European Commission

JRC TECHNICAL REPORTS

IMEP-42: Determination of PFASs in fish

Interlaboratory Comparison Report

Pieter Dehouck, Marta Dabrio, Fernando Cordeiro, Aneta Cizek-Stroh, Beatriz de la Calle

2015

EUR 27579 EN

Joint Research Centre

IMEP-42: Determination of PFASs in fish

JRC Science Hub
https://ec.europa.eu/jrc

JRC98549

EUR 27579 EN

ISBN 978-92-79-53884-1 (PDF)

ISSN 1831-9424 (online)

doi: 10.2787/063168 (online)

How to cite: Pieter Dehouck, Marta Dabrio, Fernando Cordeiro, Aneta Cizek-Stroh, Beatriz de la Calle; IMEP-42: Determination of PFASs in fish. Interlaboratory Comparison Report; EUR 27579 EN; doi: 10.2787/063168

Contents

Executive summary

The Institute for Reference Materials and Measurements of the Joint Research Centre, a Directorate General of the European Commission (EC-JRC-IRMM) operates the International Measurement Evaluation Programme (IMEP). It organises interlaboratory comparisons (ILC's) in support to European Union (EU) policies. This report presents the results of a proficiency test (PT), IMEP-42, on the determination of perfluoroalkyl substances (PFASs) in fish tissue. The exercise was organised in support to the Commission Recommendation 2010/161/EU on the monitoring of perfluoroalkylated substances in food and the Water Framework Directive 2000/60/EC.

Seventeen participants from thirteen countries registered to the exercise and all of them reported results.

The test item was fish tissue (pike-perch) containing perfluoroalkyl carboxylates as perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA) or perfluorotetradecanoic acid (PFTeDA); perfluoroalkyl sulfonates as perfluorohexanesulfonate (PFHxS), linear perfluorooctane sulfonate (L-PFOS) or branched perfluorooctane sulfonate (br-PFOS); and, perfluoroalkyl sulphonamides as perfluorooctane sulphonamide (FOSA). The test item was a candidate certified reference material (CRM) produced by IRMM under ISO Guide 34 accreditation and in line with ISO Guide 35. Laboratories with demonstrated experience in the field provided results to establish the assigned values (X_{ref}). The standard uncertainties associated to the assigned values (u_{ref}) were calculated according to the ISO Guide 35 by combining the uncertainty of the characterisation (u_{char}) with a contribution for homogeneity (u_{bb}) and for stability (u_{st}).

Laboratory results were rated with *z*- and zeta (*ζ*-) scores in accordance with ISO 13528. The standard deviation for the proficiency assessment, $\hat{\sigma}$, for all elements was set at 25 % of the respective assigned value.

The overall performance in this PT was good even though analyte dependent. High rates (78% - 100%) of satisfactory performances expressed as z-scores ≤ 2 were obtained for L-PFOS, PFDA, PFUnDA, PFDoDA, tot-PFOS and FOSA while the lowest rates of satisfactory performances (50%) were obtained for br-PFOS and PFTrDA. Many "less than X" values were reported for the three PFASs that could not be scored due to the high uncertainty on the assigned value (PFNA, PFTeDA and PFHxS). The results in this PT showed that the sensitivities of the methods used by the participants were fit for the purpose of measuring the legal limits set in legislation.

1 Introduction

The IMEP-42 study was organised to assess the world-wide performance of control laboratories on the determination of PFASs in fish.

The PT supports the Commission Recommendation 2010/161/EU on the monitoring of perfluoroalkylated substances in food [1]. In 2008 the European Food Safety Authority established a human tolerable daily intake of 150 ng kg^{-1} body weight for perfluorooctane sulfonate (PFOS) and 1500 ng kg^{-1} body weight for perfluorooctanoic acid (PFOA) [2]. However this dietary exposure assessment was limited by the lack of occurrence data of PFASs in different foodstuffs. As a follow-up the European Commission issued the Commission Recommendation 2010/161/EU in order to collect more data. In its report of 2012 EFSA concluded that there were only few quantified results among the data and that the use of analytical methods with increased sensitivity would be required to monitor a set of priority PFASs in order to increase the proportion of quantitative data and thereby the reliability of exposure assessments [3,4].

The PT also supports the implementation of the Water Framework Directive 2000/60/EC (WFD) [5] which aims at achieving a long-term high level protection from chemical pollution of the aquatic environment, covering lakes, ground water and coastal waters. The WFD established a list of priority substances. The daughter Directive 2013/39/EU [6] lays down the environmental quality standards (EQS) for priority substances and other pollutants with the aim of achieving good chemical status of surface waters. Regarding the PFASs investigated in this study, EQS are set for perfluorooctane sulfonic acid and it's derivatives at 0.65 ng L^{-1} for inland surface waters and at 9.1 ng g^{-1} for biota [6].

IMEP-42 was run in 2015 and made use of a candidate Certified Reference Material (CRM) as test item containing perfluoroalkyl carboxylates as perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA) or perfluorotetradecanoic acid (PFTeDA); perfluoroalkyl sulfonates as perfluorohexanesulfonate (PFHxS), linear perfluorooctane sulfonate (L-PFOS) or branched perfluorooctane sulfonate (br-PFOS); and, perfluoroalkyl sulphonamides as perfluorooctane sulphonamide (FOSA). The candidate CRM was produced under ISO Guide 34 accreditation and in line with the ISO Guide 35 standard [7,8]. Homogeneity and stability studies were carried out as part of the CRM production. Assigned values in this study were determined by expert laboratories. Seventeen laboratories registered for the study and all seventeen submitted results.

This report summarizes and evaluates the outcome of IMEP-42.

2 IMEP support to EU policy

IMEP is owned by the JRC – IRMM and provides support to the European measurement infrastructure in the following ways:

IMEP disseminates metrology from the highest level down to routine laboratories. These laboratories can benchmark their measurement result against the IMEP certified reference value which is established according to metrological best practice.

IMEP helps laboratories to assess their estimate of measurement uncertainty. Participants are invited to report the uncertainty on their measurement results. IMEP integrates the estimate into the scoring, and provides assistance for its interpretation.

IMEP supports EU policies by organising interlaboratory comparisons in the frame of specific EU legislation, or on request of a specific Directorate-General of the European Commission. IMEP-42 provided specific support to the following stakeholders:

- The European Cooperation for Accreditation (EA) in the frame of a Memorandum of Understanding on a number of metrological issues, including the organisation of interlaboratory comparisons. National accreditation bodies were invited to nominate a limited number of laboratories for participation in IMEP-42. Mr Richard McFarlane from the United Kingdom Accreditation Service (UKAS) liaised between EA and IMEP for this ILC.
- The Asia Pacific Laboratory Accreditation Cooperation (APLAC) in the frame of collaboration with APLAC. Mrs Cynthia Chen (APLAC PT committee) liaised between APLAC and IMEP, announcing the exercise to the accreditation bodies in the APLAC network.
- The InterAmerican Accreditation Cooperation (IAAC). Mrs Julia Sancricca and Mrs Cheryl Morton liaised between IAAC and IMEP, announcing the exercise to the accreditation bodies in the IAAC network.

3 Scope and aim

The scope of this PT was to assess the performance of laboratories world-wide in the determination and quantification of PFASs in fish.

The assessment of measurement results followed the administrative and logistic procedures of the EC-JRC-IRMM for the organisation of PTs, which is accredited according to ISO 17043:2010 [9]. This PT is identified as IMEP-42.

4 Set-up of the exercise

4.1 Time frame

The exercise was announced on the JRC webpage in May 2015 (Annex 1). Additionally, the exercise was announced to EA, to APLAC and to IAAC. These announcements were made on 25 March 2015 (Annexes 2-4).

Registration was open till 31 May 2015. The dispatch of test items was organised during the first half of June 2015. The deadline for reporting results was 31 July 2015.

4.2 Confidentiality

The following confidentiality statement was made to EA, IAAC and APLAC: "*Confidentiality of the participants and their results towards third parties is guaranteed.*" In the case of EA the following was added: "*However, IMEP will disclose details of the participants that have been nominated by EA to the EA working group for ILCs in Testing coordinator for this exercise. The EA accreditation bodies may wish to inform the nominees of this disclosure.*"

4.3 Distribution

Test items were dispatched on 16^{th} of June 2015. Each participant received one package containing:

- One glass jar containing approximately 35 g of the test item,
- The "*Sample accompanying letter*" (Annex 5),
- A *"Confirmation of receipt"* form to be sent back to IRMM after receipt of the test item (Annex 6).

4.4 Instructions to participants

Detailed instructions were given to participants in the "Sample accompanying letter" mentioned above. Measurands were defined as "perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexanesulfonate (PFHxS), linear perfluorooctane sulfonate (L-PFOS), branched perfluorooctane sulfonate (br-PFOS), total perfluorooctane sulfonate (tot-PFOS) and perfluorooctane sulphonamide (FOSA) in a fish paste".

Participants were asked to perform two or three independent measurements and to report their calculated mean (x_{lab}, the results of sulfonates to be reported on an anion basis) and its associated expanded measurement uncertainty (U_{lab}).

Participants received an individual code to access the on-line reporting interface, to report their measurement results and to complete the related questionnaire. A dedicated questionnaire was used to gather additional information related to measurements and laboratories (Annex 7).

Participants were informed that the procedure used for the analysis should resemble as closely as possible their routine procedures for this particular matrix, analyte and concentration level.

The laboratory codes were given randomly and communicated to the participants by e-mail.

5 Test item

5.1 Preparation

The test item was a candidate CRM and was produced by IRMM in close collaboration[1] with the Institute for Environmental Studies (IVM), VU University, Amsterdam, The Netherlands. The base material used for the production of the test item was pike-perch (*Lucioperca lucioperca*) fillets originating from the rivers Nieuwe Merwede and Amer in The Netherlands.

Eighty kg of pike-perch fillet naturally contaminated with PFASs were divided in three batches and sequentially finely cut and homogenised at room temperature using a Stephan cutter system (Stephan Food Service Equipment GmbH, Hameln, DE, 40L).

After 15 min of cutting and mixing, butylhydroxy toluene (BHT) 0.02% (m/m) was gradually added to the fish and the cutting and mixing process continued for a period of 2 hours. The 3 batches obtained were then merged and subsequently split again in three parts for further mixing. This process was repeated two more times to minimise any potential material heterogeneity between the sub batches. The fish paste was manually filled (> 35 g) using plastic syringes into 65 ml glass jars, and closed with a twist-off 66 lid RAB blik goudster, both items from Catalonië Glasverpakkingen BV, Tilburg, NL. The jars were then sterilized by autoclaving (1.44 bar, 121 °C, 45 min) and labelled according to the filling order prior to storage at 18 °C.

5.2 Homogeneity and stability studies

As the test item was a candidate CRM, homogeneity and stability studies were performed by the CRM producer in line with the ISO Guide 35 standard [8].

6 Reference values and their uncertainties

6.1 Assigned value X_{ref}

The assigned values were taken from the CRM producer. They were determined during the certification study of the candidate CRM by a number of expert laboratories. Both certified values (for L-PFOS, PFDA, PFUnDA, PFDoDA) and indicative values (for br-PFOS, tot-PFOS, FOSA, PFNA, PFTrDA, PFTeDA, PFHxS) were used as assigned values in this PT. For PFNA, PFTeDA and PFHXs the uncertainty on the assigned value was high and as a result they could not be scored, as discussed in chapter 6.3.

[1] European research project PERFOOD (Perfluorinated Organics in Our Diet, No. FP7-KBBE-2007-227525)

6.2 Associated uncertainty u_{ref}

The CRM producer provided the expanded uncertainties of the assigned values (U_{ref}) with a coverage factor k=2 corresponding to a level of confidence of about 95%. The assigned values (X_{ref}) and expanded uncertainties (U_{ref}) are summarised in Table 1.

6.3 Standard deviation for proficiency assessment $\hat{\sigma}$

The standard deviation for proficiency assessment, $\hat{\sigma}$, for all PFASs was set by the advisory board of this PT to 25 % of the respective assigned values, on the basis of the complexity of the analyses.

For PFNA, PFTeDA and PFHXs $u_{ref} > \hat{\sigma}$. For this reason no scorings were given to the participants for PFNA, PFTeDA and PFHXs.

Table 1. *Assigned values (X_{ref}) and associated expanded uncertainties (U_{ref}). All values are expressed in ng g^{-1}. Certified values have a grey background and indicative values are in italics. * means that the analyte was not scored.*

Analyte	**X_{ref}**	**U_{ref}**
Certified values		
L-PFOS	16.0	1.7
PFDA	1.28	0.17
PFUnDA	0.74	0.20
PFDoDA	0.97	0.21
Indicative scored values		
FOSA	*1.6*	*0.5*
tot-PFOS	*17*	*4*
br-PFOS	*0.92*	*0.25*
PFTrDA	*0.62*	*0.29*
Indicative non scored values		
*PFNA**	*0.09*	*0.05*
*PFTeDA**	*0.45*	*0.30*
*PFHxS**	*0.09*	*0.05*

7 Evaluation of results

7.1 Scores and evaluation criteria

Individual laboratory performance was expressed in terms of *z-* and *ζ-scores* in accordance with ISO 13528 [10]:

$$z = \frac{x_{lab} - X_{ref}}{\hat{\sigma}} \qquad \text{Eq. 3}$$

$$\zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u_{ref}^2 + u_{lab}^2}} \qquad \text{Eq. 4}$$

The interpretation of the *z-* and *ζ-score* is done as follows (according to ISO/IEC 17043 [8]):

Satisfactory performance,	$\lvert score \rvert \leq 2$	(green in Annexes 8-18)
Questionable performance,	$2 < \lvert score \rvert < 3$	(yellow in Annexes 8-18)
Unsatisfactory performance,	$\lvert score \rvert \geq 3$	(red in Annexes 8-18)

The z-score compares the participant's deviation from the assigned value with the standard deviation for proficiency assessment ($\hat{\sigma}$) used as common quality criterion. $\hat{\sigma}$ is defined by the PT organiser as the maximum acceptable standard uncertainty for the concerned measurands.

The ζ-score states if the laboratory result agrees with the assigned value within the respective uncertainty. The denominator is the combined uncertainty of the assigned value and the measurement uncertainty as stated by the laboratory. The ζ-score includes all parts of a measurement result, namely the expected value (assigned value), its uncertainty in the unit of the result as well as the uncertainty of the reported values. An unsatisfactory ζ-score can either be caused by an inappropriate estimation of the concentration or of its uncertainty or both.

The standard uncertainty of the laboratory (u_{lab}) was estimated by dividing the reported expanded uncertainty by the reported coverage factor, k. When no uncertainty was reported, it was set to zero ($u_{lab} = 0$). When k was not specified, the reported expanded uncertainty was considered as the half-width of a rectangular distribution; u_{lab} was then calculated by dividing this half-width by $\sqrt{3}$, as recommended by Eurachem and CITAC [11].

Uncertainty estimation is not trivial; therefore an additional assessment was provided to each laboratory reporting uncertainty, indicating how reasonable their uncertainty estimate is. The standard uncertainty from the laboratory (u_{lab}) is most likely to fall in a range between a minimum uncertainty (u_{min}), and a maximum allowed uncertainty (u_{max}, case "a"). u_{min} is set to the standard uncertainty of the assigned value (u_{ref}). It is unlikely that a laboratory carrying out the analysis on a routine basis would measure the measurand with a smaller uncertainty than the expert laboratories chosen to establish the assigned value. u_{max} is set to the standard deviation ($\hat{\sigma}$) accepted for the PT assessment.

If u_{lab} is smaller than u_{min} (case "b") the laboratory may have underestimated its uncertainty. However, such a statement has to be taken with care as each laboratory reported only measurement uncertainty, whereas the uncertainty of the reference value also includes contributions of homogeneity and stability. If those are large, measurement uncertainties smaller than u_{min} (u_{ref}) are possible and plausible.

If u_{lab} is larger than u_{max}, (case "c") the laboratory may have overestimated the uncertainty. An evaluation of this statement can be made when looking at the difference of the reported value and the assigned value: if the difference is small and the uncertainty is large, then overestimation is likely. If, however, the deviation is large but is covered by the uncertainty, then the uncertainty is properly assessed, but large. It should be pointed out that u_{max} is only a normative criterion if laid down by legislation.

7.2 General observations

Results were received from all 17 registered laboratories and all laboratories filled in the associated questionnaire. Not all laboratories reported results for all measurands. The total number of results received for the individual PFASs ranged from 7 (br-PFOS) to 15 (PFDA) as shown in Table 2.

7.3 Laboratory results and scorings

Some laboratories reported "less than X" values for some analytes. The limit values "X" reported by the laboratories usually correspond to the limits of quantification (LOQ) or limits of detection (LOD) of the applied methods. Those reporting "less than X" values were not included in the data evaluation. However, reported "less than X" values were compared with the corresponding $X_{ref} - U_{ref}$. If the reported limit value "X" is lower than the corresponding $X_{ref} - U_{ref}$, this statement is considered incorrect, since the laboratory should have detected the respective analyte. Laboratories having been identified with such cases are indicated in red in Annexes 8-18. The number of correct and incorrect "less than X" statements is summarized in Table 2. It can be observed that on a total of 17 "less than X" statements for the scored analytes, only three statements were found incorrect. It can also be observed that in total 20 "less than X" values were reported for the three analytes that could not be scored (PFNA, PFTeDA, PFHxS). Indeed, Table 1 shows that these three PFASs were present at very low concentrations. Tables 1 and 2 also show that for the PFOS present above the EQS of 9.1 ng g^{-1} set in Directive 2013/39/EU no "less than X" values were reported, indicating that the Limits of Quantification (LOQ) of the methods used are fit for the purpose of measuring the legal limit [6].

Table 2. *Total number of reported results, number of reported "less than X" values and number of correct and incorrect "less than X" values for each analyte. Certified values have a grey background and indicative values are in italics. * means that the analyte was not scored. NA: not applicable*

Analyte	Number of reported results	Number of "less than X"	Correct "less than X"	Incorrect "less than X"
L-PFOS	14	0	0	0
PFDA	15	2	1	1
PFUnDA	14	4	3	1
PFDoDA	12	4	4	0
br-PFOS	*7*	*1*	*1*	*0*
tot-PFOS	*9*	*0*	*0*	*0*
FOSA	*10*	*2*	*1*	*1*
PFTrDA	*8*	*4*	*4*	*0*
*PFNA**	*13*	*8*	*NA*	*NA*
*PFTeDA**	*8*	*4*	*NA*	*NA*
*PFHxS**	*13*	*8*	*NA*	*NA*

The overall performance of the participants regarding the z- and ζ-scores is summarized in Figure 1: for the determination of the 8 scored PFASs a range of 50 % (br-PFOS, PFTrDA) to 100 % (PFDA, PFDoDA) of satisfactory performances expressed as z-scores ≤ 2 were obtained by the participants in this exercise. Regarding the performances expressed as ζ-scores, satisfactory performances (ζ-scores ≤ 2) were obtained by 50 % (br-PFOS) to 88 % (PFDoDA, FOSA) of the participants.

The reported results for the individual PFASs are presented in Annexes 8 to 18 in the form of a table and a graph. Because of the low number of reporting laboratories, no Kernel density plots (giving the probability density function of the reported measurement results) are shown.

It can be concluded that the overall performance in this PT was good even though it was analyte dependent. High rates of satisfactory performances expressed as z-scores ≤ 2 (78% - 100%) were obtained for L-PFOS, PFDA, PFUnDA, PFDoDA, tot-PFOS and FOSA while the lowest rates of satisfactory performances expressed as z-scores ≤ 2 (50%) were obtained for br-PFOS and PFTrDA. The results obtained in this PT are in line with the values obtained by the CRM producer during the certification study of the test item. The PT also indicates that the sensitivity of the methods used by the participants is fit for the purpose of measuring the EQS set in Directive 2013/39/EU [6].

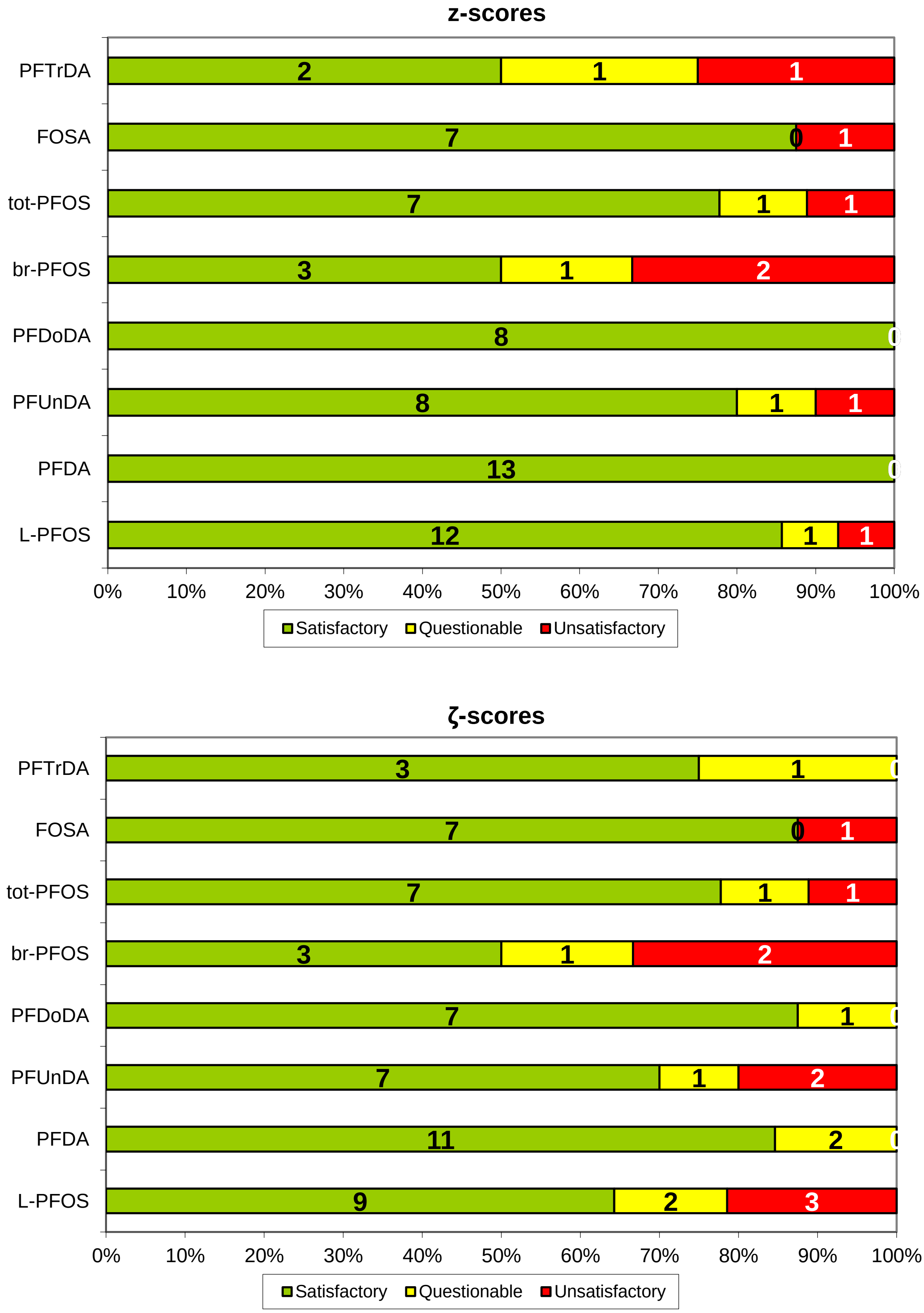


Figure 1. *Number of evaluated laboratories with satisfactory, questionable and unsatisfactory performances expressed as z and ζ-scores. (The numbers on the bars correspond to the exact number of laboratories in a certain scoring category)*

7.4 Further information extracted from the questionnaire

The associated questionnaire was answered by all 17 participating laboratories. For each laboratory some technical details about the analysis were collected. They are summarized in Annex 19 together with an overview of the z-scores.

7.4.1 Extraction

When looking at the details of the used extraction techniques, it was observed that the majority of laboratories (8) used Liquid Solid Extraction (LSE), while 5 laboratories used Solid Phase Extraction (SPE), 2 laboratories dispersive Solid Phase Extraction (d-SPE), 1 laboratory Liquid Liquid Extraction (LLE), 1 laboratory a combination of an extraction and saponification and 1 laboratory Accelerated Solvent Extraction (ASE). Annex 19 shows that all these extraction techniques lead to satisfactory performances. Methanol and/or acetonitrile were used as extraction solvents: in total 10 laboratories used methanol of which three alkalinised the methanol with potassium hydroxide or sodium hydroxide. Five laboratories used acetonitrile of which two acidified the acetonitrile with formic acid. Finally one laboratory used a mixture of methanol and acetonitrile and 1 laboratory used water in an ASE extraction. Annex 19 shows that the use of alkalinised methanol for the extraction leads to 100% satisfactory performances ($z \leq 2$). No other correlations between extraction solvent and performance could be detected.

7.4.2 Sample clean-up

Fifteen out of the 17 laboratories carried out a sample clean-up. Different techniques were used but most of them were based on SPE with anion exchange. The two laboratories that did not carry out a sample clean-up showed worse performance: only 2 out of 5 of their results were satisfactory. Moreover two out of the three incorrect "less than X" values were reported by one of these laboratories. Although based on a limited number of results, these observations show the added value of sample clean-up for this type of analysis.

7.4.3 Chromatography

Most of the laboratories performed the analysis on a reversed phase C18 column. Other phases used were C8 and pentafluorophenyl (Phenomenex Kinetex PFP). Ten laboratories protected the chromatographic column with a guard column. Only three laboratories did not make use of isotopically labelled internal standards. Four laboratories mentioned they applied an official method, but only one laboratory specified the use of the EPA 537:2009 method for "the determination of selected perfluorinated alkyl acids in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry".

7.4.4 Uncertainty statement

On the question whether the laboratories usually provide an uncertainty statement to their customers for this type of analysis 10 laboratories replied they do. In this PT exercise, all laboratories reported measurement uncertainties. These were based on uncertainty budget with ISO GUM (3 laboratories), uncertainty estimation of the method by in-house validation (9 laboratories), measurement of replicates (4 laboratories), judgement (1 laboratory) and type A statistical evaluation of QC data (1 laboratory).

8 Conclusion

The IMEP-42 PT on the determination of eleven PFASs in fish demonstrated the general competence of the participants in this analysis. However, the performance was analyte dependent. The six scored PFASs, L-PFOS, PFDA, PFUnDA, PFDoDA, tot-PFOS and FOSA showed high numbers of reported values (8-14) and high rates of satisfactory performances ($z \leq 2$). The two other scored PFASs, br-PFOS and PFTrDA, showed a lower number of reported values (4-6) and only 50% of satisfactory performances ($z \leq 2$). Finally many "less than X" values were reported for the three PFASs that could not be scored (PFNA, PFTeDA and PFHxS).

The IMEP-42 PT illustrates how PT results can indicate if the methods applied by the participants are fit for the purpose, e.g. for measuring compliance with legal limits.

Finally, PTs can provide useful information to the participants. In IMEP-42, the use of alkalinised methanol for the extraction led to 100% satisfactory performances ($z \leq 2$) and sample clean-up seemed to contribute to achieve satisfactory performance.

9 Acknowledgements

The laboratories participating in this exercise, listed below, are kindly acknowledged.

Organisation	**Country**
Umweltbundesamt GmbH	AUSTRIA
WIV-ISP	BELGIUM
SGS Belgium	BELGIUM
Maxxam Analytics International Corporation	CANADA
Health Canada	CANADA
Technical Center of Shandong Entry-Exit Inspection and Quarantine Bureau	CHINA
Institut of Chemical technology, Prague	CZECH REPUBLIC
Danish Food Administration	DENMARK
National Food Institute	DENMARK
National institute for health and welfare (THL)	FINLAND
LABERCA - ONIRIS	FRANCE
National Center for Scientific Research "Demokritos"	GREECE
Acque del Chiampo spa	ITALY
Istituto Zooprofilattico Sper. L.E.R.	ITALY
Institute of Food Safety, Animal Health and Environment BIOR	LATVIA
Intertek Testing Services Taiwan Ltd	TAIWAN
Fera Science Limited	UNITED KINGDOM

10 Abbreviations

APLAC	Asia Pacific Laboratory Accreditation Cooperation
ASE	Accelerated Solvent Extraction
BHT	butylhydroxy toluene
br-PFOS	branched perfluorooctane sulfonate
CITAC	Cooperation on international traceability in analytical chemistry
CRM	Certified Reference Material
d-SPE	dispersive Solid Phase Extraction
EA	European Cooperation for Accreditation
EQS	Environmental Quality Standards
EU	European Union
FOSA	perfluorooctane sulphonamide
IAAC	InterAmerican Accreditation Cooperation
ILC	Interlaboratory Comparison
IMEP	International Measurement Evaluation Programme
IRMM	Institute for Reference Materials and Measurements
ISO GUM	International Organisation for Standardisation – Guide to the expression of Uncertainty in Measurement
JRC	Joint Research Centre
LLE	Liquid Liquid Extraction
L-PFOS	linear perfluorooctane sulfonate
LSE	Liquid Solid Extraction
PFASs	Perfluoroalkyl substances
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFHxS	perfluorohexanesulfonate
PFNA	perfluorononanoic acid
PFTeDA	perfluorotetradecanoic acid
PFTrDA	perfluorotridecanoic acid
PFUnDA	perfluoroundecanoic acid
PT	Proficiency Test
SPE	Solid Phase Extraction

tot-PFOS	total perfluorooctane sulfonate
UKAS	United Kingdom Accreditation Service
WFD	Water Framework Directive

11 References

[1] European Commission, Commission Recommendation 2010/161/EU on the monitoring of perfluoroalkylated substances in food, OJ Eur. Union L68/22, 2010.

[2] Opinion of the Scientific panel on contaminants in the food chain on perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts, The EFSA Journal 2008, p. 1-131.

[3] Perfluoroalkylated substances in food: occurrence and dietary exposure. EFSA Journal 2012, 10 (6).

[4] J.M. Weiss, I. van der Veen, S.P.J. van Leeuwen, W. Cofino, S. Crum, J. de Boer, Analytical improvements shown over four interlaboratory studies of perfluoroalkyl substances in environmental and food samples, TrAC 43 (2013) 204-216.

[5] European Commission, Directive 2000//60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy, OJ Eur. Union L 327, 22.12.2000.

[6] European Commission, Directive 2013/39/EU on environmental quality standards in the field of water policy, OJ Eur. Union L 226, 2013.

[7] ISO Guide 34, issued by ISO-Geneva (CH), ISO-Geneva (CH).

[8] ISO Guide 35 Reference Materials – general and statistical principles for certification (2006), issued by ISO-Geneva (CH), ISO-Geneva (CH).

[9] ISO/IEC 17043:2010 "Conformity assessment – General requirements for proficiency testing", issued by the International Organisation for Standardization, Geneva

[10] ISO 13528:2005 – "Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons", issued by ISO-Geneva (CH), International Organisation for Standardization.

[11] Eurachem/CITAC, "*Quantifying Uncertainty in Analytical Measurement".* http://www.eurachem.org., 3rd Ed., 2012.

12 Annexes

Annex 1: IRMM – IMEP web announcement

IMEP-42

Description	Determination of PFASs in fish
Status	Registration Open
Year	2015
Type	Proficiency Test
Participation	Open to All
Contact	jrc-irmm-imep@ec.europa.eu
IL category	IMEP

More	The IMEP-42 exercise focuses on the analysis of perfluoroalkyl substances (PFASs) in fish. This PT is organised in support to the EU Water Framework Directive and the Commission Recommendation on the monitoring of perfluoroalkylated substances in food. IMEP-42 is open to all laboratories having experience in this kind of analyses. The cost of this interlaboratory comparison is EUR 355 per registration. **Test items and analytes** The test item to be analysed is a fish paste sample. Each participant will receive 1 sample. The measurands are linear perfluorooctane sulfonate (L-PFOS), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), branched perfluorooctane sulfonate (br-PFOS), total perfluorooctane sulfonate (tot-PFOS), perfluorooctane sulphonamide (FOSA), perfluorononanoic acid (PFNA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA) and perfluorohexanesulfonate (PFHxS) in a fish paste. **General outline of the exercise** Participants are requested to perform 1 – 3 independent analyses using the method of their choice, and to report the mean, its expanded uncertainty and coverage factor *k*. Detailed instructions will be sent together with the sample.

Registration URL	https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?sel...
Registration deadline	Sunday, 31 May 2015
Sample dispatch	First half of June 2015
Reporting of results	31/07/2015
Report to participants	End of December 2015

Keywords	food/feed

Annex 2: Invitation letter to EA

Ref. Ares(2015)1411309 - 31/03/2015

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
International Measurement Evaluation Program

Geel, 25 March 2015

UKAS
Richard Mc Farlane
21-47 High Street Feltham,
Middlesex TW13 4UN,
UK

IMEP-42: Interlaboratory comparison for the determination of PFASs in fish

Dear Mr Mc Farlane,

The Institute for Reference Materials and Measurements (IRMM) organises IMEP-42, a proficiency test for the "**Determination of perfluoroalkyl substances (PFASs) in fish**" in support to the EU Water Framework Directive and the Commission Recommendation on the monitoring of perfluoroalkylated substances in food. IMEP-42 focuses on the analysis of linear perfluorooctane sulfonate (L-PFOS), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), branched perfluorooctane sulfonate (br-PFOS), total perfluorooctane sulfonate (tot-PFOS), perfluorooctane sulphonamide (FOSA), perfluorononanoic acid (PFNA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA) and perfluorohexanesulfonate (PFHxS) in a fish paste.

In the frame of the EA-IRMM collaboration agreement, IRMM kindly invites EA to nominate laboratories for free participation. They should hold (or be in the process of obtaining) an accreditation for this type of measurement.

I suggest that you forward this invitation to the national EA accreditation bodies for its consideration. There are a limited number of samples at your disposal and the number of nominees should not exceed 5 laboratories per country.

Confidentiality of the participants and their results towards third parties is guaranteed. However, IMEP will disclose details of the participants that have been nominated by EA to the EA working group for ILCs in Testing coordinator for this exercise. The EA accreditation bodies may wish to inform the nominees of this disclosure.

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 767, Fax: (32-14) 571 865

E-mail: jrc-irmm-imep@ec.europa.eu
Web site: https://ec.europa.eu/jrc/institutes/irmm/

The registration page for laboratories appointed by EA is open until **31st of May 2015**. Distribution of the samples is foreseen for mid-June 2015. The deadline for submission of results is **31st of July 2015**.

In order to register, laboratories must:

1. **Enter** their details online: https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=1441
2. **Print** the completed form when the system asks to do so and clearly indicate on the printed form that they have been appointed by the European Cooperation for Accreditation to take part in this exercise **otherwise the laboratory will be invoiced 355 € for participation** as charged to the non-appointed laboratories.
3. **Send** the printout to both the IMEP-42 and the EA-IMEP-42 coordinators:

IMEP-42 coordinator	EA-IMEP-42 coordinator
Dr. Pieter Dehouck	Richard Mc Farlane
Fax +32 14 571 252	
E-mail jrc-irmm-imep@ec.europa.eu	E-mail Richard.McFarlane@ukas.com

Please contact me if you have any questions or comments. We are looking forward to our cooperation!

With kind regards

Pieter Dehouck
IMEP-42 Coordinator

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211
Telephone: direct line (32-14) 571 767, Fax: (32-14) 571 865

E-mail: jrc-irmm-imep@ec.europa.eu
Web site: https://ec.europa.eu/jrc/institutes/irmm/

2

Annex 3: Invitation letter to APLAC

Ref. Ares(2015)1393376 - 30/03/2015

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
International Measurement Evaluation Program

Geel, 25 March 2015

To: Cynthia Chen
APLAC PT Committee

IMEP-42: Interlaboratory comparison for the determination of PFASs in fish

Dear Mrs Chen,

The Institute for Reference Materials and Measurements (IRMM) organises IMEP-40, a proficiency test for the "**Determination of perfluoroalkyl substances (PFASs) in fish**" in support to the EU Water Framework Directive and the Commission Recommendation on the monitoring of perfluoroalkylated substances in food. IMEP-42 focuses on the analysis of linear perfluorooctane sulfonate (L-PFOS), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), branched perfluorooctane sulfonate (br-PFOS), total perfluorooctane sulfonate (tot-PFOS), perfluorooctane sulphonamide (FOSA), perfluorononanoic acid (PFNA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA) and perfluorohexanesulfonate (PFHxS) in a fish paste.

IRMM kindly invites APLAC to nominate 10 laboratories for free participation. They should hold (or be in the process of obtaining) an accreditation for this type of measurement. I suggest that you forward this invitation to a selection of specialised laboratories in this area.

In addition to the 10 laboratories mentioned above, other laboratories may take part in IMEP-42 paying a registration fee of 355 €.

Confidentiality of the participants and their results towards third parties is guaranteed.

The registration page is open until **31st of May 2015**. Distribution of the samples is foreseen for mid-June 2015. The deadline for submission of results is **31st of July 2015**.

In order to register, laboratories must:

1. **Enter** their details online:
https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=1441

Print the completed form when the system asks to do so and clearly indicate on the printed form that they have been appointed by APLAC to take part in this exercise **otherwise the laboratory will be invoiced 355 € for participation** as charged to the non-appointed laboratories.

2. **Send** the printout to both the IMEP-42 and the APLAC coordinators:

IMEP-42 coordinator Dr. Pieter Dehouck Fax +32 14 571 252 E-mail: jrc-irmm-imep@ec.europa.eu	**APLAC coordinator** Cynthia Chen E.Mail: cynthia_chen@taftw.org

Please contact me if you have any questions or comments. We are looking forward to our cooperation!

With kind regards

Pieter Dehouck

IMEP-42 Coordinator

Annex 4: Invitation letter to IAAC

Ref. Ares(2015)1393258 - 30/03/2015

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
International Measurement Evaluation Program

Geel, 25 March 2015

To: Julia Sancricca, Cheryl Morton
IAAC Lab Committee

IMEP-42: Interlaboratory comparison for the determination of PFASs in fish

Dear Mrs Sancricca, Dear Mrs Morton,

The Institute for Reference Materials and Measurements (IRMM) organises IMEP-42, a proficiency test for the **"Determination of perfluoroalkyl substances (PFASs) in fish"** in support to the EU Water Framework Directive and the Commission Recommendation on the monitoring of perfluoroalkylated substances in food. IMEP-42 focuses on the analysis of linear perfluorooctane sulfonate (L-PFOS), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), branched perfluorooctane sulfonate (br-PFOS), total perfluorooctane sulfonate (tot-PFOS), perfluorooctane sulphonamide (FOSA), perfluorononanoic acid (PFNA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA) and perfluorohexanesulfonate (PFHxS) in a fish paste.

IRMM kindly invites IAAC to nominate 10 laboratories for free participation. They should hold (or be in the process of obtaining) an accreditation for this type of measurement. I suggest that you forward this invitation to a selection of specialised laboratories in this area.

In addition to the 10 laboratories mentioned above, other laboratories may take part in IMEP-42 paying a registration fee of 355 €.

Confidentiality of the participants and their results towards third parties is guaranteed.

The registration page is open until **31st of May 2015**. Distribution of the samples is foreseen for mid-June 2015. The deadline for submission of results is **31st of July 2015**.

In order to register, laboratories must:

1. **Enter** their details online:
https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=1441

Print the completed form when the system asks to do so and clearly indicate on the printed form that they have been appointed by IAAC to take part in this exercise **otherwise the laboratory will be invoiced 355 € for participation** as charged to the non-appointed laboratories.

2. **Send** the printout to both the IMEP-42 and the IAAC coordinators:

IMEP-42 coordinator Dr. Pieter Dehouck Fax +32 14 571 252 E-mail: jrc-irmm-imep@ec.europa.eu	**IAAC coordinator** Julia Sancricca, Cheryl Morton E.Mail: secretariat@iaac.org.mx

Please contact me if you have any questions or comments. We are looking forward to our cooperation!

With kind regards

Pieter Dehouck
IMEP-42 Coordinator

Annex 5: Sample accompanying letter

Ref. Ares(2015)2443447 - 11/06/2015

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
International Measurement Evaluation Program

Geel, 11 June 2015
JRC.D5/PD/acs/Ares(2015)

«Title» «Firstname» «Surname»
«Organisation»
«Department»
«Address»
«Address2»
«Zip» «Town»
«Country»

Participation in IMEP-42, a proficiency test for the determination of PFASs in fish.

Dear «Title» «Surname»,

Thank you for participating in the IMEP-42 proficiency test for the determination of PFASs in fish. This proficiency test (PT) is organised in support to the EU Water Framework Directive and the Commission Recommendation 2010/161/EU on the monitoring of perfluoroalkylated substances in food.

Please keep this letter. You need it to report your results.

This parcel contains:
a) One glass jar containing approximately 35 g of the test item
b) A "Confirmation of Receipt" form
c) This accompanying letter.

Please check whether the bottle containing the test item remained undamaged during transport. Then, send the "Confirmation of receipt" form back (fax: +32-14-571865, e-mail: JRC-IRMM-IMEP@ec.europa.eu). Upon arrival you should store the sample in a dark place at 4°C until analysis.

The measurands are linear perfluorooctane sulfonate (L-PFOS), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), branched perfluorooctane sulfonate (br-PFOS), total perfluorooctane sulfonate (tot-PFOS), perfluorooctane sulphonamide (FOSA), perfluorononanoic acid (PFNA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA) and perfluorohexanesulfonate (PFHxS) in a fish paste.

The procedure used for the analyses should resemble as closely as possible the one that you use in routine analyses.

Retieseweg 111, B-2440 Geel - Belgium. Telephone: +32-(0)14-571 211.
Telephone: direct line +32-(0)14-571 767, Fax: +32-(0)14-571 865.

E-mail: JRC-IRMM-IMEP@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

Reporting of results
Please perform two or three independent measurements and report on the reporting website:

- the **mean** of your two or three measurement results, the results of sulfonates should be reported on anion basis,
- the associated expanded **uncertainty**,
- the **coverage factor** and
- the **technique** used.

The results should be reported in the same form (e.g. number of significant figures) as those normally reported to the customer.

The reporting website is https://web.jrc.ec.europa.eu/ilcReportingWeb

To access the webpage you need a personal password key, which is: **«Part_key»**. The system will guide you through the reporting procedure. After entering your results, please complete also the relating questionnaire.

Do not forget to submit and confirm always when required.

Directly after submitting your results and the questionnaire information online, you will be prompted to print the completed report form. Please do so, **sign the paper version and return it to IRMM by fax (at +32-14-571-865) or by e-mail.** Check your results carefully for any errors before submission, since this is your last definitive confirmation.

The **deadline** for submission of results is **31/07/2015**.

Keep in mind that collusion is contrary to professional scientific conduct and serves only to nullify the benefits of proficiency tests to customers, accreditation bodies and analysts alike.

Your participation in this project is greatly appreciated. If you have any remaining questions, please contact me by e-mail: JRC-IRMM-IMEP@ec.europa.eu

With kind regards,

Pieter Dehouck (PhD)
IMEP-42 Coordinator

Cc: F. Ulberth (SFB HoU)

Retieseweg 111, B-2440 Geel - Belgium. Telephone: +32-(0)14-571 211.
Telephone: direct line +32-(0)14-571 767, Fax: +32-(0)14-571 865.

E-mail: JRC-IRMM-IMEP@ec.europa.eu
Web site: http://irmm.jrc.ec.europa.eu

Annex 6: "Confirmation of receipt" form

Ref. Ares(2015)2443447 - 11/06/2015

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Directorate D - Institute for Reference Materials and Measurements
International Measurement Evaluation Program

Geel, 11 June 2015
JRC.D5/PD/acs/Ares(2015)

«Title» «Firstname» «Surname»
«Organisation»
«Address»
«Address2»
«Zip» «Town»
«Country»

IMEP-42

determination of PFASs in fish

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 ....

Signature ....

Please return this form to:

Dr Pieter Dehouck

IMEP-42 Coordinator
EC-JRC-IRMM
Retieseweg 111
B-2440 GEEL, Belgium

Fax : +32-14-571865
e-mail : JRC-IRMM-IMEP@ec.europa.eu

Retieseweg 111, B-2440 Geel - Belgium. Telephone: +32-(0)14-571 211.
Telephone: direct line +32-(0)14-571 767, Fax: +32-(0)14-571 865.

E-mail: JRC-IRMM-IMEP@ec.europa.eu
Web site: https://ec.europa.eu/jrc/institutes/irmm/

Annex 7: Questionnaire

1. **Experimental details about the analysis.**

1.1. What extraction technique(s) did you use?

- ☐ a) liquid liquid extraction (LLE)
- ☐ b) liquid solid extraction (LSE)
- ☐ c) solid phase extraction (SPE)
- ☐ d) dispersive solid phase extraction (dSPE)
- ☐ e) None
- ☐ f) Other

1.1.1. If "Other", specify which one.

1.2. What extraction solvents did you use?

1.3. Did you carry out a clean-up of the sample?

- a) yes
- b) no

1.3.1. If "yes", give the relevant details.

1.4. Did you use isotopically labelled internal standards?

- a) yes
- b) no

1.4.1. If "yes", specify which internal standards were used for which compounds.

Use of Internal Standards

Questions/Response table	Used Internal Standard (name)	Source of Internal Standard (supplier)
L-PFOS		
PFDA		
PFUnDA		
PFDoDA		
br-PFOS		
tot-PFOS		
FOSA		
PFNA		
PFTrDA		
PFTeDA		
PFHxS		

1.5. What chromatographic column did you use?

1.6. Did you use a guard column?

- a) yes
- b) no

1.6.1. If "yes", which one?

1.7. Does your laboratory use reference material for this type of analysis?

- a) yes
- b) no

1.7.1. If "yes", specify which one.

1.8. Did you use any PTFE or other fluoropolymers as part of your chromatographic system?

- a) yes
- b) no

1.8.1. If "yes", specify.

1.9. Did you use an official method?

- a) yes
- b) no

1.9.1. If "yes" specify which one.

1.10. Did you correct your results for recovery?

- a) yes
- b) no

1.10.1. If "no", why not?

1.11. Did you observe any interference during the analysis?

- a) yes
- b) no

1.11.1. If "yes", specify which one.

2. **What is the basis of your uncertainty estimate?**

- ☐ a) Uncertainty budget (ISO GUM)
- ☐ b) Known uncertainty of the standard method
- ☐ c) Uncertainty of the method (in-house validation)
- ☐ d) Measurement of replicates (precision)
- ☐ e) Estimation based on judgement
- ☐ f) Use of intercomparison data
- ☐ g) Other

2.1. If "Other", specify.

3. **Do you usually provide an uncertainty statement to your customers for this type of analysis?**

- a) yes
- b) no

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

- a) yes
- b) no

4.1. If "yes", specify which one.

- ISO 17025
- ISO 9001
- Other

5. **Does your laboratory take part in an inter-laboratory comparison scheme for this type of analysis?**

- a) yes
- b) no

5.1. If "yes", specify which one.

Annex 8: Results for L-PFOS

X_{ref} = 16.0 ; U_{ref} (k=2) = 1.7 ; $\hat{\sigma}$ = 4.0 (ng g^{-1})

Lab code	X_{lab}	U_{lab}	k[a]	Analytical method	u_{lab}	z-score[b]	ζ-score[b]	u_{lab}[c]
001	20.59	7.37	2	LC-MS/MS	3.685	1.15	1.21	a
002	14	5.3	2	LC-MS/MS	2.7041	-0.50	-0.71	a
003	24.06	0.0016	2	LC-MS/MS	0.0008	2.01	9.48	b
004	14.3	37	2	LC-MS/MS	18.5	-0.42	-0.09	c
005	23	2.5	1.1	LC-MS/MS	2.3364	1.75	2.82	a
006	15.81	35	v3	LC-MS/MS	20.208	-0.05	-0.01	c
008	15.793	3.159	2	LC-MS/MS	1.5795	-0.05	-0.12	a
009	45.5	15.9	v3	LC-MS/MS	9.1801	7.38	3.20	c
010	15.09	1.21	2	LC-MS/MS	0.605	-0.23	-0.87	b
011	18.51	14	2	LC-MS/MS	7	0.63	0.36	c
013	20.014	2.763	2	LC-MS/MS	1.3815	1.00	2.47	a
014	9.6	3.5	2	LC-MS/MS	1.75	-1.60	-3.29	a
015	16	1.86	2	LC-MS/MS	0.93	0.00	0.00	a
017	17.6	4.5	2	LC-MS/MS	2.25	0.40	0.67	a

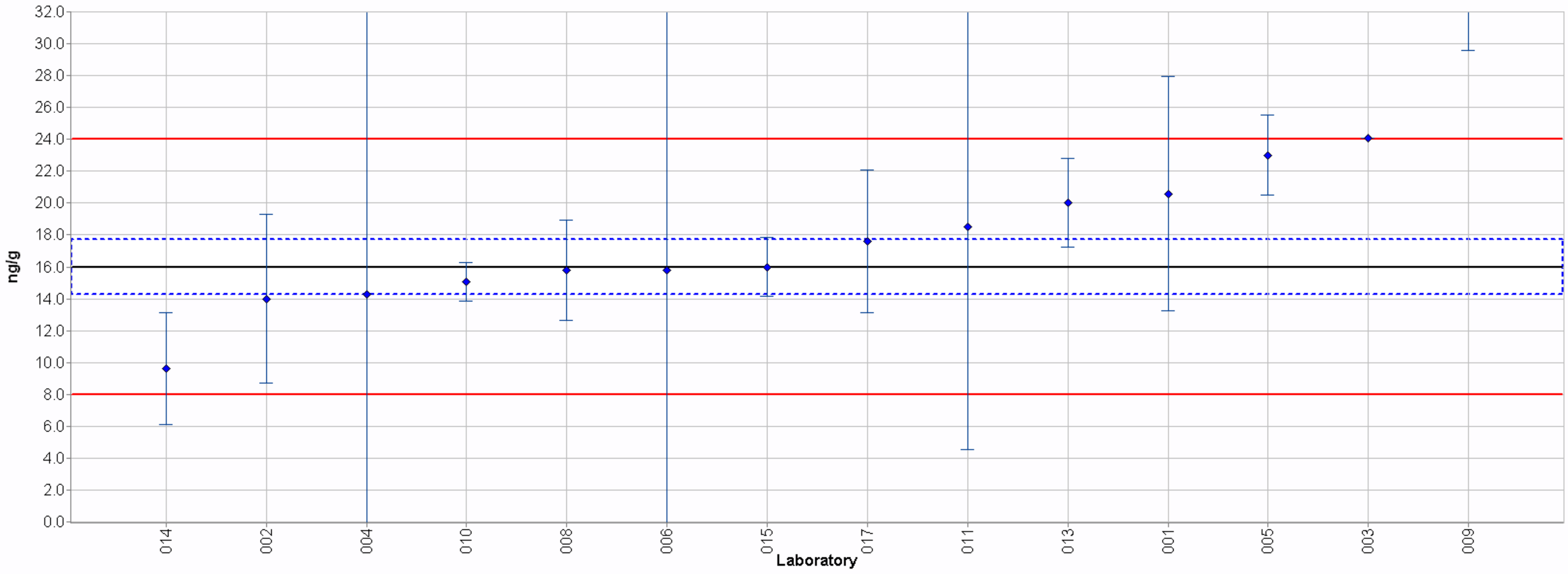
[a] √3 is set by the ILC coordinator when no expansion factor k is reported. The reported uncertainty was assumed to have a rectangular distribution with k = √3.

[b] **Satisfactory**, **Questionable**, **Unsatisfactory**

[c] a: u_{min} (u_{ref}) ≤ u_{lab} ≤ u_{max} ($\hat{\sigma}$); b: u_{lab} < u_{min}; c: u_{lab} > u_{max} ($\hat{\sigma}$)

IMEP-42: L-PFOS in fish

$X_{ref} = 16.0$; U_{Ref} (k=2) = 1.7 ; $\hat{\sigma}$ = 4.0 (ng g^{-1})



Measurement results and associated uncertainties (reported uncertainties shown).

Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\hat{\sigma}$): red lines

Annex 9: Results for PFDA

X_{ref} = 1.28 ; U_{ref} (k=2) = 0.17 ; $\hat{\sigma}$ = 0.32 (ng g^{-1})

Lab code	X_{lab}	U_{lab}	k[a]	Analytical method	u_{lab}	z-score[b]	ζ-score[b]	u_{lab}[c]
001	1.23	0	v3	LC-MS/MS	0	-0.16	-0.59	b
002	0.93	0.46	2	LC-MS/MS	0.23	-1.09	-1.43	a
003	< 1.0							
004	1.3	17	2	LC-MS/MS	8.5	0.06	0.00	c
005	1.7	0.13	1	LC-MS/MS	0.1368	1.31	2.61	a
006	1.29	35	v3	LC-MS/MS	20.208	0.03	0.00	c
008	1.244	0.249	2	LC-MS/MS	0.1245	-0.11	-0.24	a
009	1.2	0.4	v3	LC-MS/MS	0.2309	-0.25	-0.33	a
010	1.02	0.09	2	LC-MS/MS	0.045	-0.81	-2.70	b
011	1.72	19	2	LC-MS/MS	9.5	1.37	0.05	c
012	1.27	0.7	2	LC-MS/MS	0.35	-0.03	-0.03	c
013	1.674	0.484	2	LC-MS/MS	0.242	1.23	1.54	a
014	< 5.0			LC-MS/MS				
015	1.2	0.15	2	LC-MS/MS	0.075	-0.25	-0.71	b
016	1.8	0.54	v3	LC-MS/MS	0.3118	1.63	1.61	a

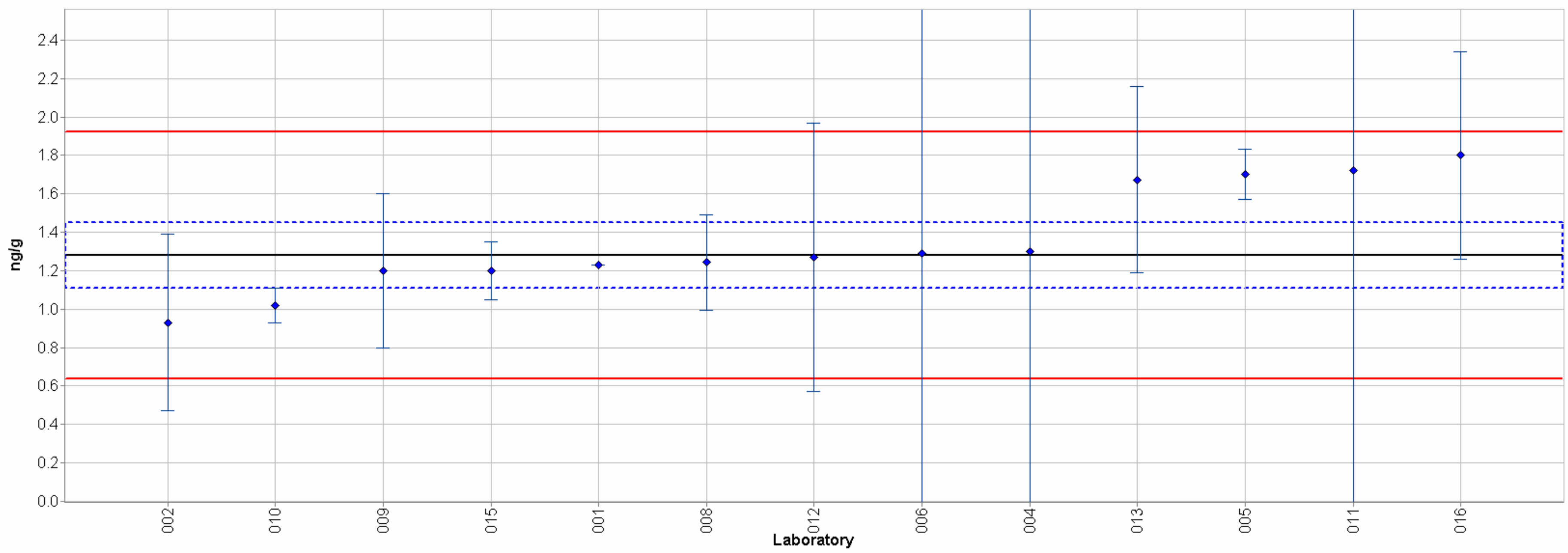
[a] √3 is set by the ILC coordinator when no expansion factor k is reported. The reported uncertainty was assumed to have a rectangular distribution with k = √3.

[b] **Satisfactory**, **Questionable**, **Unsatisfactory**

[c] *a*: u_{min} (u_{ref}) ≤ u_{lab} ≤ u_{max} ($\hat{\sigma}$); b: u_{lab} < u_{min}; c: u_{lab} > u_{max} *($\hat{\sigma}$)*

IMEP-42: PFDA in fish

X_{ref} = 1.28 ; U_{Ref} (k=2) = 0.17 ; $\hat{\sigma}$ = 0.32 (ng g^{-1})



Measurement results and associated uncertainties (reported uncertainties shown).

Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\hat{\sigma}$): red lines

Annex 10: Results for PFUnDA

X_{ref} = 0.74 ; U_{Ref} (k=2) = 0.20 ; $\hat{\sigma}$ = 0.18 (ng g^{-1})

Lab code	X_{lab}	U_{lab}	k[a]	Analytical method	u_{lab}	z-score[b]	ζ-score[b]	u_{lab}[c]
001	0.706	0	v3	LC-MS/MS	0	-0.18	-0.34	b
002	0.8	0.34	2	LC-MS/MS	0.17	0.32	0.30	a
003	< 1.0							
004	< 0.4			LC-MS/MS				
005	1.1	0.02	1.2	LC-MS/MS	0.0161	1.95	3.55	b
006	0.77	35	v3	LC-MS/MS	20.208	0.16	0.00	c
008	1.326	0.265	2	LC-MS/MS	0.1325	3.17	3.53	a
009	0.6	0.2	v3	LC-MS/MS	0.1155	-0.76	-0.92	a
011	1.11	18	2	LC-MS/MS	9	2.00	0.04	c
012	1.2	0.3	2	LC-MS/MS	0.15	2.49	2.55	a
013	1.089	0.509	2	LC-MS/MS	0.2545	1.89	1.28	c
014	< 5.0			LC-MS/MS				
015	< 1.0			LC-MS/MS				
016	1.1	0.33	v3	LC-MS/MS	0.1905	1.95	1.67	c

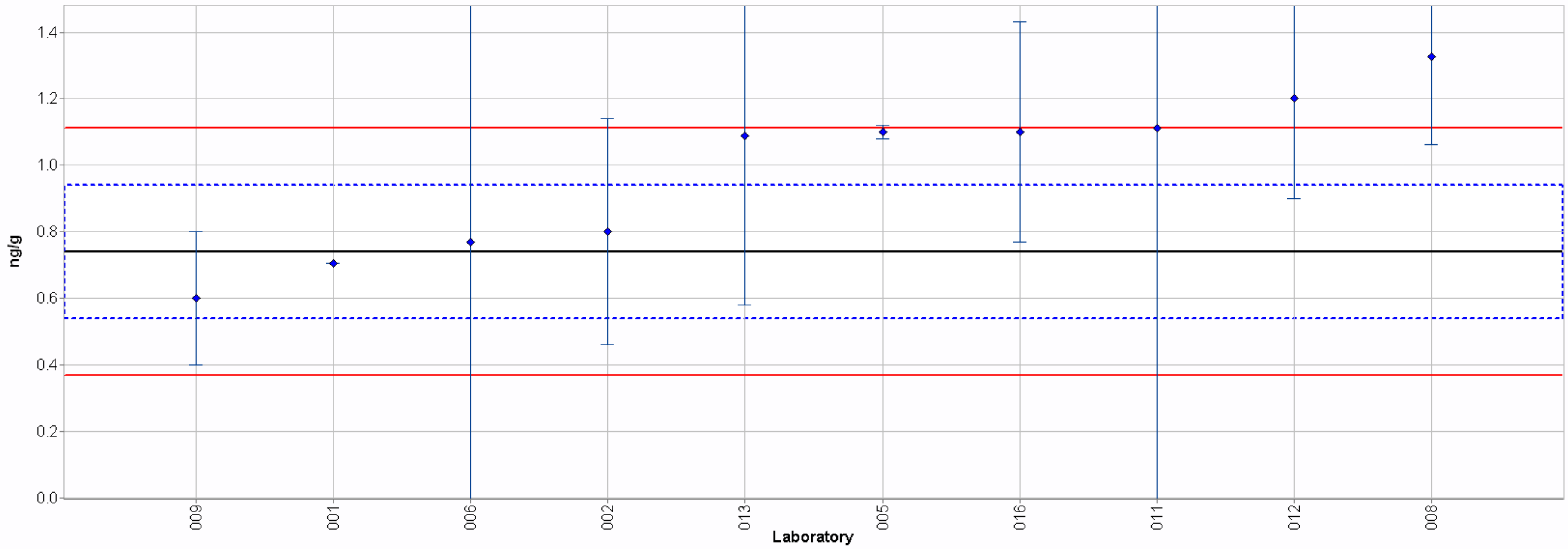
[a] √3 is set by the ILC coordinator when no expansion factor k is reported. The reported uncertainty was assumed to have a rectangular distribution with k = √3.

[b] **Satisfactory**, **Questionable**, **Unsatisfactory**

[c] *a*: u_{min} (u_{ref}) ≤ u_{lab} ≤ u_{max} ($\hat{\sigma}$); b: u_{lab} < u_{min}; c: u_{lab} > u_{max} ($\hat{\sigma}$)

IMEP-42: PFUnDA in fish

$X_{ref} = 0.74$; U_{Ref} $(k=2) = 0.20$; $\hat{\sigma} = 0.18$ (ng g^{-1})



Measurement results and associated uncertainties (reported uncertainties shown).

Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\hat{\sigma}$): red lines

Annex 11: Results for PFDoDA

X_{ref} = 0.97 ; U_{Ref} (k=2) = 0.21 ; $\hat{\sigma}$ = 0.24 (ng g^{-1})

Lab code	X_{lab}	U_{lab}	k[a]	Analytical method	u_{lab}	z-score[b]	ζ-score[b]	u_{lab}[c]
001	0.9	0	v3	LC-MS/MS	0	-0.29	-0.67	b
002	< 1.0			LC-MS/MS				
003	< 1.0							
004	0.7	12	2	LC-MS/MS	6	-1.11	-0.04	c
006	0.95	35	v3	LC-MS/MS	20.208	-0.08	0.00	c
008	0.96	0.192	2	LC-MS/MS	0.096	-0.04	-0.07	b
009	0.6	0.2	v3	LC-MS/MS	0.1155	-1.53	-2.37	a
011	< 0.0							
012	1.27	0.9	2	LC-MS/MS	0.45	1.24	0.65	c
013	1.249	0.251	2	LC-MS/MS	0.1255	1.15	1.71	a
014	< 5.0			LC-MS/MS				
015	< 1.0			LC-MS/MS				
016	1.3	0.38	v3	LC-MS/MS	0.2194	1.36	1.36	a

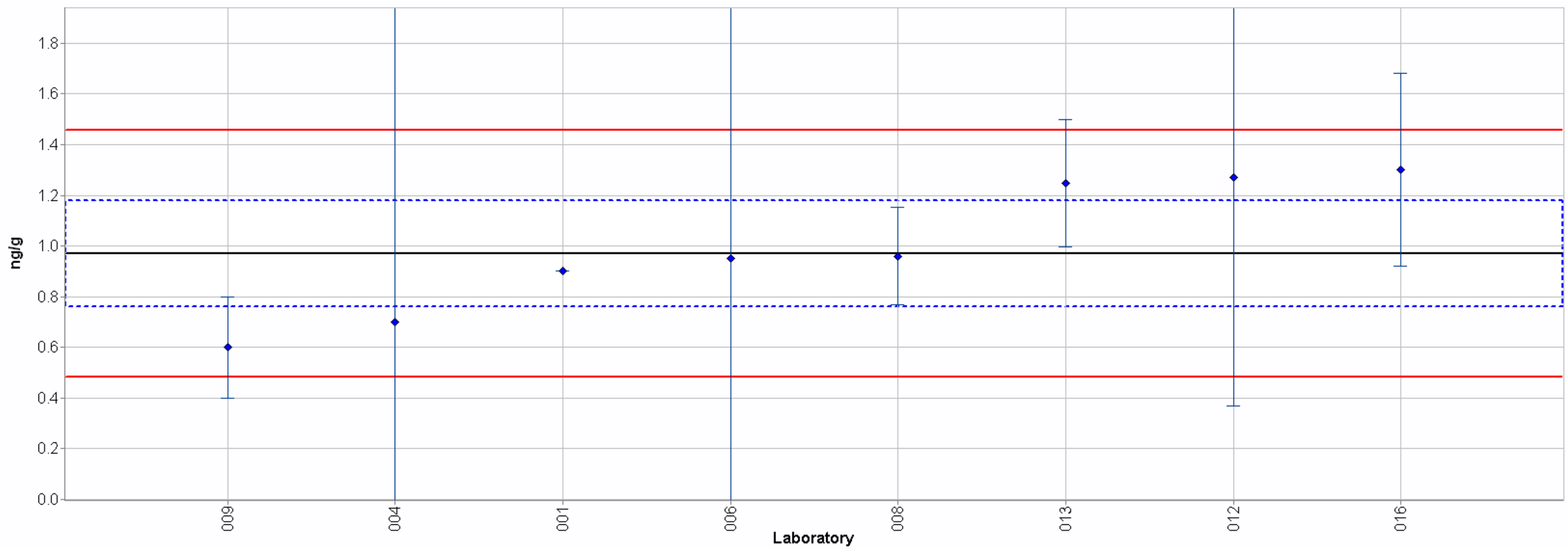
[a] √3 is set by the ILC coordinator when no expansion factor k is reported. The reported uncertainty was assumed to have a rectangular distribution with k = √3.

[b] **Satisfactory**, **Questionable**, **Unsatisfactory**

[c] *a*: u_{min} (u_{ref}) ≤ u_{lab} ≤ u_{max} ($\hat{\sigma}$); b: u_{lab} < u_{min}; c: u_{lab} > u_{max} ($\hat{\sigma}$)

IMEP-42: PFDoDA in fish

$X_{ref} = 0.97$; U_{Ref} (k=2) = 0.21 ; $\hat{\sigma}$ = 0.24 (ng g^{-1})



Measurement results and associated uncertainties (reported uncertainties shown).

Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\hat{\sigma}$): red lines

Annex 12: Results for br-PFOS

X_{ref} = 0.92 ; U_{Ref} (k=2) = 0.25 ; $\hat{\sigma}$ = 0.23 (ng g^{-1})

Lab code	X_{lab}	U_{lab}	k[a]	Analytical method	u_{lab}	z-score[b]	ζ-score[b]	u_{lab}[c]
002	0.4	0.29	2	LC-MS/MS	0.1465	-2.26	-2.70	a
003	6.48	0.0016	2	LC-MS/MS	0.0008	24.17	44.48	b
006	0.6	35	v3	LC-MS/MS	20.208	-1.39	-0.02	c
008	1.01	0.303	2	LC-MS/MS	0.1515	0.39	0.46	a
011	2.49	0	v3		0	6.83	12.56	b
014	< 5.0			LC-MS/MS				
015	0.94	0.13	2	LC-MS/MS	0.065	0.09	0.14	b

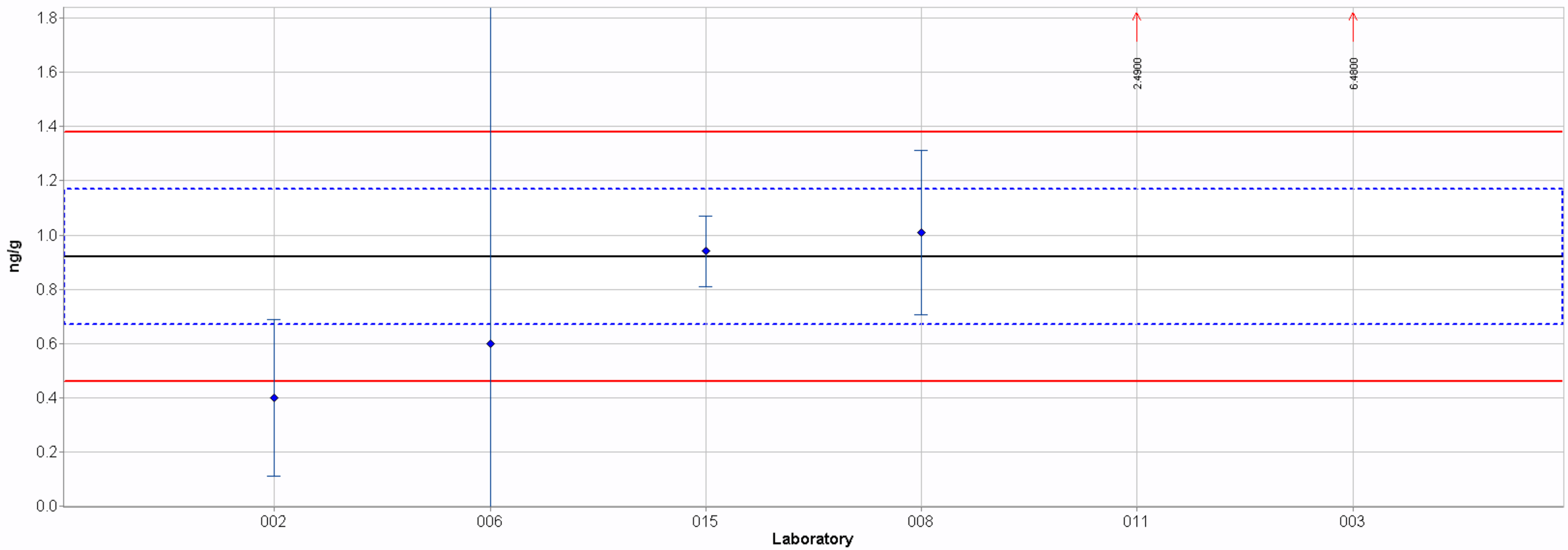
[a] √3 is set by the ILC coordinator when no expansion factor k is reported. The reported uncertainty was assumed to have a rectangular distribution with k = √3.

[b] **Satisfactory**, **Questionable**, **Unsatisfactory**

[c] *a*: u_{min} (u_{ref}) ≤ u_{lab} ≤ u_{max} ($\hat{\sigma}$); b: u_{lab} < u_{min}; c: u_{lab} > u_{max} *($\hat{\sigma}$)*

IMEP-42: br-PFOS in fish

$X_{ref} = 0.92$; U_{Ref} (k=2) = 0.25 ; $\hat{\sigma}$ = 0.23 (ng g^{-1})



Measurement results and associated uncertainties (reported uncertainties shown).

Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\hat{\sigma}$): red lines

Annex 13: Results for tot-PFOS

X_{ref} = 17 ; U_{Ref} (k=2) = 4 ; $\hat{\sigma}$ = 4.2 (ng g^{-1})

Lab code	X_{lab}	U_{lab}	k[a]	Analytical method	u_{lab}	z-score[b]	ζ-score[b]	u_{lab}[c]
002	14.4	5.3	2	LC-MS/MS	2.7041	-0.61	-0.77	a
003	30.54	0.0016	2	LC-MS/MS	0.0008	3.19	6.77	b
006	16.41	35	v3	LC-MS/MS	20.208	-0.14	-0.03	c
007	14.7	25	2	Orbitrap-MS	12.5	-0.54	-0.18	c
008	16.803	5.041	2	LC-MS/MS	2.5205	-0.05	-0.06	a
011	21	14	2	LC-MS/MS	7	0.94	0.55	c
014	11	4	2	LC-MS/MS	2	-1.41	-2.12	a
015	16	1.86	2	LC-MS/MS	0.93	-0.24	-0.45	b
016	26	7.9	v3	LC-MS/MS	4.5612	2.12	1.81	c

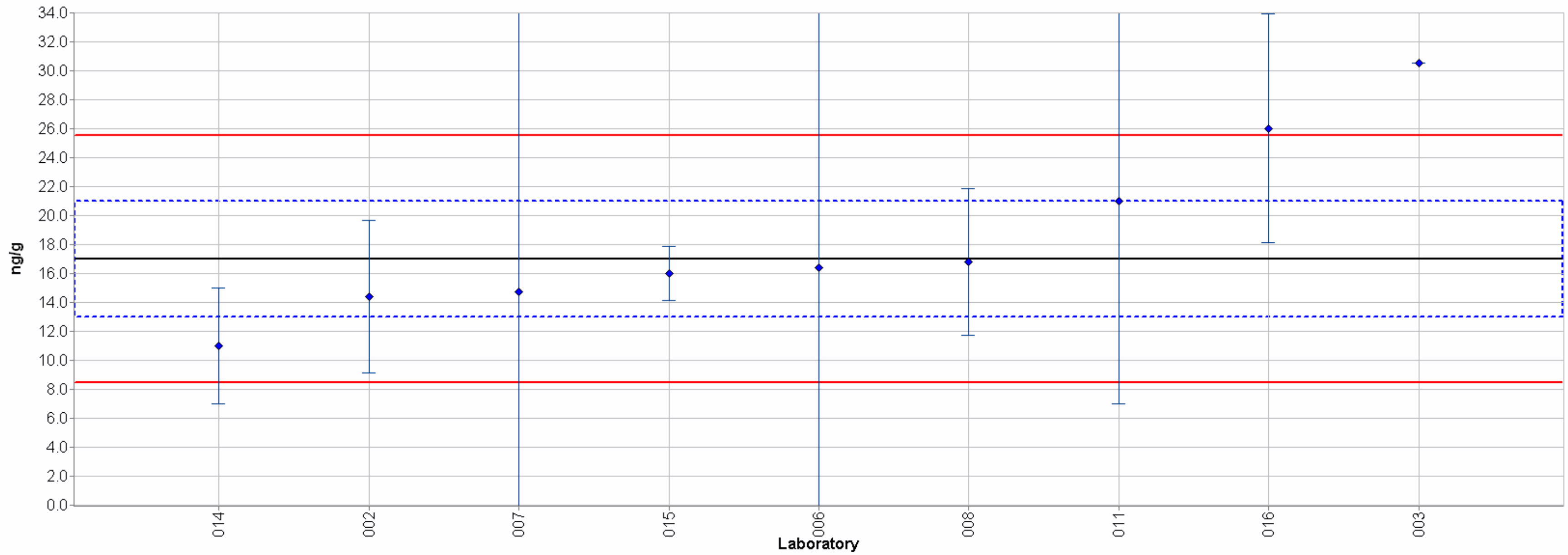
[a] √3 is set by the ILC coordinator when no expansion factor k is reported. The reported uncertainty was assumed to have a rectangular distribution with k = √3.

[b] **Satisfactory**, **Questionable**, **Unsatisfactory**

[c] *a*: u_{min} (u_{ref}) ≤ u_{lab} ≤ u_{max} ($\hat{\sigma}$); b: u_{lab} < u_{min}; c: u_{lab} > u_{max} ($\hat{\sigma}$)

IMEP-42: tot-PFOS in fish

$X_{ref} = 17$; U_{Ref} (k=2) = 4 ; $\hat{\sigma}$ = 4.2 (ng g^{-1})



Measurement results and associated uncertainties (reported uncertainties shown).

Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\hat{\sigma}$): red lines

Annex 14: Results for FOSA

X_{ref} = 1.6 ; U_{Ref} (k=2) = 0.5 ; $\hat{\sigma}$ = 0.4 (ng g^{-1})

Lab code	X_{lab}	U_{lab}	k[a]	Analytical method	u_{lab}	z-score[b]	ζ-score[b]	u_{lab}[c]
001	1.6	0	v3	LC-MS/MS	0	0.00	0.00	b
003	< 1.0							
005	1.23	0.22	1	LC-MS/MS	0.2136	-0.93	-1.13	b
006	1.29	35	v3	LC-MS/MS	20.208	-0.78	-0.02	c
008	1.408	0.352	2	LC-MS/MS	0.176	-0.48	-0.63	b
010	1.46	0.11	2	LC-MS/MS	0.055	-0.35	-0.55	b
011	1.18	50	2	LC-MS/MS	25	-1.05	-0.02	c
014	< 10.0			LC-MS/MS				
015	1.5	0.25	2	LC-MS/MS	0.125	-0.25	-0.36	b
016	5.4	1.6	v3	LC-MS/MS	0.9238	9.50	3.97	c

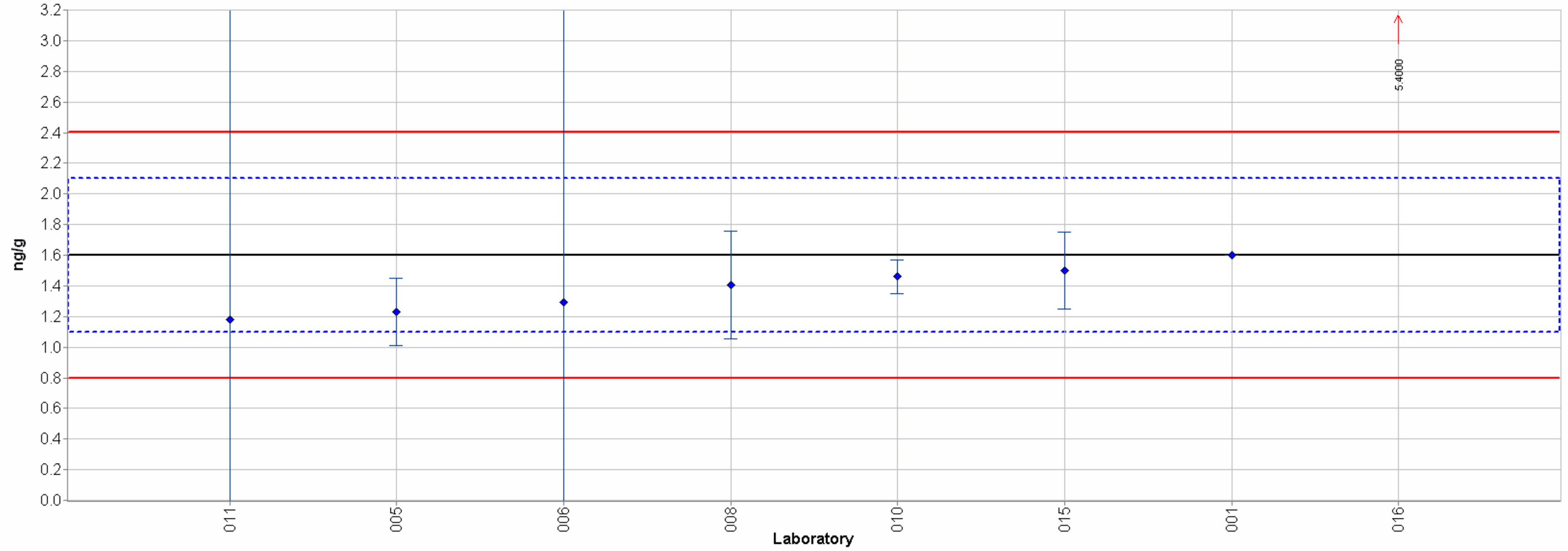
[a] √3 is set by the ILC coordinator when no expansion factor k is reported. The reported uncertainty was assumed to have a rectangular distribution with k = √3.

[b] **Satisfactory**, **Questionable**, **Unsatisfactory**

[c] *a*: u_{min} (u_{ref}) ≤ u_{lab} ≤ u_{max} ($\hat{\sigma}$); b: u_{lab} < u_{min}; c: u_{lab} > u_{max} ($\hat{\sigma}$)

IMEP-42: FOSA in fish

$X_{ref} = 1.6$; U_{Ref} $(k=2) = 0.5$; $\hat{\sigma} = 0.4$ (ng g^{-1})



Measurement results and associated uncertainties (reported uncertainties shown).

Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\hat{\sigma}$): red lines

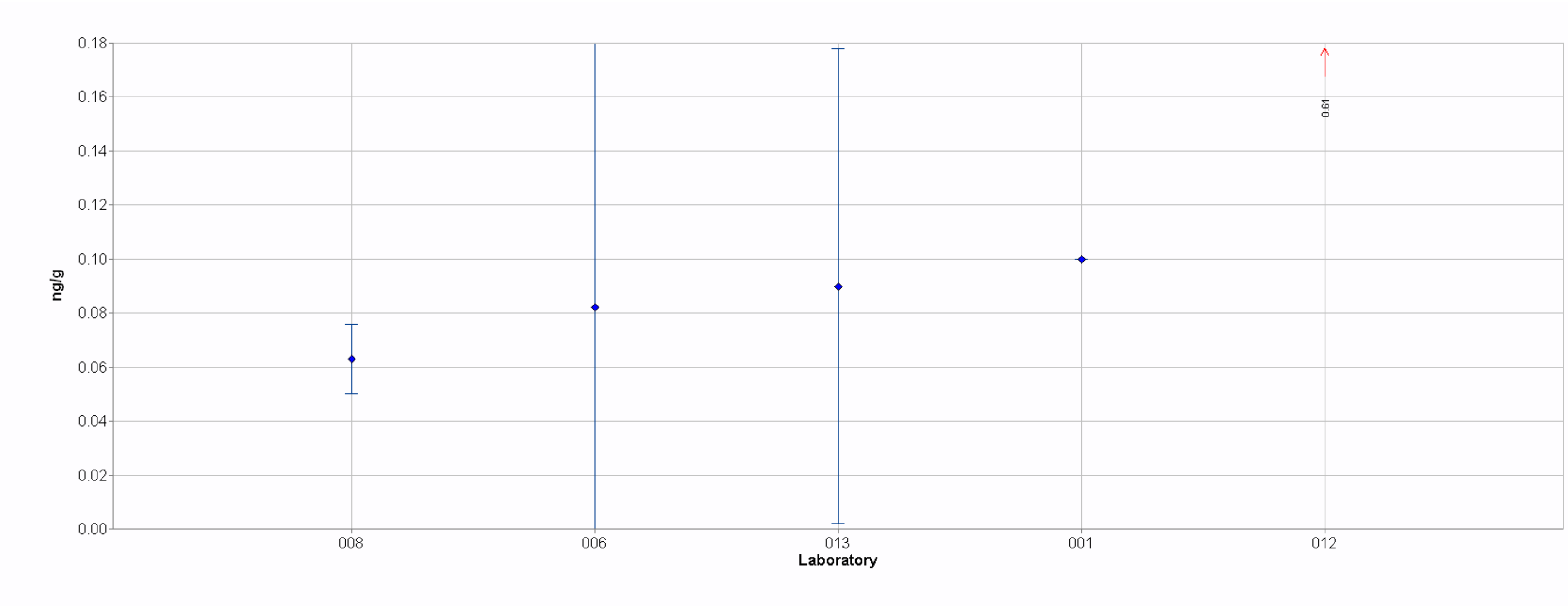
Annex 15: Results for PFNA

Results in ng g^{-1}

Lab code	X_{lab}	U_{lab}	k[a]	Analytical method	u_{lab}
001	0.1	0	v3	LC-MS/MS	0
002	< 0.8			LC-MS/MS	
003	< 1.0				
004	< 0.4			LC-MS/MS	
005	< 0.5			LC-MS/MS	
006	0.082	35	v3	LC-MS/MS	20.208
008	0.063	0.013	2	LC-MS/MS	0.0065
009	< 5.0				
012	0.61	0.2	2	LC-MS/MS	0.1
013	0.09	0.088	2	LC-MS/MS	0.044
014	< 5.0			LC-MS/MS	
015	< 1.0			LC-MS/MS	
016	< 0.5			LC-MS/MS	

[a] √3 is set by the ILC coordinator when no expansion factor *k* is reported. The reported uncertainty was assumed to have a rectangular distribution with *k* = √3.

IMEP-42: PFNA in fish



Measurement results and associated uncertainties (reported uncertainties shown).

Annex 16: Results for PFTrDA

X_{ref} = 0.62 ; U_{Ref} (k=2) = 0.29 ; $\hat{\sigma}$ = 0.15 (ng g^{-1})

Lab code	X_{lab}	U_{lab}	k[a]	Analytical method	u_{lab}	z-score[b]	ζ-score[b]	u_{lab}[c]
001	0.5	0	v3	LC-MS/MS	0	-0.77	-0.83	b
003	< 1.0							
004	< 1.0			LC-MS/MS				
006	1.13	35	v3	LC-MS/MS	20.208	3.29	0.03	c
008	0.458	0.114	2	LC-MS/MS	0.057	-1.05	-1.04	b
013	0.994	0.188	2	LC-MS/MS	0.094	2.41	2.16	b
014	< 0.0							
015	< 1.0			LC-MS/MS				
016	< 1.0			LC-MS/MS				

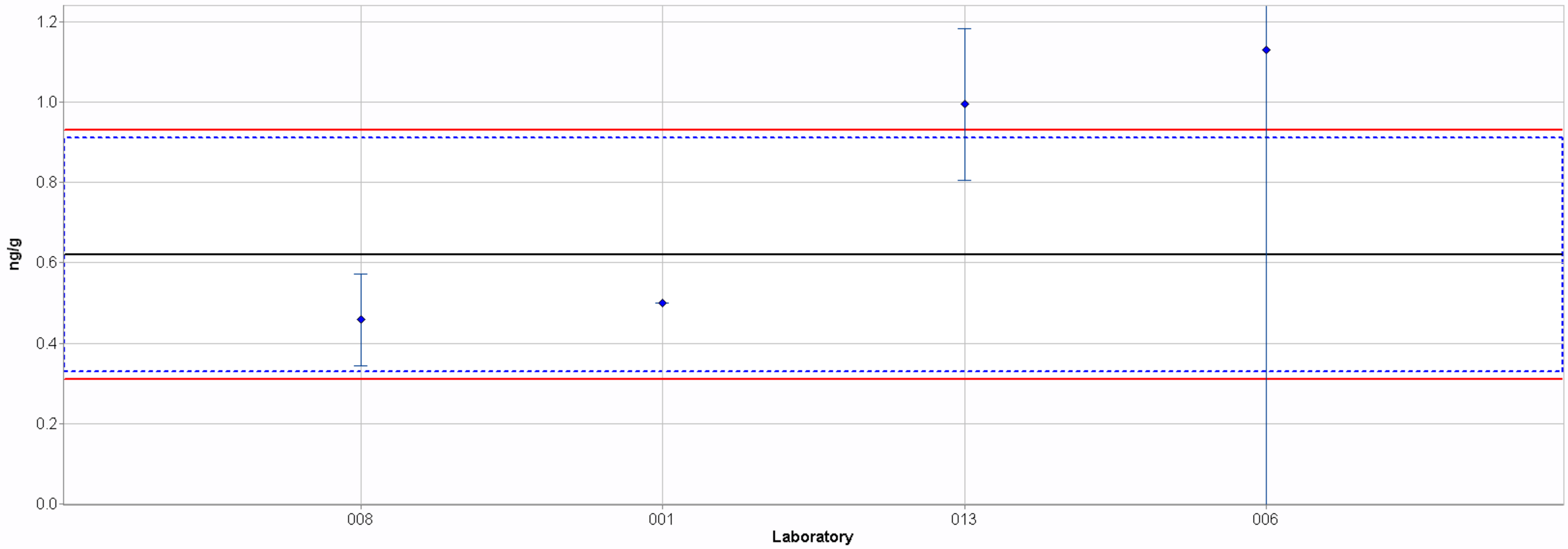
[a] √3 is set by the ILC coordinator when no expansion factor k is reported. The reported uncertainty was assumed to have a rectangular distribution with k = √3.

[b] **Satisfactory**, **Questionable**, **Unsatisfactory**

[c] a: u_{min} (u_{ref}) ≤ u_{lab} ≤ u_{max} ($\hat{\sigma}$); b: u_{lab} < u_{min}; c: u_{lab} > u_{max} ($\hat{\sigma}$)

IMEP-42: PFTrDA in fish

$X_{ref} = 0.62$; U_{Ref} (k=2) = 0.29 ; $\hat{\sigma}$ = 0.15 (ng g^{-1})



Measurement results and associated uncertainties (reported uncertainties shown).

Reference value (X_{ref}): solid black line; Reference interval ($X_{ref} \pm U_{ref}$): dashed blue lines; Target interval ($X_{ref} \pm 2\hat{\sigma}$): red lines

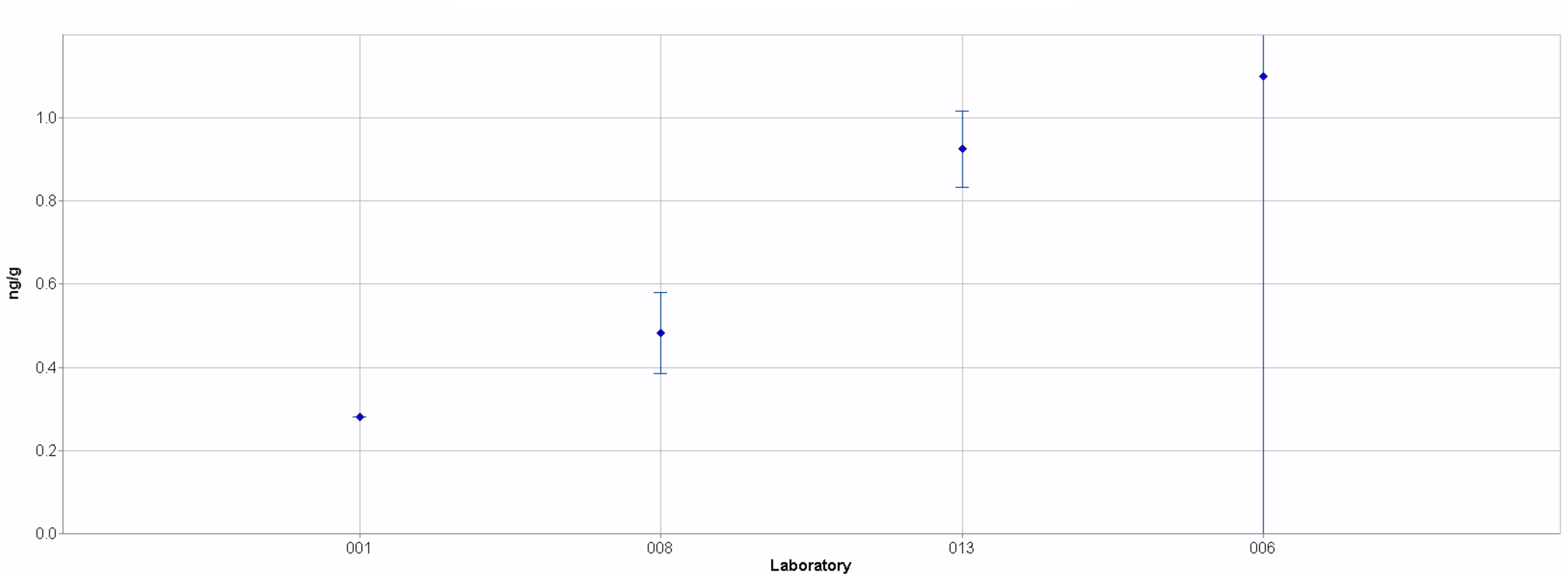
Annex 17: Results for PFTeDA

Results in ng g^{-1}

Lab code	X_{lab}	U_{lab}	k^{a}	Analytical method	u_{lab}
001	0.28	0	v3	LC-MS/MS	0
003	< 1.0				
004	< 1.0			LC-MS/MS	
006	1.1	35	v3	LC-MS/MS	20.208
008	0.483	0.097	2	LC-MS/MS	0.0485
013	0.925	0.091	2	LC-MS/MS	0.0455
014	< 0.0				
015	< 1.0			LC-MS/MS	
016	< 0.5			LC-MS/MS	

[a] √3 is set by the ILC coordinator when no expansion factor *k* is reported. The reported uncertainty was assumed to have a rectangular distribution with *k* = √3.

IMEP-42: PFTeDA in fish



Measurement results and associated uncertainties (reported uncertainties shown).

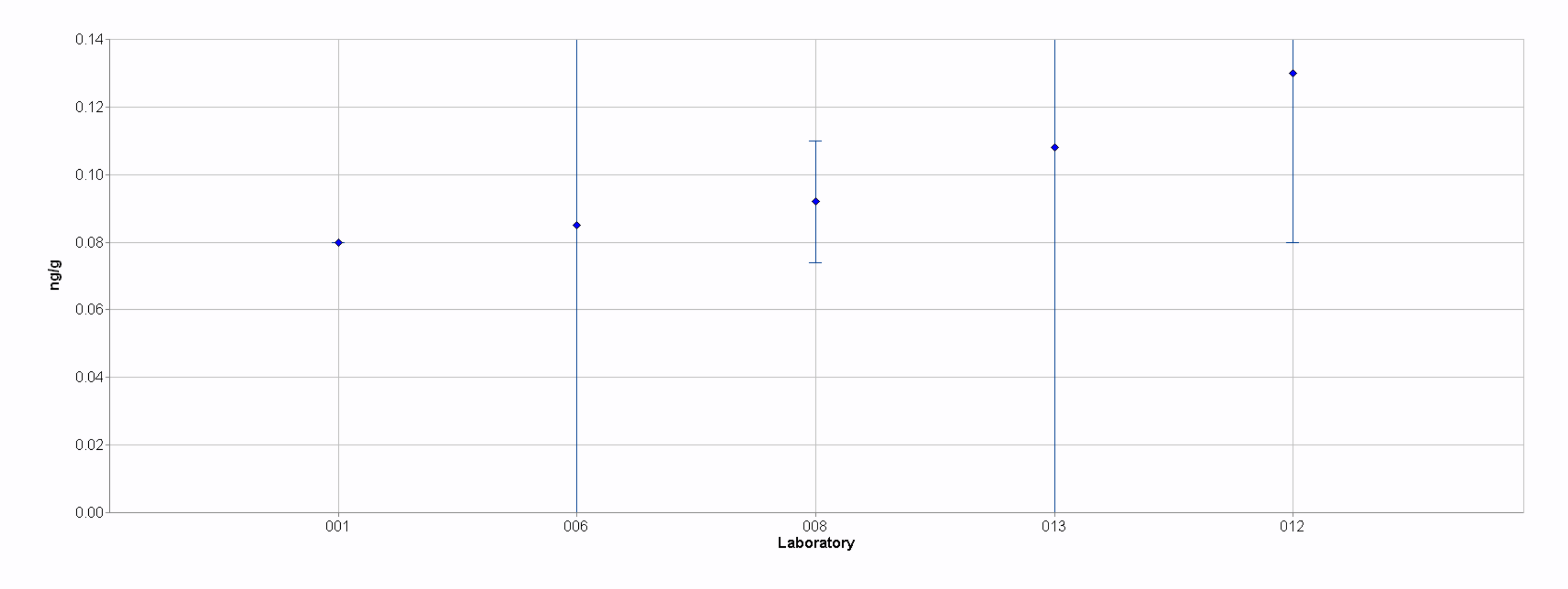
Annex 18: Results for PFHxS

Results in ng g^{-1}

Lab code	X_{lab}	U_{lab}	k^a	Analytical method	u_{lab}
001	0.08	0	v3	LC-MS/MS	0
002	< 0.8			LC-MS/MS	
003	< 1.0				
004	< 0.4			LC-MS/MS	
005	< 0.5			LC-MS/MS	
006	0.085	35	v3	LC-MS/MS	20.208
007					
008	0.092	0.018	2	LC-MS/MS	0.009
009	< 5.0				
012	0.13	0.05	2	LC-MS/MS	0.025
013	0.108	0.108	2	LC-MS/MS	0.054
014	< 5.0			LC-MS/MS	
015	< 1.0			LC-MS/MS	
016	< 0.5			LC-MS/MS	

[a] √3 is set by the ILC coordinator when no expansion factor *k* is reported. The reported uncertainty was assumed to have a rectangular distribution with $k = \sqrt{3}$.

IMEP-42: PFHxS in fish



Measurement results and associated uncertainties (reported uncertainties shown).

Annex 19: Summary of z-scores and questionnaire data

Lab code	L-PFOS	PFDA	PFUnDA	PFDoDA	br-PFOS	tot-PFOS	FOSA	PFTrDA	Extraction	Extraction solvent	Clean-up	Clean-up details	Chromatographic column	Guard column	Type of guard column
001	1.15	-0.16	-0.18	-0.29			0.00	-0.77	LSE	methanol	a) yes	SPE anion exchange	Hypersil Gold 100*2,1 mm	a) yes	hypersil gold
002	-0.50	-1.09	0.32		-2.26	-0.61			SPE	Acetonitrile-methanol	a) yes	HybridSPE Phospholipid Ultra 30mg/1mL SPE Tubes	C18 15cm	b) no	
003	2.01				24.17	3.19			LSE	Methanol	b) no		ZORBAX Eclipse XDB-C18	b) no	
004	-0.42	0.06		-1.11					LSE	Methanol KOH 0.01M	a) yes	SPE OASIS WAX 150mg + SPE ENVICARB 500mg	Acquity UPLC BEH C18 2.1*100 mm 1.7µm Waters	a) yes	PFC isolator waters
005	1.75	1.31	1.95				-0.93		extraction-saponification	methanol	a) yes	SPE	Kinetex C18 2.6 µm 100 x 2.1 mm with ultra HPLC inline filter 0.5 µm 0.004inch id	b) no	
006	-0.05	0.03	0.16	-0.08	-1.39	-0.14	-0.78	3.29	dSPE	Methanol	a) yes	dispersive solid phase extraction	C18	b) no	
007						-0.54			LSE	NaOH/MeOH	a) yes	SPE on weak anion exchanger	Kinetex 1.7 micron C18 50 x 3.0mm	b) no	
008	-0.05	-0.11	3.17	-0.04	0.39	-0.05	-0.48	-1.05	LSE	acetonitrile	a) yes	dispersive solid phase extraction using sorbent C18	BEH (C18) (100 x 2.1 mm; 1.7 um)	a) yes	ACQUITY UPLC Col. In-Line Filter Kit
009	7.38	-0.25	-0.76	-1.53					SPE	ACETONITRILE FORMIC ACID	a) yes		beh c18	b) no	
010	-0.23	-0.81					-0.35		dSPE	2% formic acid in acetonitrile	a) yes	PSA+C18	Agilent Poroshell 120 SB-C18	b) no	
011	0.63	1.37	2.00		6.83	0.94	-1.05		SPE	MeOH	a) yes		C-18	a) yes	
012		-0.03	2.49	1.24					LSE,SPE	Methanol	b) no		FluoroSep RP Octyl Phase	a) yes	C8 guard column
013	1.00	1.23	1.89	1.15				2.41	LSE	acetonitrile	a) yes	SPE-WAX	Phenomenex Gemini-NX C18, 3um (150 x 2mm)	a) yes	Phenomenex security guard standard Gemini-NX
014	-1.60					-1.41			LLE	Methanol	a) yes	Filtration	Acquity UPLC BEH C18 1.7 µm, 50 mm x 2.1 mm	a) yes	Symmetry C18 3.5 µm, 100 mm x 2.1 mm)
015	0.00	-0.25			0.09	-0.24	-0.25		SPE	10mM KOH in Methanol	a) yes	weak anion exchange SPE	Phenomenex Kinetex PFP, 2.1x100mm; 2.6um	a) yes	Phenomenex SecurityGuard ULTRA Cartridge, PFP
016		1.63	1.95	1.36		2.12	9.50		LSE	Acetonitrile	a) yes	with charcoal	Phenomenex LUNA 5u C18(2) 100A 100x2mm	a) yes	C18
017	0.40								ASE	H2O	a) yes	SPE C18	Hipyrity advance	a) yes	C18

LA-NA-27579-EN-N

JRC Mission

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new methods, tools and standards, and sharing its know-how with the Member States, the scientific community and international partners.

Serving society
Stimulating innovation
Supporting legislation

Publications Office

doi: 10.2787/063168

ISBN 978-92-79-53884-1