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JRC REFERENCE MATERIALS REPORT

CERTIFICATION OF THE MASS FRACTION OF PERFLUORALKYL SUBSTANCES (PFASs) IN FISH TISSUE (PIKE-PERCH): IRMM-427

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Abstract

This report describes the production of IRMM-427, a fish material certified for the mass fraction of perfluoroalkyl substances (PFASs). The material was produced following ISO Guide 34:2009.
The starting material for the CRM is naturally contaminated pike-perch fillets originating from the rivers Nieuwe Merwede and Amer in the Netherlands. After converting the tissue into a paste, the material was sterilized by autoclavation at 120 °C and the jars stored at 18 °C. Between-unit homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006. Within-unit homogeneity was quantified to determine the minimum sample intake.
The material was characterised by an intercomparison among laboratories of demonstrated competence and in most cases adhering to ISO/IEC 17025:2005. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.
Uncertainties of the certified values were estimated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) and include uncertainties related to possible inhomogeneity, instability and characterisation.
The material is intended for the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The CRM is available in glass jars containing approximately 35 g of fish paste. The minimum amount of sample to be used is 1 g.

CERTIFICATION REPORT

Certification of the mass fraction of perfluoroalkyl substances (PFASs) in fish tissue (pike-perch): IRMM-427

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Summary

This report describes the production of IRMM-427, a fish material certified for the mass fraction of perfluoroalkyl substances (PFASs). The material was produced following ISO Guide 34:2009 [1].

The starting material for the CRM is naturally contaminated pike-perch fillets originating from the rivers Nieuwe Merwede and Amer in the Netherlands. After converting the tissue into a paste, the material was sterilized by autoclavation at 120 °C and the jars stored at 18 °C. Between-unit homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006 [2]. Within-unit homogeneity was quantified to determine the minimum sample intake.

The material was characterised by an intercomparison among laboratories of demonstrated competence and in most cases adhering to ISO/IEC 17025:2005 [3]. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.

Uncertainties of the certified values were estimated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [4] and include uncertainties related to possible inhomogeneity, instability and characterisation.

The material is intended for the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The CRM is available in glass jars containing approximately 35 g of fish paste. The minimum amount of sample to be used is 1 g.

The following values were assigned:

	Mass fraction	
	Certified value [2] [ng/g]	Uncertainty [3] [ng/g]
Linear perfluorooctane sulfonate (L-PFOS) [1]	16.0	1.7
Perfluorodecanoic acid (PFDA) [1]	1.28	0.17
Perfluoroundecanoic acid (PFUnDA) [1]	0.74	0.20
Perfluorododecanoic acid (PFDoDA) [1]	0.97	0.21
	Indicative value [2] [ng/g]	Uncertainty [3] [ng/g]
Branched perfluorooctane sulfonate (br-PFOS) [1]	0.92	0.25
Total perfluorooctane sulfonate (tot-PFOS) [1]	17	4
Perfluoroctane sulfonamide (FOSA) [1]	1.6	0.5
Perfluorononanoic acid (PFNA) [1]	0.09	0.05
Perfluorotridecanoic acid (PFTrDA) [1]	0.62	0.29
Perfluorotetradecanoic acid (PFTeDA) [1]	0.45	0.30
Perfluorohexanesulfonate (PFHxS) [1]	0.09	0.05

1) As defined by using liquid chromatography mass spectrometry.

2) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory with a method of determination including liquid chromatography mass spectrometry. Sulfonates are expressed on an anion basis. The certified/ values and their uncertainties are traceable to the International System of Units (SI).

3) The uncertainty of the certified / indicative value is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

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Glossary

ANOVA	Analysis of variance
BHT	Butylhydroxytoluene
br-PFOS	Branched perfluorooctane sulfonate
CRM	Certified reference material
EC	European Commission
EFSA	European Food Safety Authority
EN	European norm (standard)
EQS	Environmental quality standard
ESI	Electrospray ionisation
EU	European Union
FOSA	Perfluorooctane sulfonamide
GUM	Guide to the Expression of Uncertainty in Measurements
IRMM	Institute for Reference Materials and Measurements of the JRC
ISO	International Organization for Standardization
JRC	Joint Research Centre
k	Coverage factor
LC-MS	Liquid chromatography-mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
LSE	Liquid solid extraction
MS	Mass spectrometry
$MS_{between}$	Mean square between-unit from an ANOVA
MSDS	Material safety data sheet
MS_{within}	Mean square within-unit from an ANOVA
MTBE	Methyl tert-butyl ether
n	Number of replicates per unit
N	Number of samples (units) analysed
n.a.	Not applicable
n.c.	Not calculated
n.d.	Not detectable
n.r.	Not reported
PERFOOD	Perfluorinated organics in our diet, project No. FP7-KBBE-2007-227525
PFASs	Perfluoroalkyl substances
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid

PFHpS	Perfluoroheptane sulfonate
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFP	Pentafluorophenyl
PFTrDA	Perfluorotridecanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFUnDA	Perfluoroundecanoic acid
POPs	Persistent organic pollutants
PTFE	Polytetrafluoroethylene
SI	International System of Units
s_{meas}	Standard deviation of measurement data; an additional index "rel" is added as appropriate
SPE	Solid phase extraction
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
s_{wb}	Within-unit standard deviation
T	Temperature
t	Time
$\bar{t}$	Time elapsed at time point i
t_i	Mean of all t_i
$t_{\alpha, df}$	Critical t-value for a t-test, with a level of confidence of 1-α and df degrees of freedom
t_{sl}	Proposed shelf life
TDCA	Taurodeoxycholic acid
tot-PFOS	Total perfluorooctane sulfonate
u	Standard uncertainty
U	Expanded uncertainty
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
u_c	Combined standard uncertainty; an additional index "rel" is added as appropriate
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate

U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
u_{Δ}	Combined standard uncertainty of measurement result and certified value
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added as appropriate
u_{t}	Standard uncertainty of trueness
WFD	Water Framework Directive
$\overline{x}$	Arithmetic mean
$\overline{x}_{ns}$	Arithmetic mean of all results of normal stock samples
$\overline{x}_{ref}$	Arithmetic mean of results of reference samples
α	Significance level
Δ_{meas}	Absolute difference between mean measured value and the certified value
$\nu_{s,meas}$	Degrees of freedom for the determination of the standard deviation s_{meas}
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}

1 Introduction

1.1 Background

Perfluoroalkyl substances (PFASs) are highly fluorinated aliphatic substances that contain one or more carbon atoms on which all the hydrogen substituents have been replaced by fluorine atoms in such a manner that they contain the perfluoroalkyl moiety C_nF_{2n+1} (Figure 1) [5].

These anthropogenic compounds bring together both water- and lipid-repellent properties in combination with a high chemical and thermal stability [5]. The complementarity of these properties makes PFASs and their related compounds useful for a large variety of industrial and commercial applications. Fire fighting foams, textiles, products from photographic industry, semiconductors, coating additives, cleaning products and pesticides are some examples [5,6].

The high stability of the compounds, resistance to biodegradation, atmospheric photooxidation, direct photolysis and hydrolysis result in persistency in the environment. For that reason PFASs such as perfluorooctane sulfonic acid (PFOS) (Figure 1) and its salts were recently integrated within the list of persistent organic pollutants (POPs). In the European Union (EU), their use is currently restricted by regulation [7, 8] that covers provisions regarding production, placing on the market and use of chemicals, management of stockpiles and wastes, and measures to reduce unintentional release of POPs.

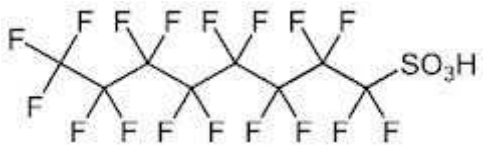

Figure 1: Example of the molecular structure of a PFASs, linear perfluorooctane sulfonic acid, L-PFOS

The high persistence of PFASs triggers effects of bioaccumulation in the trophic chain as well. Several adverse health effects such as hepatotoxicity, developmental toxicity, neurobehavioral toxicity, immunotoxicity, reproductive toxicity, lung toxicity, hormonal effects, besides a weak genotoxic and carcinogenic potential, have been demonstrated in experimental studies in animals [5,6]. Potential pathways of exposure include ingestion of food and water, the use of commercial products or inhalation from a long-range air transport [9]. Despite numerous studies to elucidate toxicological effects, levels of exposure and metabolic aspects, there is no EU legislation currently available on maximum PFASs levels in foodstuffs. Whereas for environment, the European Commission recently proposed, through the Water Framework Directive (WFD), to include PFOS in the list of priority hazardous substances to be monitored in the EU water bodies and set an environmental quality standard (EQS) of 0.65 ng/L for inland surface waters as well as 9.1 ng/g for biota [10].

In 2008 the European Food Safety Authority (EFSA) elaborated an opinion setting the human tolerable daily intake to 150 ng/kg and 1500 ng/kg body weight for PFOS and perfluorooctanoic acid (PFOA), respectively [11]. More recently, a dietary intake estimation conducted by EFSA concluded that because of a high frequency of non-quantifiable results (<LOQ), the calculation of a more realistic dietary exposure was prevented. Consequently

EFSA recommended the improvement of the sensitivity of analytical methods as a tool to increase the proportion of quantifiable results and thereby the reliability of exposure assessments for PFASs [6].

Over the last ten years a number of international interlaboratory studies have been sequentially conducted in the frame of EU-funded projects to assess the overall performance of laboratories on the analysis of PFASs [12,13,14]. The outcome of the exercises allowed a gradual improvement of the analytical methods after identification of critical factors within the process. The use of well-characterised calibrants, the use of mass-labeled internal standards or minimising matrix effects were named as key elements [11,12,13]. Still comparability of results between different laboratories may remain challenging, e.g. when different sources of standards are employed.

Analytical method validation requires the assessment of performance characteristics such as precision and trueness. The most appropriate tool for evaluating trueness is the use of certified reference materials (CRMs).

To improve comparability and harmonisation of analytical results, the production of CRMs for PFASs was included as part of the activities for the European research project PERFOOD (Perfluorinated Organics in Our Diet, No. FP7-KBBE-2007-227525). In this context, the IRMM was requested to produce two CRMs for perfluoroalkyl substances (PFASs) in fish tissue and drinking water respectively. The task was performed in close collaboration with the Institute for Environmental Studies (IVM), VU University, Amsterdam, The Netherlands.

1.2 Choice of the material

The base material employed for CRM IRMM-427 was pike-perch (*Lucioperca lucioperca*) fillets originating from the rivers Nieuwe Merwede and Amer in The Netherlands. The fish was selected after pre-screening experiments conducted by LC-MS/MS on different naturally contaminated species where the presence of a number of PFASs (Table 1) was investigated (results not shown). Special attention was given to select fish containing mass fraction levels around 0.2-20 ng/g for the most analysed and earlier used PFAS, L-PFOS, and for perfluorodecanoic acid (PFDA) as to be in a low range of concentrations although still detectable with a guaranteed level of confidence (> LOQ). After processing and thermal sterilization, the jars were stored at 18 °C.

1.3 Design of the project

The project was designed in collaboration between IRMM and the Institute for Environmental Studies (IVM), VU University Amsterdam, The Netherlands, under the auspices of the PERFOOD European project.

Fish tissue naturally contaminated with different PFASs was selected as base material for the CRM processing. The resulting fish paste was employed as candidate CRM for the characterisation of a number of PFASs, listed in Table 1, with a particular focus on two compounds, L-PFOS and PFDA.

A laboratory intercomparison was planned for the characterisation of the candidate reference material involving a number of expert laboratories that participate in the PERFOOD consortium. The number of laboratories, seven, was found to be critically low for the success of the project. Therefore IRMM selected six additional laboratories with ISO 17025 [3] accreditation in the relevant field, to take part in the material certification campaign. The laboratories were instructed to apply their own validated analytical methodology for the determination of PFASs. Together with the samples of IRMM-427, the laboratories received

ampoules containing a solution of one of the target compounds, PFDA, for calibration purposes (see description in Section 7).

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

Institute for Environmental Studies[1] (IVM), VU University, Amsterdam, NL

2.2 Processing

Institute for Environmental Studies[1] (IVM), VU University, Amsterdam, NL

With the assistance of European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.3 Homogeneity study

Institute for Environmental Studies[1] (IVM), VU University, Amsterdam, NL
(measurements under the scope of ISO/IEC 17025 accreditation Dutch Accreditation Council, L476)

2.4 Stability study

Institute for Environmental Studies[1] (IVM), VU University, Amsterdam, NL
(measurements under the scope of ISO/IEC 17025 accreditation Dutch Accreditation Council, L476)

2.5 Characterisation

3M Company- Environmental Laboratory, Maplewood MN, USA
(measurements under the scope of ISO/IEC 17025 accreditation American Association for Laboratory Accreditation, certificate number 2052.01)

AXYS Analytical Services Ltd., Sidney B.C., Canada
(measurements under the scope of ISO/IEC 17025 accreditation Canadian Association for Laboratory Accreditation Inc. CALA, A2637)

Department of Applied and Environmental Sciences[1], Stockholm University, SE
(measurements under the scope of ISO/IEC 17025 accreditation SWEDAC, 11-2501-51.1295)

Eurofins GfA Lab Service GmbH, Hamburg, DE
(measurements under the scope of ISO/IEC 17025 accreditation Certificate DAP-PL-1053.99)

Fraunhofer Institute for Process Engineering and Packaging[1], IVV, Freising, DE
(measurements under the scope of ISO/IEC 17025 accreditation DAkkS D-PL-11140-04-00)

Institute for Biodiversity and Ecosystem Dynamics-Earth Surface Science[1] (IBED-ESS), University of Amsterdam, NL

Institute for Environmental Studies[1] (IVM), VU University, Amsterdam, NL
(measurements under the scope of ISO/IEC 17025 accreditation Dutch Accreditation Council, L476)

[1] Laboratory associated to PERFOOD consortium

Institute of Chemical Technology[1], Prague, CZ
(measurements under the scope of ISO/IEC 17025 accreditation Czech Accreditation Institute, No. 319/2009)

National Institute or Nutrition and Seafood Research, Bergen, NO
(measurements under the scope of ISO/IEC 17025 accreditation Norsk Akkreditering, TEST 050)

Norwegian Institute for Air Research[1], Tromsø, NO
(laboratory under the scope of ISO/IEC 17025 accreditation Norsk Akkreditering, TEST 008)

RIKILT Wageningen UR, Wageningen, NL
(laboratory under the scope of ISO/IEC 17025 accreditation Dutch Accreditation Council, L014)

VITO, Mol, BE
(measurements under the scope of ISO/IEC 17025 accreditation BELAC nr. 045-TEST)

Federal Institute for Materials Research and Testing, BAM, Berlin, DE (qNMR analysis)
(measurements under the scope of ISO/IEC 17025 accreditation DAP-PL-2614.14)

3 Material processing and process control

3.1 Origin of the starting material

The base material employed for CRM IRMM-427 was pike-perch (*Lucioperca lucioperca*) fillets originating from the rivers Nieuwe Merwede and Amer in The Netherlands. The fish was naturally contaminated with PFASs, as confirmed by preliminary LC-MS/MS analysis targeting a number of PFASs listed in Table 1 (results not shown).

3.2 Processing

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 (Figure 2), and closed with a twist-off 66 lid RAB blik goudster, both items from Catalonië Glasverpakkingen BV, Tilburg, NL. The jars, referring in this report to the term "unit", were then sterilized by autoclavation (1.44 bar, 121 °C, 45 min) and labelled according to the filling order prior to storage at 18 °C.

Figure 2: Glass jars filled with fish paste before closing and autoclavation

Table 1: PFASs compounds investigated in the fish material and their abbreviations

PFAS Compounds	Abbreviation
Perfluorononanoic acid	PFNA
Perfluorodecanoic acid	PFDA
Perfluoroundecanoic acid	PFUnDA
Perfluorododecanoic acid	PFDoDA
Perfluorotridecanoic acid	PFTrDA
Perfluorotetradecanoic acid	PFTeDA
Perfluorohexane sulfonate	PFHxS
Perfluoroheptane sulfonate	PFHpS
Linear perfluorooctane sulfonate	L-PFOS
Total perfluorooctane sulfonate	tot-PFOS
Branched perfluorooctane sulfonate	br-PFOS
Perfluoroctane sulfonamide	FOSA

3.3 Process control

Process control consisted of preliminary analytical measurements by LC-MS/MS to ensure suitable levels of target PFASs in the fish material (results not shown). During processing, particle size analysis (PSA) was performed on the content of two randomly selected jars, obtaining particle sizes below 1 mm for the fish paste. Autoclavation tape was placed inside in a handful of jars to verify that the core temperature had indeed reached 121 °C during the sterilization process. The data obtained ensured that the process control was adequate.

4 Homogeneity

A key requirement for any reference material (RM) is the equivalence between the various units. In this respect, it is relevant whether the variation between units is significant compared to the uncertainty of the certified value. In contrast to that it is not relevant if the variation between units is significant compared to the analytical variation. Consequently, ISO Guide 34 [1] requires RM producers to quantify the between-unit variation of the property values. This aspect is covered in between-unit homogeneity studies.

The within-unit inhomogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. Quantification of within-unit inhomogeneity is therefore necessary to determine the minimum sample intake.

4.1 Between-unit homogeneity

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all units of the material, within the stated uncertainty.

The number of selected units corresponds to approximately the cubic root of the total number of the produced units. The 14 units were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. For this, the batch was divided into 14 groups (with a similar number of units) and one unit was selected randomly from each group. Three independent samples were taken from each selected unit, and analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Fish samples were prepared by methanol liquid solid extraction (LSE) followed by a clean-up procedure involving active carbon [15]. Briefly, one gram of sample was added to 3 g Na_2SO_4 and mixed with a metal spoon. At that moment the isotopically labelled PFASs employed as internal standards were added and allowed to equilibrate overnight. Methanol was added and, after shaking for 30 min, the sample was centrifuged. The upper part of the extract was collected and the step was repeated. The extract was cleaned up using active carbon, shaking the mixture and subsequently centrifuging. The resulting extract was reconstituted with water/methanol (1/1, v/v) prior to injection into the LC-MS/MS system. Measurements were performed under repeatability conditions and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence. Reporting data results from the formal target analytes of the certification project (L-PFOS and PFDA) was mandatory whereas the analysis and reporting of other PFASs compounds detected in the sample was provided on voluntary basis. The analytical results obtained are shown as Tables in Annex A. The dry mass fraction was additionally determined for each unit by oven drying with an average result of 21.9 % and a standard deviation of 0.4 %.

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. No trends in the filling sequence or the analytical sequence were visible for any of the PFASs compounds tested, to a 95 % confidence level. The dataset was assessed for consistency using single and double Grubbs outlier tests at a confidence level of 99 % on the individual results and on the unit means. Two outlying individual results were detected for PFTrDA with the double Grubbs test. One outlying unit mean was detected for FOSA with the single Grubbs test. The outliers were retained for the evaluation since no technical reason could be found for excluding those particular results.

Quantification of between-unit inhomogeneity was accomplished by analysis of variance (ANOVA), which can separate the between-unit variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual samples are representative for the whole unit.

Evaluation by ANOVA requires unit means which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. Distribution of the unit means was visually tested using histograms and normal probability plots. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations. The results of all statistical evaluations are given in Tables 2 and 3.

Table 2: Results of the statistical evaluation of the homogeneity studies at a 95% confidence level (trends) and a 99 % confidence level (outliers)

Measurand	Trends		Outliers		Distribution	
	Analytical sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
PFNA	no	no	none	none	normal	normal
PFDA	no	no	none	none	normal	normal
PFUnDA	no	no	none	none	normal	normal
PFDoDA	no	no	none	none	normal	normal
PFTrDA	no	no	two	none	normal	unimodal
PFTeDA	no	no	none	none	normal	normal
PFHxS	no	no	none	none	normal	unimodal
PFHpS[1)]	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
tot-PFOS	no	no	none	none	normal	normal
L-PFOS	no	no	none	none	normal	normal
br-PFOS	no	no	none	none	normal	normal
FOSA	no	no	none	one	normal	normal

[1)] Mass fraction levels below LOQ

One has to bear in mind that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and therefore subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean square within groups (MS_{within}), resulting in negative arguments under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [16]. u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

Method repeatability ($s_{wb,rel}$), between-unit standard deviation ($s_{bb,rel}$) and $u^*_{bb,rel}$ were calculated as:

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad \textbf{Equation 1}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad \textbf{Equation 2}$$

$$u^*_{bb,rel} = \frac{\sqrt{\frac{MS_{within}}{n}}\sqrt[4]{\frac{2}{v_{MSwithin}}}}{\bar{y}} \qquad \textbf{Equation 3}$$

MS_{within} mean square within-unit from an ANOVA

$MS_{between}$ mean square between-unit from an ANOVA

$\bar{y}$ mean of all results of the homogeneity study

n mean number of replicates per unit

$v_{MSwithin}$ degrees of freedom of MS_{within}

However, a different approach was adopted for FOSA, for which 1 outlying unit mean was detected. In this case the between-unit inhomogeneity was modelled as a rectangular distribution limited by the largest outlying unit mean and the rectangular standard uncertainty associated with homogeneity was estimated by:

$$u_{rec} = \frac{\left| outlier - \bar{y} \right|}{\sqrt{3} \cdot \bar{y}} \qquad \textbf{Equation 4}$$

$\bar{y}$ mean of all results of the homogeneity study

It should be mentioned that the outlying unit mean for FOSA is a result of outlying individual values and does not necessarily reflect the real distribution of the compound in the material.

Table 3: Results of the homogeneity study for PFASs in IRMM-427

Measurand	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{rec,rel}$ [%]	$u_{bb,rel}$ [%]
PFNA	11.01	n.c	3.28	n.a	3.28
PFDA	6.43	n.c	1.92	n.a	1.92
PFUnDA	6.80	n.c	2.03	n.a	2.03
PFDoDA	4.74	n.c	1.42	n.a	1.42
PFTrDA	9.50	6.22	2.84	n.a	6.22
PFTeDA	19.61	9.29	5.85	n.a	9.29
PFHxS	15.60	4.35	4.66	n.a	4.66
tot-PFOS	2.40	0.68	0.72	n.a	0.72
L-PFOS	2.63	0.20	0.78	n.a	0.78
br-PFOS	4.80	n.c	1.43	n.a	1.43
FOSA	5.74	2.11	1.71	6.61	6.61

n.c.: cannot be calculated as $MS_{between} < MS_{within}$

The homogeneity study showed no outlying unit means or trends in the filling for the majority of the compounds tested. In those cases the between-unit standard deviation s_{bb} can be used as estimate of u_{bb}. As u^*_{bb} sets the limits of the study to detect inhomogeneity, the larger value of s_{bb} and u^*_{bb} is adopted as uncertainty contribution to account for potential inhomogeneity.

An outlying unit mean was found for one compound, FOSA. In this case the inhomogeneity quantified as u_{rec} is large compared to the values of s_{bb} and u^*_{bb} for other compounds. But it is still sufficiently small to make the material useful. Therefore, for FOSA, u_{rec} was used as estimate of u_{bb}.

4.2 Within-unit homogeneity and minimum sample intake

The within-unit homogeneity is closely correlated to the minimum sample intake. Due to this correlation, individual aliquots of a material will not contain the same amount of analyte. The minimum sample intake is the minimum amount of sample that is representative for the

whole unit and thus should be used in an analysis. Using sample sizes equal or above the minimum sample intake guarantees the certified value within its stated uncertainty.

Homogeneity and stability experiments were performed using a 1 g sample intake. This sample intake gives acceptable repeatability for target analytes, demonstrating that the within-unit inhomogeneity no longer contributes to analytical variation at this sample intake.

The minimum sample intake was additionally assessed from the results of the characterisation study, using the method information supplied by the participants (Annex D). The smallest sample intake that still yielded results with acceptable accuracy to be included in the respective studies was considered as a suitable minimum sample intake. Using the data from Annex E, it can be observed that sample intakes of 0.2 g or 0.5 g, used by labs L10 and L11, respectively, gave acceptable accuracy for several analytes. However laboratories employing such sample intakes did not report values for all target PFASs. For that reason, it was decided to take a conservative approach and establish 1 g as minimum sample intake.

5 Stability

Time, temperature and light were regarded as the most relevant influences on stability of the materials. Materials are stored and dispatched in the dark, thus eliminating practically the possibility of degradation by light. Additionally the material was sterilized by autoclavation to preclude microbial growth. Therefore, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish conditions for storage (long-term stability) as well as conditions for dispatch to the customers (short-term stability). During transport, especially in summer time, temperatures up to 60 °C could be reached and stability under these conditions must be demonstrated if transport at ambient temperature will be applied.

The stability studies were carried out using an isochronous design [17]. In that approach, samples are stored for a certain time at different temperature conditions. Afterwards, the samples are moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples are analysed simultaneously under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions greatly improves the sensitivity of the statistical tests applied to the stability studies.

5.1 Short-term stability study

For the short-term stability study, units were stored at 18 °C and 60 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to 4 °C. Two units per storage time and temperature were selected using a random stratified sampling scheme. From each unit, three samples were measured by LC-MS/MS as described in the homogeneity section. The measurements were performed under repeatability conditions, and in a randomised sequence to be able to differentiate any potential analytical drift from a trend over storage time. Reporting data results from the formal target analytes of the certification project (L-PFOS and PFDA) was mandatory whereas the analysis and reporting of other PFASs compounds detected in the sample was voluntary. The results were not corrected for the water content. The results of the measurements are shown in Annex B.

The obtained data were evaluated individually for each temperature. The individual results were screened for outliers using the single and double Grubbs tests at a confidence level of 99%. No outlying results were found for any of the PFASs tested at both temperatures (Table 4), with the exception of PFTrDA. Since this could not be technically explained, all data were retained for the estimation of u_{sts} at 60 °C.

The data were evaluated against storage time and regression lines of mass fraction versus time were calculated. The slopes of the regression lines were tested for statistical significance, to test for potential increases/decreases of the analyte's mass fraction due to shipping conditions. The slopes of the regression lines were not significantly different from zero at a 95 % confidence level, at any of the temperatures tested (Table 4). Only for PFTrDA, a statistically significant positive slope was observed, influenced by the above mentioned outlying result. As the analyte cannot be created in the sample, a positive trend could only be due to degradation of the matrix. This, however, should be observed for all measurands, which was not the case. The observed trend was therefore regarded as a statistical artefact.

The material can be dispatched without further precautions under ambient conditions.

Table 4: Results of the short-term stability tests at a 95% confidence level (trends) and a 99 % confidence level (outliers)

Measurand	Outliers		Trends	
	18 ºC	60 ºC	18 ºC	60 ºC
PFNA	none	none	no	no
PFDA	none	none	no	no
PFUnDA	none	none	no	no
PFDoDA	none	none	no	no
PFTrDA	none	one	no	yes
PFTeDA	none	none	no	no
PFHpS	none	none	no	no
PFHxS[1)]	n.a.	n.a.	n.a.	n.a.
tot-PFOS	none	none	no	no
L-PFOS	none	none	no	no
br-PFOS	none	none	no	no
FOSA	none	none	no	no

[1)] Mass fraction levels below LOQ

5.2 Long-term stability study

For the long-term stability study, units were stored at 18 °C for 0, 8, 16 and 24 months. The reference temperature was set to 4 °C. Two units per storage time were selected using a random stratified sampling scheme. From each unit, three samples were measured by LC-MS/MS. The measurements were performed under repeatability conditions in a random sequence to be able to differentiate any potential analytical drift from a trend as a function of storage time. The methodology employed included liquid solid extraction (LSE) of the sample followed by a clean-up procedure (involving active carbon) and LC-MS/MS as described in the homogeneity section. As for the homogeneity and short-term stability studies, results from PFASs other than L-PFOS and PFDA are reported on voluntary basis, which justifies data gaps existing for PFTeDA, PFHxS and tot-PFOS. In those cases, available data from 8 and/or 12-month long-term stability studies are alternatively employed. Results were not corrected for the water content. The results of the measurements are shown in Annex C.

The obtained data were evaluated individually for each time. The results were screened for outliers using the single and double Grubbs tests at a confidence level of 99%. One

technically unexplained outlier was observed for PFDoDA, PFTrDA and FOSA, all three corresponding to the analysis of the same sub sample (Table 5). Since no technical explanation was associated to the outliers, the values were retained for evaluation.

Furthermore, the data were plotted against storage time and linear regression lines of mass fraction versus time were calculated. The slope of the regression lines was tested for statistical significance, to test for potential increases/decreases of the analyte's mass fraction due to storage conditions. For all PFASs compounds, the slopes of the regression lines were not significantly different from zero at a 95 % confidence level (Table 5).

The material can therefore be stored at 18 ºC.

Table 5: Results of the long-term stability tests at a 95% confidence level (trends) or a 99 % confidence level (outliers), for 24 months

Measurand	Outliers	Trends
	18 ºC	18 ºC
PFNA[2)]	none	no
PFDA	none	no
PFUnDA	none	no
PFDoDA	one	no
PFTrDA	one	no
PFTeDA[2)]	none	no
PFHxS[3)]	none	no
PFHpS[1)]	n.a.	n.a.
tot-PFOS[3)]	none	no
L-PFOS	none	no
br-PFOS	none	no
FOSA	one	no

[1)] Mass fraction levels below LOQ

[2)] Data retrieved from available results corresponding to a eight-month long-term stability study.

[3)] Data retrieved from available results corresponding to a 12-month long-term stability study.

5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can rule out degradation of materials completely, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means that, even under ideal conditions, the outcome of a stability study can only be "degradation is 0 ± x % per time".

Uncertainties of stability during dispatch and storage were estimated for each PFAS, as described in [18]. In this approach, the uncertainty of the linear regression line with a slope of zero is calculated. The uncertainty contributions u_{sts} and u_{lts} are calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{tt} \qquad \textbf{Equation 5}$$

$$u_{lts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{sl} \qquad \textbf{Equation 6}$$

RSD	relative standard deviation of all results of the stability study
t_i	time elapsed at time point *i*
$\bar{t}$	mean of all t_i
t_{tt}	chosen transport time (1 week at 60 ºC)
t_{sl}	chosen shelf life (24 months at 18 ºC)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated from the 60 °C studies. The uncertainty describes the possible change during a dispatch at 60 °C lasting for one week.
- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the 18 °C studies. The uncertainty contribution describes the possible degradation during 24 months storage at 18 °C.

The results of these evaluations are summarised in Table

Table 6.

Table 6: Uncertainty associated with stability during dispatch and storage. $u_{sts,rel}$ was calculated for a temperature of 60 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of 18 °C and 24 months

Measurand	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]
PFNA	2.25	27.49[1)]
PFDA	0.68	4.71
PFUnDA	1.06	11.60
PFDoDA	0.69	9.76
PFTrDA	3.54	18.44
PFTeDA	3.57	22.22[1)]
PFHxS	n.a.	28.53[2)]
tot-PFOS	0.34	4.43[2)]
L-PFOS	0.34	2.80
br-PFOS	1.13	9.28
FOSA	0.79	8.68

[1)]Estimated from available data from an eight-month long-term stability study

[2)]Estimated from available data from a 12 month long-term stability study

After the certification study campaign, the material will be included in the IRMM's regular stability monitoring programme, to control its further stability.

6 Characterisation

The material characterisation is the process of determining the certified value of a reference material.

This was based on an intercomparison of results from expert laboratories. The mass fractions of PFASs in the material, as supplied, without correction for dry mass, were determined in different laboratories that applied different measurement procedures. This was done to demonstrate the absence of a measurement bias. Due to the nature of the analytes, however, all participants used liquid chromatographic methods for the measurements. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty.

6.1 Selection of participants

Six laboratories participating in the PERFOOD project consortium took part in the characterisation study of the material. Six additional laboratories were selected by IRMM based on criteria that comprised both technical competence and quality management aspects. Each of them was asked about their quality system and requested to deliver documented evidence of their proficiency in the field of PFASs measurements in relevant matrices by submitting results of intercomparison exercises and/or details on their method validation. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 was obligatory. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

6.2 Study setup

Each laboratory received 3 units of the candidate CRM and was requested to provide 6 independent results, 2 per unit. The units were selected using a random stratified sampling scheme and covered the whole batch. The sample preparations and measurements had to be spread over three days to ensure intermediate precision conditions. An independent calibration was required for each day of analysis. The water content was determined in each unit although results are reported on wet mass basis.

Besides some specific instructions, including a request to report sulfonate compounds expressed on anion basis, the laboratories were provided with a solution of PFDA (2.5 µg/mL in methanol) for calibration purposes. Neat crystals employed for the individual calibration solution preparation (Chiron AS, Trondheim, NO) were analysed by qNMR for purity assessment (Annex F).

All laboratories were requested to submit results for L-PFOS and PFDA whereas the reporting of additional PFASs was optional.

6.3 Methods used

Methods applied for characterisation involved different sample extraction protocols followed by LC separation on chromatographic columns having C18 or fluorinated stationary phase. Detection was performed by mass spectrometry using negative electron spray ionisation (ESI).

All methods used during the characterisation study are summarised in Annex D, Tables 1 and 2. The laboratory code (e.g. L01) is a random number and does not correspond to the order of laboratories provided in Section 2. The lab-method code consists of a number assigned to each laboratory (e.g. L01).

6.4 Evaluation of results

The characterisation campaign resulted in up to 12 datasets per PFAS compound. All individual results of the participants, grouped per measurand, are displayed in tabular and graphical form in Annex E. No results were submitted by L06.

6.4.1 Technical evaluation

The obtained data were first checked for compliance with the requested analysis protocol and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- appropriate validation of the measurement procedure
- compliance with the analysis protocol: sample preparations and measurements performed on three days and water content determination.
- absence of values given as below limit of quantification.

Based on the above criteria, no datasets were rejected.

6.4.2 Statistical evaluation

The technically accepted datasets were tested for normality of dataset means using kurtosis/skewness tests and normal probability plots. They were also tested for outlying means using the single and double Grubbs tests and for outlying standard deviations using the Cochran test, both at a 99 % confidence level. Standard deviations within (s_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in

Table 7.

Table 7: Statistical evaluation of the technically accepted datasets for IRMM-427. *p*: number of technically valid datasets

Measurand	*p*	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [ng/g]	*s* [ng/g]	$s_{between}$ [ng/g]	s_{within} [ng/g]
PFNA	7	none	three	yes	0.086	0.015	0.014	0.008
PFDA	12	none	none	yes	1.346	0.198	0.194	0.096
PFUnDA	7	none	none	yes	0.744	0.122	0.119	0.065
PFDoDA	7	none	none	yes	0.970	0.112	0.107	0.080
PFTrDA	4	none	none	n.d.	0.615	0.156	0.147	0.126
PFTeDA	6	none	none	n.d.	0.445	0.203	0.201	0.079
PFHxS	6	none	two	n.d.	0.088	0.011	0.009	0.018
tot-PFOS	6	none	one	yes	17.474	3.536	3.481	1.522
L-PFOS	12	none	two	yes	16.013	2.423	2.372	1.210
br-PFOS	5	none	one	n.d.	0.920	0.191	0.181	0.149
FOSA	5	none	none	n.d.	1.589	0.276	0.268	0.162

n.d. not determined, p < 7

The laboratory means follow normal distributions for all PFASs where more than 6 datasets are available. For PFTrDA, PFTeDA, PFHxS, br-PFOS, tot-PFOS and FOSA the number of data sets available are not sufficient for the proper application of the skewness/kurtosis test. Instead the visual observation of the normal probability plots does not seem to reveal distributions other than normal. None of the data contains outlying means for any of the PFASs analysed (single and double Grubbs tests at a confidence level of 99 %). Still the datasets are consistent and the mean of laboratory means is a good estimate of the true value. Standard deviations between laboratories are for the majority of the PFASs considerably larger than the standard deviation within laboratories, showing that confidence intervals of replicate measurements are unsuitable as estimate of measurement uncertainty.

The statistical evaluation flags a number of outlying variances for various PFASs including PFNA, PFHxS, L-PFOS, tot-PFOS and br-PFOS. This merely reflects the fact that different methods have different intrinsic variability. As all measurement methods were found technically sound, all results were retained.

It should be borne in mind that the methods used in the characterisation are different from lab to lab and are methods routinely applied for measuring PFASs in fish tissue. The agreement of results from different methods demonstrates that the processing did not affect any properties relevant for these methods and that IRMM-427 behaves like a real sample.

The uncertainty related to the characterisation is estimated as the standard error of the mean of laboratory means (

Table 8).

Table 8: Uncertainty of characterisation for PFASs in IRMM-427

Measurand	*p*	Mean [ng/g]	*s* [ng/g]	u_{char} [ng/g]
PFNA	7	0.086	0.015	0.006
PFDA	12	1.346	0.198	0.057
PFUnDA	7	0.744	0.122	0.046
PFDoDA	7	0.970	0.112	0.042
PFTrDA	4	0.615	0.156	0.078
PFTeDA	6	0.445	0.203	0.083
PFHxS	6	0.088	0.011	0.005
tot-PFOS	6	17.474	3.536	1.444
L-PFOS	12	16.013	2.423	0.699
br-PFOS	5	0.920	0.191	0.085
FOSA	5	1.589	0.276	0.124

7 Characterisation of calibrating solutions

An independent solution of PFDA, employed as common calibrant during the IRMM-427 characterisation study was purchased from Chiron AS, Trondheim, Norway. The solution was prepared gravimetrically by dissolving amounts of neat crystalline PFDA in methanol to obtain a concentration of 2.5 µg/mL.

Supplementary amounts of the neat crystalline PFDA material employed during preparation of the solutions were kindly provided by Chiron to IRMM for further assessment of its purity.

The purity analysis was performed by qNMR (Federal Institute for Materials Research and Testing (BAM), DE). Four replicate analyses of the compound were carried out with a precision level of 0.5 %. Conditions of the analysis performed and results obtained are detailed in Annex F.

8 Value Assignment

Certified and indicative values were assigned.

Certified values are values that fulfil the highest standards of accuracy. General procedures at IRMM require pooling of not less than 6 datasets to assign certified values. Full uncertainty budgets were established in accordance with the 'Guide to the Expression of Uncertainty in Measurement' [4].

Indicative values are values where either the uncertainty is deemed too large or where too few independent datasets were available to allow certification. Uncertainties are evaluated according to the same rules as for certified values.

8.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in

Table 9 was assigned as certified value for each parameter.

The assigned uncertainty consists of uncertainties related to characterisation, u_{char} (Section 6), potential between-unit inhomogeneity, u_{bb} (Section 4.1) and potential degradation during transport (u_{sts}) and long-term storage, u_{lts} (Section 5.3). For some of the compounds the uncertainty related to inhomogeneity and/or degradation during transport was found to be negligible, i.e. smaller than 1/3 of other uncertainty contributions and therefore not accounted. The different contributions were combined to estimate the expanded, relative uncertainty of the certified value ($U_{CRM, rel}$) with a coverage factor *k* as:

$$U_{CRM,rel} = k \cdot \sqrt{u^2_{char,rel} + u^2_{bb,rel} + u^2_{sts,rel} + u^2_{lts,rel}} \text{ ..} \qquad \textbf{Equation 7}$$

- u_{char} was estimated as described in Section 6.4
- u_{bb} was estimated as described in Section 4.1
- u_{sts} was estimated as described in Section 5.3.
- u_{lts} was estimated as described in Section 5.3

Because of the sufficient degrees of freedom of the different uncertainty contributions, a coverage factor *k* of 2 was applied, to obtain the expanded uncertainties. The certified values and their uncertainties are summarised in Table 9, where negligible uncertainty contributions are highlighted in italic.

Table 9: Certified values and their uncertainties for IRMM-427

Measurand	Certified value [ng/g]	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [ng/g] [1)]
PFDA[2)]	1.28	4.24	1.92	*0.68*	4.71	13.2	0.17
L-PFOS	16.0	4.37	*0.78*	*0.34*	2.80	10.4	1.7
PFUnDA	0.74	6.20	*2.03*	*1.06*	11.6	26.3	0.20
PFDoDA	0.97	4.35	1.42	0.69	9.76	21.4	0.21

[1)] Expanded (*k* = 2) and rounded uncertainty

[2)] Value corrected according to the result obtained for the purity of the PFDA employed as common calibrant.

The mean value obtained for PFDA during the certification study was multiplied with a correction factor. The correction takes into account the purity of PFDA used as common calibrant, which was assessed by qNMR in parallel to the certification campaign of the material (Section 7 and Annex F). The uncertainty contribution of the PFDA purity to the certified value for the material was considered negligible compared to the rest of the contributors and therefore not accounted for the total expanded uncertainty estimation.

8.2 Indicative values and their uncertainties

Indicative values were assigned for PFNA, PFTrDA, PFTeDA, PFHxS, br-PFOS, tot-PFOS and FOSA. Although the methodology applied for their determination was analogous to that employed for determination of other PFASs, the total uncertainty associated to the assigned mass fraction value was considered too large and, in some cases, the number of valid data sets was lower than 6. For tot-PFOS and br-PFOS an additional source of variability of the results is introduced by the constituents of the calibration solutions used by the labs during the characterisation studies. Approaches vary between the use of L-PFOS alone, of technical

mixtures or of some individual br-PFOS that can influence the final concentration. In the fish tissue material IRMM-427 br-PFOS represents only a minor fraction within tot-PFOS, where the major component is L-PFOS. However the isomeric composition of PFOS may be different in different fish samples, therefore values corresponding to tot-PFOS and br-PFOS in IRMM-427 are assigned as indicative.

Indicative values may not be used as certified values. The uncertainty budgets were set up as for the certified values and are listed together with the assigned values in Table

Table 10, where negligible contributions are highlighted in italic.

Table 10: Indicative values and their uncertainties for IRMM-427

Measurand	Indicative value [ng/g]	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [ng/g] [1)]
PFNA	0.09	6.51	*3.28*	*2.25*	27.49	56.5	0.05
PFTrDA	0.62	12.68	6.22	*3.54*	18.44	46.5	0.29
PFTeDA	0.45	18.66	9.29	*3.57*	22.22	60.9	0.30
PFHxS	0.09	5.19	4.66	n.a	28.53	57.1	0.05
br-PFOS	0.92	9.28	1.43	*1.13*	9.28	26.4	0.25
tot-PFOS	17	8.26	*0.72*	*0.34*	4.43	18.7	4
FOSA	1.6	7.77	6.61	*0.79*	8.68	26.8	0.5

[1)]: Expanded (k = 2) and rounded uncertainty

9 Metrological traceability and commutability

9.1 Metrological traceability

Identity

PFASs are chemically clearly defined analytes. Identity was confirmed by mass spectrometry. The participants used different methods for the sample preparation as well as for the final determination, demonstrating absence of measurement bias. The measurands are therefore structurally defined and independent of the measurement method.

Quantity value

Only validated methods were used for the determination of the assigned values. Different calibrants of known purity and specified traceability of their assigned values were used and all relevant input parameters were calibrated. For one of the compounds a common calibrant characterised for its purity was employed during the certification campaign. The individual results are therefore traceable to the SI, as it is also confirmed by the agreement among the technically accepted datasets. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are traceable to the SI as well.

9.2 Commutability

Many measurement procedures include one or more steps which select specific (or specific groups of) analytes from the sample for the subsequent whole measurement process. Often the complete identity of these 'intermediate analytes' is not fully known or taken into account. Therefore, it is difficult to mimic all analytically relevant properties of real samples within a CRM. The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is summarised in a concept called 'commutability of a reference material'. There are various definitions that define this concept. For instance, the CLSI Guideline C53-A53A [19] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and is therefore a crucial characteristic when applying different measurement methods. When the commutability of a CRM is not established, the results from routinely used methods cannot be legitimately compared with the certified value to determine whether a bias does exist in calibration, nor can the CRM be used as a calibrant.

IRMM-427 was produced from naturally contaminated fish tissue containing a mixture of PFASs at different levels. The analytical behaviour will be the same as for a routine fish tissue sample.

10 Instructions for use

10.1 Safety information

For laboratory use only. The usual laboratory safety measures apply.

10.2 Storage conditions

The materials shall be stored at 4 °C ± 3 °C in the dark. The user is reminded to close tightly any jars immediately after taking a sample to maintain the original moisture.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially for opened jars.

10.3 Preparation and use of the material

Before use, the content of the jar must be re-homogenised. As the jar may not provide sufficient space for re-homogenisation, the content may be transferred to a suitable, clean container. Make sure that all the paste together with the fluid, if any, is transferred. Homogenise manually (by spoon, spatula e.g.) or using a blender until a fine homogeneous paste is achieved. Use only clean lab ware and tools. A sub-sample should be taken immediately after re-homogenisation.

The use of PTFE or other fluoropolymers during sample extraction and analyses must be avoided [20]. In case the analytical system employed for the PFASs determination does contain PTFE or other fluoropolymers, the PFASs leaching from these polymers may be retained by an additional column installed just prior to the injection valve. The replacement of fluoropolymer tubing by non-fluorinated polymer tubing or stainless steel reduces as well leaching of PFASs and therefore decreases potential contamination of the sample extract. In addition, care must be taken to distinguish the analytical signal of L-PFOS from that of the bile acid taurodeoxycholic acid (TDCA), which may be found as an interfering signal in the MS measurement.

10.4 Minimum sample intake

The minimum sample intake representative for all parameters is 1 g.

10.5 Use of the certified value

The main purpose of this material is to assess method performance, i.e. for checking accuracy of analytical results/calibration. As with any reference material, it can be used for establishing control charts and validation studies.

Use as a calibrant

It is not recommended to use this matrix material as calibrant. If used nevertheless, the uncertainty of the certified value shall be taken into account in the estimation of the measurement uncertainty.

Comparing an analytical result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1, www.erm-crm.org [21]).

For assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is described here in brief:

- Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).
- Combine measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}): $u_{\Delta} = \sqrt{u_{meas}^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty (u_{Δ}), using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %
- If $\Delta_{meas} \leq U_{\Delta}$ then no significant difference exists between the measurement result and the certified value, at a confidence level of approximately 95 % .

Use in quality control charts

The materials can be used for quality control charts. Different CRM-units will give the same result as inhomogeneity was included in the uncertainties of the certified values.

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12 References

1 ISO Guide 34, General requirements for the competence of reference materials producers, International Organization for Standardization, Geneva, Switzerland, 2009

2 ISO Guide 35, Reference materials – General and statistical principles for certification, International Organization for Standardization, Geneva, Switzerland, 2006

3 ISO/IEC 17025, General Requirements for the Competence of Testing and Calibration Laboratories; International Organization for Standardization, Geneva, Switzerland, 2005

4 ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement, (GUM 1995), International Organization for Standardization, Geneva, Switzerland, 2008

5 R.C. Buck, J. Franklin, U. Berger, J.M. Conder, I.T. Cousins, P. de Voogt, A.A. Jensen, K. Kannan, S.A. Mabury and S.P.J. van Leeuwen, Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. Integrated Environmental Assessment and Management, 2011. **7**(4): p. 513–541.

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

7 COMMISSION REGULATION (EU) No 756/2010 of 24 August 2010 amending Regulation (EC) No 850/2004 of the European Parliament and of the Council on persistent organic pollutants as regards Annexes IV and V in Official Journal of the European Union. 2010.

8 COMMISSION REGULATION (EU) No 757/2010 of 24 August 2010 amending Regulation (EC) No 850/2004 of the European Parliament and of the Council on persistent organic pollutants as regards Annexes I and III in COMMISSION REGULATION (EU) No 757/2010. 2010, Official Journal of the European Union: Official Journal of the European Union.

9 Draf toxicological profile for perfluoralkyls. U.S. Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry (ATSDR), 2009.

10 Proposal for a directive fo the European Parliament and of the council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, COM(2001) 876, European Commission, Editor. 2011.

11 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.

12 S.P.J. van Leeuwen, W. Cofino, S. Crum. Analytical improvements shown over four interlaboratory studies of perfluoroalkyl substances in environmental and food samples. TrAC Trends in Analytical Chemistry, 2013. 43(0): p. 204-216.

13 S.P.J. van Leeuwen, C.P. Swart, I. van der Veen, J. de Boer, Significant improvements in the analysis of perfluorinated compounds in water and fish: Results form an interlaboratory method evaluation study. Journal of Chromatography A, 2009. 1216(3) 401-409.

14 G. Lindstrom, A. Karrman, B. van Bavel, Accuracy and precision in the determination of perfluorinated chemicals in human blood verified by interlaboratory comparison. Journal of Chromatography A, 2009. 1216(3) 394-400

15 C.R. Powley, S.W. George, M.H. Russel, R.A. Hoke, R.C. Buck. Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. Chemosphere 70 (2008) 664-67

16 T.P.J. Linsinger, J. Pauwels, A.M.H. van der Veen, H. Schimmel, A. Lamberty, Homogeneity and stability of reference materials, Accred. Qual. Assur. 6 (2001) 20-25

17 A. Lamberty, H. Schimmel, J. Pauwels, The study of the stability of reference materials by isochronous measurements, Fres. J. Anal. Chem. 360 (1998) 359-361

18 T.P.J. Linsinger, J. Pauwels, A. Lamberty, H. Schimmel, A.M.H. van der Veen, L. Siekmann, Estimating the Uncertainty of Stability for Matrix CRMs, Fres. J. Anal. Chem. 370 (2001) 183-188

19 H. Vesper, H. Emons, M. Gnezda, C. P. Jain, W. G. Miller, R. Rej, G. Schumann, J. Tate, L. Thienpont, J. E. Vaks, Characterization and Qualification of Commutable Reference Materials for Laboratory Medicine; Approved Guideline, CLSI document C53-A, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2010

20 A. Ballesteros-Gomez, S. Rubio, S. van Leeuwen, Tetrahydrofuran-water extraction, in-line clean-up and selective liquid chromatography/tandem mass spectrometry for the quantitation of perfluorinated compounds in food at the low picogram per gram level. J. Chromatogr A, 2010. 1217(24) 5913-5921

21 T.P.J. Linsinger, ERM Application Note 1: Comparison of a measurement result with the certified value, www.erm-crm.org (last accessed on 11/10/2014)

ANNEX A: Results from the homogeneity study for PFASs in IRMM-427 expressed in ng/g wet material. Outlying results are highlighted in italic.

PFNA [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	0.072	0.061	0.076
101	0.074	0.074	0.074
203	0.063	0.070	0.075
310	0.056	0.079	0.066
396	0.068	0.073	0.054
465	0.074	0.079	0.058
569	0.066	0.065	0.067
660	0.059	0.063	0.066
738	0.062	0.074	0.060
832	0.073	0.074	0.075
917	0.080	0.070	0.059
996	0.073	0.079	0.075
1096	0.063	0.079	0.064
1194	0.068	0.072	0.087

PFDA [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	0.998	1.055	1.062
101	0.897	1.057	1.011
203	1.071	1.076	0.969
310	1.071	1.077	1.044
396	0.944	1.089	1.068
465	1.014	1.160	1.057
569	0.975	0.980	1.028
660	0.943	1.022	1.014
738	0.961	1.095	1.008
832	0.922	1.087	1.054
917	1.012	1.073	1.004
996	1.024	0.986	1.063
1096	1.177	1.142	0.993
1194	0.983	1.044	1.187

PFUnDA [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	0.539	0.599	0.551
101	0.519	0.545	0.558
203	0.581	0.557	0.559
310	0.559	0.557	0.534
396	0.499	0.595	0.573
465	0.510	0.603	0.538
569	0.497	0.632	0.563
660	0.558	0.571	0.510
738	0.524	0.578	0.528
832	0.525	0.537	0.589
917	0.531	0.609	0.595
996	0.543	0.532	0.565
1096	0.583	0.635	0.536
1194	0.495	0.557	0.558

PFTrDA [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	0.447	0.624	*0.370*
101	0.535	0.565	0.503
203	0.575	0.643	0.643
310	0.579	0.658	0.578
396	0.515	0.587	0.571
465	0.618	0.625	0.617
569	0.573	0.578	0.574
660	0.631	0.531	0.607
738	0.636	0.529	0.550
832	0.556	0.490	0.593
917	0.636	0.606	0.632
996	0.583	0.583	0.549
1096	0.686	0.597	0.579
1194	*0.419*	0.472	0.568

PFDoDA [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	0.835	0.875	0.828
101	0.892	0.839	0.937
203	0.871	0.903	0.913
310	0.889	0.840	0.817
396	0.925	0.952	0.861
465	0.900	0.848	0.873
569	0.881	0.850	1.005
660	0.906	0.952	0.864
738	0.885	0.861	0.867
832	0.876	0.837	0.927
917	0.832	0.912	0.919
996	0.923	0.938	0.916
1096	0.887	0.955	0.847
1194	0.867	0.915	0.866

PFTeDA [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	0.163	0.310	0.087
101	0.306	0.277	0.205
203	0.338	0.421	0.300
310	0.249	0.299	0.266
396	0.273	0.308	0.329
465	0.298	0.334	0.340
569	0.306	0.237	0.287
660	0.270	0.261	0.315
738	0.331	0.258	0.279
832	0.285	0.225	0.250
917	0.327	0.313	0.354
996	0.249	0.366	0.335
1096	0.261	0.339	0.266
1194	0.162	0.208	0.369

PFHxS [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	0.103	0.079	0.067
101	0.099	0.079	0.080
203	0.045	0.068	0.076
310	0.097	0.092	0.081
396	0.084	0.086	0.071
465	0.088	0.077	0.060
569	0.086	0.071	0.083
660	0.080	0.071	0.064
738	0.076	0.059	0.073
832	0.061	0.094	0.073
917	0.077	0.079	0.079
996	0.091	0.071	0.109
1096	0.095	0.082	0.077
1194	0.072	0.089	0.097

L-PFOS [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	17.13	16.69	16.56
101	17.90	17.15	16.94
203	16.90	15.99	17.11
310	16.97	16.65	15.78
396	17.01	16.77	16.65
465	16.53	16.84	16.21
569	16.96	16.78	16.75
660	16.77	17.03	16.80
738	16.87	16.81	16.58
832	16.79	16.70	17.26
917	16.72	16.12	17.13
996	17.39	16.53	16.88
1096	17.36	17.72	16.65
1194	15.60	16.61	17.16

br-PFOS [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	0.986	1.067	0.950
101	0.989	1.060	0.959
203	0.947	1.053	1.013
310	1.031	0.964	0.979
396	1.022	1.019	0.962
465	1.007	1.051	0.957
569	0.884	0.985	0.969
660	1.052	1.031	1.027
738	1.009	0.985	1.041
832	0.983	1.013	0.980
917	0.968	0.952	1.053
996	0.997	1.057	1.024
1096	1.123	1.047	0.979
1194	0.906	0.985	1.053

tot-PFOS [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	17.78	17.49	17.28
101	18.53	17.95	17.66
203	17.61	16.79	17.75
310	17.66	17.38	16.53
396	17.76	17.51	17.37
465	17.27	17.57	16.90
569	17.43	17.47	17.38
660	17.47	17.83	17.60
738	17.61	17.56	17.37
832	17.48	17.47	17.99
917	17.45	16.83	17.80
996	18.13	17.31	17.64
1096	18.14	18.49	17.42
1194	16.29	17.34	17.82

FOSA [ng/g]

IRMM-427 unit n.	Rep. 1	Rep. 2	Rep. 3
37	1.551	1.448	1.458
101	1.601	1.519	1.498
203	1.410	1.430	1.508
310	1.578	1.393	1.517
396	1.788	1.624	1.594
465	1.564	1.410	1.485
569	1.499	1.427	1.475
660	1.514	1.501	1.478
738	1.634	1.407	1.564
832	1.544	1.410	1.508
917	1.560	1.442	1.412
996	1.467	1.490	1.515
1096	1.337	1.436	1.664
1194	1.378	1.539	1.310

ANNEX B: Analytical results obtained from the short-term stability study of PFASs in IRMM-427. Three replicates per unit of IRMM-427 were carried out and the results are expressed as ng/g wet material for each individual PFAS compound. Outlying results are highlighted in italics.

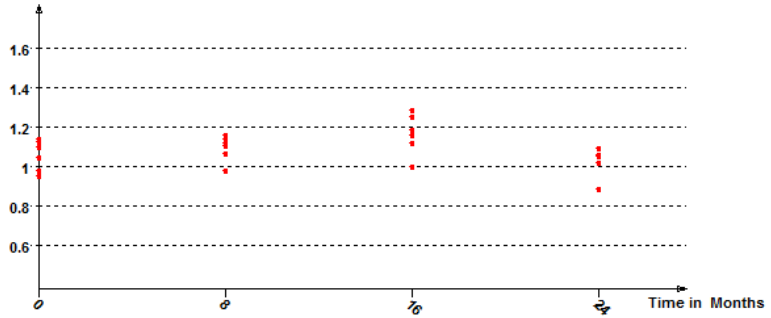
Storage T [ºC]	Storage t [weeks]	IRMM-427 unit n.	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFHxS	PFHpS	tot-PFOS	l-PFOS	br-PFOS	FOSA
4	0	26	0.059	0.969	0.565	0.912	0.331	0.166	< LOQ	0.029	14.83	13.97	0.947	1.431
4	0	26	0.052	1.022	0.533	0.906	0.265	0.154	< LOQ	< LOQ	14.90	13.94	1.103	1.267
4	0	26	< LOQ	0.995	0.491	0.800	0.238	0.236	< LOQ	0.036	14.51	13.68	0.925	1.461
4	0	667	0.066	0.916	0.556	0.890	0.304	0.218	< LOQ	0.018	15.67	14.87	1.030	1.342
4	0	667	0.058	0.998	0.605	0.917	0.317	0.247	< LOQ	0.025	15.45	14.63	1.023	1.324
4	0	667	0.058	0.955	0.534	0.885	0.286	0.244	< LOQ	0.028	14.64	13.70	0.986	1.416
18	1	72	0.049	0.999	0.601	0.885	0.298	0.235	< LOQ	0.020	14.02	13.06	1.022	1.439
18	1	72	0.058	0.961	0.520	0.889	0.267	0.172	< LOQ	0.032	15.11	14.15	1.094	1.380
18	1	72	0.038	0.956	0.639	0.923	0.268	0.214	< LOQ	0.029	15.38	14.34	1.170	1.523
18	1	681	0.053	0.943	0.488	0.860	0.310	0.164	< LOQ	0.014	15.47	14.61	1.006	1.534
18	1	681	0.038	1.031	0.614	1.034	0.295	0.176	< LOQ	0.019	15.19	14.24	1.025	1.577
18	1	681	0.046	0.945	0.460	0.967	0.261	0.256	< LOQ	0.045	15.17	14.22	1.112	1.612
18	2	134	0.066	1.082	0.606	0.861	0.411	0.148	< LOQ	0.029	15.21	14.33	1.002	1.555
18	2	134	0.049	1.102	0.561	0.921	0.263	0.190	< LOQ	0.025	14.56	13.72	0.974	1.375
18	2	134	0.060	1.075	0.616	0.855	0.256	0.135	< LOQ	0.068	15.14	14.13	1.165	1.516
18	2	735	0.050	1.064	0.578	0.991	0.369	0.183	< LOQ	0.043	14.76	13.83	1.070	1.455
18	2	735	0.065	0.989	0.557	0.905	0.308	0.220	< LOQ	0.041	15.27	14.34	1.050	1.333
18	2	735	0.075	1.079	0.654	0.950	0.306	0.276	< LOQ	0.056	14.39	13.42	1.049	1.446
18	4	146	0.052	1.020	0.614	0.847	0.304	0.167	< LOQ	0.024	14.82	14.03	0.879	1.515
18	4	146	0.047	1.115	0.569	0.870	0.290	0.227	< LOQ	0.031	15.32	14.48	1.027	1.332
18	4	146	0.048	0.904	0.465	0.867	0.345	0.220	< LOQ	0.011	15.11	14.20	0.993	1.294
18	4	775	0.085	0.971	0.502	0.875	0.334	0.240	< LOQ	0.029	15.15	14.41	0.884	1.452
18	4	775	0.044	1.028	0.556	0.877	0.301	0.207	< LOQ	0.036	15.51	14.58	1.027	1.459
18	4	775	0.040	0.897	0.544	0.904	0.265	0.184	< LOQ	0.047	15.00	14.02	1.126	1.334
60	1	182	0.065	1.020	0.555	0.886	0.395	0.166	< LOQ	0.034	14.61	13.81	0.916	1.402
60	1	182	0.050	1.020	0.608	0.897	0.283	0.089	< LOQ	0.013	15.04	14.14	1.007	1.567

60	1	182	0.047	0.950	0.525	0.970	0.333	0.213	< LOQ	0.020	15.40	14.48	1.069	1.367
60	1	835	0.055	1.021	0.570	0.890	0.533	0.217	< LOQ	0.031	15.14	14.45	0.918	1.349
60	1	835	0.052	0.956	0.542	0.823	0.324	0.181	< LOQ	0.031	15.35	14.53	1.079	1.374
60	1	835	0.066	0.944	0.456	0.877	0.339	0.239	< LOQ	0.055	15.64	14.59	1.192	1.414
60	2	261	0.060	1.034	0.537	0.923	0.475	0.192	< LOQ	0.047	15.07	14.38	0.900	1.228
60	2	261	0.041	0.926	0.583	0.936	0.357	0.138	< LOQ	< LOQ	15.00	14.10	1.034	1.405
60	2	261	< LOQ	1.101	0.603	0.944	0.319	0.162	< LOQ	< LOQ	15.96	14.91	0.913	1.218
60	2	880	0.056	1.090	0.577	0.919	0.391	0.144	< LOQ	0.059	14.97	14.08	0.969	1.320
60	2	880	0.057	1.004	0.583	0.985	0.323	0.131	< LOQ	0.035	14.69	13.89	0.883	1.346
60	2	880	0.058	1.035	0.677	0.842	0.277	0.230	< LOQ	< LOQ	15.14	14.18	1.071	1.289
60	4	284	0.068	0.979	0.566	0.832	*0.720*	0.247	< LOQ	0.048	14.82	14.01	0.860	1.352
60	4	284	0.039	1.027	0.550	0.916	0.358	0.101	< LOQ	0.013	15.35	14.42	1.040	1.405
60	4	284	0.072	0.922	0.531	0.932	0.340	0.258	< LOQ	0.024	14.86	13.93	1.048	1.339
60	4	900	0.047	1.049	0.576	0.960	0.560	0.249	< LOQ	0.035	15.25	14.38	0.975	1.299
60	4	900	0.063	0.980	0.540	0.890	0.401	0.169	< LOQ	0.029	14.75	13.88	0.985	1.315
60	4	900	0.063	1.015	0.540	0.920	0.348	0.186	< LOQ	0.032	14.97	14.01	1.094	1.446

ANNEX C: Results of a 24-month long-term stability study at 18 ºC for PFASs (expressed in ng/g wet material) in IRMM-427. Exceptionally for PFTeDA, PFHxS, PFNA and tot-PFOS, available data for stability studies performed at eight or 12 months were alternatively employed. Six independent replicates per time-point were measured. Outliers detected are highlighted in italic. For illustration, mass fractions [ng/g] of individual replicates *vs.* exposure time [month] at 18 ºC are represented for each PFAS compound below the corresponding data table.

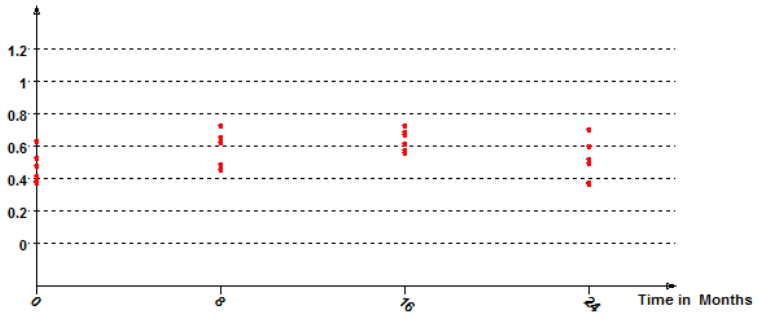
PFDA [ng/g]

	t [month]			
Replicate	0	8	16	24
1	1.1240	1.1380	1.1180	0.8846
2	0.9515	1.1170	0.9988	1.0580
3	1.0500	1.1610	1.1860	1.0510
4	0.9787	1.0690	1.2880	1.0190
5	1.1380	0.9789	1.1590	1.0200
6	1.0996	1.1080	1.2510	1.0930



PFUnDA [ng/g]

	t [month]			
Replicate	0	8	16	24
1	0.4807	0.7253	0.6179	0.365
2	0.3771	0.6542	0.5768	0.7039
3	0.3831	0.6215	0.6741	0.4986
4	0.5312	0.6231	0.6905	0.3761
5	0.4137	0.4898	0.5600	0.5985
6	0.6331	0.4597	0.725	0.5225

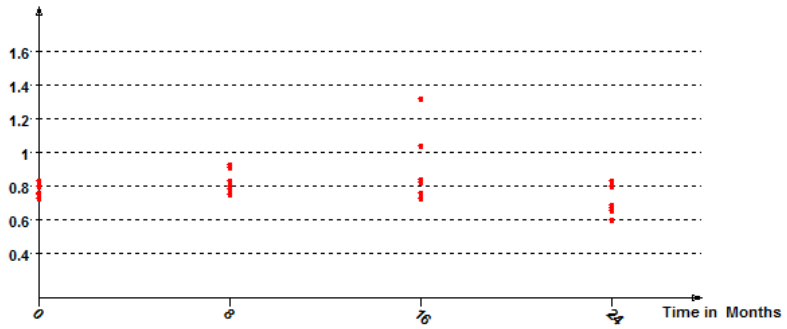


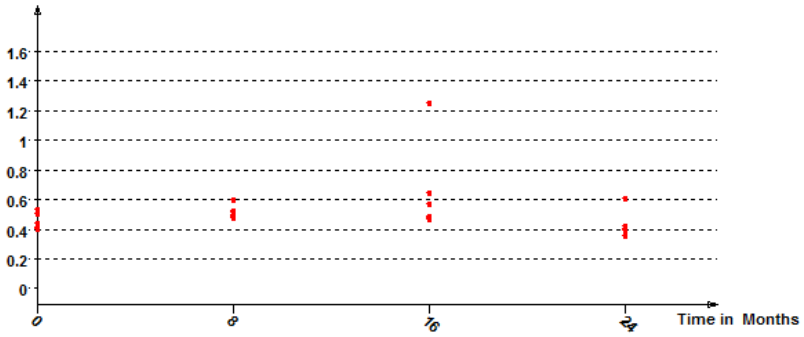
PFDoDA [ng/g]

	t [month]			
Replicate	0	8	16	24
1	0.7623	0.9271	0.7599	0.6586
2	0.7990	0.7845	0.8234	0.6718
3	0.7312	0.8284	0.8418	0.5990
4	0.7551	0.9117	1.0400	0.6865
5	0.7305	0.7991	0.7256	0.7996
6	0.8309	0.7524	*1.3232*	0.8308

PFTrDA [ng/g]

	t [month]			
Replicate	0	8	16	24
1	0.5113	0.6022	0.4861	0.3961
2	0.4080	0.4801	0.4676	0.4211
3	0.4132	0.5287	0.6516	0.3633
4	0.5384	0.4947	0.5727	0.4064
5	0.4449	0.5203	0.4786	0.4216
6	0.4191	0.4893	*1.2534*	0.607



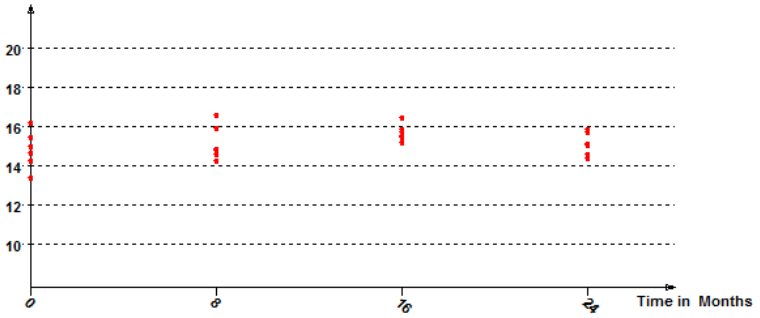


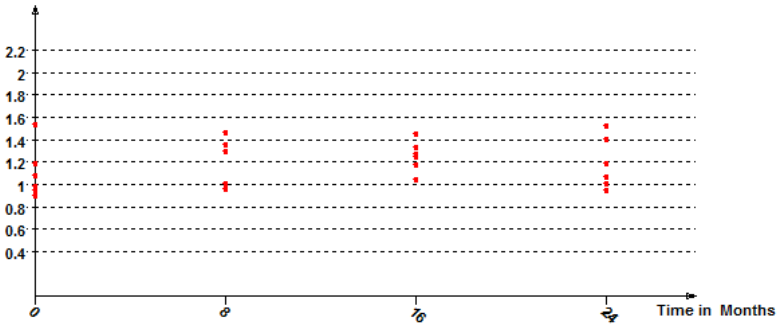
L-PFOS [ng/g]

	t [month]			
Replicate	0	8	16	24
1	15.44	16.61	16.47	14.61
2	14.97	15.93	15.73	15.74
3	13.39	14.77	15.49	14.41
4	14.65	14.84	15.87	15.06
5	14.25	14.61	15.55	15.14
6	16.17	14.25	15.17	15.85

br-PFOS [ng/g]

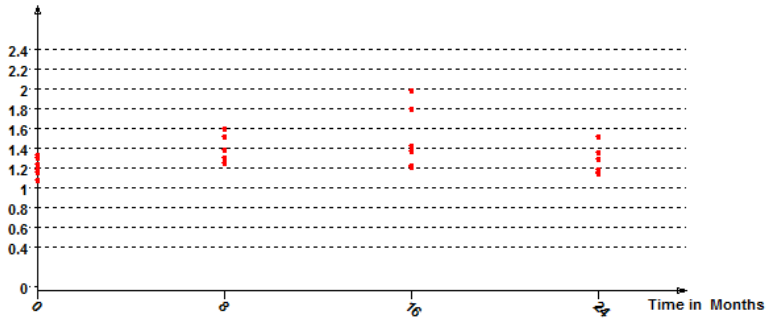
	t [month]			
Replicate	0	8	16	24
1	0.983	1.306	1.459	1.011
2	1.090	1.471	1.275	1.525
3	0.9551	1.360	1.051	0.954
4	1.197	0.969	1.340	1.074
5	0.9057	1.006	1.247	1.408
6	1.534	1.006	1.18	1.188





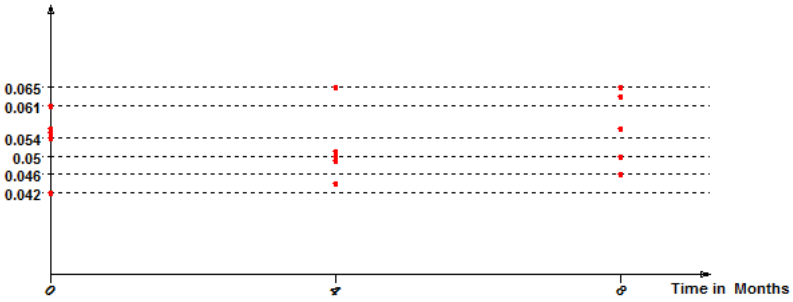
FOSA [ng/g]

	t [month]			
Replicate	0	8	16	24
1	1.335	1.605	1.422	1.161
2	1.078	1.387	1.233	1.359
3	1.245	1.311	1.806	1.293
4	1.183	1.515	1.380	1.150
5	1.166	1.250	1.216	1.186
6	1.312	1.248	*1.988*	1.514



PFNA [ng/g]

	t [month]		
Replicate	0	4	8
1	0.042	0.044	<LOQ
2	0.055	0.065	0.056
3	<LOQ	0.049	0.065
4	0.056	<LOQ	0.063
5	0.061	0.051	0.050
6	0.054	0.050	0.046



PFTeDA [ng/g]

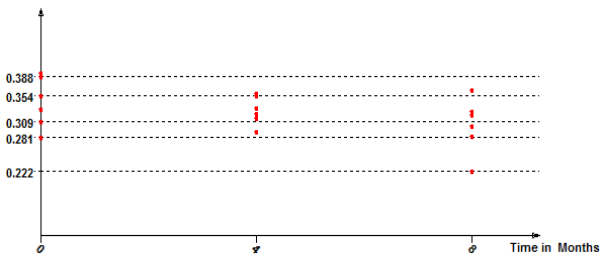
	t [month]		
Replicate	0	4	8
1	0.281	0.354	0.364
2	0.355	0.315	0.283
3	0.330	0.292	0.222
4	0.388	0.332	0.301
5	0.394	0.322	0.326
6	0.309	0.358	0.320

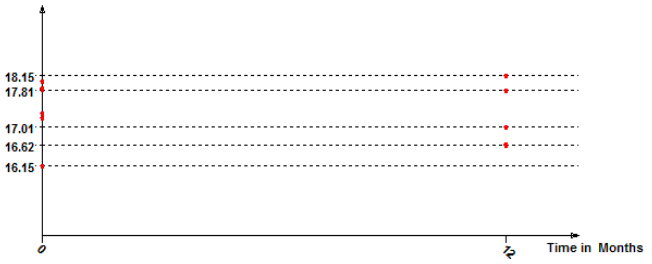
PFHxS [ng/g]

	t [month]	
Replicate	0	12
1	0.103	0.072
2	0.116	0.076
3	0.072	0.107
4	0.130	0.056
5	0.112	0.124
6	0.119	0.089

Tot-PFOS [ng/g]

	t [month]	
Replicate	0	12
1	17.21	16.63
2	16.15	16.64
3	17.86	17.01
4	17.83	16.62
5	18.01	17.81
6	17.32	18.15





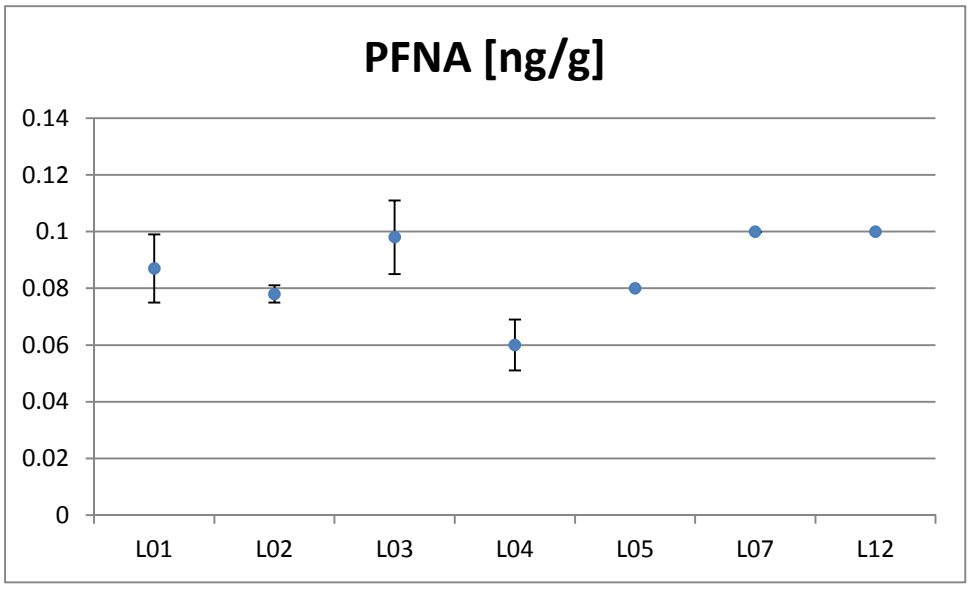
ANNEX D: Table 2. m/z ion transitions employed by participant laboratories for quantification (in bold) and identification of PFASs compounds in the certification study of IRMM-427

Lab. code	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFHxS	PFHpS	PFOS	FOSA
L01	**463>419** 463>219	**513>469** 513>269	**563>519** 563>269	**613>569** 613>169	**663>619** 663>169	**713>669** 713>169	**398.9>80** 398.9>99	**449>80** 449>99	**499>80** 499>99	**498>78** 498>498
L02	**463>419**	**513>469**	**563>519**	**613>569**		**713>669**	**399->80** 399->99	**449>80** 449>99	**499>80** 499>99	
L03	**463>419**	**513>469**	**563>519**	**613>569**		**712.9>669**	**398.9->80**		**498.9>99** 498.9>80	**497.9>78**
L04	**463>419** 463>219	**513>468.9** 513>219	**562.9>518.9** 562.9>268.9	**613>568.9** 613>318.9	**663>618.9**	**712.9>668.9** 712.9>368.9	**399>80** 399>99	**449>80** 449>99	**499>80** 499>99	**498.1>78** 498.1>168.9
L05	**463.1>419** 463>219	**513.1>469** 513.1>219	**563>519** 563.01>269	**613>569** 613.01>169		**713.1>669** 713.1>169	**398.8>79.9** 398.8>98.9		**499>79.9** 499>98.9	**498>78** 498>477.9
L06										
L07	**463>419** 463>219	**513>469** 513>269	**563>519** 563>269	**613>569** 613>319	**663>619** 663>369	**713>669** 713>369	**399>80** 399>99		**499>80** 499>99	
L08		**513>469** 513>219							**499>80** 499>99	
L09		**513>469**							**499>80**	
L10		**513>468.8** 513>268.9							**498.9>80** 498.9>98.9	
L11	**463>419** 463>219 463>169	**513>469** 513>269 513>219					**399>80** 399>99		**499>80** 499>99 499>130	
L12	**463>419** 463>219	**513>469** 513>269	**563>519** 563>269	**613>569** 613>169	**663>619** 663>169	**713>669** 713>169	**399>99** 399>80		**499>99** 499>80	**498>78** 498>478
L13		**512.9>219.1** 512.9>469							**499>99** 499>80	

ANNEX E: Characterisation data reported for PFASs in IRMM-427 by participant laboratories during the certification study. Error bars in the graphs represent the standard deviation of the results for the individual laboratories.

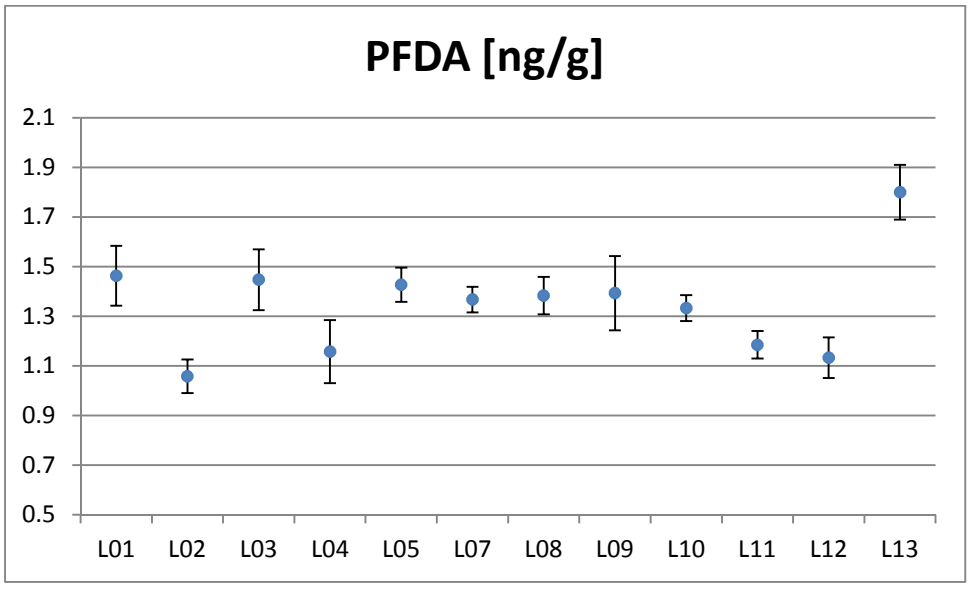
Analytical results of the certification study for PFNA in IRMM-427

	PFNA (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	0.08	0.1	0.09	0.08	0.1	0.07	0.087	0.012
L02	0.082	0.074	0.081	0.079	0.077	0.075	0.078	0.003
L03	0.1	0.1	0.1	0.12	0.08	0.09	0.098	0.013
L04	0.07	0.05	0.06	0.07	0.06	0.05	0.06	0.009
L05	0.08	0.08	0.08	0.08	0.08	0.08	0.08	
L07	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0
L08								
L09								
L10								
L11								
L12	0.1	0.1	0.1				0.1	0
L13								



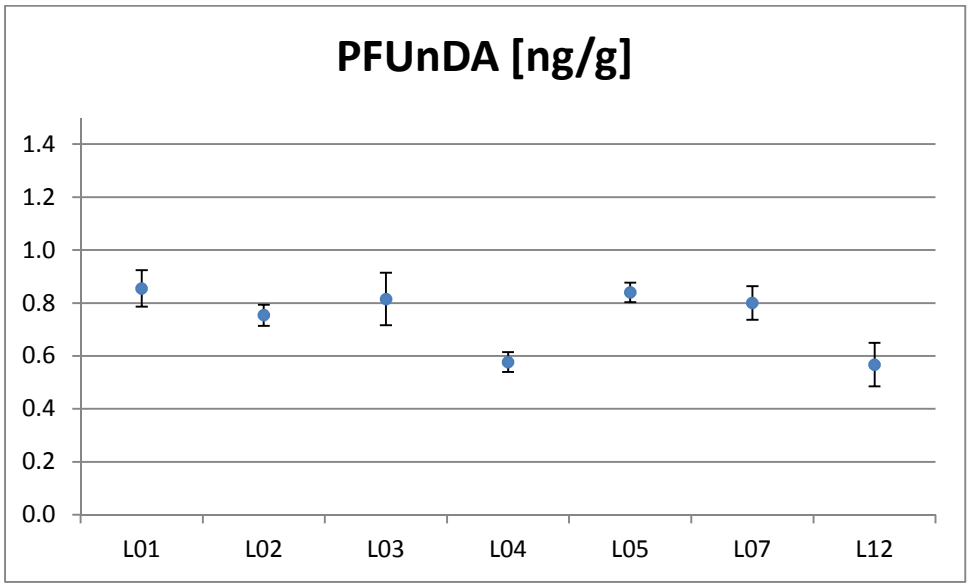
Analytical results of the certification study for PFDA in IRMM-427

	PFDA (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	1.35	1.53	1.6	1.29	1.46	1.55	1.463	0.121
L02	1.13	1.144	0.988	1.066	1.035	0.987	1.058	0.068
L03	1.56	1.56	1.3	1.37	1.55	1.34	1.447	0.123
L04	1.06	1.2	1.28	1.01	1.08	1.32	1.158	0.127
L05	1.36	1.53	1.34	1.45	1.45	1.43	1.427	0.069
L07	1.4	1.4	1.4	1.3	1.4	1.3	1.367	0.052
L08	1.4	1.4	1.3	1.4	1.5	1.3	1.383	0.075
L09	1.57	1.54	1.4	1.33	1.36	1.16	1.393	0.15
L10	1.4	1.3	1.4	1.3	1.3	1.3	1.333	0.052
L11	1.1	1.22	1.17	1.15	1.25	1.22	1.185	0.055
L12	1.1	1	1.2	1.1	1.2	1.2	1.133	0.082
L13	1.7	1.8	2	1.7	1.8	1.8	1.8	0.11



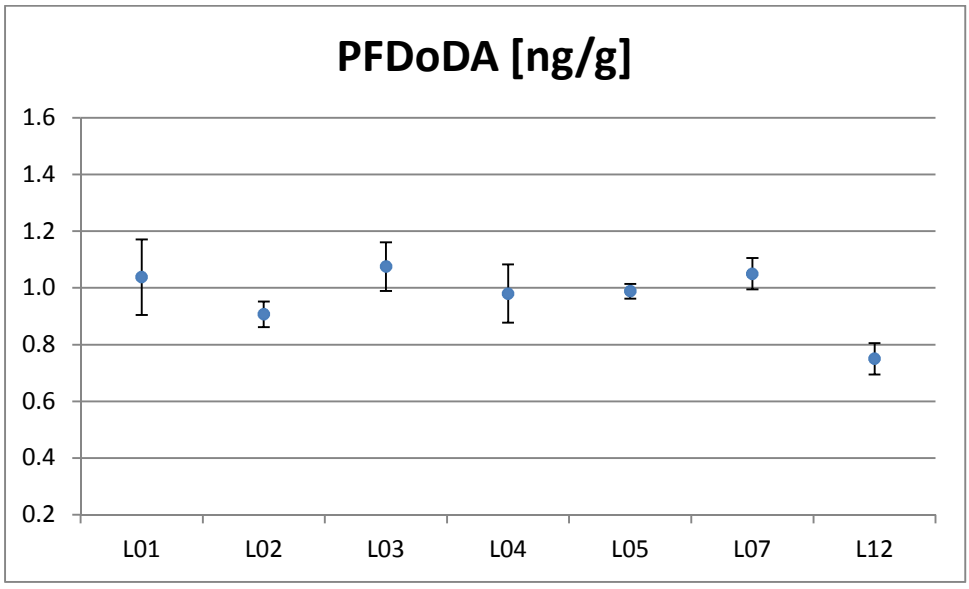
Analytical results of the certification study for PFUnDA in IRMM-427

	PFUnDA (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	0.73	0.91	0.9	0.87	0.9	0.82	0.855	0.069
L02	0.717	0.797	0.805	0.709	0.755	0.743	0.754	0.04
L03	0.88	0.84	0.75	0.72	0.97	0.73	0.815	0.099
L04	0.51	0.6	0.59	0.58	0.62	0.56	0.577	0.038
L05	0.87	0.87	0.79	0.87	0.8	0.84	0.84	0.037
L07	0.8	0.8	0.8	0.8	0.9	0.7	0.8	0.063
L08								
L09								
L10								
L11								
L12	0.5	0.5	0.7	0.5	0.6	0.6	0.567	0.082
L13								



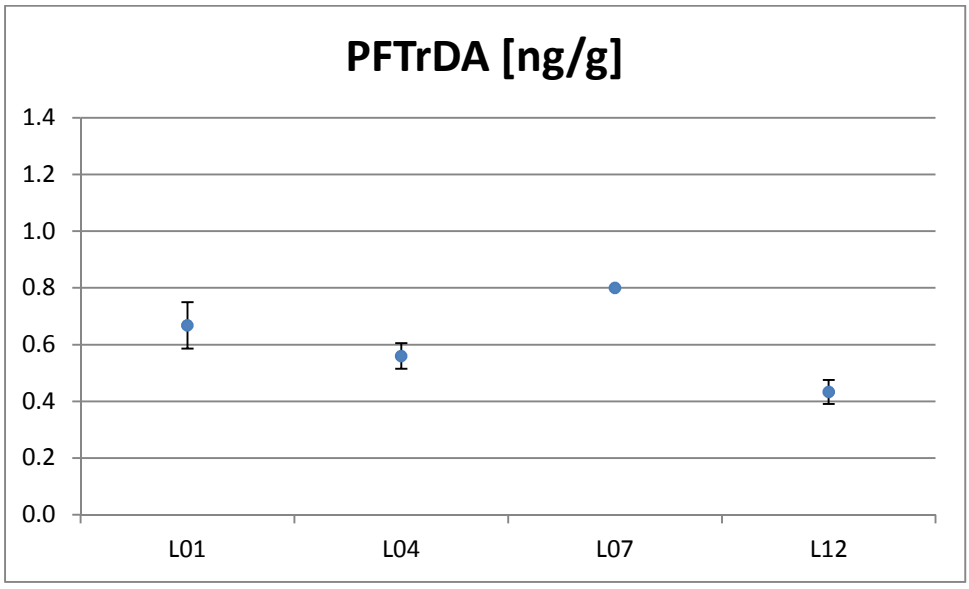
Analytical results of the certification study for PFDoDA in IRMM-427

	PFDoDA (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	1.13	0.83	1.12	0.98	0.98	1.19	1.038	0.133
L02	0.895	0.98	0.938	0.898	0.86	0.872	0.907	0.045
L03	1.08	1.12	1.14	0.97	1.17	0.97	1.075	0.086
L04	0.96	0.86	1.09	0.94	0.91	1.12	0.98	0.103
L05	0.97	1.02	0.96	0.97	1.02	0.99	0.988	0.026
L07	1.1	1	1	1	1.1	1.1	1.05	0.055
L08								
L09								
L10								
L11								
L12	0.8	0.8	0.7	0.8	0.7	0.7	0.75	0.055
L13								



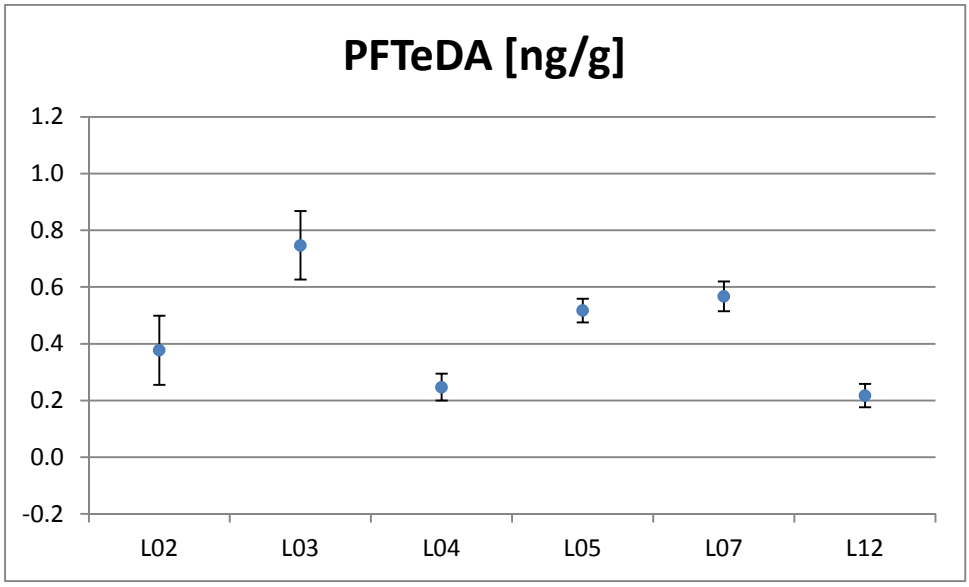
Analytical results of the certification study for PFTrDA in IRMM-427

	PFTrDA (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	0.61	0.51	1.06	0.62	0.53	0.68	0.668	0.202
L02								
L03								
L04	0.53	0.48	0.75	0.49	0.48	0.63	0.56	0.109
L05								
L07	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0
L08								
L09								
L10								
L11								
L12	0.5	0.4	0.3	0.6	0.4	0.4	0.433	0.103
L13								



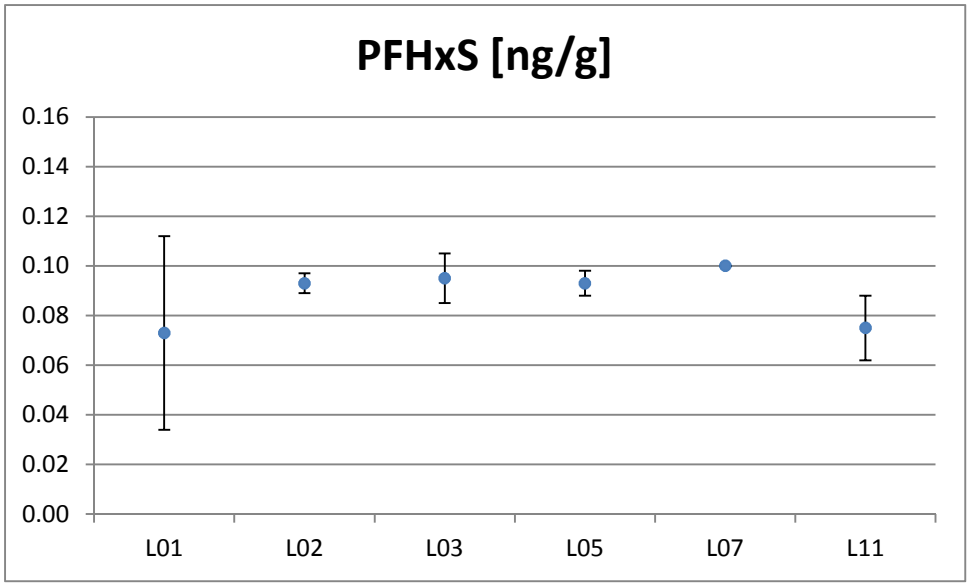
Analytical results of the certification study for PFTeDA in IRMM-427

	PFTeDA (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01								
L02	0.322	0.391	0.6	0.287	0.263	0.4	0.377	0.122
L03	0.68	0.78	0.88	0.58	0.88	0.68	0.747	0.121
L04	0.22	0.27	0.29	0.17	0.29	0.24	0.247	0.047
L05	0.49	0.55	0.47	0.49	0.58	0.52	0.517	0.042
L07	0.6	0.6	0.5	0.6	0.6	0.5	0.567	0.052
L08								
L09								
L10								
L11								
L12	0.2	0.2	0.2	0.3	0.2	0.2	0.217	0.041
L13								



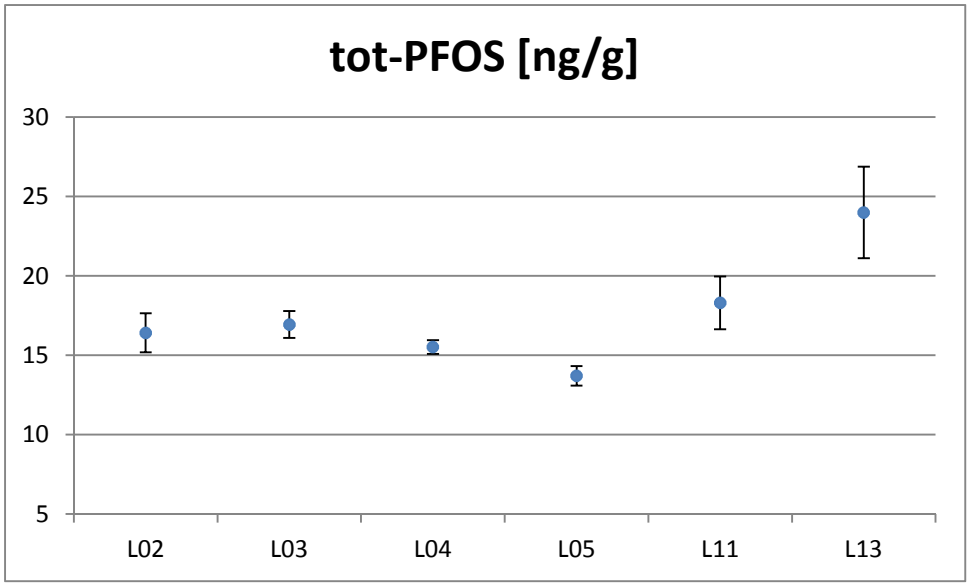
Analytical results of the certification study for PFHxS in IRMM-427

	PFHxS (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	0.06	0.05	0.15	0.05	0.05	0.08	0.073	0.039
L02	0.087	0.098	0.093	0.093	0.092	0.097	0.093	0.004
L03	0.1	0.11	0.09	0.08	0.1	0.09	0.095	0.01
L04								
L05	0.09	0.1	0.09	0.09	0.1	0.09	0.093	0.005
L07	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0
L08								
L09								
L10								
L11	0.062	0.077	0.089	0.057	0.076	0.089	0.075	0.013
L12								
L13								



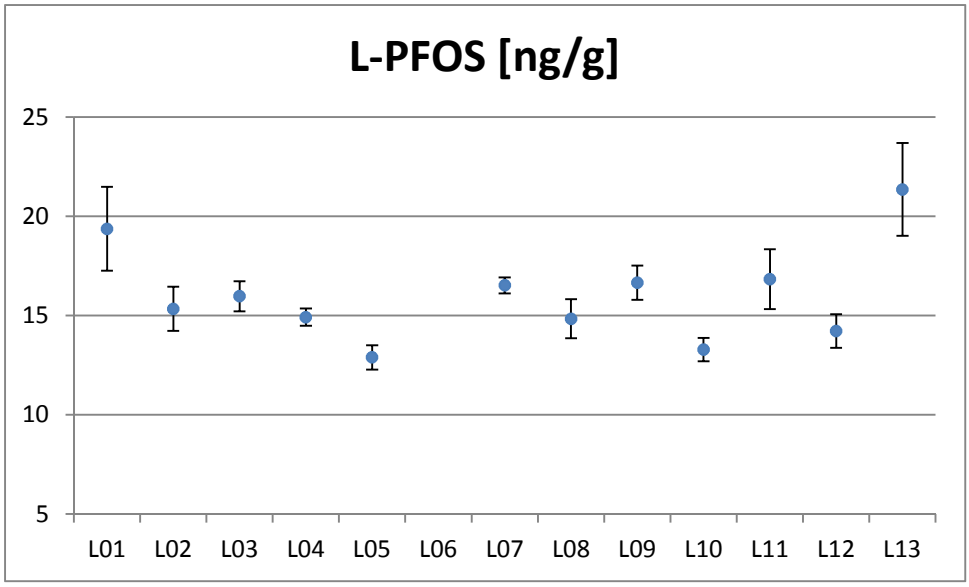
Analytical results of certification study for tot-PFOS in IRMM-427

	tot-PFOS (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01								
L02	16.65	17.1	18.38	15.56	15.06	15.7	16.408	1.222
L03	18.1	17.8	16.2	16.6	16.9	16	16.933	0.852
L04	15.6	15.2	15.8	15.9	14.8	15.8	15.517	0.431
L05	14.31	13.27	14.43	13.71	12.8	13.7	13.703	0.616
L07								
L08								
L09								
L10								
L11	17.1	19.7	18.3	17.5	20.8	16.4	18.3	1.667
L12								
L13	25.9	22.1	27.4	24.4	24.8	19.3	23.983	2.887



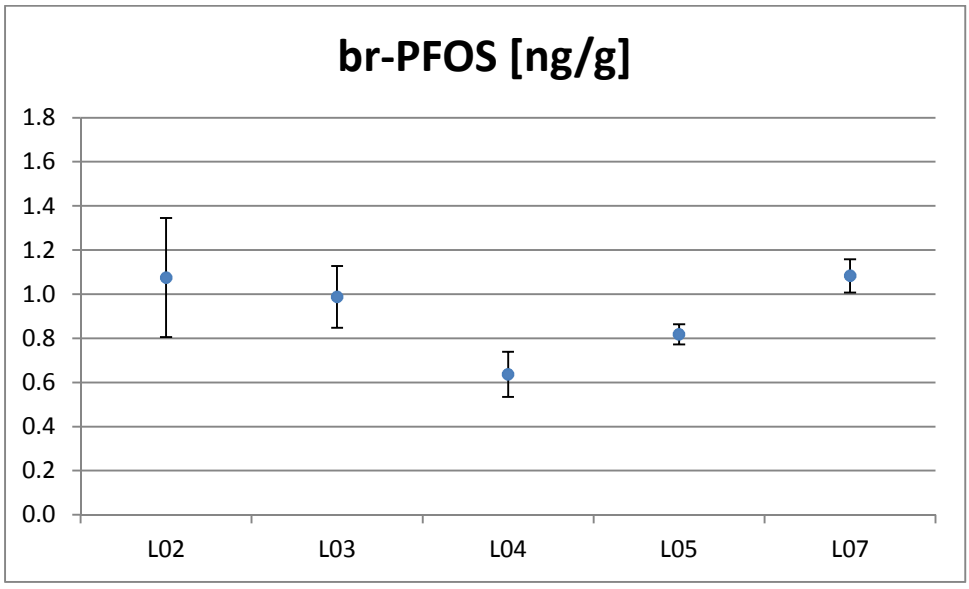
Analytical results of the certification study for L-PFOS in IRMM-427

	L-PFOS (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	20.4	20.2	17.9	18.4	16.7	22.6	19.367	2.117
L02	15.89	15.82	17.02	14.81	14.02	14.44	15.333	1.113
L03	17.1	16.7	15.4	15.7	15.7	15.2	15.967	0.758
L04	15.1	14.6	15.1	15.4	14.2	15.1	14.917	0.436
L05	13.41	12.49	13.66	12.89	11.99	12.89	12.888	0.606
L07	16.4	16	16.8	16.9	16.9	16.1	16.517	0.407
L08	15	16	15	15	13	15	14.833	0.983
L09	15.8	18	16.9	15.6	16.7	16.9	16.65	0.869
L10	13.2	13.8	12.8	12.9	14.2	12.8	13.283	0.588
L11	15.8	18.1	16.8	16.1	19.1	15.1	16.833	1.507
L12	14.6	13.8	15.1	12.8	14.1	14.9	14.217	0.847
L13	22.3	19.7	24	22	22.6	17.5	21.35	2.343



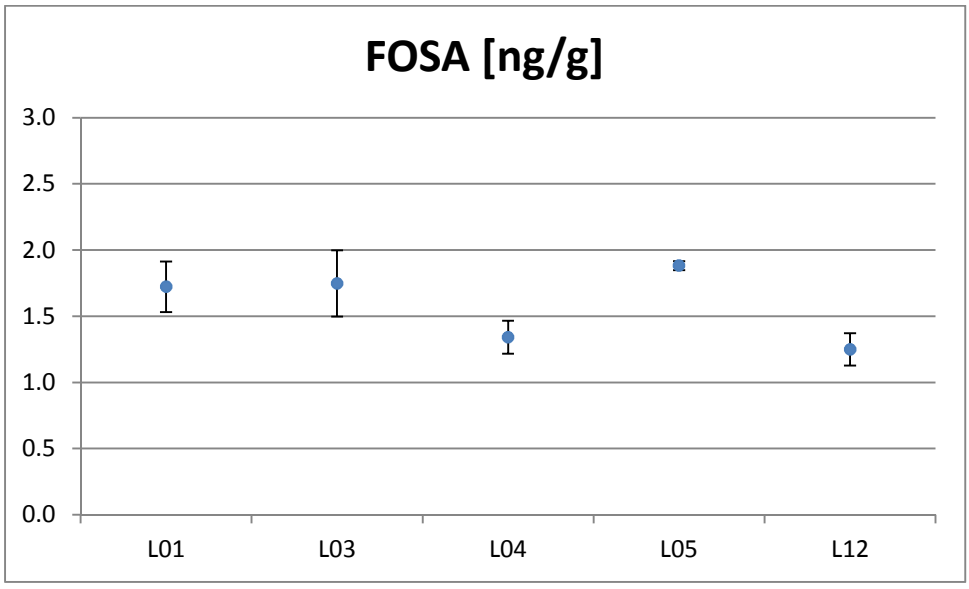
Analytical results of the certification study for br-PFOS in IRMM-427

	Br-PFOS (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01								
L02	0.76	1.28	1.36	0.75	1.04	1.26	1.075	0.27
L03	0.99	1.11	0.84	0.98	1.18	0.83	0.988	0.14
L04	0.51	0.72	0.65	0.51	0.73	0.7	0.637	0.102
L05	0.9	0.78	0.77	0.82	0.82	0.82	0.818	0.046
L07	1.1	1.1	1	1.1	1.2	1	1.083	0.075
L08								
L09								
L10								
L11								
L12								
L13								



Analytical results of the certification study for FOSA in IRMM-427

	FOSA (ng/g) in IRMM-427							
Laboratory code	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	mean	standard deviation
L01	1.73	1.65	1.74	1.6	2.08	1.54	1.723	0.191
L02								
L03	2.02	1.8	1.48	1.78	1.99	1.42	1.748	0.251
L04	1.43	1.19	1.46	1.45	1.2	1.32	1.342	0.124
L05	1.93	1.89	1.86	1.9	1.83	1.88	1.882	0.034
L07								
L08								
L09								
L10								
L11								
L12	1.1	1.4	1.3	1.1	1.3	1.3	1.25	0.122
L13								



ANNEX F: Conditions and results from the qNMR analysis of neat crystalline PFDA used for preparation of a PFDA common calibrant solution used during the certification study of IRMM-427. Purity results, expressed as mass fractions, correspond to average values obtained for 4 replicate analyses.

Compound	Purity [mg/g]	*U* (k=2)	H_2O [mol/mol F-DA]
PFDA	951.6	1.7	1.8

qNMR	Instrumental conditions	Units
Spectrometer	Bruker DMX 400	
Wave frequency	376.47	MHz
Spectral width	15015	Hz
Relaxation delay	40	s
Data acquisition time	8.7	s
Number of scans	256	
Excitation 90 19F pulse	12.7	µs
Solvent	DMSO-d6	
Quantity standard	4-F Benzoic acid (4-BZA)	
T	333.2	K

European Commission
EUR 27429 – Joint Research Centre – Institute for Reference Materials and Measurements

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Authors: Marta Dabrio Ramos, Ike van der Veen, Jean Charoud-Got, Hakan Emteborg, Jana Weiss, Heinz Schimmel

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