Report on collaborative trial

Animal feedingstuffs - Determination of iodine in animal feed by ICPMS

DTU Food
National Food Institute

Report on collaborative trial

Animal feedingstuffs – Determination of iodine in animal feed by ICPMS

CEN/TC 327/WG4 Heavy metals, minerals and trace elements
Work item: Iodine in animal feed

Jens J. Sloth
February 2017

National Food Institute
Technical University of Denmark

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Animal feedingstuffs – Determination of iodine in animal feed by ICPMS

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Collaborative trial report

Animal feedingstuffs
Mandate: M522
Project responsible: CEN TC327
Secretariat: NEN (organisational work on behalf of CEN TC327)
Work item: Iodine in animal feed
Project leader: Dr. Jens J. Sloth, National Food Institute DTU, Denmark

Content

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

Date: 13. November 2015

1. Introduction

Iodine occurs in nature primarily as iodide and iodate. Its mineral forms occur ubiquitously in igneous rocks and soils, most commonly as impurities in saltpeter and natural brines. Iodine is an essential trace element for animals and humans. The only known role of iodine in metabolism is its incorporation into the thyroid hormones, thyroxine (T4; 3,5,3,5-tetraiodothyronine) and triiodothyronine (T3; 3,5,3-triiodothyronine) as well as the precursor iodothyrosines. Both hormones have multiple functions as regulators of cell activity (energy metabolism) and growth and as transmitters of nervous stimuli and play an important role in brain development. The iodine requirements for animals vary typically between 0,1-1,1 mg/kg feed. Within the different species the requirements are influenced by physiological demands for growth, reproduction or lactation and also by dietary factors (e.g. goitrogens). In many cases iodine supplementation or daily ration is necessary due to the low iodine content of plantbased feed ingredients (EFSA, 2005). Iodine may be present as a natural constituent of animal feed and feed materials or be added to the complete feed as a nutritional additive (EC1831/2003).

2. Project background and time frame

In 2012 a tender for a project with the aim to develop a European standard method for the determination of iodine in animal feedingstuffs NEN on behalf of CEN TC327. DTU Food was selected as project leader. A service contract between NEN and DTU Food was signed in 2013 and the project official start date was 01-01-2013. The project outline, including method principle (method principle to be based on EN15111:2007), test sample selection and design of collaborative trial was presented by the project leader and discussed and agreed upon within the CEN TC327/WG4 expert group at meetings in Brussels 02/02-15 . The collaborative trial was conducted in 2015 with participants from 14 different laboratories from 7 different countries. The results from the collaborative trial were presented and discussed by the project leader at the members of CEN TC327/WG4 at a meeting in Bruxelles 26. October 2015 and comments were received from the expert group members of WG4. The present report has been prepared in November 2015.

3. Scope

The aim of the present project is to develop a European standard method (EN) for the determination of iodine in animal feedingstuffs. The method should be based on the method principle outlined in

EN15111:2007 and use alkaline extraction followed by iodine-selective determination by ICPMS. Furthermore a collaborative trial was conducted to evaluate the performance characteristics of the method using ISO 5725-2.

4. Sample material – preparation and homogeneity testing

Several different sample materials were evaluated as candidate test materials for the collaborative trial. The selected test samples included several (certified) reference materials and proficiency test materials, for which suitable homogeneity already has been verified by the supplier. For two of these test materials target values for iodine has been established from previous use in a proficiency testing scheme and these values were used to evaluate the accuracy of the present methodology (VDLUFA, 2012). All bottles were clearly labelled and numbered consecutively according to bottling order. The following Table 1 provides an overview of the sample materials selected for the collaborative trial.

Table 1 Overview of sample material for the collaborative trial and their expected approximate concentrations.

No	Sample ID	Sample type	Target value (mg/kg)
Sample 1	BCR-CD200	Seaweed meal	4-800
Sample 2	VDLUFA 5	Mineral premixture	0,72
Sample 3	IMEP-32-3	Fish meal	1-2
Sample 4	VDLUFA 6	Plantbased ingredient	12,65
Sample 5	IMEP-32-1	Complete feed (marine based)	2-4
Sample 6	FAPAS T04249QC	Animal feed (cereal based)	2-4
Sample 7	CaIO3	Mineral additive	high
Sample 8	Iodine solution	Iodine solution	0,25 (mg/l)

Samples 1, 2, 4 and 6 are certified or reference samples and thus the homogeneity was presumed to have been verified previously. Sample 7 was a pure calcium iodate salt with very fine particles and homogeneity was hence assumed. Sample 8 was a solution made from a certified iodine stock solution and hence considered homogeneous. These assumptions were furthermore discussed and accepted at the CEN WG4 meeting at 26/10-2015 in Brussels. The samples were bottled in small

dark glass containers in order not to reveal the identity of the samples to the participants. For samples 3 and 5 homogeneity tests have been performed successfully demonstrating sufficient homogeneous sample material (Annex 8). Both samples were obtained from a previous collaborative trial (IMEP-32) on inorganic arsenic in animal feed. The homogeneity of sample 6 was first assumed to be satisfactory and the samples were shipped to the participant. However, the results obtained indicated poor homogeneity and this inhomogeneity was subsequently verified by internal analysis at DTU Food, which confirmed insufficient homogeneity of sample 6 and hence this sample material was not included further in the method evaluation. This conclusion was taken in agreement with CEN TC327/WG4 at the meeting 26/10-2015. Furthermore the WG4 group decided not to include sample 7 in the evaluation. Sample 7 is a pure iodine salt and by experience the ICPMS method is not the suited method for such a material. The results for samples 6 and 7 are included in the present report for information only, but are not presented in the EN.

5. Participant invitation and information

The method was tested in a collaborative trial with 14 participating laboratories from 7 different countries (Denmark, Norway, Germany, Ireland, Czech Republic, Austria and The Netherlands). 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 1st of July 2015 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 the time period from August to October 2015.

6. Method principle

The method is based on the same principles as the CEN standard EN15111:2007 for determination of total iodine in foodstuffs (CEN TC275/WG10). The method procedure uses a simple and easy-to-use alkaline extraction with extraction solvent (tetramethyl ammonium hydroxide; TMAH) in an oven at slightly elevated temperature (90°C) for 3 hours. The determination of iodine is

subsequently performed with Inductively Coupled Plasma Mass Spectrometry (ICPMS) using tellurium (Te) as internal standard. A matrix-matched external calibration curve is used for the quantification of the iodine content in the sample extracts.

7. Results and statistical evaluation

7.1. Results

Fourteen laboratories signed up to participate in the collaborative trial. The reported results from the participating laboratories can be found in annex 6.

7.2. Laboratories compliance

Initial evaluation of the results revealed that the results reported by laboratory 3 were consistently deviating from the level reported by the other participants. Hence, laboratory 3 was judged as a non-compliant lab and the results of laboratory 3 were discarded from the statistical evaluation based on section 7.2.5 in ISO 5725-2. This decision was supported by the CEN TC327/WG4 expert group at the meeting in Brussel 26/10-2015.

Table 2 List of non-compliant laboratories

Non-compliant lab	**Reason**
L03	Abnormal test results

7.3. Outlier identification

Following the initial identification of non-compliant laboratories, results from the remaining 13 laboratories were subjected to statistical analysis following international standard recommendations ISO5725-2. First step was to identify outliers (1% confidence level) and stragglers (5% confidence level) by the Cochran and Grubbs tests. Table 3 provides an overview of the outlying results identified and the outlier/straggler type.

Table 3 Overview of outliers and stragglers identified by the Cochran and Grubbs tests.

No	**Sample type**	**Outlier lab**	**Outlier/Straggler type**
1	Seaweed meal	L01	Cochran outlier
2	Mineral premixture	L05, L07	Cochran outliers

3	Fish meal	L07	Cochran outlier
4	Plantbased ingredient	L08	Grubbs outlier
5	Compound feed (marine based)	L06	Cochran outlier
6	Animal feed (cereal based)	Not homogenous	
7	Mineral additive	L01 L07	Cochran outlier Cochran outlier
8	Solution	L05 L11	Grubbs outlier Grubbs outlier

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

7.4. Statistical evaluation of the results

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

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

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

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

An overview of the method performance characteristics can be found in Table 4.

In the following the data for samples 6 and 7 will not be commented as these samples were excluded from the evaluation as previously discussed.

The relative standard deviation under repeatability conditions (within-laboratory), RSD_r was in the range from 2,3 – 14,1 % and the relative standard deviation under reproducibility conditions (between-laboratory), RSD_R was in the range 7,7 – 22,2 %. These values are satisfactory and indicate that the method has a satisfactory precision. Samples 2 and 4 have previously been used in a VDLUFA proficiency trial with the following target values:

Sample 2: 12,65 mg/kg (overall mean = 13,58 mg/kg)

Sample 4: 0,72 mg/kg (overall mean = 0,70 mg/kg)

Both overall mean values are in good agreement with the target values indicating satisfactory accuracy of the method. Furthermore, sample 8 is an in-house produced iodine standard solution at 250 µg/l (made from certified iodine stock solution). The overall mean for sample 8 is 266 µg/l, which is also in good agreement with target value.

The method working range was established in the concentration range 0,70 mg/kg to 631 mg/kg. HorRat values in the range of 0,40-1,33 were obtained, which is very satisfactory and all below the guideline value of 2.

Table 5 Method performance characteristics from the collaborative trial.

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8 OBS ug/L
No of labs		14	14	14	14	14	14	13	14
Non-compliant labs		1	1	1	1	1	1	1	1
No of Cochran stragglers		0	0	0	0	0	0	0	0
No of Cochran outliers		1	2	1	0	1	0	1	1
No of Grubbs stragglers		0	0	0	0	0	0	0	0
No of Grubbs outliers		0	0	0	1	0	0	0	1
No of valid labs		12	11	12	12	12	13	11	11
Outlier percentage	%	7,1	14,3	7,1	7,1	7,1	0,0	7,7	14,3
Overall mean	mg kg^{-1}	631	13,58	1,34	0,70	3,39	2,90	323221	266
Sr	mg kg^{-1}	14,56	0,61	0,10	0,10	0,33	0,82	4575	6,21
RSDr	%	2,31	4,52	7,73	14,12	9,62	29,80	1,42	2,33
rL	mg kg^{-1}	40,77	1,72	0,29	0,28	0,91	2,29	12810	17,38
SR	mg kg^{-1}	48,25	1,11	0,10	0,16	0,59	1,62	54693	20,89
RSDR	%	7,65	8,14	7,73	22,23	17,36	59,23	16,92	7,85
RL	mg kg^{-1}	135,10	3,09	0,29	0,44	1,65	4,54	153139	58,48
Horwitz value according to Horwitz	%	6,04	10,75	15,21	16,69	13,24	13,66	2,37	19,36
HorRat value according to Horwitz	-	1,27	0,76	0,51	1,33	1,31	4,34	7,13	0,41
Horwitz value according to Thompson		6,06	10,80	15,31	16,86	13,31	13,63	1,76	19,52
HorRat value according to Thompson		1,26	0,75	0,50	1,32	1,30	4,35	9,62	0,40

8. Conclusion

A method for the determination of iodine in animal feedingstuffs was developed at DTU Food. The method principle is based on alkaline extraction with dilute TMAH followed by determination of iodine by ICPMS.

The method performance characteristics were assessed in a collaborative trial with 14 participating laboratories on five different feed samples within the concentration range of 0,70 – 631 mg kg^{-1} in addition to an aqueous iodine solution. 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 iodine in animal feedingstuffs.

9. Acknowledgements

Dr. Rie R. Rasmussen (DTU Food) has been an immense help with the practical work on the method development. Ms Marianne Hansen and Mrs Annette Landin (DTU Food) has skilfully conducted most of the practical work with the characterisation of test materials as well as preparation and shipping of test materials to the participants. Finally and important all the participating laboratories are thanked for their voluntary participation in the collaborative trial, for their production of good results and their useful comments on the method. Thanks go also to the members of the CEN TC327/WG4 expert group for their constructive comments and encouragement during the project period.

10. References

- Horwitz W., Albert R. The Horwitz Ratio (HorRat): A Useful Index of Method Performance with Respect to Precision. J. AOAC Int. 2006, 89, 1095–1109
- Thompson M. Recent Trends in Inter-Laboratory Precision at ppb and sub-ppb Concentrations in Relation to Fitness for Purpose Criteria in Proficiency Testing. Analyst (Lond.), 2000, 125, 385–386
- IMEP-32, 2011, Determination of Inorganic Arsenic in Animal Feed of Marine Origin: A Collaborative Trial Report, https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/imep-32-determination-inorganic-arsenic-animal-feed-marine-origin-collaborative-trial-report (last accessed on 30/11/15)
- VDLUFA, 2012, Auswertung der Ringanalyse 150/2011/Q des Arbeitskreises Anorganik der Fachgruppe Umwelt- und Spurenanalytik des VDLUFA „Jodbestimmung in Futter- und Lebensmitteln mit ICP-MS nach unterschiedlichen Aufschluss Extraktionsverfahren“

Annex 1. Invitation letter to collaborative trial

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

Invitation for participation in collaborative studies:

Determination of iodine in animal feeding stuffs by ICPMS following alkaline extraction

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Denmark, June 2015

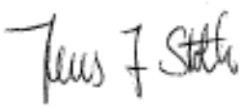
Dear colleague,

You are hereby invited to participate in a collaborative study of a method for the determination of iodine in animal feed. The method is based on the same principles as in EN15111:2007 Foodstuffs – Determination of trace elements – Determination of iodine by ICP-MS (inductively coupled plasma mass spectrometry), where the iodine content is determined by ICPMS following alkaline extraction with TMAH.

The method principles and the set-up of the collaborative trial have been discussed and agreed upon in the CEN TC327/WG4 expert group, where also the results of the collaborative trial will be evaluated and the final version of the standard will be drafted together with the project leader.

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 back to me. Your efforts are very much appreciated thanks in advance.

Best regards,



Dr. Jens J. Sloth (senior scientist)
Project-leader of the collaborative trial

Introduction:

An international collaborative study will be conducted under the CEN leadership to evaluate a method for the determination of iodine in animal feeds. The proposed method has been discussed and approved within CEN/TC 327/WG 4. The National Food Institute at the Technical University in Denmark (DTU Food) will organize this collaborative trial.

Principle of the method:

Extraction of iodine is done by alkaline extraction with a solution of diluted TMAH. Iodine is determined by using inductively coupled plasma mass spectrometry (ICPMS). A description of the method procedure to be followed will be sent. The method is based on the principles in EN15111:2007 Determination of iodine in foodstuffs by ICPMS.

Samples:

The set-up and execution of the collaborative study will be done according to international standards, e.g. the IUPAC protocol (for the design, conduct and interpretation of method-performance studies) (Pure&Appl.Chem, 1995, 67, 331] and ISO5725-2.

Feed samples with unknown concentrations (broad concentration range) will be sent to the participating laboratories for analysis and the data will be used for the subsequent statistical validation of the proposed method. The laboratories are asked to perform duplicate analysis of all samples and to provide information regarding e.g. experience level of the lab, the instrumentation used, any deviations from method procedure etc.

Requirements to the participating laboratories:

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

- Conventional/drying oven - capable of controlling the temperature at 90 +/- 3 °C
- Inductively Coupled Plasma Mass Spectrometer (ICPMS instrument)
- Reagents needed: TMAH, Iodine stock solution, Tellurium stock solution

Time schedule:

- Estimated time for dispatch of samples – **first half of July 2015**
- Deadline for submission of results: **11. September 2015.**
- Discussion of results will subsequently take place in CEN TC327/WG 4
- Report on the outcome of the collaborative trial will be sent to participants

Organisation of the study:

The studies are organised by the National Food Institute at the Technical University of Denmark (DTU Food).

The contact address is:
Jens J. Sloth (project leader)
E-mail: jjsl@food.dtu.dk
Phone: +45 3588 7625
National Food Institute
Mørkhøj Bygade 19
DK-2860 Søborg
Denmark

Participation in the study:

Please send the attached registration form to jjsl@food.dtu.dk

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

Registration form for collaborative study:

Animal feeding stuffs – Determination of iodine in animal feedingstuffs by ICPMS following alkaline extraction

Yes, I will participate in the collaborative study ☐ or **No**, cannot participate ☐

Does the lab perform iodine analysis of food and feed? **YES** ☐ **NO** ☐

If yes, what method is used? **EN15111:2007** ☐

Other ☐ **(describe shortly)**

Approx number of samples/year for iodine? <50 ☐; >50 ☐

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

Please send this registration form as soon as possible by email to: **jjsl@food.dtu.dk**

Annex 2 Accompanying letter to participants

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

July 2015
/jjsl

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

Dear participant,

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

The deadline for submission of results is Friday 11/09/2015

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

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

or

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

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

Best regards,

Jens J. Sloth

INSTRUCTIONS – Collaborative trial on iodine 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 concentration range of iodine in the samples.

Sample ID	Sample type	Approx sample amount (g/bottle)	Recomm. test portion size for analysis (g)	Concentration range (mg/kg)
CENFEEDiodine – S1	Seaweed meal	1,5	0,3	100-1000
CENFEEDiodine – S2	Mineral premixture	3	0,5	5-20
CENFEEDiodine – S3	Marine based feed ingredient	3	0,5	0,5-5
CENFEEDiodine – S4	Plantbased feed ingredient	2,5	0,5	0,1-2
CENFEEDiodine – S5	Complete feed (marine based)	3	0,5	0,5-5
CENFEEDiodine – S6	Complete feed (cereal based)	3	0,5	0,5-5
CENFEEDiodine – S7	Mineral iodine-salt additive	3	0,3	high
CENFEDDiodine – S8	Aqueous standard	5 ml/tube	Note: Dilute 10 times prior to analysis	5-50 µg/l

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

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

B: Analysis of samples

For the collaborative study please perform two independent measurements on the same day and please remember to follow the draft method procedure carefully

PLEASE REMEMBER - THIS IS A STUDY OF THE METHOD - NOT OF THE LABORATORY PERFORMANCE. THE METHOD MUST BE STRICTLY FOLLOWED AS DESCRIBED.
It is very important that you report any deviation from the method. Comments to the method procedure description are also welcome.

C: Standard solution (CENiodine 8)

The standard solution (CENFEEDiodine – S8) contains a known amount of iodine. Please dilute the solution 10 times prior to measurement. Dilute with the same solution as you use for dilution of calibration standards.

D: Reporting of results

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

Furthermore please fill in the questionnaire. This information is valuable for the subsequent evaluation of the results. Remember to note all deviations and if anything unexpected happens during analysis.

E: Method procedure and other forms

You will per email receive the following:

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

F: Summary

Please provide the following:

1. Confirmation upon receipt of samples to jjsl@food.dtu.dk
2. A reporting scheme with the results from the analysis of the samples following the method protocol
3. Fill in the questionnaire
4. Report any deviation and unexpected observations

G: Thanks for your contribution – highly appreciated

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

Results scheme

CEN TC327/WG4 Collaborative trial on determination of iodine 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})
CENFEEDiodine – sample 1				
CENFEEDiodine – sample 2				
CENFEEDiodine – sample 3				
CENFEEDiodine – sample 4				
CENFEEDiodine – sample 5				
CENFEEDiodine – sample 6				
CENFEEDiodine – sample 7				

CENFEEDiodine – sample 8			µg/l	µg/l
Reagent blank	-------	------	µg/l	µg/l

Deadline for submission of results: **Friday 11. September 2015**
Remember to fill in the questionnaire.

Please send to: jjsl@food.dtu.dk

Annex 4. Questionnaire

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

Please complete this questionnaire.

Laboratory name: _____

1. **Method related questions**

1.1 Which equipment did you use?
ICPMS: _____
Drying oven for extraction: _____
Oven temperature (°C): _____
Any comments?:
_____
_____

1.2 What type of calibration was used to quantify the iodine content of the samples? (external calibration, standard addition, addition calibration)_____

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?
CENFEEDiodine-sample 1: _____
CENFEEDiodine-sample 2: _____
CENFEEDiodine-sample 3: _____
CENFEEDiodine-sample 4: _____
CENFEEDiodine-sample 5: _____
CENFEEDiodine-sample 6: _____
CENFEEDiodine-sample 7: _____
CENFEEDiodine-sample 8: _____

1.5 How and for how long time did you store the sample extracts in the time period from extraction to analysis?
_____

1.6 Did you analyse any reference materials together with the samples? If yes, which and provide the recovery obtained._____

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 Please provide the concentration of the sample blank solution (µg/l)?_____

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

1.11 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 (or in 6):
_____
_____

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 together with the results of the analysis.
Thanks for your time ☺

Annex 5. List of participating laboratories

Lab	City	Country	contact person
LTZ Augustenberg	Karlsruhe	Germany	Dr. Klaus Michels
NIFES	Bergen	Norway	Dr Heidi Amlund
AGES (Österreichische Agentur für Gesundheit und Ernährungssicherheit)	Linz	Austria	Dr Gerhard Liftinger
Central Institute for Supervising and testing in Agriculture (UKZUZ)	Brno	Czech Republic	Eva Cizmarova
Thüringer Landesanstalt für Landwirtschaft (Tll)	Jena	Germany	Katrin Spörl
Statliche Betriebsgesellschaft fûr umwelt und landwirtschaft (BfUL)	Nossen	Germany	Ralf Klose
RIKILT	Wageningen	The Netherlands	Martijn van der Lee
Danish Veterinary and Food Administration	Lystrup	Denmark	Inge Rokkjær
DTU Food	Søborg	Denmark	Jens Sloth
Muva Kempten	Kempten	Germany	Ingo Piccon
State Veterinary Institute Olomouc, Laboratory Kromieriz	Kromeriz	Czech Republic	Alena Simakova
Landesanstalt für Landwirtschaftliche Chemie (Universität Hohenheim)	Stuttgart	Germany	Dr Sonja Schlosser
Landeslabor Berlin-Brandenburg	Berlin	Germany	Dr Susanne Pieper
The State Laboratory	Celbridge Co. Kildare	Ireland	John Fields

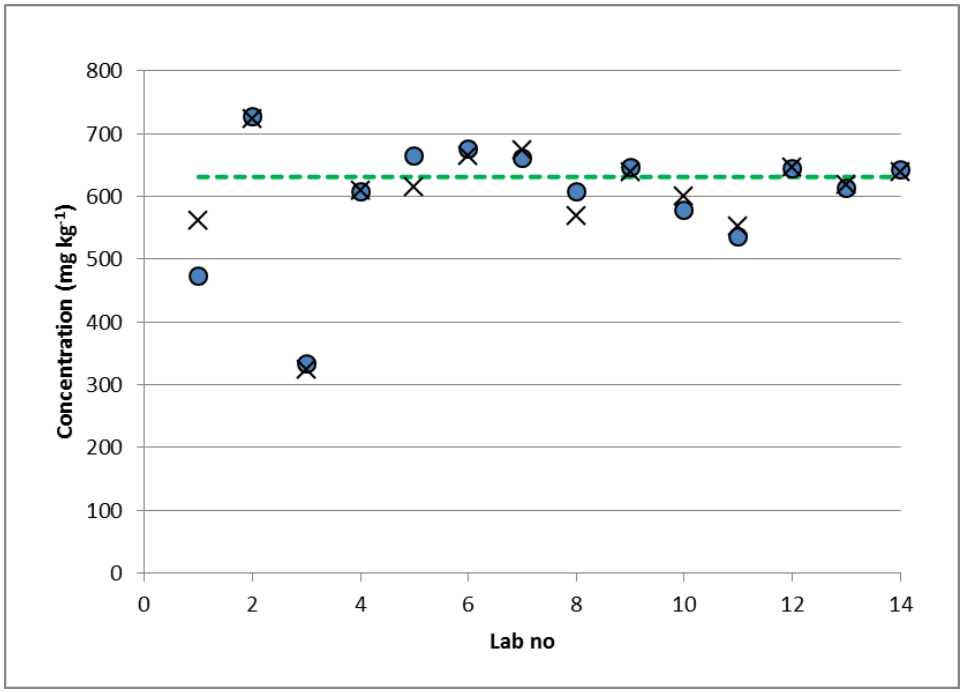
Annex 8 Results reported by the participating laboratories

Lab No	sample 1		sample 2		sample 3		sample 4		sample 5		sample 6		sample 7		sample 8	
1	473	561	11,9	12,5	1,18	0,882	0,68	0,69	2,07	2,22	6,01	7,94	296338	334739	305	305
2	727,46	723,41	14,54	13,5	1,38	1,35	0,72	0,75	4,14	3,15	2,8	1,58	345361,5	338977,5	256,6	252,5
3	334	323,8	7,816	7,708	0,81	0,799	0,326	0,328	1,865	1,783	6	5,78	2118	2138	0,157	0,157
4	607	609	15,3	15,4	1,52	1,52	1,06	0,83	3,19	3,81	2,65	3,57	271700	262900	280	282
5	664	615	17,5	13,9	1,63	1,95	0,6	0,73	4,37	4,35	2,48	2,04	448490	446000	305	258
6	676	665	13,3	13,8	1,1	1,31	0,69	0,61	2,63	7,69	4,7	2,01	338000	326000	270	271
7	661	674	13,9	22,1	5,83	4,76	0,653	0,978	3,58	3,19	1,62	1,72	366000	293000	270	274
8	607	568	14,5	15	1,36	1,34	0,124	0,052	3,05	3,97	0,954	0,392	259229	249320	223	221
9	645,5	638,5	13,58	14,24	1,456	1,317	0,697	0,63	3,64	3,608	3,255	3,967	323600	324800	270,9	271,1
10	577	599	12,6	12,4	1,07	1,06	0,669	0,498	3,31	3,17	4,22	3,78	330000	334000	266	269
11	535	552	15,3	13,5	1,19	1,18	0,569	0,456	3,57	3,7	3,14	3,35	270000	275000	526	521
12	644	645	13,4	13,3	1,47	1,48	0,885	0,957	3,58	3,65	2,15	2,28	343644	344396	273	271
13	613	618	12,7	12,5	1,35	1,33	0,75	0,772	3,23	3,35	2,04	2,77	318000	315000	253	261
14	642	639	11,9	13,5	1,19	1,19	0,54	0,49	2,56	2,89	1,25	2,74	nd	nd	267	240

- Lab 3 was considered non-compliant and not included in the statistical evaluation.

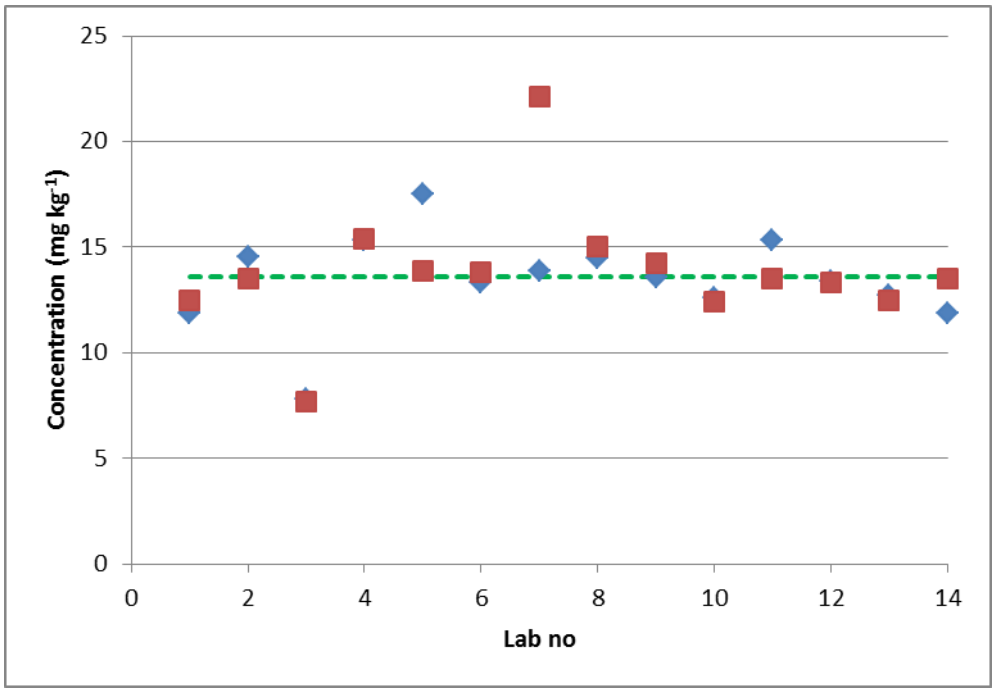
Annex 9 Plots of results from compliant laboratories

Sample 1 Seaweed meal (mean value +/- u_{obs} = 631 +/- 47 mg/kg)



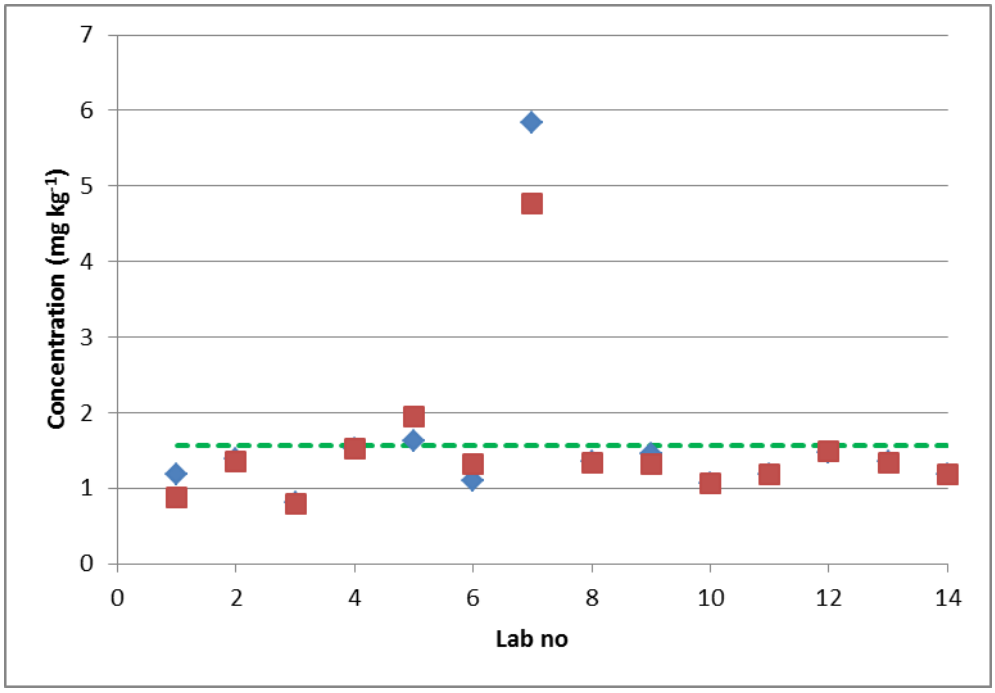
L01 is a Cochran outlier.
L03 is non-compliant lab and not included in the statistical evaluation.

Sample 2 Mineral premixture (mean value +/- u_{obs} = 13,6 +/- 1,1 mg/kg)



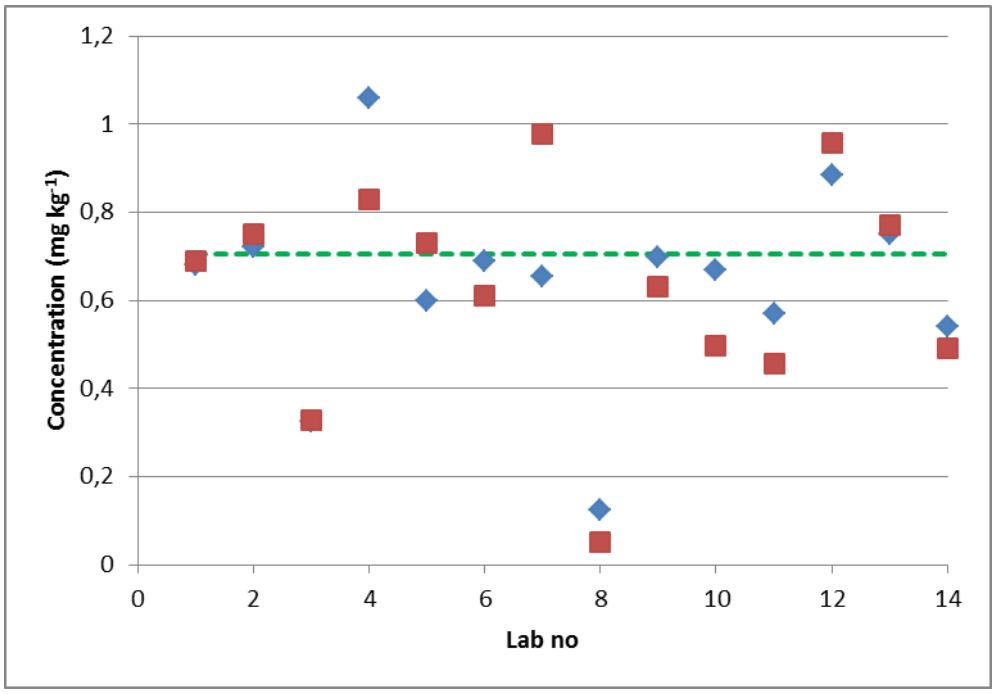
L05 and L07 are Cochran outliers.
L03 is non-compliant lab and not included in the statistical evaluation.

Sample 3 Fish meal (mean value +/- u_{obs} = 1,34 +/- 0,22 mg/kg)



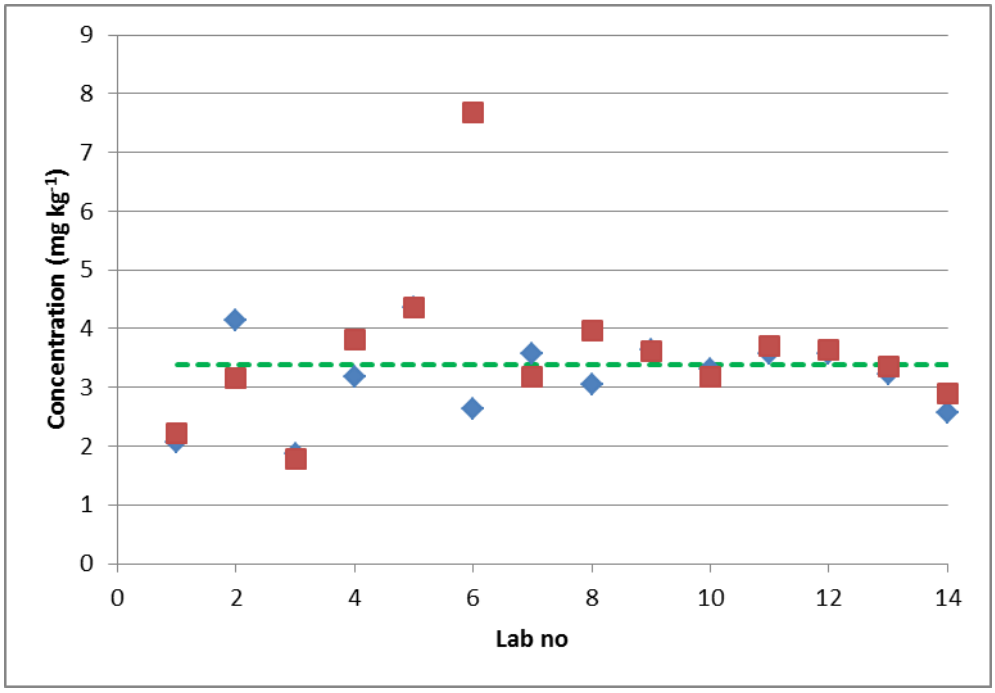
L07 is a Cochran outlier.
L03 is non-compliant lab and not included in the statistical evaluation.

Sample 4 Plantbased ingredient (mean value +/- u_{obs} = 0,70 +/- 0,15 mg/kg)



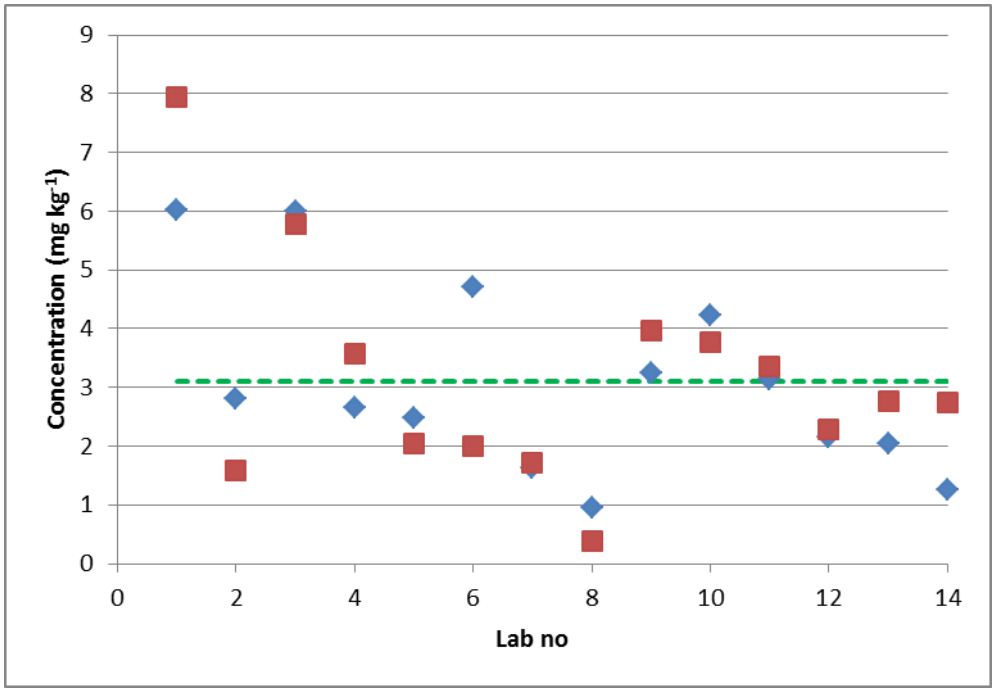
L08 is a Grubbs outlier.
L03 is non-compliant lab and not included in the statistical evaluation.

Sample 5 Complete feed (mean value +/- u_{obs} = 3,39 +/- 0,58 mg/kg)



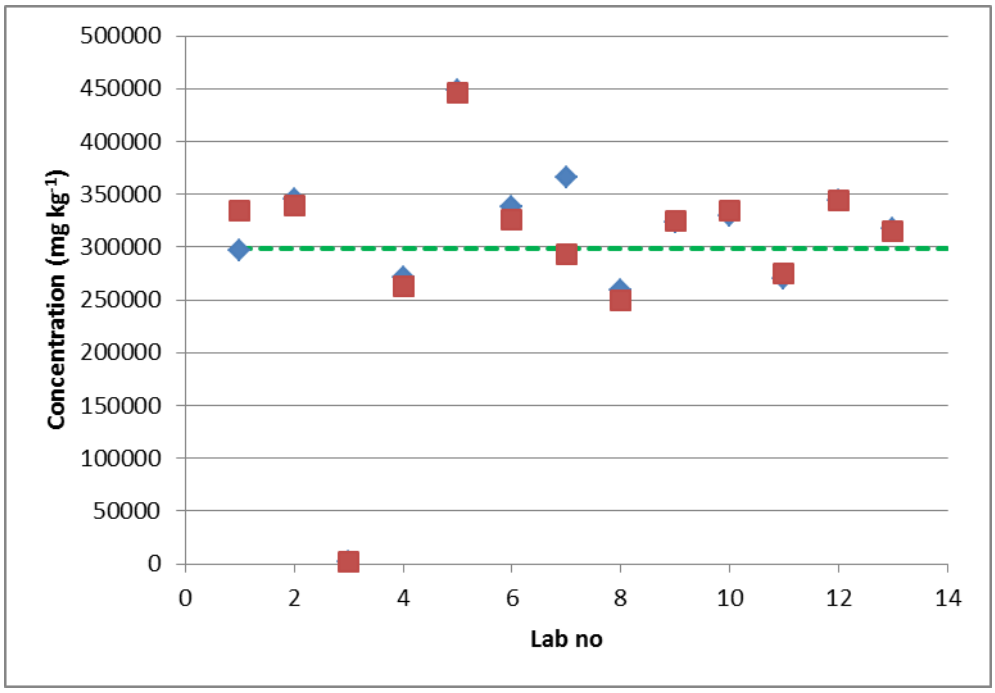
L06 is a Cochran outlier.
L03 is non-compliant lab and not included in the statistical evaluation.

Sample 6 Animal feed (mean value +/- u_{obs} = 2,90 +/- 1,60 mg/kg) – *for information only*



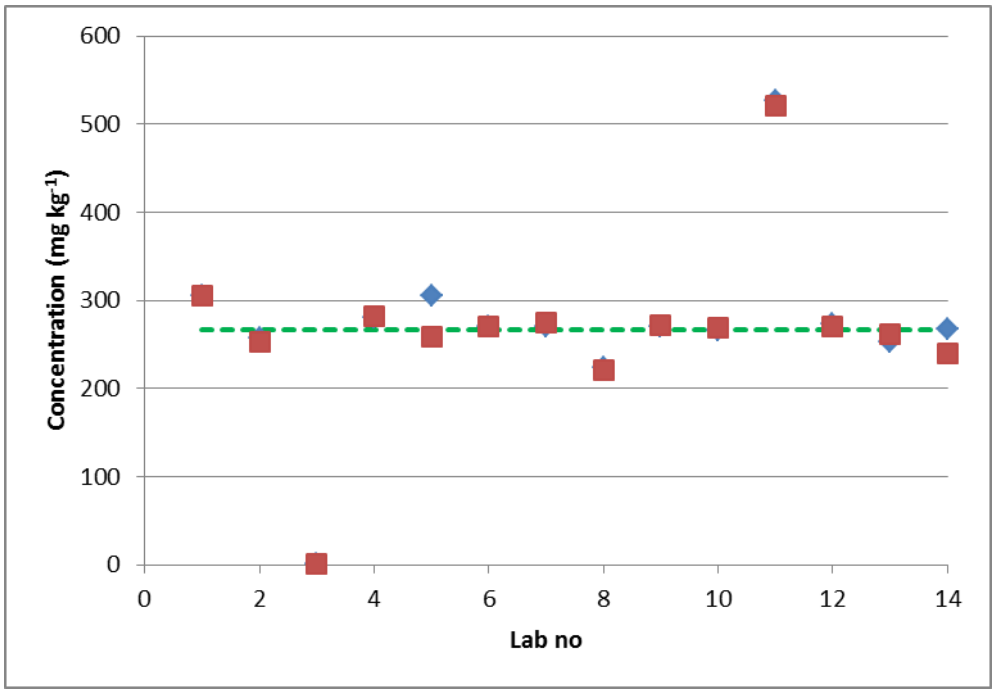
L08 is a grubbs straggler.
L03 is non-compliant lab and not included in the statistical evaluation.

Sample 7 $CaIO_3$ (mean value +/- u_{obs} = 332220+/- 53244 mg/kg) – *for information only*



L01 and L07 are Cochran outliers.
L03 is non-compliant lab and not included in the statistical evaluation.

Sample 8 Iodine solution (mean value +/- u_{obs} = 266 +/- 20 mg/kg)



L05 is a grubbs outlier. L11 is a grubbs outlier.
L03 is non-compliant lab and not included in the statistical evaluation.

Annex 10 Results from the homogeneity testing of sample 3 and sample 5

Sample 3

Results of the homogeneity and stability studies

Sample	IMEP32-3	iodine
Bottle ID	Replicate 1	Replicate 2
1	**1,28**	**1,43**
4	**1,28**	**1,27**
9	**1,33**	**1,31**
11	**1,15**	**1,29**
14	**1,28**	**1,3**
15	**1,3**	**1,33**
20	**1,32**	**1,27**
23	**1,23**	**1,31**
31	**1,31**	**1,31**
37	**1,25**	**1,33**
Grand Mean	**1,294**	
Number of bottles, m	**10**	

S_{DD} Sum of Squares	Cochran Test	Cochran value
0,059	0,380	0,602

Cochran outlier test

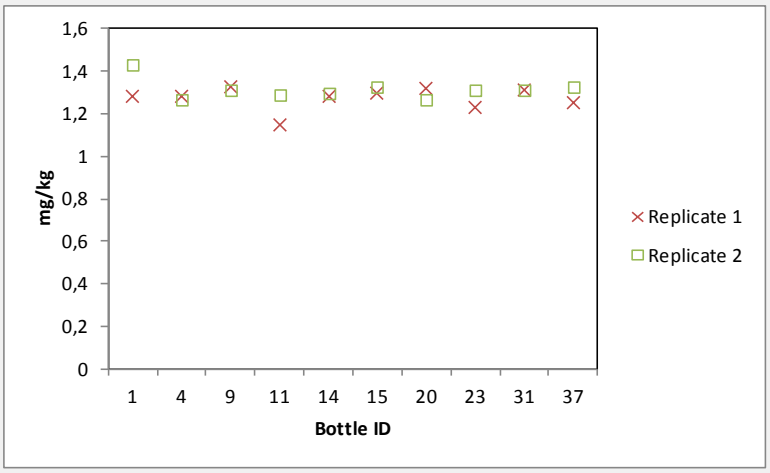
Conclusion:
no analytical outliers

	Sum_i	$Difference_i$	D^2	$Vs = (Si-S^-)^2/(m-1)$
	2,710	-0,150	0,02250	0,00165
	2,550	0,010	0,00010	0,00016
	2,640	0,020	0,00040	0,00030
	2,440	-0,140	0,01960	0,00243
	2,580	-0,020	0,00040	0,00001
	2,630	-0,030	0,00090	0,00020
	2,590	0,050	0,00250	0,00000
	2,540	-0,080	0,00640	0,00026
	2,620	0,000	0,00000	0,00011
	2,580	-0,080	0,00640	0,00001
$\sum$	**25,880**	-0,420	**0,05920**	**0,00513**
S^-	2,588			
$S_{an}^2 = \sum D^2/2m$	**0,002960**			
$S_{sam}^2 = (Vs/2-S_{an}^2)/2$	**0,0000000**			
F1	*1,88*			
F2	*1,01*			
σ-hat (%)	**15**			
$\sigma^2_{all} = (0,3\sigma_p)^2$	0,003391			
$c = F1\sigma^2_{all}+F2S_{an}^2$	0,009364			
$S_{sam}^2 < c$	**passed**			

Homogeneity test

Conclusion:
material homogeneous

Visual appraisal of results:



Sample 5

Results of the homogeneity and stability studies

Sample	IMEP32-1	iod
Bottle ID	Replicate 1	Replicate 2
5	3,08	3,03
9	3,05	2,7
12	2,79	3,39
16	2,86	3,08
19	2,96	2,95
20	3,19	3,15
23	3,36	3,59
32	3,46	2,88
33	2,82	3,23
36	4,44	3,32
Grand Mean	3,167	
Number of bottles, m	10	

S_{DD} Sum of Squares	Cochran Test	Cochran value
2,347	0,534	0,602

	Sum_i	$Difference_i$	D^2	$Vs = (Si-\bar{S})^2/(m-1)$
	6,110	0,050	0,00250	0,00553
	5,750	0,350	0,12250	0,03777
	6,180	-0,600	0,36000	0,00260
	5,940	-0,220	0,04840	0,01716
	5,910	0,010	0,00010	0,01988
	6,340	0,040	0,00160	0,00001
	6,950	-0,230	0,05290	0,04230
	6,340	0,580	0,33640	0,00001
	6,050	-0,410	0,16810	0,00890
	7,760	1,120	1,25440	0,22626
$\sum$	**63,330**	**3,16650**	**2,34690**	**0,36040**
$\bar{S}$	6,333			
$S_{an}^2 = \sum D^2/2m$	**0,117345**			
$S_{sam}^2 = (Vs/2-S_{an}^2)/2$	**0,0314278**			
F1	*1,88*			
F2	*1,01*			
σ-hat (%)	**15**			
$\sigma^2_{all} = (0,3\sigma_p)^2$	0,020304			
$c = F1\sigma^2_{all}+F2S_{an}^2$	0,156690			
$S_{sam}^2 < c$	passed			

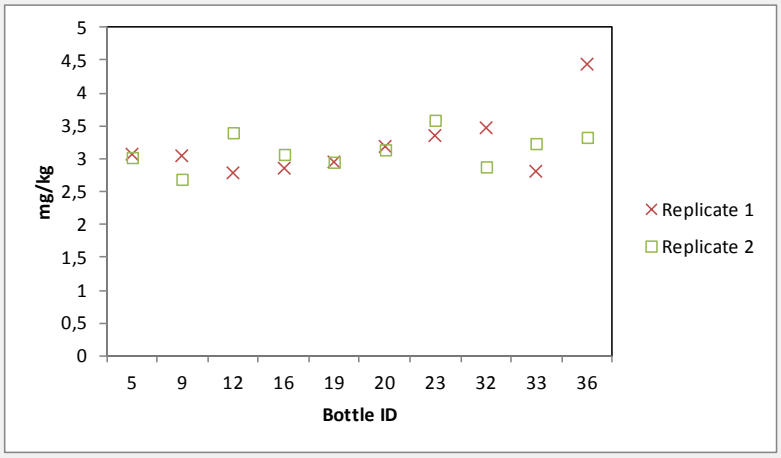
Cochran outlier test

Conclusion:
no analytical outliers

Homogeneity test

Conclusion:
material homogeneous

Visual appraisal of results:



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