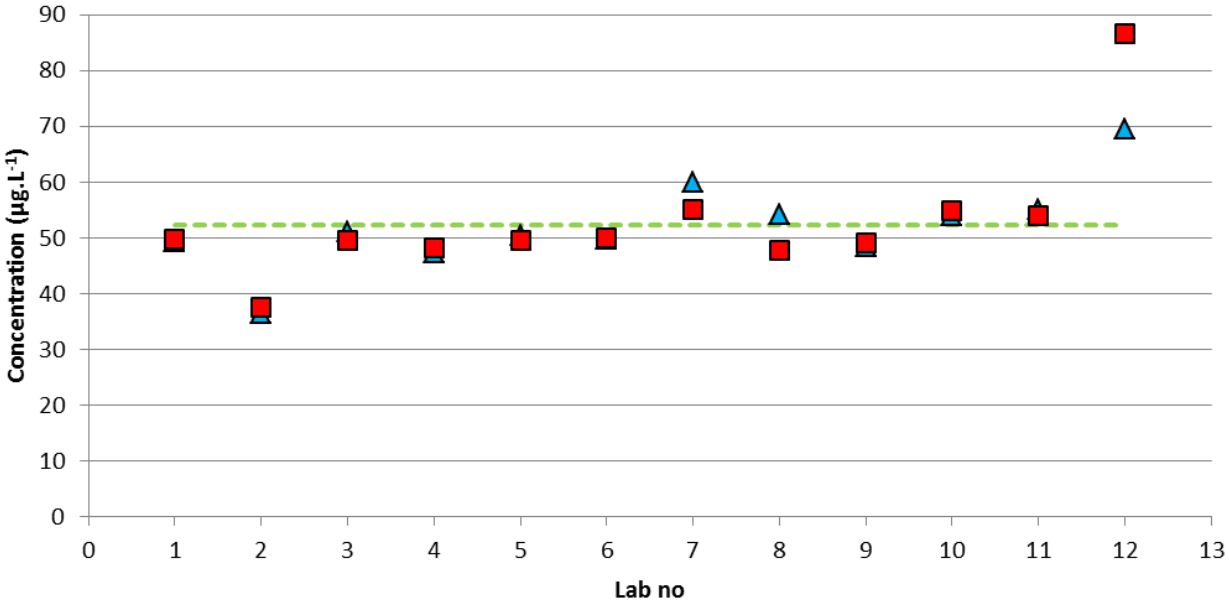
No outliers/stragglers

Sample s7 Complete Feed 2 (Vegetable) (mean value +/- u_{obs} = 0.312 +/- 0.048 mg/kg)



L08 is a Cochran straggler

Sample s8 Aqueous Standard (mean value +/- u_{obs} = 52.40 +/- 9.71 µg/L)



L12 is a Cochran outlier and L2 is a Grubbs straggler

Annex 10 Results from the homogeneity testing of samples s1. s5 and s7

Sample s1 – Rice Meal

Sample	S1 Rice Meal	
Bottle ID	Replicate 1	Replicate 2
50	0.182	0.155
23	0.161	0.153
3	0.160	0.148
45	0.150	0.153
39	0.157	0.145
10	0.142	0.148
15	0.152	0.148
40	0.150	0.152
26	0.149	0.148
12	0.153	0.142
Grand Mean	0.152	
Number of bottles, m	10	

S_{DD} Sum of Squares	Cochran Test	Cochran value
0.001	0.561	0.602

Cochran outlier test

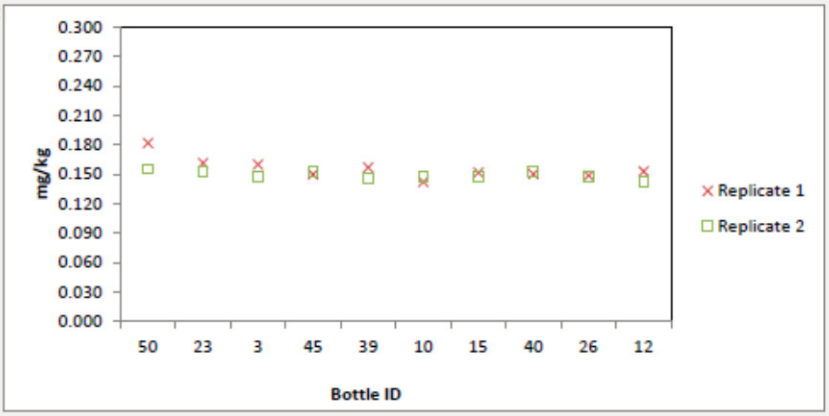
Conclusion:
no analytical outliers

	Sum_i	$Difference_i$	D^2	$Vs = (Si-\bar{S})^2/(m-1)$
	0.337	0.026	0.00069	0.00011
	0.314	0.009	0.00008	0.00001
	0.307	0.012	0.00015	0.00000
	0.303	-0.004	0.00001	0.00000
	0.302	0.012	0.00014	0.00000
	0.290	-0.006	0.00003	0.00002
	0.299	0.004	0.00002	0.00000
	0.303	-0.002	0.00000	0.00000
	0.296	0.001	0.00000	0.00001
	0.295	0.010	0.00011	0.00001
$\sum$	3.047	0.063	0.00123	0.00017
$\bar{S}$	0.305			
$S_{an}^2 = \sum D^2/2m$	0.000062			
$S_{sam}^2 = (Vs/2-S_{an}^2)/2$	0.0000119			
F1	*1.88*			
F2	*1.01*			
σ-hat (%)	15			
$\sigma^2_{all} = (0{,}3\sigma_p)^2$	0.000047			
$c = F1\sigma^2_{all}+F2S_{an}^2$	0.000151			
$S_{sam}^2 < c$	passed			

Homogeneity test

Conclusion:
material homogeneous

Visual appraisal of results:



Sample s5 – Plant Based

Sample	S5 Plant Based					
Bottle ID	Replicate 1	Replicate 2	Sum_i	$Difference_i$	D^2	$Vs = (Si-\bar{S})^2/(m-1)$
37	1.857	1.715	3.572	0.143	0.02039	0.12321
46	2.910	2.690	5.600	0.220	0.04847	0.10574
5	2.170	2.015	4.185	0.155	0.02389	0.02147
18	1.796	1.847	3.643	-0.051	0.00261	0.10713
49	1.626	1.756	3.382	-0.130	0.01703	0.17176
12	1.771	5.903	7.674	-4.132	17.07671	1.03328
33	1.572	1.745	3.317	-0.173	0.02997	0.19000
1	1.695	2.051	3.747	-0.356	0.12665	0.08567
22	1.732	1.806	3.538	-0.074	0.00553	0.13137
14	1.782	5.809	7.591	-4.027	16.21472	0.97741
Grand Mean	2.312		**46.249**	**-8.427**	**33.56597**	**2.94705**
Number of bottles, m	10					

$\sum$	**46.249**
$\bar{S}$	4.625
$S_{an}^2 = \sum D^2/2m$	**1.678298**
$S_{sam}^2 = (Vs/2-S_{an}^2)/2$	**0.0000000**
F1	*1.88*
F2	*1.01*
σ-hat (%)	**15**
$\sigma^2_{all} = (0{,}3\sigma_p)^2$	0.010829
$c = F1\sigma^2_{all}+F2S_{an}^2$	1.715439
$S_{sam}^2 < c$	passed

S_{DD} Sum of Squares	Cochran Test	Cochran value
33.566	0.509	0.602

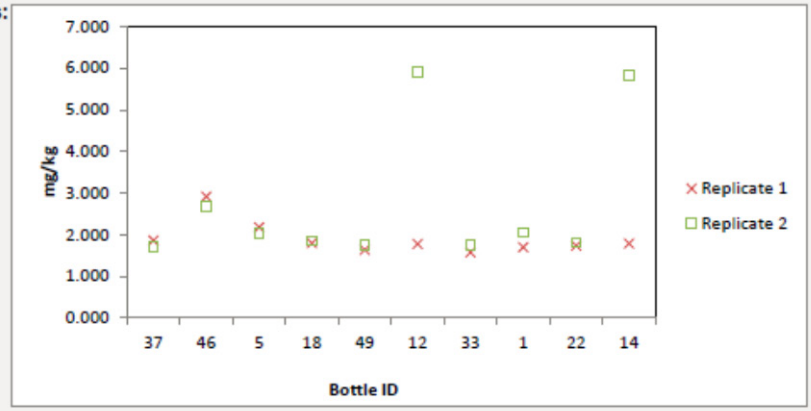
Cochran outlier test

Conclusion:
no analytical outliers

Homogeneity test

Conclusion:
material homogeneous

Visual appraisal of results:



Sample s7 – Complete Feed 2 (Vegetable)

Sample	S7 Complete Feed 2 (Vegetable)						
Bottle ID	Replicate 1	Replicate 2		Sum_i	$Difference_i$	D^2	Vs = $(Si-\bar{S})^2/(m-1)$
43	0.339	0.329		0.668	0.011	0.00011	0.00000
28	0.335	0.334		0.669	0.002	0.00000	0.00000
7	0.332	0.335		0.667	-0.003	0.00001	0.00000
9	0.329	0.337		0.666	-0.009	0.00008	0.00000
20	0.327	0.339		0.666	-0.011	0.00013	0.00000
41	0.329	0.331		0.660	-0.002	0.00000	0.00000
37	0.333	0.329		0.662	0.003	0.00001	0.00000
14	0.328	0.332		0.660	-0.004	0.00002	0.00000
4	0.323	0.336		0.660	-0.013	0.00017	0.00000
49	0.331	0.331		0.661	0.000	0.00000	0.00000
Grand Mean	0.332		$\sum$	6.641	-0.026	0.00053	0.00001
Number of bottles, m	10		$\bar{S}$	0.664			
			$S_{an}^2 = \sum D^2/2m$	0.000026			
			$S_{sam}^2 = (Vs/2-S_{an}^2)/2$	0.0000000			

S_{DD} Sum of Squares	Cochran Test	Cochran value		
0.001	0.319	0.602	F1	*1.88*
			F2	*1.01*
			σ-hat (%)	15
			$\sigma^2_{all} = (0,3\sigma_p)^2$	0.000223
			$c = F1\sigma^2_{all}+F2S_{an}^2$	0.000446
			$S_{sam}^2 < c$	passed

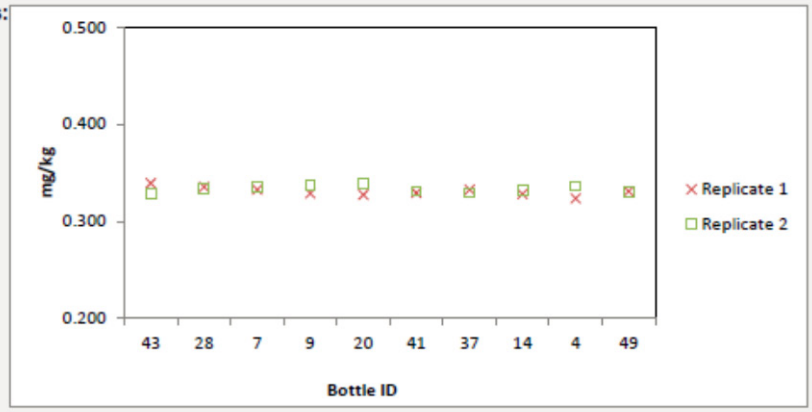
Cochran outlier test

Conclusion:
no analytical outliers

Homogeneity test

Conclusion:
material homogeneous

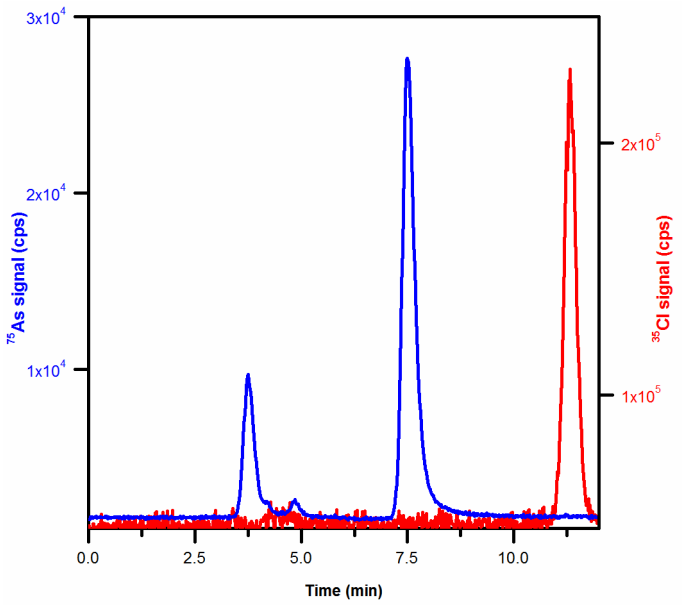
Visual appraisal of results:



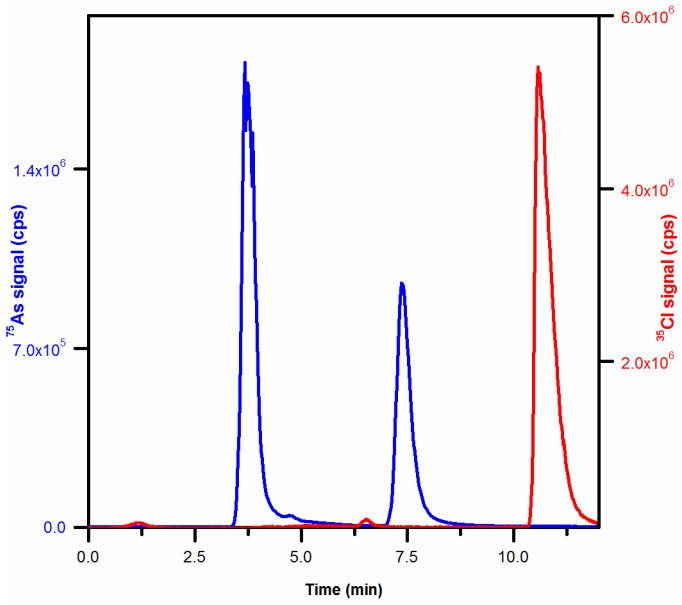
Annex 11 Examples of chromatograms

The following figures show typical chromatograms obtained from the analysis of the eight 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 7-8 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.

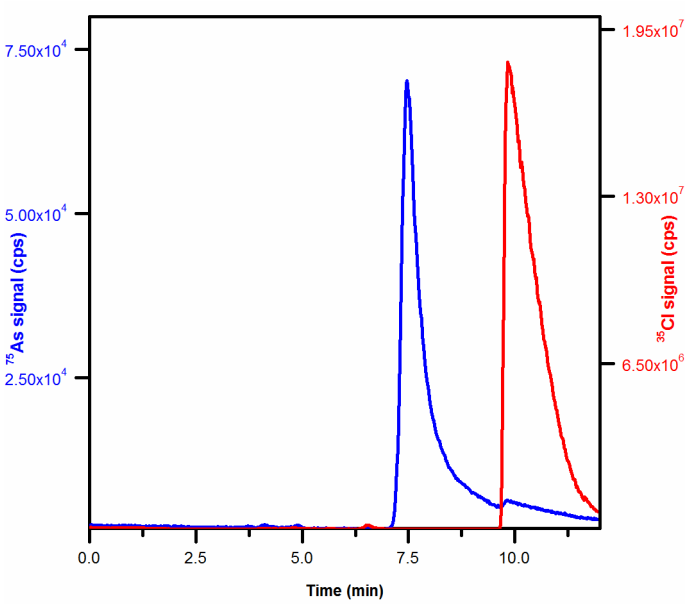
Sample s1 – Rice Meal



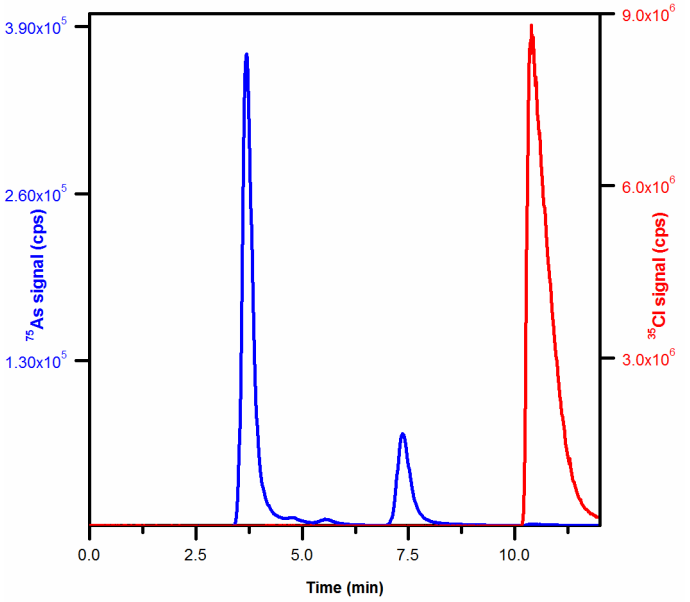
Sample s2 – Seaweed Meal



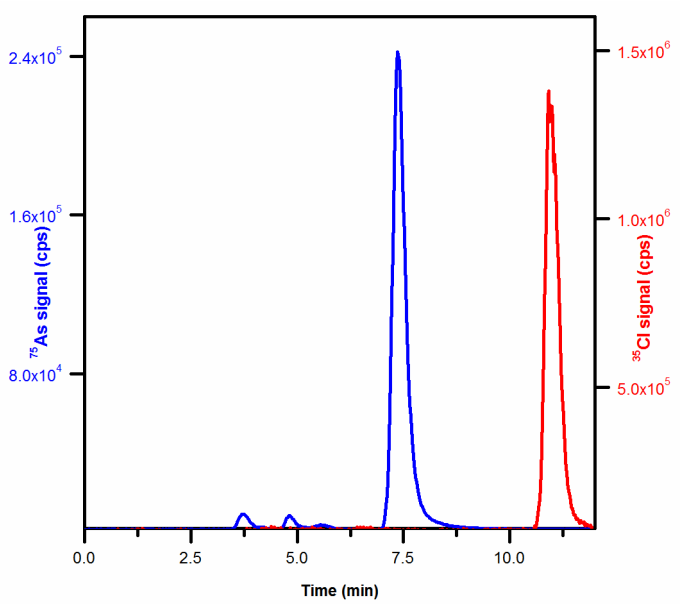
Sample s3 – Mineral Feed



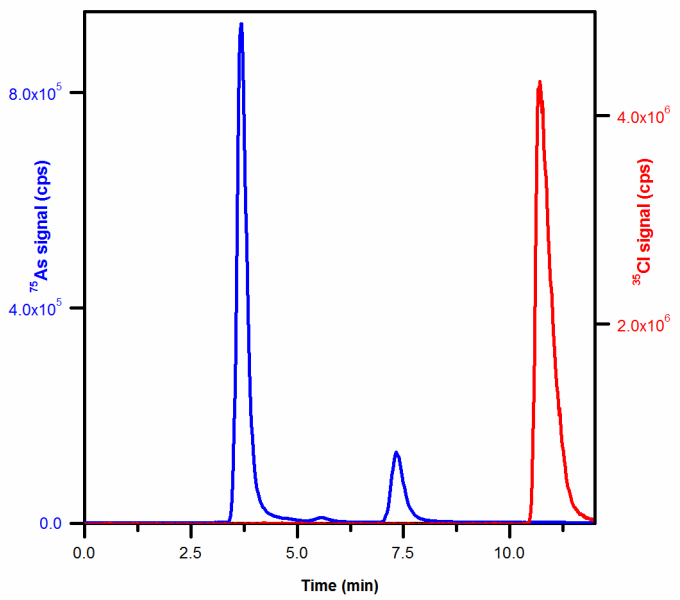
Sample s4 – Fish Meal



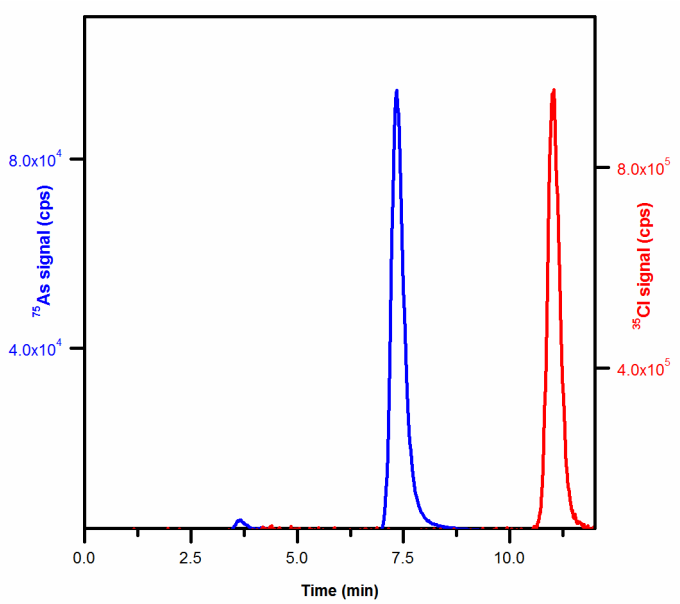
Sample s5 – Fish Meal



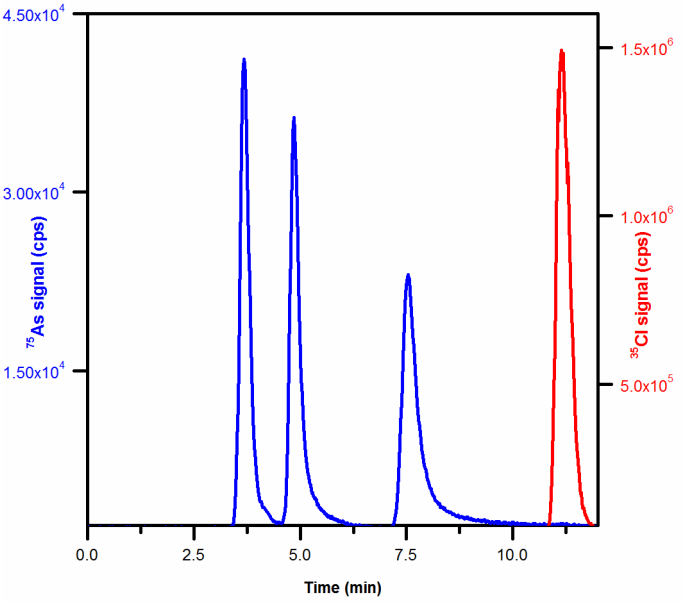
Sample s6 – Complete Feed 1 (Marine)



Sample s7 – Complete Feed 2 (Vegetable)



Sample s8 – Aqueous Standard



Annex 12 Additional results

One laboratory (L03) sent in three datasets corresponding to three different measurement modes by using their ICPMS (Agilent 8900 ICP-QQQ-MS).

- The main dataset used for the collaborative trial (L03) acquired upon measurement in O_2 mode was used (which is the conventional and validated ICPMS method used by L03 for measurement of iAs)
- The second data set (L03a) was acquired upon measurement in no gas mode
- The third data set (L03b) was acquired upon measurement in He mode

In all 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.

		s1	s2	s3	s4	s5	s6
L03	Mass shift mode	0.15	9.44	1.07	0.48	1.42	0.76
	(O2 mode)	0.15	9.36	1.07	0.49	1.68	0.83
L03a	No gas mode	0.14	8.41	0.94	0.41	0.92	0.64
		0.14	8.48	1.03	0.45	1.08	0.60
L03b	He mode	0.13	8.49	0.99	0.43	1.32	0.72
		0.14	8.63	1.03	0.46	1.45	0.77
Overall mean	*(this study)*	0.15	9.69	0.98	0.45	1.76	0.80

Fødevareinstituttet
Danmarks Tekniske Universitet
Kemitorvet
2800 Lyngby

Tlf. 35 88 77 00

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