IMEP-32: Determination of inorganic arsenic in animal feed of marine origin

A Collaborative Trial Report

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IMEP

EURL European Union Reference Laboratory Heavy Metals in Feed and Food

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IMEP-32: Determination of inorganic arsenic in animal feed of marine origin

A Collaborative Trial Report
April 2011

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Content

1 Abstract

A collaborative study, IMEP-32, was conducted in accordance with international protocols to determine the performance characteristics of an analytical method for the determination of inorganic arsenic in animal feed of marine origin. The method would support Directive No 2002/32/EC of the European Parliament and the Council on undesirable substances in animal feed [1] where it is indicated 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 is based on solid phase extraction (SPE) separation of inorganic arsenic from organoarsenic compounds followed by detection with hydride generation atomic absorption spectrometry (HG-AAS). The collaborative study investigated different types of samples of marine origin, including complete feed (unspiked and spiked), fish meal (unspiked and spiked), fish fillet (spiked) and a lobster hepatopancreas (unspiked). In total seven samples were investigated within the concentration range of 0.07 – 2.6 mg kg^{-1}.

The test samples were dispatched to 23 laboratories in 12 different countries. Nineteen participants reported results. The performance characteristics are presented in this report. All method performance characteristics obtained in the frame of this collaborative trial indicates that the proposed SPE-HG-AAS standard method is fit for the intended analytical purpose.

2 IMEP support to EU policy

The International Measurement Evaluation Programme® IMEP is owned by the Joint Research Centre - Institute for Reference Materials and Measurements (JRC-IRMM). IMEP provides support to the European measurement infrastructure in the following ways:

- IMEP promotes metrology from the highest level down to the field laboratories. These laboratories can benchmark their measurement results against the IMEP certified reference value. This value is established according to metrological best practice.
- IMEP helps laboratories participating in proficiency tests (PTs) to assess their estimate of measurement uncertainty. The participants are invited to report the measurement uncertainty. The designation of this ILC is IMEP32 "Determination of inorganic arsenic in feed". IMEP integrates the estimate into the scoring, and provides assistance for the interpretation.
- IMEP supports EU policies by organising interlaboratory comparison exercises (ILC) in the frame of specific EU Directives, or on specific requests from Directorate-General.

This collaborative trial was organized by CEN TC327 WG4 and coordinated by the Danish Technical University Food (DTU Food) in collaboration with IRMM.

IMEP is accredited according to ISO Guide 43-1.

3 Introduction

Arsenic is an ubiquitous element, introduced to the environment from natural sources such as volcanic activity and weathering of minerals or anthropogenic sources such as ore smelting, burning of coal, pesticide use and the use of growth promoters. A large variety of different naturally arsenic species exist, especially in the marine environment, where more than 50 different species have been identified [2]. The toxicity of the species varies very much, with inorganic arsenic being the most toxic species, the simple methylated forms showing intermediate toxicity, whereas most organoarsenic compounds (e.g. arsenobetain) are considered non-toxic. In 2005 EFSA issued an opinion related to Arsenic as undesirable substance in animal feed [3]. In the report the difference in toxicity of the various compounds was emphasized and it was stated that: *"Analytical data from the Member States on total arsenic in feed materials do not indicate arsenic levels of concern in materials others than fish-derived products, for which further data on chemical speciation are needed, to identify the actual levels of inorganic arsenic"*. The EU Directive 2002/32/EC [1] (and later amendments) on undesirable substances in animal feed, which provides maximum levels for a range of undesirable substances in animal feed. Arsenic is one of the chemical parameters included in that Directive. However, in the EU-directive the maximum levels are only for the total content of arsenic, not for the species of toxicological interest, namely inorganic arsenic. However, a footnote indicates 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. This analysis is of particular importance for the seaweed species Hizikia fusiforme"*. One of the reasons for not having implemented maximum levels for inorganic arsenic instead of total arsenic is a lack of standardized methods for control purposes. Hence, the present project with the aim of developing a CEN method for the determination of inorganic arsenic in animal feed of marine origin was initiated.

In 2006 an enquiry was sent to the members of the TC327/WG1 (now WG4) to obtain information about their preference for which analytical technique the CEN method should be based on. Two options were given, either HPLC-ICPMS or SPE-HG-AAS. A large majority of the WG members voted for the last option. The method development work was carried out at the National Food Institute at the Technical University of Denmark.

4 Scope and aim

The scope and aim of the present project was to develop a standard method based on SPE-HG-AAS for the determination of inorganic arsenic in animal feedingstuffs of marine origin to be used in the frame of the feed control authorities carried out in support to the Directive 2002/32/EC. The standardization included an interlaboratory validation study (collaborative trial) to establish the performance characteristics of the method, accordingly to the IUPAC recommendations [4].

The assessment of the measurement results was undertaken on the basis of requirements laid down in the ISO Standards (ISO 5725-2 [5] and ISO 13528 [6]). Furthermore, the administrative and logistic procedures of IMEP were applied.

5 Time frame

The method was developed and further validated at DTU Food in 2008-2010. The collaborative trial was announced via CEN, EU-RL-HM (European Union Reference Laboratory for heavy metals in feed and food) and VDLUFA network in September 2010 (Annex 1). The registration was opened till 30th September 2010. Samples were dispatched on 8th October 2010. The deadline for submission of results was 17th November 2010. The homogeneity and stability studies were carried out in February - May 2010.

6 Material

6.1. Preparation of the test samples

The test samples listed in Table 1 were included in the collaborative trial.

Samples 1-7 were obtained from the company NUTRECO (The Netherlands) from the company normal sample flow. The samples were freeze dried. Several samples were spiked with a solution of arsenate (As_2O_5 in H_2O, titrisol ampoule from Merck) to achieve the target concentration which covers the working range of the analytical method (0.07 to 2.6 mg kg^{-1}). Once spiked, the material was homogenized and freezedried. IRMM performed the final homogenization, grinding, sieving, filtering and bottling of the material. All samples were filled into 50 mL brown glass bottles and stored at 4 °C until dispatch. The control sample is the certified reference material (TORT-2) which was purchased from the National Research Council in Canada (NRCC, Ontario, Canada). From previous analysis of this CRM at DTU Food it was known that this material had a natural content of inorganic arsenic of about 0.5-0.6 mg kg^{-1}. The CRM was assumed to be stable for total As and in

the experience of the DTU and the EU-RL-HM, inorganic As should be considered to have an adequate stability and homogeneity if total As is.

Table 1 Test samples for the collaborative trial

Sample no	Sample type	Spiked / unspiked
IMEP32-1	Complete fish feedingstuff	Unspiked
IMEP32-2	Complete fish feedingstuff	Spiked
IMEP32-3	Fish meal	Unspiked
IMEP32-4	Fish meal	Spiked
IMEP32-5	Fish fillet	Spiked
IMEP32-7	Fish meal	Spiked
IMEP32 Control sample	CRM TORT-2 Lobster Hepatopancreas	Unspiked

6.2. Homogeneity

Homogeneity studies were carried out by DTU Food using anion-exchange chromatography high performance liquid chromatography, coupled with an inductively coupled plasma - mass spectrometry instrument (HPLC-ICP-MS). The HPLC-ICP-MS system consisted of an Agilent 1100 HPLC system with an autosampler and a quaternary pump with vacuum degasser. The analytical column was an ION-120 anion exchange column (Transgenomic). A sample volume of 25 µL was injected. Isocratic elution was applied using 40 mmol l^{-1} $(NH_4)_2CO_3$ with a flow rate of 1.0 mL min^{-1}. The experimental design used for the assessment of the homogeneity of the test samples complied with the requirements set by the ISO 13528 [6]. These tests compare the between bottle standard deviation with the target standard deviation of an exercise, which was set to 15 % of each assigned value. The tests indicate that all the test samples were sufficiently homogeneous for the inorganic arsenic analysis (Annex 2). The between-bottle standard uncertainty, u_{bb} (expressed as a percentage) was calculated using the SoftCRM software [7] and fall in the range of 1.8 to 3.2 %.

6.3. Stability

An isochronous stability study [8, 9] was carried out by IRMM at three temperatures (4, 18 and 60 °C) with the aim to:

- Measure all samples under repeatability conditions (thus avoiding the need to combine the repeatability with long term reproducibility conditions).
- Quantify the potential degradation during the entire interlaboratory comparison study (approximately two months).

All measurements for the stability studies were carried out by DTU Food. No significant degradation for any of the test samples was evidenced.

The evaluation of the stability of the test materials was made using the SoftCRM 2.0 software [7]. The materials proved to be stable at 18 °C for a length covering the whole time frame of the exercise. Annex 3 shows the results from the stability test at 18 °C considering a shelf life of seven weeks. The participants were instructed to store the material at a maximum temperature of 4° C after receipt.

6.4. Sample distribution

The samples were dispatched to the participants by IRMM on the 8th of October 2010. Each participant received one package containing:

- Twelve bottles, each containing ~ 5-10 gram of the test material (two bottles for each type of sample type).
- One bottle containing a control sample
- A pack of SPE cartridges (32 pcs)
- A portion of anti-foam agent (silicone oil)
- A letter accompanying the sample (Annex 4)
- A confirmation receipt form (Annex 5)
- Reporting scheme for the results (Annex 6)
- Questionnaire to be answered and returned together with the results (Annex 7)

A copy of the method protocol to be followed strictly was distributed to the participant by email on the 7th October 2010.

7 Participant invitation, registration and information

A call for participation was published via the CEN, EU-RL-HM and VDLUFA networks (Annex 1). The letter accompanying the samples provided the general instructions for participants, i.e. the measurand, type of samples, analytical method to use, deadlines, etc (Annex 4). Twenty three laboratories from 12 different countries signed up for participation in this exercise (all from European Countries). Results were reported by 19 participants. A list of the Participants, who sent results, is given in Annex 8. The measurand was defined as inorganic arsenic (i.e. the sum of arsenite (As(III) and arsenate (As(V)). The following instructions were sent to the participants;

"For the collaborative study please perform two independent measurements per bottle on two different days (one bottle/day) following the draft method procedure. Please report to original substance (no dry matter correction) in mg As kg^{-1} as inorganic arsenic with at least 3 significant figures. Report the values in the accompanying results form and send it to the coordinator".

Furthermore the following message was given:

"IMPORTANT! 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".

Participants used the provided form to report their measurement results and to complete the related questionnaire (Annex 7). The questionnaire was used to obtain additional information related to measurements and laboratories.

8 Statistical analysis

Statistical evaluation of the data was performed following international standard recommendations (ISO 5725-2 [5] and ISO 13528 [6]).

The following tests were performed;

1) Analysis of variance, ANOVA, to confirm that no statistically significant difference existed, for any of the test samples, between the two individual bottles provided to the participants, i.e. no statistical significant between-day effect (reproducibility). Since this was the case, all four measurements were pooled for further calculations
2) Check for laboratory outliers within the series of independent replicates applying the Grubbs-internal test (repeatability)
3) Check for outliers in the laboratory precision (variance) applying the Cochran test. This test compares the highest laboratory internal repeatability variance with the sum of reported variances from all the participants
4) Check for outliers in the laboratory mean applying the Grubbs test. This test checks for laboratory means deviating significantly from the total mean calculated from all data reported from all participants.

9 Method principle

Extraction of inorganic As is done by microwave assisted acidic extraction with a mixture of dilute hydrochloric acid and hydrogen peroxide. Inorganic arsenic is selectively separated from organic arsenic compounds using solid phase extraction (SPE) and thereafter determined by hydride

generation atomic absorption spectrometry (HG-AAS). A description of the method protocol to be followed was sent to the laboratories

10 Results

10.1. Laboratories compliance

Nineteen out of the twenty three participating laboratories reported results using the reporting scheme. In addition the laboratories also filled in the questionnaire fully or partly. Some of the laboratories were classified as non-compliant laboratories due to reported technical problems and/or to reporting several technically doubtful results. The laboratories that were judged as non-compliant are listed in Table 2 together with the reasoning for doing so. A compilation of the information extracted from the questionnaire can be found in Annex 9. Annex 10 summarises all the reported results.

Table 2 List of non-compliant laboratories.

Non-compliant lab	**Reason**
L03	Reported about instrumental problems
L04	Reported about presence of foam in the gas/liquid separator
L06	Data excluded due to too large systematic error.* Results are generally too low.
L07	Reported about presence of intensive foam generation in the gas/liquid separator
L08	Data excluded due to too large systematic error.* Results are generally too low.
L09	Data excluded due to too large systematic error.* Results are generally too high.
L10	Water-bath was used for the extraction instead of microwave oven extraction
L13	Reported about big problems with control of the SPE dropping speed. Speed was probably too fast.
L17	Reported on problems with the pre-reduction step. Achieved brown and very turbid solutions after pre-reduction.

* The data were discarded on the basis of §7.2.5 in ISO 5725-2 [5] which states that *"When several unexplained abnormal test results occur at different levels with the same laboratory, then that laboratory may be considered to be an outlier, having too high a within-laboratory variance and/or too large a systematic error in the level of its test results. It may then be reasonable to discard some of all of the data from such an outlying laboratory"*

10.2. Outlier identification

After the initial identification of non-compliant laboratories, results from the remaining ten laboratories were subjected to statistical analysis following the procedures described in section 8 and outlying results were identified. Table 3 provides an overview of the outlying results and the outlier type. In all cases the number of outliers is below the threshold recommended by the AOAC guideline [10] where a maximum outlier rate of 2/9 is established.

Table 3 Statistical data evaluation (scrutinizing data for outlier identification)

Sample	Initial N° of Laboratories	Outlier Laboratory	N° of outlier results	Outlier type
IMEP 32 -1	10	L01 [b)] L01 **	4	Cochran Grubb's (mean)
IMEP 32-2	10	L21 *	1	Grubb's (internal)
IMEP 32-3	10	L01 [b)]	4	Cochran
IMEP 32-4	10	L01 [a)]	0	Cochran
IMEP 32-5	10	-	0	-
IMEP 32-7	10	L01 [b)]	4	Cochran
IMEP 32 Control sample	10	-	0	-

Cochran test; this test compares the highest laboratory internal repeatability variance with the sum of reported variances from all the participants,

a) Straggler (test statistics greater than its 5 % critical value but less or equal to its 1 % critical value)

b) Outlier (test statistic is greater than its 1 % critical value)

Grubbs test;

* Grubb's internal outlier refers to a single replicate being statistically significantly different from the other replicates (within a laboratory).

** Grubb's applied to the averaged reported means (highest and lowest values)

10.3. Statistical evaluation of the results

All the remaining measurements were used to evaluate all relevant performance characteristics related to the trueness and to the precision of the method under validation shown in Table 4, on which, the following information and method performance characteristics are given:

- 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) [10, 11],
- The percentage of identified and excluded outliers

Accuracy

The analytical recovery of the method is evaluated by comparison of the data with the reference value of the control sample. A reference value (x_{ref}) of 0.599 mg kg^{-1} was determined with an expanded uncertainty of 0.07 mg kg^{-1} (k = 2) for the control sample using HPLC-ICP-MS analysis at DTU Food. From the present study a value of 0.544 ± 0.162 mg kg^{-1} was obtained from the averaged values of measurements carried out for TORT-2. This gives a recovery of 90.8 %. When considering the estimated uncertainty value on x_{ref} and x_{obs} as confidence intervals for the control sample, it can be seen that no statistically significant difference can be detected and hence no significant bias.

Precision

RSD_r values were from 5.4 to 17.5 % and the RSD_R values ranged from 13.2 to 31.9 % (excluding sample IMEP 32-1).

Method working range

The concentration of the test samples was from 0.1-2.7 mg kg^{-1}. For IMEP 32-1 the concentration of inorganic arsenic is much lower (0.07 mg kg^{-1}) and evaluated not to be within the working range of the method, where relative high values of RSD_r and RSD_R (22.8 % and 57.6 %, respectively) were obtained. Hence the working range of the method is proposed to be from 0.1 to 2.6 mg kg^{-1}. In the EU directive on undesirable substances in feed [1] an indicative maximum level for inorganic arsenic is stated at 2 ppm (i.e. 2 mg kg^{-1}) in footnote no. 9. The obtained lower concentration value for the working range (0.1 mg kg^{-1}) is well below the indicative maximum level.

Table 4: Method performance characteristics from the collaborative trial on the determination of iAs in feed (following ISO 5725-2).

Matrix	Units	IMEP 32-1	IMEP 32-2	IMEP 32-3	IMEP 32-4	IMEP 32-5	IMEP 32-7	IMEP 32 Control Sample
N° of participating laboratories		10	10	10	10	10	10	10
Remaining data after outlier elimination		29	35	28	36	36	30	34
N° of remaining laboratories		9	10	9	10	10	9	10
Outliers	%	12.1	2.8	12.5	0.0	0.0	11.8	0.0
Overall mean $X_{obs} \pm u_{obs}$	mg Kg^{-1}	0.071 ± 0.041	0.713 ± 0.117	0.189 ± 0.060	1.062 ± 0.140	2.643 ± 0.506	0.432 ± 0.066	0.544 ± 0.162
S_r	mg Kg^{-1}	0.016	0.054	0.014	0.105	0.277	0.023	0.095
RSD_r	**%**	**22.8**	**7.6**	**7.5**	**9.9**	**10.8**	**5.4**	**17.5**
r_L	mg Kg^{-1}	0.046	0.153	0.040	0.294	0.776	0.065	0.266
S_R	mg Kg^{-1}	0.041	0.117	0.060	0.140	0.506	0.066	0.162
RSD_R	**%**	**57.6**	**16.4**	**31.9**	**13.2**	**19.1**	**15.3**	**29.7**
R_L	mg Kg^{-1}	0.115	0.327	0.169	0.391	1.416	0.185	0.453
σ_H	mg Kg^{-1}	0.017	0.120	0.039	0.168	0.365	0.078	0.095
HorRat		**2.4**	**1.0**	**1.6**	**0.8**	**1.4**	**0.8**	**1.7**

The Horwitz value was estimated according to the modified Horwitz equation [Analyst, 2000, **125**, 385-386].

12 Conclusion

A method for the determination of inorganic arsenic (sum of arsenite (As(III) and arsenate (As(V)) in animal feedingstuff of marine origin was developed at DTU Food. The method principle is based on SPE separation of inorganic arsenic from organoarsenic compounds followed by determination by HG-AAS.

The method performance characteristics were assessed in a collaborative trial IMEP-32, including six samples within the concentration range of 0.1 - 2.6 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 determination of inorganic arsenic in animal feed of marine origin, i.e. is fit for its intended analytical purpose.

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Finally and importantly all the laboratories are thanked for their voluntary participation in this project. Their efforts are highly appreciated.

Abbreviations

AAS Atomic Absorption Spectrometry
DTU Food National Food Institute at the Technical University of Denmark
EFSA European Food Safety Authority
HG Hydride Generation
IMEP International Measurement Evaluation Programme
IRMM Institute for Reference Materials and Measurements
JRC Joint Research Centre
SPE Solid Phase Extraction

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List of Annexes

Annex 1 Invitation letter

CEN / TC 327 `Animal feeding stuffs:
Working group 4 'Heavy metals, trace elements and minerals'

Invitation for participation in collaborative studies:
Determination of inorganic arsenic (iAs) by SPE-HG-AAS after microwave assisted extraction in marine feeding stuffs

Copenhagen, 9th September 2010

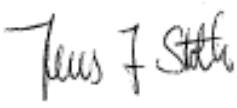
Dear colleague,

You are hereby invited to participate in a collaborative study of a method for the determination of inorganic arsenic in animal feed of marine origin. The method principles are based on microwave assisted extraction, selective separation by solid phase extraction (SPE) and element-specific determination by hydride generation atomic absorption spectrometry (HG-AAS).

There has recently been drawn much attention on the content of inorganic arsenic in food and feed. Emphasis has from official side been pointing at the need for selective methods for the determination of inorganic arsenic [1, 2]. In the EU directive on undesirable substances in animal feedingstuffs maximum levels for total arsenic are laid down for a range of feed products [3]. However, it is also stressed that the responsible operator should be able to document that the inorganic arsenic concentration is below 2 mg kg^{-1} [3]. The participation in this collaborative study will provide knowledge to your laboratory on the measurement of inorganic arsenic and furthermore provide a unique set of samples with elevated concentrations of inorganic arsenic to be used for quality assurance purposes.

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 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)

References:

[1] Scientific Opinion on Arsenic in Food EFSA journal 109, 7, 1351.
[2] Joint FAO/WHO Expert Committee on Food Additives, 72th meeting Rome, February 2010
[3] Commission Directive 2009/141/EC of 23 November 2009

Introduction:

An international collaborative study will be conducted under the CEN leadership to evaluate a method for the determination of inorganic arsenic (iAs) marine animal feeds. The proposed method was discussed within CEN/TC 327/WG 4. The National Food Institute at the Technical University in Denmark will organize this collaborative trial. CEN members and other interested laboratories are invited to participate.

Principle of the method:

Extraction of inorganic As is done by microwave assisted acidic extraction with a mixture of dilute hydrochloric acid and hydrogen peroxide. Inorganic arsenic is selectively separated from organic arsenic compounds using solid phase extraction (SPE) and thereafter determined by hydride generation atomic absorption spectrometry (HG-AAS). A description of the method procedure to be followed will be sent.

Samples:

The set-up and execution of the collaborative study will be done according to the IUPAC protocol (for the design, conduct and interpretation of method-performance studies) (Pure & Appl. Chem, 1995, 67, 331]. According to this guideline in minimum 5 different samples should be analysed in more than 8 (valid) laboratories.

5-6 marine samples with unknown concentrations (concentration range ?? LOQ - 4 mg kg^{-1}) will be sent out for the statistical validation of the CEN methods. Furthermore for lab training one control sample (with known concentration) will be sent to the participating laboratories. SPE cartridges will be provided together with the samples.

Requirements to the participating laboratories:

The following equipment and reagents should be available at the participating labs.

- Microwave oven - preferably capable of controlling the temperature
- Vacuum chamber for solid phase extraction (Vac Elut system or similar)
  (NOTE: SPE cartridges will be provided together with the samples)
- HG-AAS apparatus with an arsenic specific lamp
- Reagents: $NaBH_4$, KI, HCl, H_2O_2, acetic acid, $(NH_4)_2CO_2$, methanol, ascorbic acid

Time schedule:

- Estimated time for dispatch of samples – **before 8. October 2010**
- Deadline for submission of results: **15. November 2010.**
- Discussion of results will subsequently take place in CEN TC327/WG 4
- Reports to participants will be sent out after WG discussions

Organisation of the study:

The studies are organised by CEN/TC 327/WG 4. The National Food Institute at the Technical University of Denmark (DTU Food) will be in charge to organize.

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

Participation in the study:

If you want to participate, please send your reply to the email-address indicated (use the attached registration form), **before September 30, 2010.**

If you have any questions please send a mail to: jjsl@food.dtu.dk or call +45 35887625

Registration form Collaborative study: Animal feeding stuffs - Determination of inorganic arsenic by SPE-HG-AAS after microwave assisted extraction in marine feedingstuffs

Yes, I will participate in the CEN/TC 327 collaborative study in feeds for:

☐ Inorganic arsenic by SPE-HG-AAS

Name contact person	
E-Mail	
Organisation	
Postal address	
City and postal zip code	
Country	

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

Annex 2 Homogeneity tests

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 7	
Measurement results (mg Kg^{-1})												
Bottle N°	**R_1**	**R_2**	**R_1**	**R_2**	**R_1**	**R_2**	**R_1**	**R_2**	**R_1**	**R_2**	**R_1**	**R_2**
1	0,04	0,02	0,85	0,81	0,19	0,18	1,42	1,40	3,55	3,07	0,44	0,45
2	0,03	0,03	0,83	0,84	0,19	0,19	1,44	1,37	3,01	3,63	0,44	0,46
3	0,04	0,03	0,84	0,87	0,19	0,19	1,40	1,46	3,45	3,38	0,51	0,48
4	0,03	0,03	0,84	0,87	0,19	0,20	1,40	1,41	3,47	3,41	0,47	0,47
5	0,02	0,04	0,85	0,87	0,17	0,19	1,46	1,49	3,49	3,74	0,51	0,45
6	0,04	0,05	0,83	0,81	0,17	0,19	1,37	1,45	3,31	3,43	0,46	0,50
7	0,03	0,03	0,86	0,86	0,18	0,20	1,33	1,37	3,37	3,39	0,45	0,45
8	0,02	0,03	0,86	0,86	0,19	0,20	1,37	1,38	3,36	3,27	0,43	0,48
9	0,03	0,03	0,83	0,83	0,18	0,19	1,43	1,37	3,56	3,22	0,51	0,45
10	0,03	0,03	0,81	0,81	0,21	0,18	1,36	1,38	3,47	4,02	0,44	0,47
Mean	**0,03**		**0,84**		**0,19**		**1,40**		**3,43**		**0,47**	
σ (15 %)	**0,005**		**0,126**		**0,028**		**0,210**		**0,515**		**0,0699**	
Homogeneity test according to the ISO 13528 (values in mg Kg^{-1})												
0.3 σ	**0,001**		**0,038**		**0,008**		**0,063**		**0,154**		**0,021**	
S_x	**0,0053**		**0,0169**		**0,0058**		**0,0312**		**0,1419**		**0,0171**	
S_w	**0,0074**		**0,0134**		**0,0112**		**0,0303**		**0,2374**		**0,0261**	
S_S	**0,0007**		**0,0140**		**0,0000**		**0,0227**		**0,0000**		**0,0000**	
$S_S \leq 0.3\sigma$?	**Yes**		**Yes**		**Yes**		**Yes**		**Yes**		**Yes**	
Test result	**Passed**		**Passed**		**Passed**		**Passed**		**Passed**		**Passed**	

Notes: R1, R2

Notes: R_1, R_2 refers to replicate 1 and 2 respectively. For all the other abbreviations see the respective references.
The standard deviation for the proficiency assessment σ in use in this table was calculated as a fraction of the mean obtained from the homogeneity studies and not as a fraction of the reference value.

S_x = std of sample averages
S_w = within-sample std

S_S = between-sample std

S_{All} = 0.3 σ allowable std (criterion)

Bottle ID are arbitrarily numbered as from 1 to 10 and do not correspond to the real bottle ID as analysed.

Annex 3 Stability tests

	Sample 1 Weeks			
	Results in mg Kg^{-1}			
Bottle	**0**	**3**	**5**	**8**
1	**0,02**	**0,04**	**0,03**	**0,04**
2	**0,03**	**0,02**	**0,04**	**0,01**
Slope =	0,000			
SE Slope =	0,001			
Intercept =	0,028			
SE Intercept =	0,007			
Correlation Coefficient =	0,002			
Slope of the linear regression significantly <> 0 (95%) :	**No**			
Slope of the linear regression significantly <> 0 (99%) :	**No**			
Test results	**Stable**			

	Sample 2 Weeks			
	Results in mg Kg^{-1}			
Bottle	**0**	**3**	**5**	**8**
1	**0,82**	**0,83**	**0,85**	**0,91**
2	**0,84**	**-**	**0,85**	**-**
Slope =	0,008			
SE Slope =	0,003			
Intercept =	0,820			
SE Intercept =	0,012			
Correlation Coefficient =	0,713			
Slope of the linear regression significantly <> 0 (95%) :	**No**			
Slope of the linear regression significantly <> 0 (99%) :	**No**			
Test results	**Stable**			

	Sample 3 Weeks			
	Results in mg Kg^{-1}			
Bottle	**0**	**3**	**5**	**8**
1	**0,18**	**0,18**	**0,19**	**0,19**
2	**0,20**	**0,20**	**0,19**	**0,19**
Slope =	0,000			
SE Slope =	0,001			
Intercept =	0,190			
SE Intercept =	0,005			
Correlation Coefficient =				
Slope of the linear regression significantly <> 0 (95%) :	**No**			
Slope of the linear regression significantly <> 0 (99%) :	**No**			
Test results	**Stable**			

	Sample 4 Weeks			
	Results in mg Kg^{-1}			
Bottle	**0**	**3**	**5**	**8**
1	**1,39**	**1,42**	**1,39**	**1,33**
2	**1,38**	**1,35**	**1,3**	**1,45**
Slope =	-0,001			
SE Slope =	0,006			
Intercept =	1,379			
SE Intercept =	0,031			
Correlation Coefficient =	0,001			
Slope of the linear regression significantly <> 0 (95%) :	**No**			
Slope of the linear regression significantly <> 0 (99%) :	**No**			
Test results	**Stable**			

	Sample 5 Weeks			
	Results in mg Kg^{-1}			
Bottle	**0**	**3**	**5**	**8**
1	**3,2**	**3,18**	**3,11**	**3,27**
2	**3,36**	**3,29**	**3,32**	**2,99**
Slope =	-0,018			
SE Slope =	0,014			
Intercept =	3,288			
SE Intercept =	0,07			
Correlation Coefficient =	0,218			
Slope of the linear regression significantly <> 0 (95%) :	**No**			
Slope of the linear regression significantly <> 0 (99%) :	**No**			
Test results	**Stable**			

	Sample7 Weeks			
	Results in mg Kg^{-1}			
Bottle	**0**	**3**	**5**	**8**
1	**0,43**	**0,45**	**0,44**	**0,45**
2	**0,43**	**0,42**	**0,43**	**0,44**
Slope =	0,002			
SE Slope =	0,001			
Intercept =	0,429			
SE Intercept =	0,006			
Correlation Coefficient =	0,269			
Slope of the linear regression significantly <> 0 (95%) :	**No**			
Slope of the linear regression significantly <> 0 (99%) :	**No**			
Test results	**Stable**			

Annex 4 Letter accompanying the samples

To the participants of the
collaborative trial on inorganic As
in marine feed by SPE-HG-AAS

October 2010
/jjsl

CEN TC327/WG4 Collaborative trial on the determination of inorganic arsenic in marine feed by SPE-HG-AAS

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 feeds. Your participation is a very important contribution and very much appreciated.

You receive the following items:

1) A total of 14 bottles with the following samples:
   - *6 marine samples in duplicate for the actual study*
   - *1 control sample with known concentration for QA purposes (approx 1 g)*
   - *1 bottle with antifoam agent (silicone oil) (see 4.17 in method procedure)*
2) Confirmation form for receipt of samples (to be returned to coordinator)
3) A package with 32 SPE columns to be used for the analysis
4) Reporting scheme for the results
5) Questionnaire to be answered and returned together with the results.

Please check whether the bottles containing the test material remained undamaged during transport. Then, please send the "Confirmation of receipt" form back (e-mail: jjsl@food.dtu.dk; fax: +45-3588 7448). You should store the samples in a dark and cold place (at maximum 4 ºC) until analysis.

The method procedure description will be sent to you by email.

For the collaborative study please perform two independent measurements per bottle on two different days (one bottle/day) following the draft method procedure. Please report to original substance (no dry matter correction ?? no moisture content correction?) in **mg As kg^{-1} as inorganic arsenic** with at least 3 significant figures. Report the values in the accompanying results form and send it to the coordinator (jjsl@food.dtu.dk). Check your results carefully for any errors before submission.

For QA purposes a control sample has been included. This is a marine sample with inorganic arsenic concentration of approximately 0.5 mg As kg^{-1} as inorganic arsenic. Please also analyse the control sample in duplicate on each of the days and report the results in the results scheme. Please also report the results from the reagent blank solution (in µg/L) as well as the calibration equation obtained from the two calibration curves run before and after the samples, respectively. This information will be very helpful in the evaluation of the results.

The deadline for submission of results is <u>Wednesday 17/11/2010</u>.

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

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

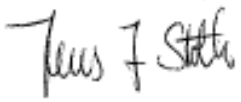
Jens J. Sloth: email: jjsl@food.dtu.dk or phone +45 3588 7625

or

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

Thank you for very much your collaboration,

Best regards,



Jens J. Sloth

Annex 5 Sample receipt confirmation form

To the participants of the collaborative trial on inorganic arsenic in feed

IMEP-32

Confirmation of receipt of the samples

Please return this form at your earliest convenience.
This confirms that the sample package arrived.
In case the package is damaged,
please state this on the form and contact us immediately.

ANY REMARKS …….....
…….....

Date of package arrival …….....

Laboratory .....

Signature …….....

Please return this form to (email preferred):

National Food Institute
Mørkhøj Bygade 19
DK-2860 Søborg
Denmark
Attn: Jens J. Sloth

Fax : +45 3588 7448

e-mail : jjsl@food.dtu.dk

Annex 6 Results reply form

Results scheme

CEN TC327/WG4 Collaborative trial on inorganic arsenic in marine feed

Laboratory:_____

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

DAY1

Sample	Bottle no	Result replicate 1 (mg kg^{-1})	Result replicate 2 (mg kg^{-1})
IMEP32-1			
IMEP32-2			
IMEP32-3			
IMEP32-4			
IMEP32-5			
IMEP32-7			
Control sample			

Procedural blank	-	µg/L	µg/L
		Slope	Intercept
Calibration curve equation (first in sequence)			
Calibration curve equation (last in sequence)			

DAY2

Sample	Bottle no	Result replicate 1 (mg kg^{-1})	Result replicate 2 (mg kg^{-1})
IMEP32-1			
IMEP32-2			
IMEP32-3			
IMEP32-4			
IMEP32-5			
IMEP32-7			
Control sample			

Procedural blank	-	µg/L	µg/L
		Slope	Intercept
Calibration curve equation (first in sequence)			
Calibration curve equation (last in sequence)			

Deadline for submission of results: **17. November 2010**

Please remember to fill in the questionnaire.

Send to: jjsl@food.dtu.dk

Annex 7 Questionnaire

CEN TC327/WG4 Collaborative trial on the determination of inorganic arsenic in marine feed by SPE-HG-AAS

Please complete this questionnaire.

1. Method related questions

1.1 Which HG-AAS instrument did you use? _____

1.1.1 Please provide the settings:

Slit/band width (nm)? _____

Temperature of cell (°C)? _____

Electrical heated or flame heated? _____

Wavelength (nm)? _____

Background correction? _____

Measurement time? _____

1.2 Did you use flow mode or batch mode for the hydride generation step?_____

1.3 Did you use a linear or a quadratic calibration curve?_____

1.4 Which working range have you used? Indicate lowest and highest standard

_____

_____

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

IMEP 32-1:

_____

IMEP 32-2:

_____

IMEP 32-3:

_____

IMEP 32-4:

_____

IMEP 32-5:

_____

IMEP 32-7:

_____

Control sample:_____

1.6 Did you apply a recovery factor for correction of the results? If yes how?

_____

_____

1.7 Have you identified any interference(s)?_____

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 SPE experiments 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 carry out HG-AAS 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** _____

5. **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:** _____

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

   O No O Yes
   If yes, which accreditation body: _____

7. **Do you have any comments? Please let us know:**

_____
_____

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

Annex 8 List of Participants

Participating laboratory	Country	Contact person	Email adress
Central Institute for Supervising and Testing in Agriculture (CISTA)	Czech Republic	Eva Niedobová	eva.niedobova@ukzuz.cz
State Veterinary Institute Olomouc	Czech Republic	Alena Simakova	asimakova@svuol.cz
UKZUZ - NRL RO Praha	Czech Republic	Jaroslava Petrova	jaroslava.petrova@ukzuz.cz
DTU Food	Denmark	Jens J. Sloth	jjsl@food.dtu.dk
Agricultural Research Centre, Laboratory for Residues and Contaminants	Estonia	Merike Toome	merike.toome@pmk.agri.ee
Finnish Food Safety Authority EVIRA	Finland	Eija-Riitta Venäläinen	eija-riitta.venalainen@evira.fi
Bioanalytik Weihenstephan -TUM	Germany	Dr. Jürgen Danier	juergen.danier@tum.de
Landeslabor Berlin-Brandenburg, FBII-4	Germany	Dr. Christine Meier	Christine.meier@landeslabor-bbb.de
LTZ Augustenberg	Germany	Dr. Klaus Michels	klaus.michels@ltz.bwl.de
Muva Kempten	Germany	Ingo Piccon	ingo.piccon@muva.de
Staatliches Betriebsgesellshaft für Umwelt und Landwirtschaft	Germany	Dr. Ralf Klose	ralf.klose@smul.sachsen.de
Staatliches Veterinäruntersuchungsamt	Germany	Annette Poschner	annette.poschner@svua-arnsberg.nrw.de
Thüringer Landesanstalt für Landwirtschaft	Germany	Rita Kirmse	rita.kirmse@tll.thueringen.de
University of Hohenheim LA Chemie	Germany	Dr. Holger Hrenn	hrenn@lachemie.uni-hohenheim.de
Central Agricultural Office, Food and Feed Safety Directorate, Feed Investigation NRL	Hungary	Mr. Jozsef Dömsödi	kozplab@ommi.hu
National Food and Veterinary Assessment Institute	Lithuania	Arunas Jankauskas	ajankauskas@vet.lt
National Research Institute of Animal Production, National Laboratory for Feedingstuffs	Poland	Waldemar Korol	korol@clpp.lublin.pl
Laboratório Nacional de Investigação Veterinária (LNIV INRB)	Portugal	Gabriela Assis	gabriela.assis@lniv.min-agricultura.pt
MasterLab B.V.	The Netherlands	Theo Vrijenhoek	theo.vrijenhoek@nutreco.com

Annex 9 Information extracted from the questionnaire

Lab	HG technique used	Laboratory experience		Quality system and accreditation		Comments
		SPE	HGAAS	Lab	Accreditation	
L01	HG-GF-AAS Electrical heated, Ir coated tube (2100°C)	No answer	Yes 0-50 samples/year	Yes, ISO17025	No	No further comments
L02	Electrical heated cell (900°C)	No for inorganic analysis Yes for mycotoxin and vitamin analysis	Yes >1000 samples in 2009 (As+Se)	Yes, ISO17025	yes	Pre-reduction solution: 5 g KI+5 g ascorbic acid to 100 mL with water 1 mL aliquot after SPE elution with 1,25 mL 0,5 M HCl + 2,5 mL pre-reduction solution + 2,5 mL 30% HCl in 25 mL flask, wait for 2h at room temperature, then filled to mark with water.
L03	Flame heated quartz cell (T not reported)	No	No	Yes, ISO17025	No	Because we were having some equipment problems, we didn't have time to perform the determinations in replicate. The microwave digestion and SPE extraction were made in two different days but the HG-AAS determination was performed in the same day.
L04	Flame heated quartz cell (900-1000°C)	No	20y of experience, but last 2 years ICPMS is used instead of HGAAS	Yes, ISO17025+ISO9000	Yes	Foam in gas/liquid separator
L06	Flame heated quartz cell (1800°C)	No	Yes 50-200 samples/year	Yes, ISO17025	Yes	Quartz cell temperature 1800°C – flame heated with acetylene.
L07	Electrical heated cell (900°C)	No	Yes >200 samples/year	Yes, ISO17025	Yes	The centrifugation of sample S1+S2 were bad (turbid extracts). Intensive foam formation during hydride generation. Two gas/liquid separators were used.
L08	Electrical heated cell (840°C)	No	Yes >200 samples/year	Yes, ISO17025	Yes	Filtering with glass microfiber filter instead of centrifuging (section 6.2).
L09	Flame heated quartz cell (~1000°C)	Yes ~50 samples/year	Yes 50-200 samples/year	Yes, ISO17025	Yes (for Se determination)	No further comments
L10	Flame heated quartz cell (T not known)	No	Yes >200 samples/year (for As and Se)	Yes, ISO17025	No	Temperature of the quartz cell was not known. Waterbath (25 min at 95°C) was used instead of microwave oven for extraction of samples. Additional filtration (Whatman No. 42 filter) after the centrifugation step (6.2)
L11	Electrical heated cell (900°C)	Yes 50-200 samples/year	Yes >200 samples/year	Yes, ISO17025	Yes	No further comments
L12	FIAS-THGA coupling (HG-GF-AAS)	No	No	Yes, ISO17025	Not for iAs determination	No comments

L13	HG-GF-AAS Electrical heated, Ir coated tube (400/2100°C)	No for inorganic analysis Yes for pesticide analysis	Yes >200 samples/year	Yes, ISO17025	yes	Big problems with the vacuum chamber for control of dropping speed of the SPE cartridges. For most of the samples the dropping speed was probably too fast. Used GF-HG-AAS.
L14	HG-GF-AAS Electrical heated, Ir coated tube (T not reported)	Yes >200 samples/year	No	Yes, ISO17025	no	Recovery factor derived from the analysis of an arsenic standard solution was used for correction of results.
L17	HG-GF-AAS Electrical heated (T not reported)	No	Yes ~500 samples/year	Yes, ISO17025	yes	In my opinion there was something wrong with our prereduction. The measurement solutions turned into brown and turbid solutions af the pre-reduction with potassium iodide/ascorbic acid and hydrochloric acid. We carried out the As determination after filtration.
L18	Electrical heated cell (900°C)	Yes 0-50 samples/year	Yes >200 samples/year	Yes, ISO17025	yes	Extraction done in a heated electrical digestion apparatus with open vessels
L19	Electrical heated cell (900°C)	No	Yes 50-200 samples/year	Yes, ISO17025	yes	V1=25 mL Pre-reduction solution 10 g KI and 10 g Ascorbic acid in 200 mL water
L21	Electrical heated cell (900°C)	No	Yes >200 samples/year	Yes, ISO17025	yes	No further comments
L22	Electrical heated cell (900°C)	No for inorganic analysis Yes for HPLC analysis	Yes 50-200 samples/year	Yes, ISO17025+ISO9000	yes	T in extraction step: 105°C Centrifuge speed: 3900 rpm Conc of HG reagents: 1 g NaBH4 + 0,25 g NaOH to 500 mL water 242 mL HCL to 1000 mL water
L23	Electrical heated cell (900°C)	yes	Yes 50-200 samples/year	Yes ISO17025	yes	No further comments

Annex 10 Results

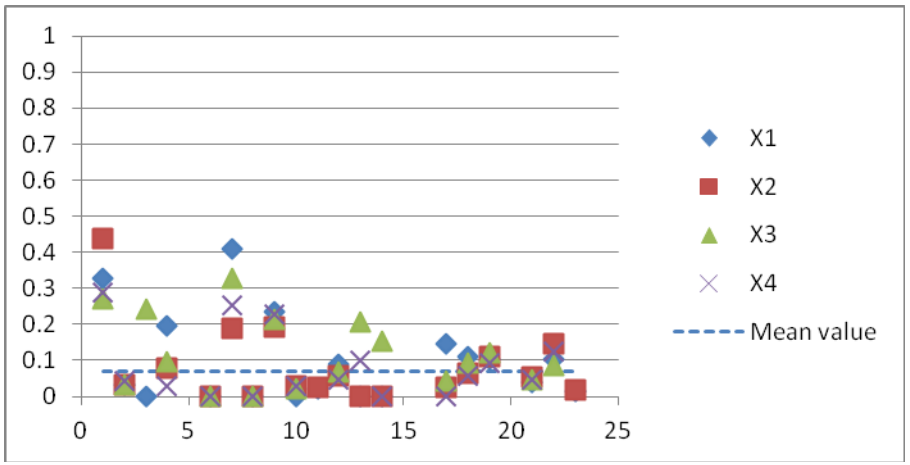
IMEP 32-1 Fish feed (unspiked)

	Day 1		Day 2	
Lab no	X1	X2	X3	X4
1	0,326	0,439	0,271	0,287
2	0,0336	0,032	0,032	0,0414
3	<0		0,242	
4	0,197	0,078	0,097	0,028
5				
6	<0,01	<0,01	<0,01	<0,01
7	0,411	0,188	0,329	0,254
8	0	0	0	0
9	0,235	0,191	0,215	0,227
10	0	0,03	0,02	0,03
11	0,0223	0,0262		
12	0,09	0,058	0,068	0,047
13	0	0	0,206	0,1
14	<0,106	<0,106	0,154	<0,108
15				
16				
17	0,146	0,024	0,044	0
18	0,109	0,0656	0,0919	0,0569
19	0,105	0,109	0,1195	0,092
20				
21	0,038	0,0525	0,046	0,047
22	0,1047	0,1474	0,0869	0,124
23	0,014	0,017		

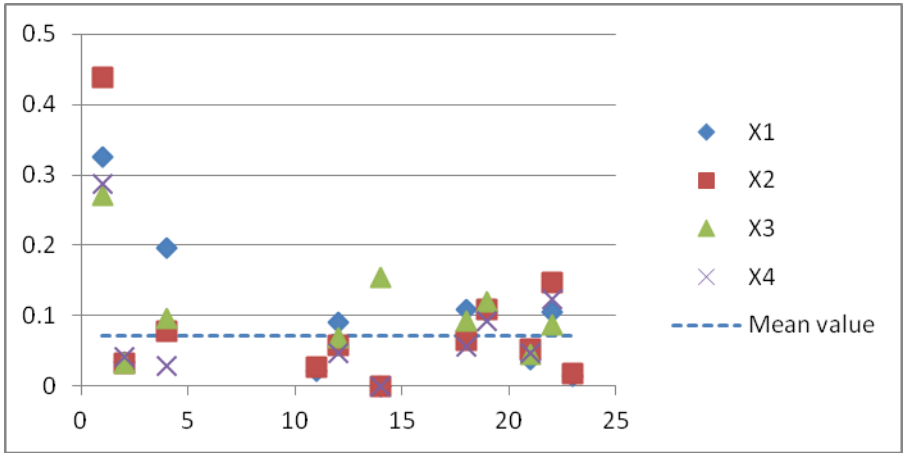
Empty cells = no results reported

Mean value 0,071 mg kg^{-1}

Plot with all reported results



Plot after discarding results from non-compliant laboratories



L01 is Cochran outlier (all results)

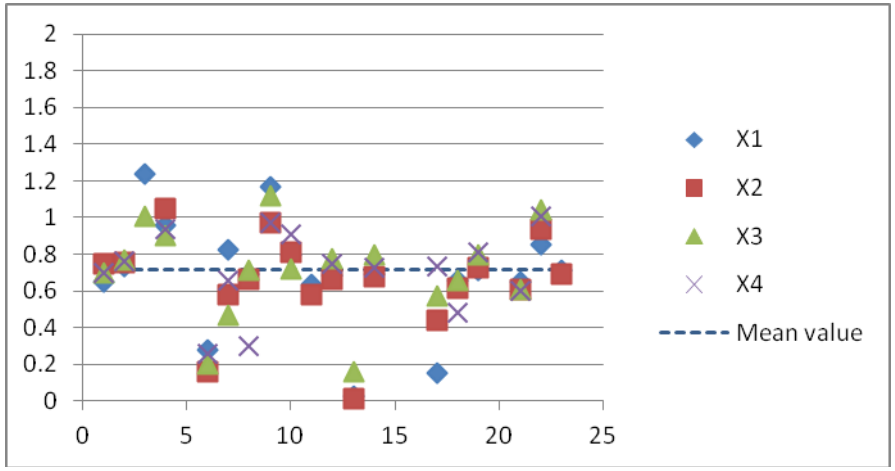
IMEP 32-2 Fish feed (spiked)

	Day 1		Day 2	
Lab no	X1	X2	X3	X4
1	0,651	0,749	0,698	0,695
2	0,734	0,755	0,771	0,763
3	1,238		1,006	
4	0,955	1,046	0,901	0,936
5				
6	0,28	0,16	0,2	0,26
7	0,827	0,582	0,465	0,653
8	0,69	0,66	0,71	0,3
9	1,17	0,971	1,12	0,972
10	0,8	0,81	0,72	0,91
11	0,637	0,58		
12	0,686	0,66	0,775	0,747
13	0,0294	0,0154	0,157	
14	0,693	0,675	0,796	0,728
15				
16				
17	0,149	0,437	0,573	0,73
18	0,656	0,613	0,656	0,481
19	0,709	0,723	0,8	0,812
20				
21	0,647	0,604	0,604	0,599
22	0,8502	0,9388	1,0405	1,0036
23	0,711	0,689		

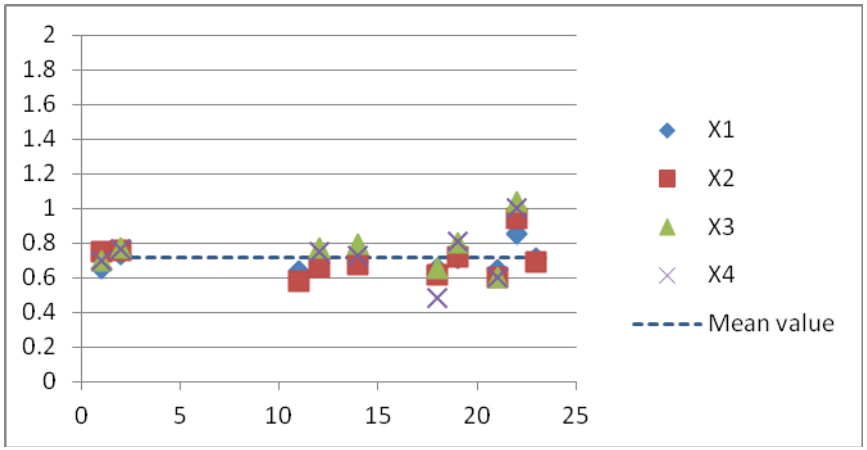
Empty cells = no results reported

Mean value 0,713 mg kg^{-1}

Plot with all reported results



Plot after discarding results from non-compliant laboratories



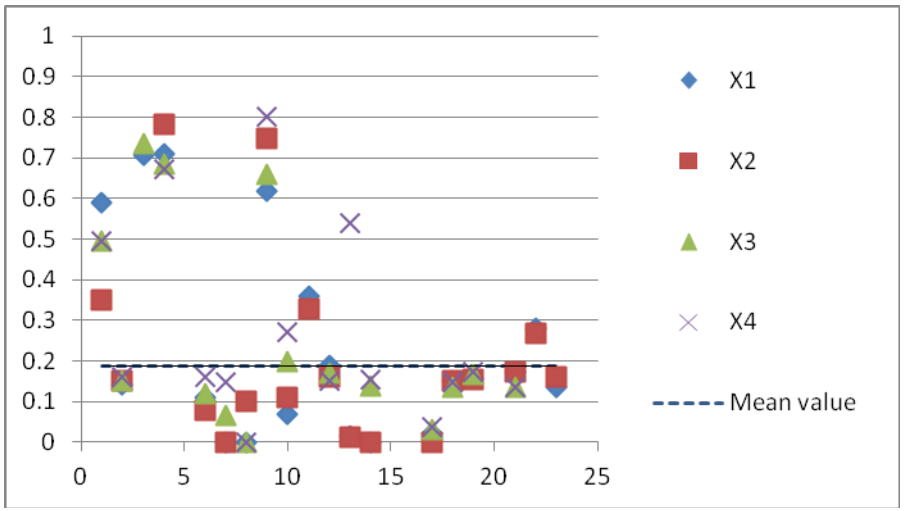
L21 is Grubbs outlier (X1)

IMEP 32-3 Fish meal (unspiked)

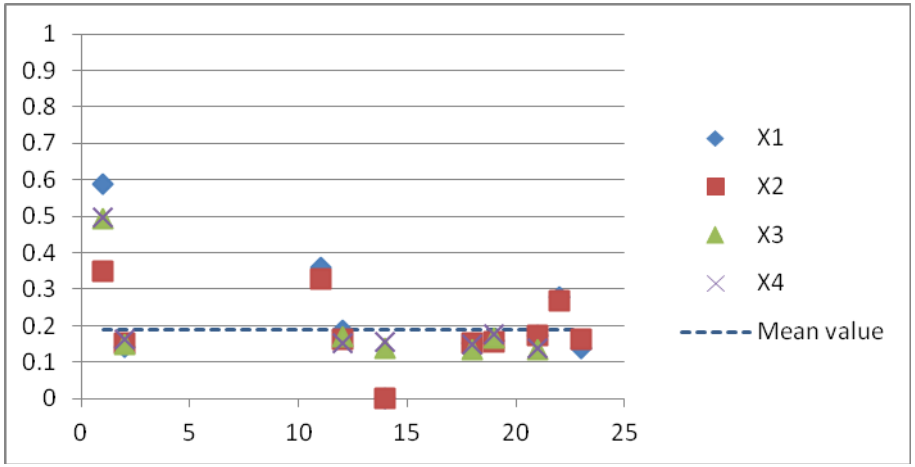
	Day 1		Day 2	
Lab no	X1	X2	X3	X4
1	0,59	0,35	0,495	0,496
2	0,142	0,153	0,15	0,162
3	0,708		0,736	
4	0,71	0,783	0,685	0,673
5				
6	0,11	0,08	0,12	0,16
7	<0,05	<0,05	0,066	0,148
8	0	0,1	0	0
9	0,619	0,748	0,659	0,802
10	0,07	0,11	0,2	0,27
11	0,361	0,328		
12	0,188	0,162	0,171	0,151
13	0,0155	0,0125		0,5396
14	<0,106	<0,106	0,139	0,155
15				
16				
17	0,021	0	0,031	0,039
18	0,153	0,153	0,136	0,149
19	0,159	0,155	0,166	0,175
20				
21	0,174	0,173	0,136	0,137
22	0,2804	0,2667		
23	0,137	0,162		

Empty cells = no results reported

Plot with all reported results



Plot after discarding results from non-compliant laboratories



Mean value	0,189	mg kg^{-1}	L01 is Cochran outlier (all results)

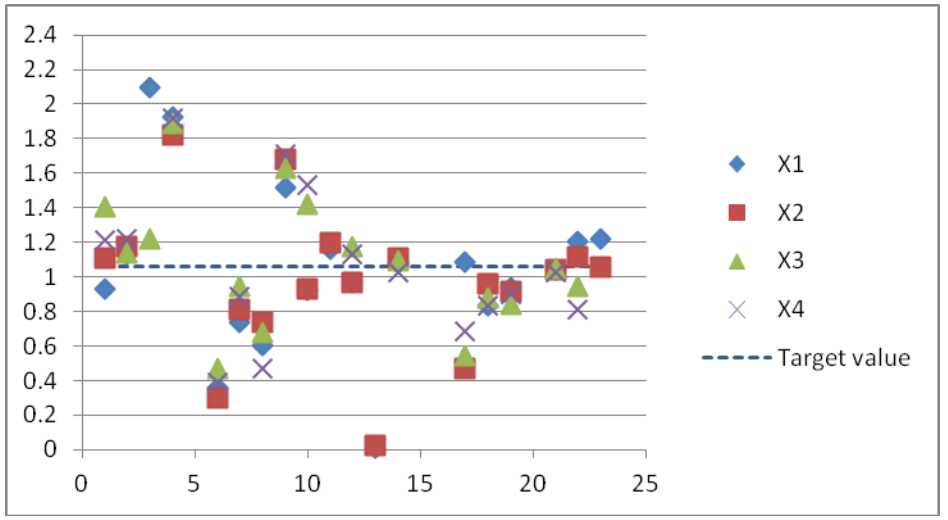
IMEP 32-4 **Fish meal (spiked)**

	Day 1		Day 2	
Lab no	X1	X2	X3	X4
1	0,928	1,106	1,403	1,216
2	1,132	1,172	1,138	1,223
3	2,097		1,22	
4	1,924	1,818	1,892	1,921
5				
6	0,36	0,3	0,47	0,39
7	0,739	0,809	0,945	0,889
8	0,6	0,74	0,68	0,47
9	1,52	1,68	1,63	1,71
10	0,92	0,93	1,42	1,53
11	1,16	1,2		
12	0,968	0,967	1,175	1,133
13	0,0094	0,0247		
14	1,08	1,11	1,09	1,03
15				
16				
17	1,084	0,47	0,543	0,685
18	0,831	0,963	0,875	0,831
19	0,941	0,916	0,842	0,902
20				
21	1,04	1,04	1,05	1,03
22	1,2067	1,1164	0,9472	0,8113
23	1,218	1,055		

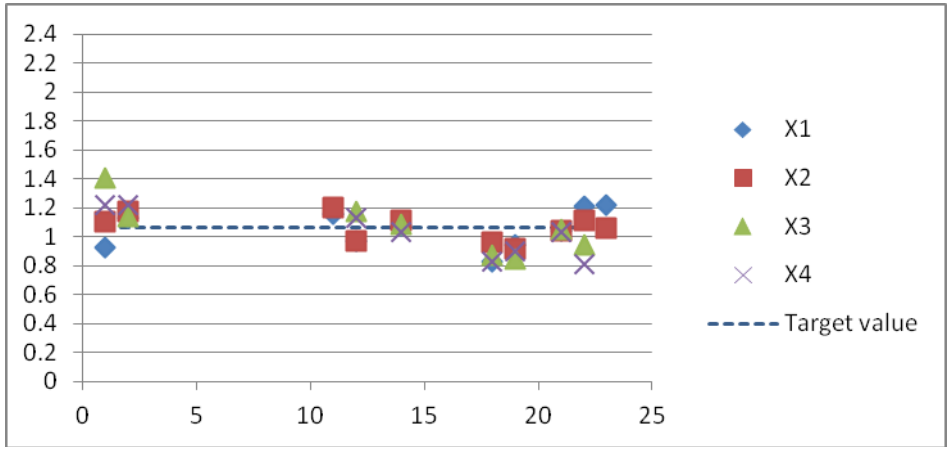
Empty cells = no results reported

Target value 1,062 mg kg^{-1}

Plot with all reported results



Plot after discarding results from non-compliant laboratories



L01 is a Cochran straggler - no outliers identified.

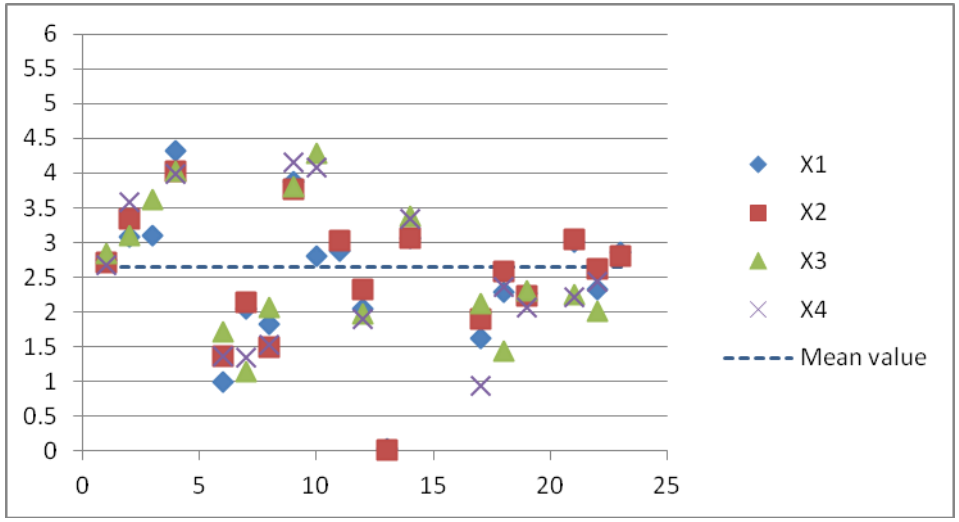
IMEP 32-5 **Fish fillet (spiked)**

	Day 1		Day 2	
Lab no	X1	X2	X3	X4
1	2,748	2,72	2,838	2,679
2	3,079	3,333	3,099	3,577
3	3,094		3,623	
4	4,314	4,024	4,02	3,987
5				
6	1	1,36	1,71	1,37
7	2,055	2,14	1,148	1,337
8	1,83	1,5	2,06	1,53
9	3,87	3,76	3,81	4,16
10	2,8		4,29	4,08
11	2,88	3,02		
12	2,055	2,328	1,97	1,908
13	0,0261	0,0184		
14	3,06	3,07	3,37	3,35
15				
16				
17	1,618	1,908	2,125	0,939
18	2,28	2,58	1,44	2,36
19	2,27	2,23	2,31	2,06
20				
21	3,01	3,05	2,25	2,22
22	2,3316	2,6155	2,002	2,4279
23	2,858	2,801		

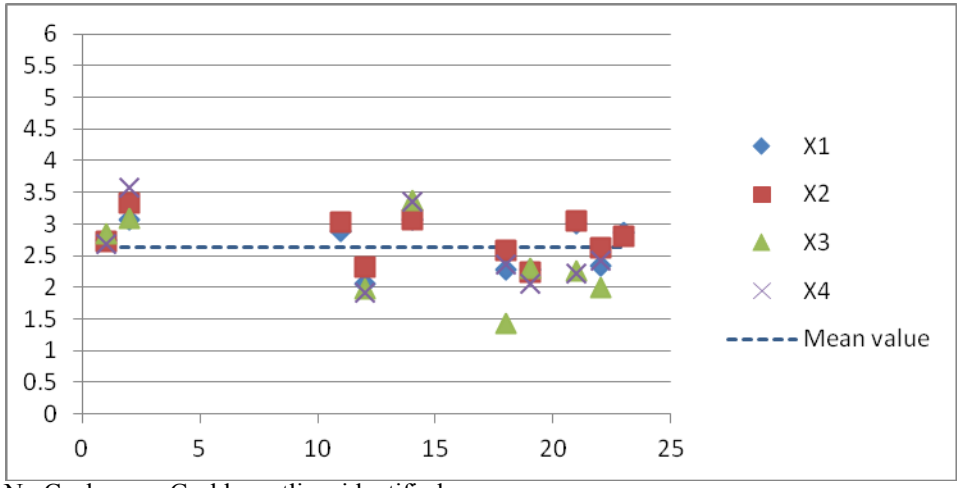
Empty cells = no results reported

Mean value 2,643 mg kg^{-1}

Plot with all reported results



Plot after discarding results from non-compliant laboratories



No Cochran or Grubbs outliers identified.

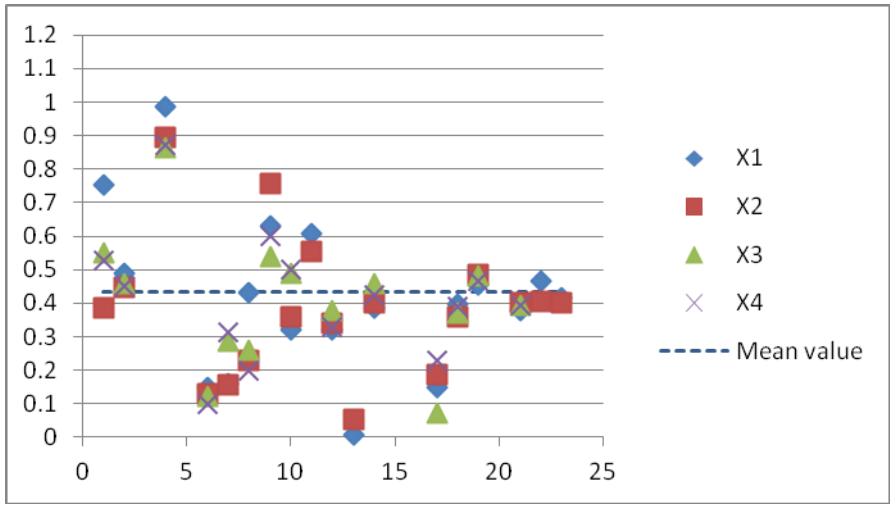
IMEP 32-7 **Fish meal (spiked)**

	Day 1		Day 2	
Lab no	X1	X2	X3	X4
1	0,752	0,387	0,549	0,527
2	0,49	0,447	0,458	0,451
3	1,454		1,206	
4	0,987	0,895	0,865	0,871
5				
6	0,15	0,13	0,12	0,1
7	0,159	0,155	0,286	0,315
8	0,43	0,23	0,26	0,2
9	0,632	0,755	0,537	0,599
10	0,32	0,36	0,49	0,5
11	0,609	0,554		
12	0,319	0,339	0,377	0,328
13	0,0054	0,0533		
14	0,386	0,401	0,459	0,423
15				
16				
17	0,147	0,188	0,074	0,23
18	0,398	0,359	0,372	0,389
19	0,454	0,485	0,48	0,466
20				
21	0,378	0,402	0,393	0,393
22	0,4674	0,4055		
23	0,416	0,4		

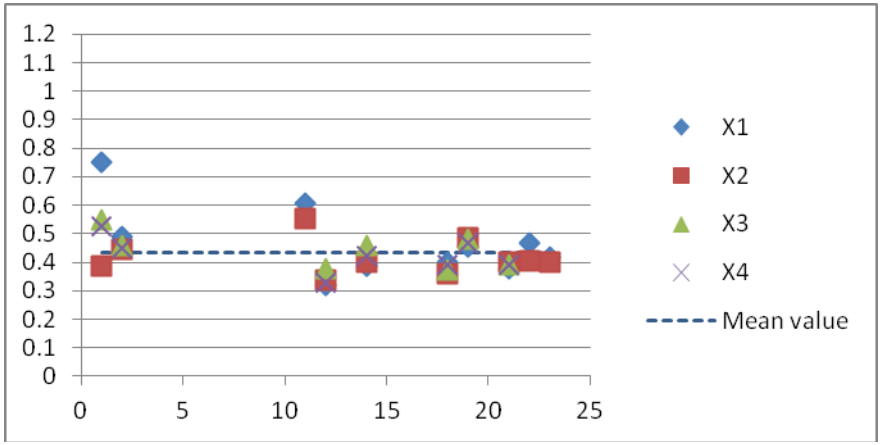
Empty cells = no results reported

Mean value 0,432 mg kg^{-1}

Plot with all reported results



Plot after discarding results from non-compliant laboratories



L01 is a Cochran outlier (all results)

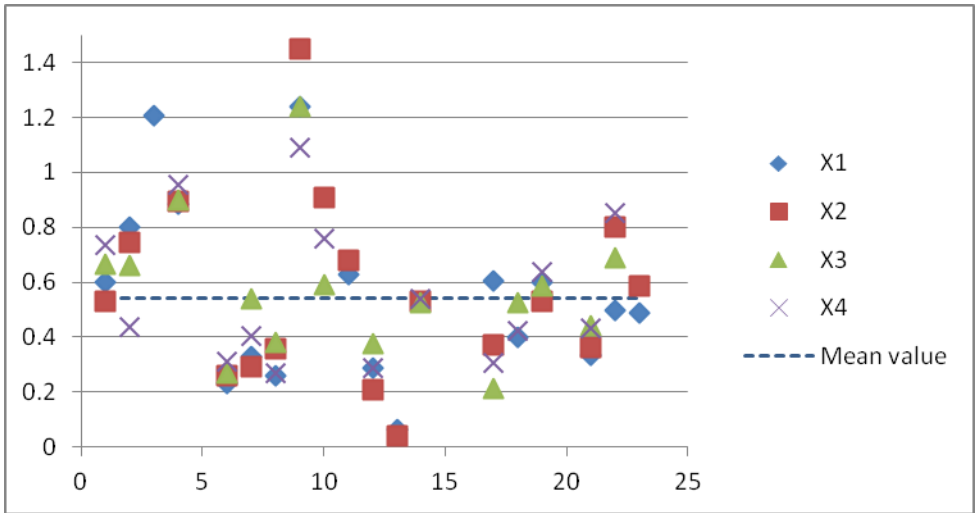
Control sample **TORT-2 Lobster Hepatopancreas**

	Day 1		Day 2	
Lab no	X1	X2	X3	X4
1	0,598	0,531	0,664	0,736
2	0,801	0,743	0,66	0,438
3	1,209		1,715	
4	0,887	0,895	0,899	0,956
5				
6	0,23	0,26	0,27	0,31
7	0,33	0,291	0,539	0,406
8	0,26	0,36	0,38	0,27
9	1,24	1,45	1,24	1,09
10		0,91	0,59	0,76
11	0,627	0,68		
12	0,287	0,21	0,375	0,288
13	0,0645	0,0386		
14		0,529	0,524	0,539
15				
16				
17	0,604	0,37	0,211	0,305
18	0,398		0,525	0,424
19	0,602	0,53	0,588	0,637
20				
21	0,335	0,361	0,441	0,434
22	0,496	0,8022	0,6867	0,8522
23	0,490	0,585		

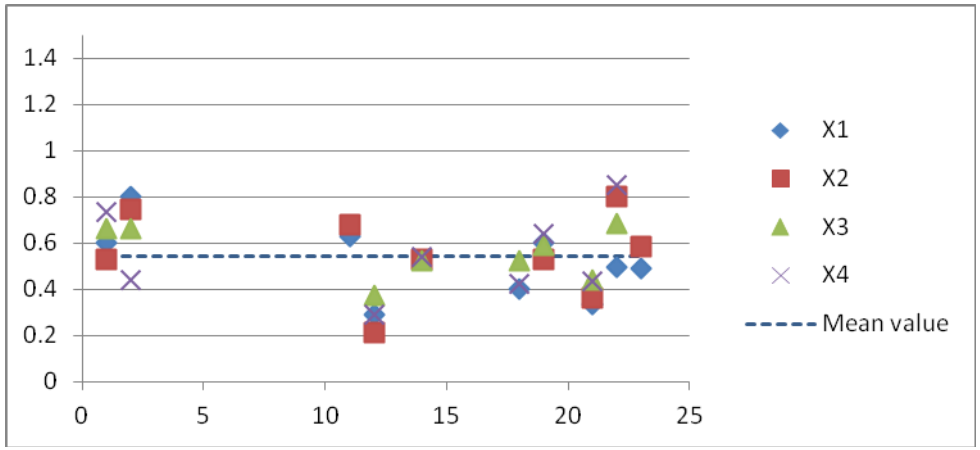
Empty cells = no results reported

Mean value 0,544 mg kg^{-1}

Plot with all reported results



Plot after discarding results from non-compliant laboratories



No Cochran or Grubbs outliers identified.

European Commission

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Abstract

A collaborative study, IMEP-32, was conducted in accordance with international protocols to determine the performance characteristics of an analytical method for the determination of inorganic arsenic in animal feed of marine origin. The method would support Directive No 2002/32/EC of the European Parliament and the Council on undesirable substances in animal feed where it is indicated 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 is based on solid phase extraction (SPE) separation of inorganic arsenic from organoarsenic compounds followed by detection with hydride generation atomic absorption spectrometry (HG-AAS). The collaborative study investigated different types of samples of marine origin, including complete feed (unspiked and spiked), fish meal (unspiked and spiked), fish fillet (spiked) and a lobster hepatopancreas (unspiked). In total seven samples were investigated within the concentration range of 0.07 – 2.6 mg kg^{-1}.
The test samples were dispatched to 23 laboratories in 12 different countries. Nineteen participants reported results. The performance characteristics are presented in this report. All method performance characteristics obtained in the frame of this collaborative trial indicates that the proposed SPE-HG-AAS standard method is fit for the intended analytical purpose.

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