PFOS and PFOA in Ground Water
UME EnvCRM 01

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ABBREVIATIONS

ANOVA	analysis of variance
α	significance level
BAM	Bundesanstalt für Materialforschung und -prüfung (BAM), Germany
CRM	certified reference material
EMPIR	European Metrology Programme for Innovation and Research
HDPE	high density polyethylene
ID MS	isotope dilution Mass Spectrometry
IS	internal standard
ISO	International Organization for Standardization
$MS_{between}$	mean square between-bottle from ANOVA
MS_{within}	mean square within-bottle from ANOVA
n	number of replicates per unit
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LC-HRMS	Liquid chromatography high resolution mass spectrometry
L-PFOA	Linear perfluorooctanoic acid
L-PFOS	Linear perfluorooctane sulfonate
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutane sulfonic acid
PFDA	Perfluorodecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFPA	Perfluoropentanoic acid
PFAS	Perfluorinated alkyl substances
PTFE	polytetrafluoroethylene
QC	quality control
RSD	relative standard deviation
s	standard deviation
s_{bb}	between-bottle standard deviation
SGT	single Grubbs' test
SI	International System of Units
s_{wb}	within-bottle standard deviation
SYKE	Finnish Environment Institute, Finland
u_{bb}	standard uncertainty related to possible between-bottle heterogeneity
u^*_{bb}	standard uncertainty of heterogeneity that can be hidden by method repeatability
UME	TÜBİTAK National Metrology Institute of Turkey, Turkey
u_{char}	standard uncertainty related to characterisation
u_{lts}	standard uncertainty related to long term stability
UW	University of Warsaw, Poland

The subscript "rel" is added when a variable is expressed in relative terms (e.g. as percent)

ABSTRACT

Perfluoro alkyl substances (PFAS) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been used in numerous industrial applications and products. Because of their high stability, and resistance to biodegradation, atmospheric photooxidation, direct photolysis and hydrolysis, they are persistent in the environment. As persistent, bioaccumulative and toxic compounds, PFOS and PFOA are included in Stockholm Convention's Annex B and A, respectively, and their production, use and import in the EU are restricted with regulations (2019/1021/EU and 2020/784/EU). PFOS is identified as a priority hazardous substance in the EU's Water Framework Directive (2013/39/EU).

This report describes the production of a ground water reference material: UME EnvCRM 01, certified for PFOS and PFOA mass fractions. The material was produced in accordance with requirements of ISO 17034 standard.

The raw material for the CRM is ground water collected from a well at Kartal - Istanbul, Turkey. The well supplies significant amount of ground water for swimming pools and irrigation of gardens in Istanbul. The material was spiked with a mixture of PFAS, bottled and sterilized by gamma irradiation before storage at (5 ± 3) °C.

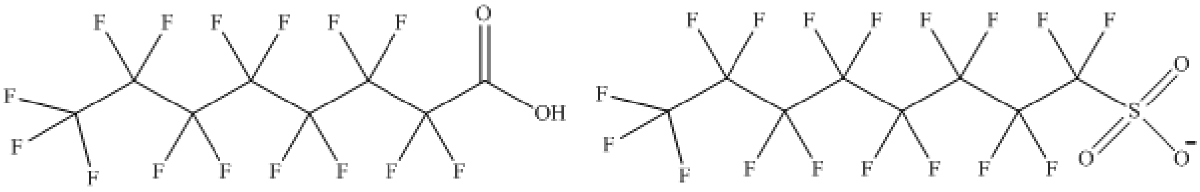
Homogeneity, stability and characterisation of the material were assessed in accordance with ISO Guide 35. The material was characterized by an interlaboratory comparison among competent laboratories.

Uncertainties of the certified values were calculated in accordance with GUM “Guide to the Expression of Uncertainty in Measurement” and includes characterization, homogeneity, stability components.

The material is intended for method development and validation in determination of L-PFOS and L-PFOA mass fractions in waters and for quality control purposes. The CRM is available in HDPE bottles containing approximately 98 g of material all of which is suggested to be used at once.

INTRODUCTION

The measurands to be certified are the mass concentration of linear forms for perfluoro-n-octanoic acid (L-PFOA) as acidic form and linear perfluoro-1-octane sulfonate (L-PFOS) as anion form in ground water. The structures of PFASs are shown in Figure 1.



L-PFOA L-PFOS

Figure 1. Structures of L-PFOA and L-PFOS

Perfluoro alkyl substances (PFASs) such as PFOS and PFOA have been used in numerous industrial applications and products. Because of their high stability, and resistance to biodegradation, atmospheric photooxidation, direct photolysis and hydrolysis, they are extremely persistent in the environment. As persistent, bioaccumulative and toxic compounds, PFOS and PFOA are included in Stockholm Convention's Annex B and A, respectively, and their production, use and import in the EU are restricted (2019/1021/EU [1] and 2020/784/EU [2]). PFOS is identified as a priority hazardous substance in the EU Water Framework Directive (2013/39/EU) [3]. The annual average environmental quality standard (AA-EQS) of PFOS in inland and other surface waters is 0.65 ng/L and 0.13 ng/L, respectively. The maximum allowable concentration EQS (MAC-EQS) is 36 µg/L in inland surface water and 7.2 µg/L in other surface waters.

PFOS and PFOA have been used in numerous industrial applications and products, for example in Aqueous Film-Forming Foams (AFFFs). The use of PFOS-containing AFFFs has been banned since June 2011 in the EU (EU directive 2013/39/EU). However, there are countless areas (e.g. fire training sites, airports), where AFFFs have been used, and in consequence, areas that are polluted with PFOS and PFOA. As relatively water soluble, effectively non-degradable compounds, PFOS and PFOA migrate to ground water. They are not removed in the conventional drinking water treatment, and therefore cause health risks in polluted areas. The EU Drinking Water Directive is under revision, and the European Commission has proposed a limit value of 100 ng/L for the sum of 20 PFASs, including PFOS and PFOA (Document ST 6060/1/2020 REV 1) [4].

Laboratories performing sampling and tests in this field need matrix CRMs enabling appropriate quality control. National metrology institutes and designated institutes with proven metrological capabilities for the production and certification of such materials are necessary for the provision of quality data. The EMPIR joint research project ENVCRM [5] developed capacity to produce CRMs for environmental analysis by transferring the theoretical and practical know-how between the partners and combining their skills to focus on environmental CRM production according to ISO 17034:2016 [6].

UME EnvCRM 01, the production of which was carried out by a project consortium described in this report, is intended to be used as a quality assurance and quality control tool especially by the laboratories involved in the mandatory monitoring prescribed by relevant environment legislations.

The parameters aimed to be certified in UME EnvCRM 01 are the following: mass fractions of PFOS and PFOA. The values are stated as mass concentrations in nanograms per kilogram (ng/kg). The target concentration levels for all analytes were decided to meet laboratories' needs.

PARTICIPANTS

Laboratory/organisations involved in the production and their contributions are presented in Table 1.

Table 1. Laboratory/organisations involved and their contributions

Activity	Laboratory / Organization
Project management and data evaluation	TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, TURKEY
Preliminary measurements	TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, TURKEY SYKE, Finnish Environment Institute, Helsinki, FINLAND UW, University of Warsaw, Warsaw, POLAND
Sampling and processing	TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, TURKEY
Sterilization	GAMMA PAK Sterilizasyon Sanayi ve Ticaret A.Ş., Çerkezköy - Tekirdağ, TURKEY
Homogeneity study	TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, TURKEY
Stability studies	TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, TURKEY SYKE, Finnish Environment Institute, Helsinki, FINLAND
Characterization study	TÜBİTAK UME, National Metrology Institute, Gebze - Kocaeli, TURKEY SYKE, Finnish Environment Institute, Helsinki, FINLAND

MATERIAL PROCESSING

Ground water raw material was collected at 40°55'16.8" N, 29°12'43.6" E from a 250 m deep well at Kartal – Istanbul, Turkey. This well is supplying significant amount of ground water to be used for swimming pools and irrigation of gardens in Istanbul. Prior to collection carboys (10 L, HDPE), were cleaned with deionized water (Resistivity: > 15 MΩ·cm) and dried at ambient temperature. Ground water sample were collected directly from the well to the cleaned carboys. Then collected samples (total of 200 Liters in 22 carboys) were transported to TÜBİTAK UME for further processing.

For preliminary measurements, subsamples from 3 different carboys were selected and sent to SYKE (Finland). Results of this measurement are summarized in Table 2.

Table 2. Natural and target concentration levels of PFAS in ground water

Parameter	Preliminary Measurement Result (ng/L)	Target Level (ng/L)
L-PFOA	< 0.1	5.0
L-PFOS	< 0.1	5.0

Results showed that the candidate raw material has almost none of the target analytes, thus it was decided to spike this material to reach target levels.

Anion and cation content of the raw material was also investigated by TÜBİTAK UME and UW. Results of this measurement are summarized in Table 3.

Table 3. Preliminary analysis results of ground water by TÜBİTAK UME and UW

Parameter	Preliminary Measurement Result (mg/L)
Ca^{2+}	126.5 ± 0.2*
Cl^-	114.81 ± 0.04**
F^-	0.164 ± 0.013**
Mg^{2+}	44.9 ± 0.1*
Na^+	45.1 ± 0.4*
NO_3^-	1.50 ± 0.03**
K^+	2.2 ± 0.1*
SO_4^{2-}	213.9 ± 0.1**

*: ± std. dev, *n* = 2, **: ± std. dev, *n* = 10

Material in carboys were combined in homogenization tanks (2 x 110 L, HDPE) by filtering through cloth filter and then 0.8/0.2 µm filter (Pall Corp., AcroPac 1000, USA). Spike mixture (prepared from a mixture of PFAC's, Wellington) was added. Homogenization performed by circulating the content between tanks (HDPE) for 3 hours. A pressurized air driven circulation pump (DEBEM, SDB 71722, Italy) was used to transfer the content between the tanks via PVC pipes.

Filling and capping were done using automated filling machine (FARMATEK, FTED 1-150, Turkey) (Fig. 6). Total 1180 units, each ~98 mL were filled and capped within 4 hours. Homogenized ground water sample was dispensed into 125 mL low particulate narrow-mouth HDPE Bottles (Nalgene, Cat No: 382099-0125, USA). It was critical to precisely control the filled amount to the bottles due to the fact that the candidate CRM is intended to be used for single use with all of its content. For this purpose, the variation of the filled amount in the bottles throughout the whole filling process was checked gravimetrically using a balance (Sartorius, MSA524S-100-DA, Germany) and the standard deviation was calculated as 0.3 g (*n* = 24).

Capped bottles were sterilized by ^{60}Co γ-irradiation with 25 kGy dose. The irradiation caused the expected change in color of the medium level transparent HDPE bottles to pale yellow. After this step, the bottles were stored at 4 °C in the dark. All stages of processing is summarized as a flow diagram in Annex 1.

HOMOGENEITY

Homogeneity study between the units is performed to show that the assigned values are valid for all units within the stated uncertainty. Homogeneity study between the units is performed with a number of samples representing the whole batch. In this project, 24 units were selected by using random stratified sampling. For the evaluation of homogeneity, since samples are for single use and evaluation of MS_{within} is not possible, approach described in "Alternative Strategies" section of ISO Guide 35:2017 [7] is performed on the bulk material left from production of the candidate reference material and was kept in a 10 L HDPE tank at reference temperature (+4 °C) until the analysis. MS_{within} is calculated from the measurement results variance of 24 subsamples prepared from this bulk material. For each subsample three instrumental repetitions was performed. $MS_{between}$ is calculated from the measurement results variance of 24 units selected by stratified sampling scheme covering the whole batch. The measurements were performed under repeatability conditions, using validated methods and according to a random sequence to clarify the possible trends in analytical sequence and filling order. The evaluation of homogeneity and calculation of uncertainty between and within bottles was performed by Equation (1) and (2) using approach described in "Alternative Strategies" section of ISO Guide 35:2017 which is recommended for single use samples. There was one outlier result in both measurements for PFOS (between bottle) and PFOA (within bottle), since there is no technical explanation they were included in the calculations.

The s_{bb} is calculated using Equation (1) given below:

$$s_{\mathrm{bb}}^2 = \frac{MS_{between} - MS_{within}}{n},\ n = 1 \qquad (1)$$

Alternatively u_{bb}^* is calculated using Equation (2) as described by Linsinger *et.al.* [8].

$$u_{\mathrm{bb}}^* = \sqrt{\frac{MS\ \mathrm{within}}{n}} \mathrm{x} \sqrt[4]{\frac{2}{df_{MS\ \mathrm{within}}}}\ \ n=1 \qquad (2)$$

where,

u_{bb}: Standard uncertainty related to possible between-bottle heterogeneity,
MS_{between}: Mean of square of variance between units,
MS_{within}: Mean of square of variance within the unit,
n: Number of measurements within unit,
df: Degree of freedom.

The between bottles homogeneity, s_{bb} and u_{bb} values are given in Table 4.

Table 4. Results of the homogeneity study

	L-PFOA	**L-PFOS**
$s_{\mathrm{bb,rel}}(\%)$	4.19	$MS_{between} < MS_{within}$
$u_{\mathrm{bb,rel}}^*(\%)$	1.94	4.54
$\boldsymbol{u_{\mathrm{bb,rel}}}$**(%)**	**4.19**	**4.54**

As a conservative approach, the larger value of $\mathrm{s_{bb,rel}}$ and $\mathrm{u_{bb,rel}^*}$ was accepted as the estimate for the relative between units homogeneity uncertainty, $u_{\mathrm{bb,rel}}^*$.
For PFOS, the s_{bb} can not be calculated using Equation (1) since $MS_{between} < MS_{within}$.

The plotted data used for the evaluation of homogeneity are given in Annex 2.

STABILITY

Stability studies were performed with isochronous design. For the short term stability (STS) test, two different temperatures (+18 °C and +60 °C), and four time points (0, 1, 2 and 4 weeks) were tested. 28 units were selected by using a stratified sampling scheme covering whole batch. 24 samples were subjected to the test temperatures for the specified time intervals. For the long term stability test (LTS), 16 units for each laboratory (TÜBİTAK UME and SYKE) were tested at +18 °C for 0, 3, 6 and 12 months time points.

Units were moved to +4 °C (reference temperature) after completion of the test time. All units were analyzed at the same time. Samples were prepared based on the single use for each unit and were analyzed using an LC-MS/MS method by SYKE and LC-HRMS by TÜBİTAK UME under the repeatability conditions to determine the mass concentration of L-PFOA and L-PFOS.

Short Term Stability Results:

The results obtained from isochronous measurements were first grouped according to the time period and then evaluated for each time point. These evaluations were carried out for both temperatures, separately.

The data for each temperature were first examined by single Grubbs test for both 95% and 99% confidence intervals to find out outliers. Number of detected outliers are given in the Table 5. Since no technical reason can be found to reject these data, all outliers were included in the calculations.

Values calculated for each time point were plotted against the time. The relationship between variables were analyzed in order to determine if any significant change exists with the testing time (regression analysis). It was found that the slopes were significant for both PFOS and PFOA reported by SYKE (except for 60 °C in 99 % confidence interval). The trend graphs of short term stability are shown in Annex 3. The relative short term stability uncertainty, $u_{\mathrm{sts,rel}}$ for each parameter is then calculated using Equation (3) for the required transfer time as described in [9]:

$$u_{sts,rel} = \frac{RSD}{\sqrt{\sum(t_i - \bar{t})^2}} \times t \qquad (3)$$

where,

RSD : relative standard deviation of the points on the regression line as described in B.17 [7],

t_i : time point for each replicate expressed in weeks ,

$\bar{t}$: mean of all time points expressed in weeks,

t : maximum time suggested for transfer (1 week).

As a conservative approach, the relative uncertainty contribution for short term stability, $u_{\mathrm{sts,rel}}$ is accepted as the highest value obtained from two institute's and at two different test temperatures (shown in bold, Table 5).

Table 5. Short Term Stability (STS) test results

Parameter (Lab)	18 °C $u_{sts,rel}$ for 1 week (%)	60 °C $u_{sts,rel}$ for 1 week (%)	Number of outliers in 95% confidence interval [1]		Number of outliers in 99% confidence interval [1]		Any significant trend in 95% confidence interval?		Any significant trend in 99% confidence interval?	
			18°C	60 °C	18 °C	60 °C	18 °C	60 °C	18 °C	60 °C
L-PFOS (TÜBİTAK UME)	4.3 [2]	6.0 [2]	1	1	1	1	No	No	No	No
L-PFOS (SYKE)	1.2 [3]	1.1	-	-	-	-	Yes	Yes	Yes	No
L-PFOA (TÜBİTAK UME)	1.2	1.4	1	-	-	-	No	No	No	No
L-PFOA (SYKE)	1.1 [3]	1.6 [3]	-	-	-	-	Yes	Yes	Yes	Yes

[1] Single Grubbs Test
[2] Technical problem for this measurement was reported
[3] u_{sts} is calculated by taking into account the degradation (u_{lts}= slope of reg. line/√3)

The material is found to be stable at 18 °C and 60 °C for up to 1 week. Thus, the samples can be safely dispatched under conditions where the temperatures do not exceed 60 °C for up to 1 week, i.e. at ambient temperature without applying any cooling elements.

Long Term Stability Results:

Shelf life of the CRM has been determined through long term stability measurements. For the measurements, four units for each of the months of 0, 3, 6 and 12 have been stored at +18 °C and transferred to reference temperature (+4 °C) after each period of time to be measured isochronously afterwards. Four units, designated as reference units, of the month 0 was stored at +4 °C.

The relative long term stability uncertainty, $u_{lts,rel}$ for each parameter is then calculated using equation (4) for the required shelf life as [8]:

$$u_{lts,rel} = \frac{RSD}{\sqrt{\Sigma\left(t_i - \bar{t}\right)^2}} \times t \tag{4}$$

where

RSD : the relative standard deviation of the points on the regression line as described in B.17 [7],
t_i : the time point for each replicate expressed in months,
$\bar{t}$: the average of all time points expressed in months,
t : the proposed shelf life at 18 °C (12 months).

The uncertainty contribution u_{lts} was calculated for 12 months (t) at 18 °C. As a conservative approach, the relative uncertainty contribution for long term stability, $u_{lts,rel}$ is accepted as the highest value obtained from two institute's (shown in bold, Table 6). The graphs for long term stability are given in Annex 4.

Table 6. Long Term Stability (LTS) test results

Parameter (Lab)	18 °C $u_{lts,rel}$ for 12 m (%)	Number of outliers in 95% confidence interval	Number of outliers in 99% confidence interval	Any significant trend in 95% confidence interval?	Any significant trend in 99% confidence interval?
L-PFOS (TÜBİTAK UME)	1.5	1	1	No	No
L-PFOS (SYKE)	**2.7**	-	-	No	No
L-PFOA (TÜBİTAK UME)	1.1*	-	-	Yes	No
L-PFOA (SYKE)	**2.3**	-	-	No	No

*Slope is found to be significant at 95 % confidence interval, u_{lts} is calculated by taking into account the degradation (u_{lts}= slope of reg. line/√3)

CHARACTERIZATION

According to ISO 17034, the characterization and the value assignment can be carried out in different ways. The approach chosen in this project is; characterization of a non-operationally defined measurand using two methods of demonstrable accuracy in two competent laboratories. The participating laboratories were partners of the EnvCRM project consortium [4]. The detailed information about the laboratories is given in the participants section. The participating laboratories used validated methods (potentially primary methods such as ID-LC MS and ID-LC MS/MS).

Each laboratory received five bottles of samples which were selected from the whole set of samples to represent the whole produced batch. The samples were selected randomly from the set of samples by the random stratified sampling technique. Each laboratory was asked to report at least three independent measurement results, together with their associated measurement uncertainty values and the approach used for the estimation of measurement uncertainty. In the reports, the details of the reference materials used in the calibration were also requested in order to assure the traceability of the reported results.

Data obtained from characterization study revealed normal distribution and measurement uncertainties were calculated according to the "Guide to the Expression of Uncertainty in Measurements (GUM)" and "EURACHEM/CITAC Guide Quantifying Uncertainty in Analytical Measurement" documents, with Equations (5, 6, 7) stated by M. S. Lenson et al [10]. Value assignment of the material performed by arithmetic averaging two method results.

$$u(B) = \frac{|x_{ID\ LC\ MS} - x_{ID\ LC\ MS/MS}|}{2\sqrt{3}} \qquad (5)$$

$$u(X) = \sqrt{(\tfrac{1}{2})^2 u^2(\mathrm{ID\ LC\ MS}) + (\tfrac{1}{2})^2 u^2(\mathrm{ID\ LC\ MS/MS})} \qquad (6)$$

$$u_{char} = \sqrt{u^2(X) + u^2(B)} \qquad (7)$$

here,
$u(B)$: the standard uncertainty based on the difference on the difference of results of two methods,
$u(X)$: the standard uncertainty obtained by combining uncertainties of two methods,
u_{char} : the standard uncertainty of characterisation by two methods.

All data related to the characterization study are given in Annex 6.

PROPERTY VALUE AND UNCERTAINTY ASSIGNMENT

Assigned values and uncertainties of the CRM was evaluated by applying approach in the characterization and uncertainty data that contribute to the homogeneity and stability assessments.

Data obtained in the characterization study were checked for normal distribution and outliers. Distributions were found to be normal and no outlier was detected.

Mean value of characterization results is assigned as the property value of the reference materials. Absence of bias for the assigned value was further confirmed by dataset obtained from the international comparison study "CCQM-K156 "Determination of PFOA and PFOS in Ground Water" organized by CIPM CCQM Organic Analysis Working Group [11]. Graphs of the results reported by the participating laboratories to the key comparison study is given in Annex 7.

Equation (8) is used to calculate the combined expanded uncertainty of CRMs:

$$\mathrm{U_{CRM}} = k\sqrt{\mathrm{u_{char}^2} + \mathrm{u_{bb}^2} + \mathrm{u_{lts}^2} + \mathrm{u_{sts}^2}} \qquad (8)$$

Uncertainty value on CRM certificate includes uncertainty contribution from characterization (u_{char}), homogeneity (u_{bb}), long term stability (u_{lts}) and short term stability. Expansion of uncertainty value of CRMs wase done with a coverage factor (*k* = 2) representing 95 % confidence level. Certified values, uncertainties and relative percent contribution of each component on uncertainty is given in Table 7 and Table 8.

Table 7. Certified values and uncertainties

Analyte	**Certified value (ng/kg)**	**U_{CRM} (µg/kg, *k*=2)**	**$U_{CRM,rel}$ (%, *k*=2)**	**$u_{char,rel}$ (%)**	**$u_{bb,rel}$ (%)**	**$U_{sts,rel}$ (%)**	**$U_{lts,rel}$ (%)**
L-PFOA	4.8	0.7	13.9	4.76	4.19	1.60	2.33
L-PFOS	3.8	0.6	15.3	5.40	4.54	1.24	2.65

Table 8. Percent contribution of each uncertainty component to U_{CRM}

Analyte	**u_{bb} (%)**	**u_{lts} (%)**	**u_{sts} (%)**	**u_{char} (%)**
L-PFOA	36.4	11.3	5.3	47.0
L-PFOS	35.4	12.1	2.6	49.9

INFORMATIVE VALUES

Density and pH were measured on two different units by UW, results for these measurements are given in Table 9.

Table 9. Informative values for river water measured by UW

Parameter	**Measured Value, *n* = 2**
Density (at 20 °C)	1.0046 ± 0.0010* g/cm^3
pH (at 20 °C)	7.72 ± 0.06*

* ± represents standard deviation

Other PFAS content were measured on five different units by SYKE, results for these measurements are given in Table 10.

Table 10. Informative values for other PFAS by SYKE

Parameter (Cas No)	**Measured Value ± Uncertainty (ng/kg, *k*=2)**
PFBA (375-22-4) [1]	3.9 ± 1.2
PFBS (375-73-5) [2]	3.3 ± 1.0
PFDA (335-76-2) [1]	3.9 ± 1.0
PFHpA (375-85-9) [2]	4.0 ± 1.0
PFHxA (307-24-4) [1]	4.3 ± 1.1
PFHxS (355-46-4) [1]	4.0 ± 1.0
PFNA (375-95-1) [1]	4.3 ± 1.3
PFPA (2706-90-3) [2]	3.6 ± 1.0

[1] Unweighted mean of ID LC MS/MS measurements (*n* = 5)
[2] Unweighted mean of IS LC MS/MS measurements (*n* = 5)

COMMUTABILITY

Commutability is the mathematical relationship of the equation between the reference material and the results produced by the different measurement methods that can be used to measure the routine samples it represents [12]. UME EnvCRM 01 was produced from ground water spiked with a mixture of PFAS. The analytical behavior will be the same as for a routine sample of ground water.

TRACEABILITY

The metrological traceability of the CRM was ensured by using SI traceable calibration standards and using reference methods i.e. ID LC MS by the participating laboratories. The laboratories were asked to provide detailed information about the calibration standards and reference methods used in the measurements. The details about the measurement methods, calibration standards and quality control materials used by the participating laboratories are given in Annex 5.

INSTRUCTIONS FOR USE

Shipping conditions

This material can be safely dispatched under conditions where the temperature do not exceed 60 °C for up to one week, i.e. at ambient temperature without applying any cooling elements.

Storage conditions

The material should be stored at (18 ± 4) °C in dark and clean environment. The bottle should be shaken before opening. In order to prevent contamination, it is recommended that the bottle should be opened in a clean environment and pipette should not be inserted into the bottle. TÜBİTAK UME cannot be held responsible for changes that might happen to the material at customer's premises due to noncompliance with the instructions for use, and the storage conditions given.

Safety precautions

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

Minimum sample intake

The material was bottled considering single use therefore, measurement should be conducted using the entire content (~ 98 g) of the bottle. After transferring the content for measurement, for possible remaining sticking content, it is recommended to wash inside surface of the bottle and cap with a solvent such as methanol and add this wash solution to the clean-up process at elution step.

Use of Certified Value

For assessing the method performance, the measured values of the CRM are compared with the certified values [13]. The procedure can be described briefly as:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).
- Combine measurement uncertainty (u_{meas}) with the standard uncertainty of the certified value (u_CRM) using Equation (9):

$$u_\Delta=\sqrt{u_\mathrm{meas}^2+u_\mathrm{CRM}^2} \tag{9}$$

- Calculate the expanded uncertainty (U_Δ) from the combined uncertainty (u_Δ) using a coverage factor of two (k = 2), corresponding to a confidence level of approximately 95 %.

If $\Delta m \leq U_\Delta$, then it is assumed that there is no significant difference between the measurement result and the certified value at a confidence level of approximately 95%.

An online application: CRM Result Evaluation-CRM RE to evaluate measurement results and automatically create quality control charts is available through the link: https://rm.ume.tubitak.gov.tr/en/crm_re/

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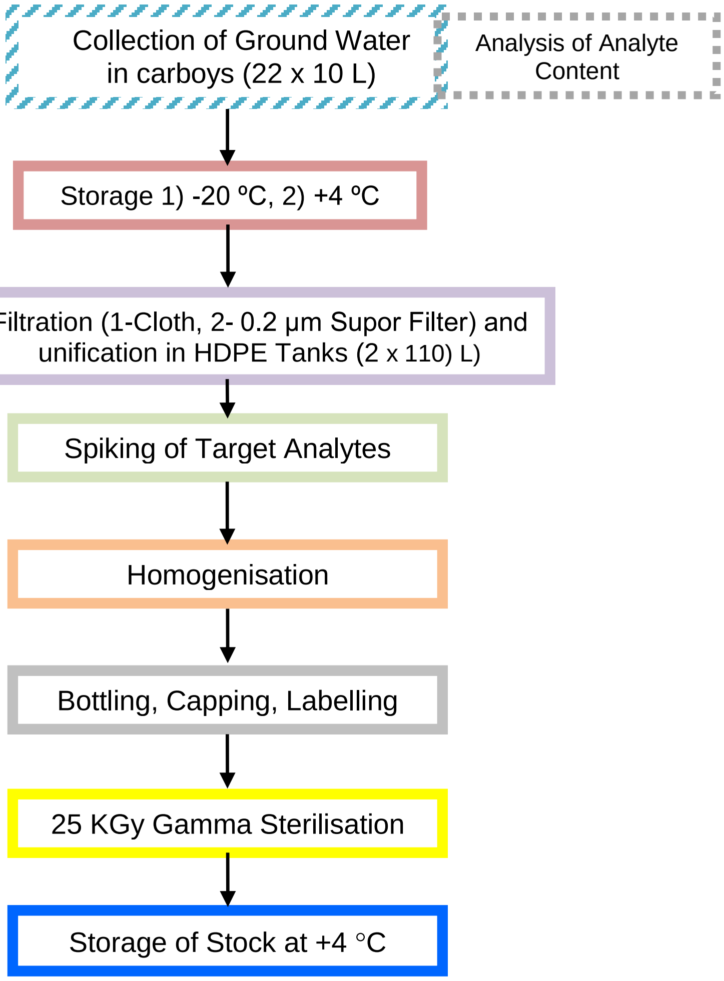
REVISION HISTORY

Date	Remarks
10.02.2021	First issue.

ANNEX 1. Flow Diagram for the Preparation of the Ground Water CRM



ANNEX 2. Graphs for Homogeneity Studies

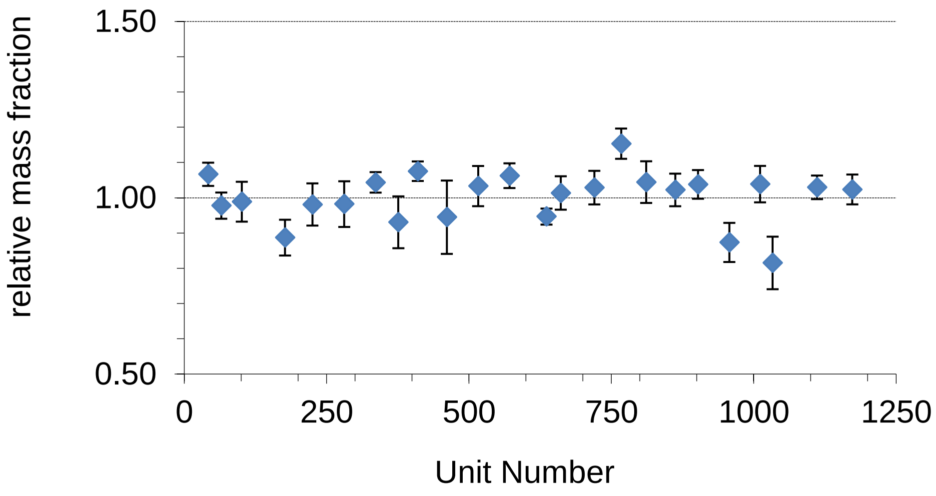


Figure A2.1. Between units homogeneity plot for L-PFOA

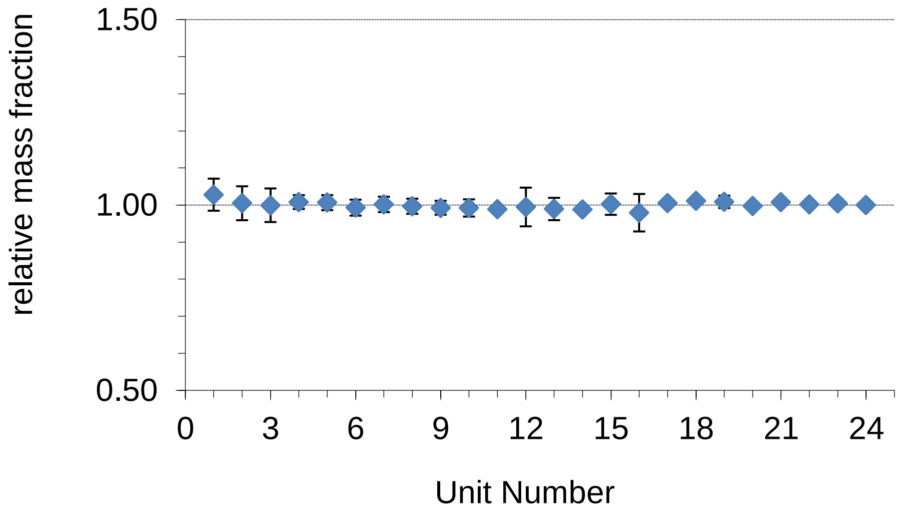


Figure A2.2. Within unit homogeneity plot for L-PFOA

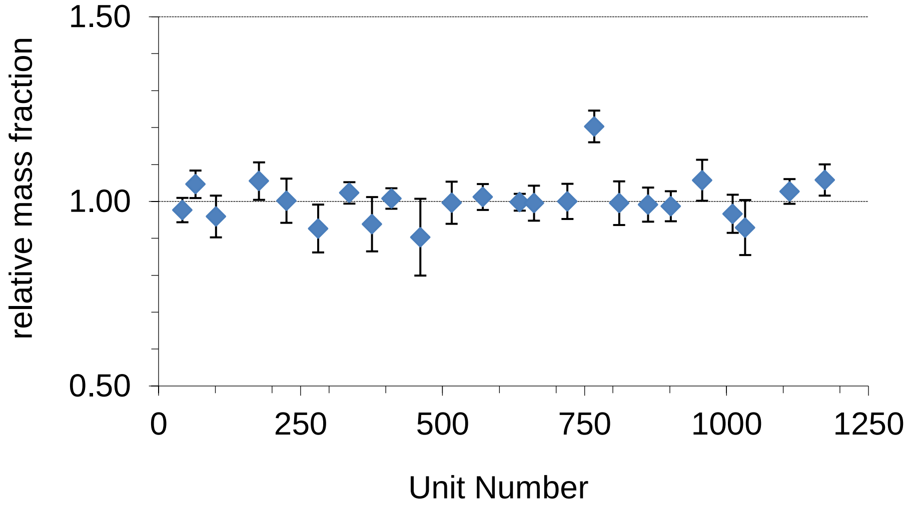


Figure A2.3. Between units homogeneity plot for L-PFOS

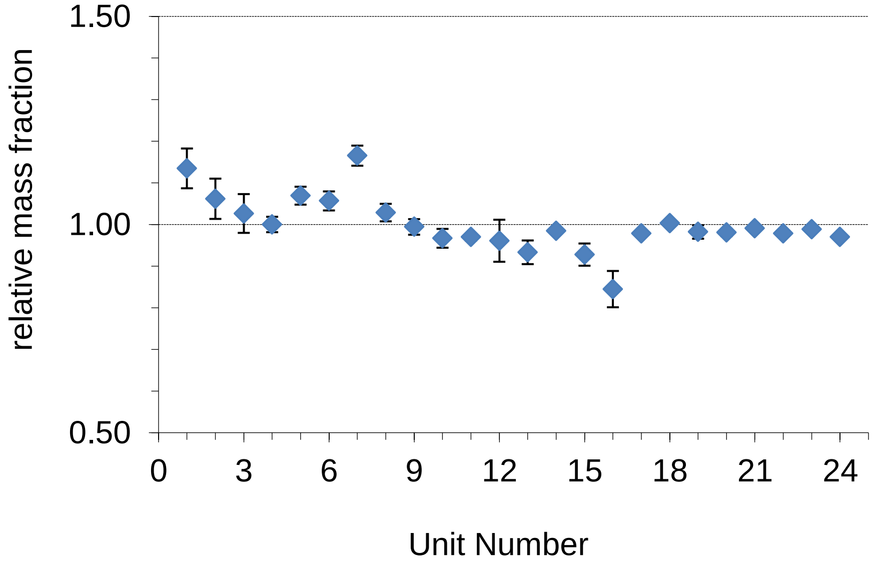


Figure A2.4. Within unit homogeneity plot for L-PFOS

ANNEX 3. Graphs for Short Term Stability Studies

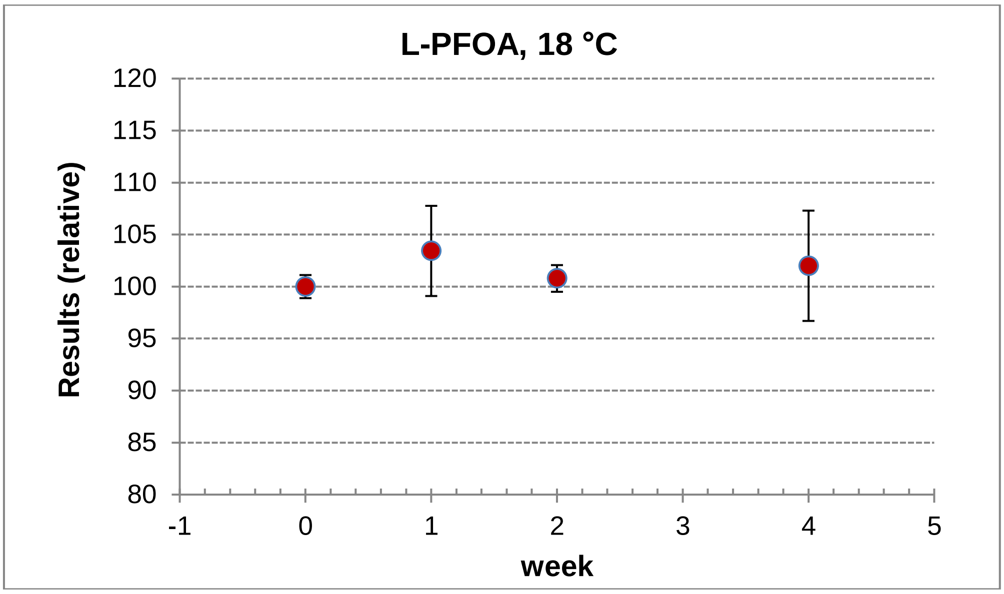


Figure A3.1. Short Term Stability Plot for L-PFOA at 18 °C by TÜBİTAK UME

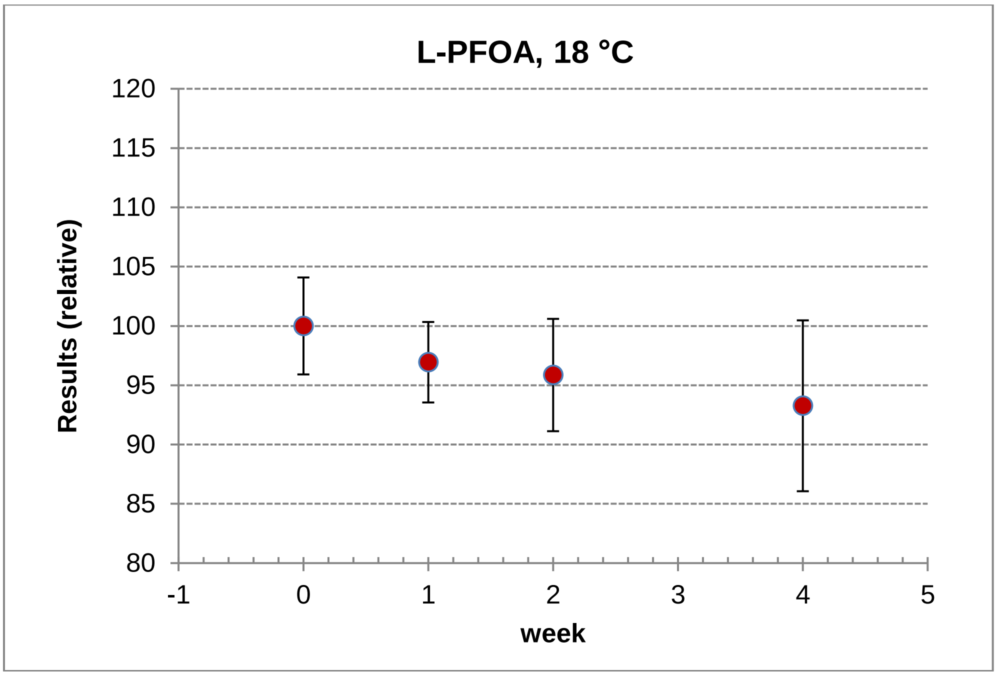


Figure A3.2. Short Term Stability Plot for L-PFOA at 18 °C by SYKE

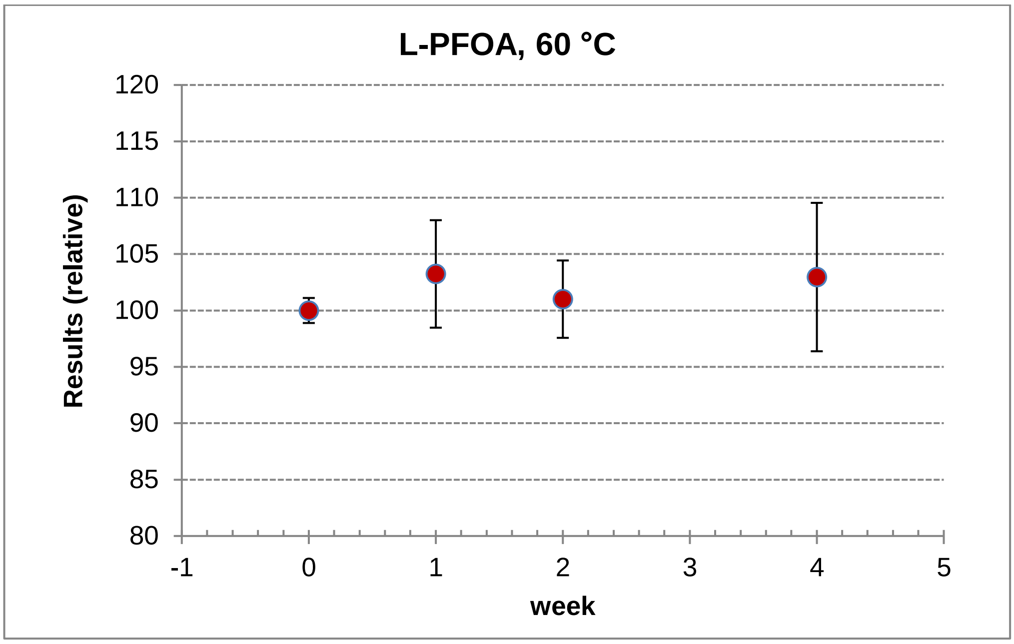


Figure A3.3. Short Term Stability Plot for L-PFOA at 60 °C by TÜBİTAK UME

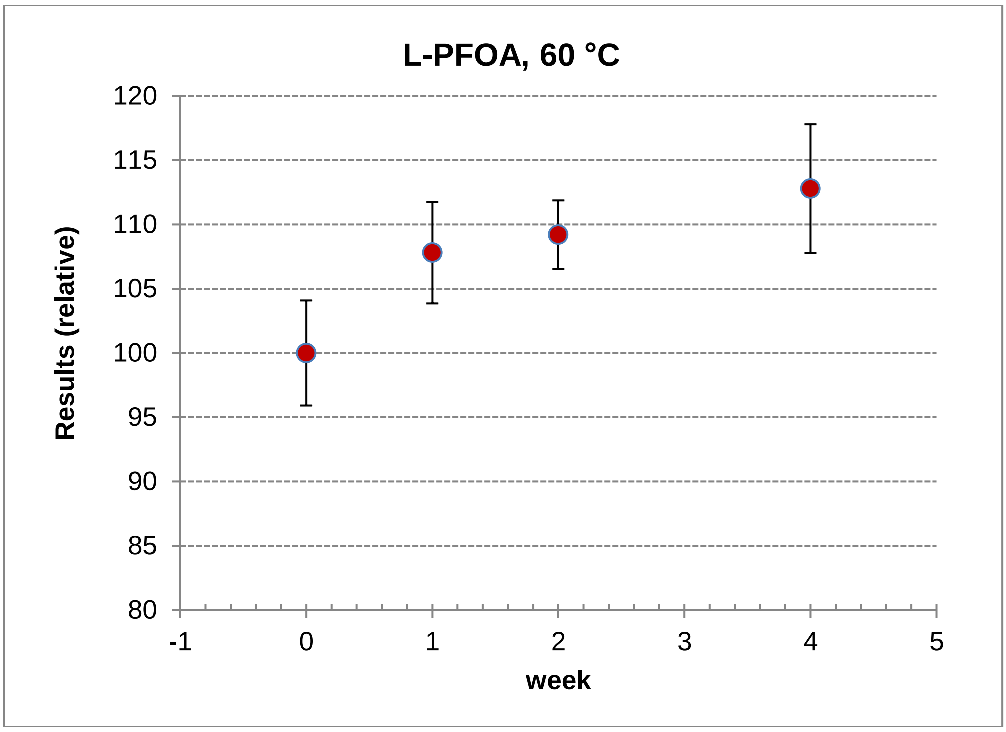


Figure A3.4. Short Term Stability Plot for L-PFOA at 60 °C by SYKE

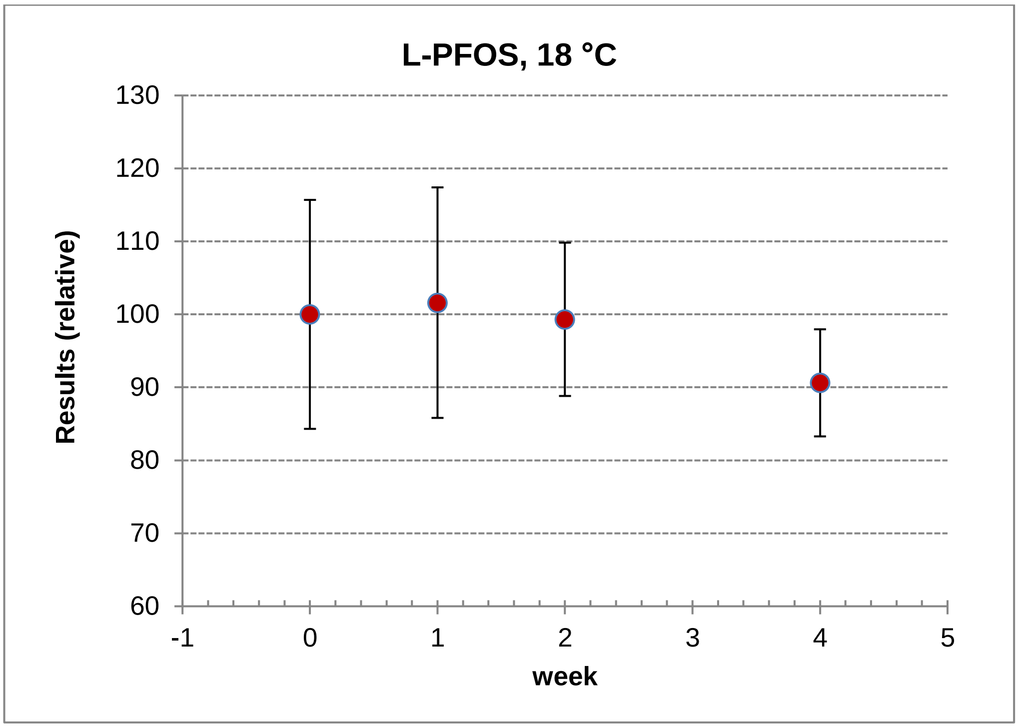


Figure A3.5. Short Term Stability Plot for L-PFOS at 18 °C by TÜBİTAK UME

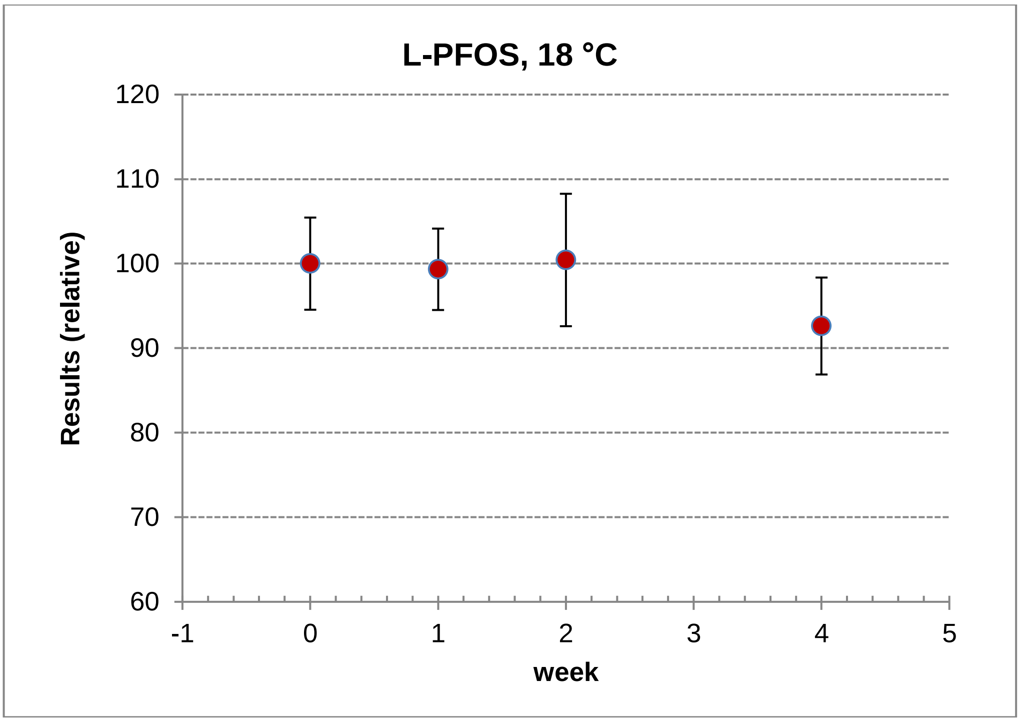


Figure A3.6. Short Term Stability Plot for L-PFOS at 18 °C by SYKE

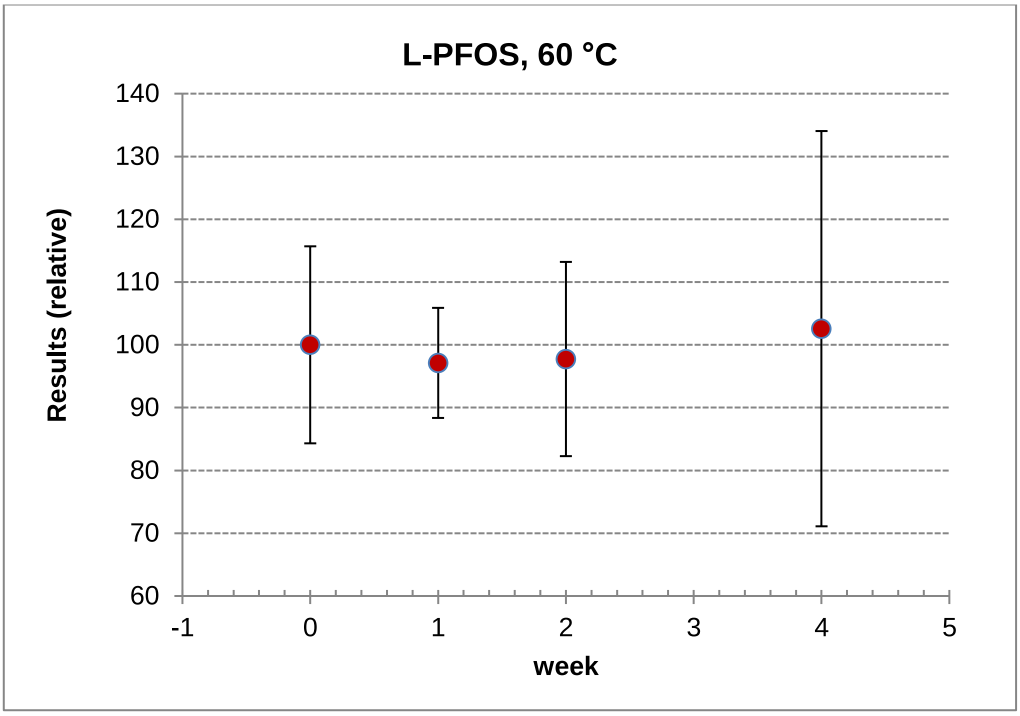


Figure A3.7. Short Term Stability Plot for L-PFOS at 60 °C by TÜBİTAK UME

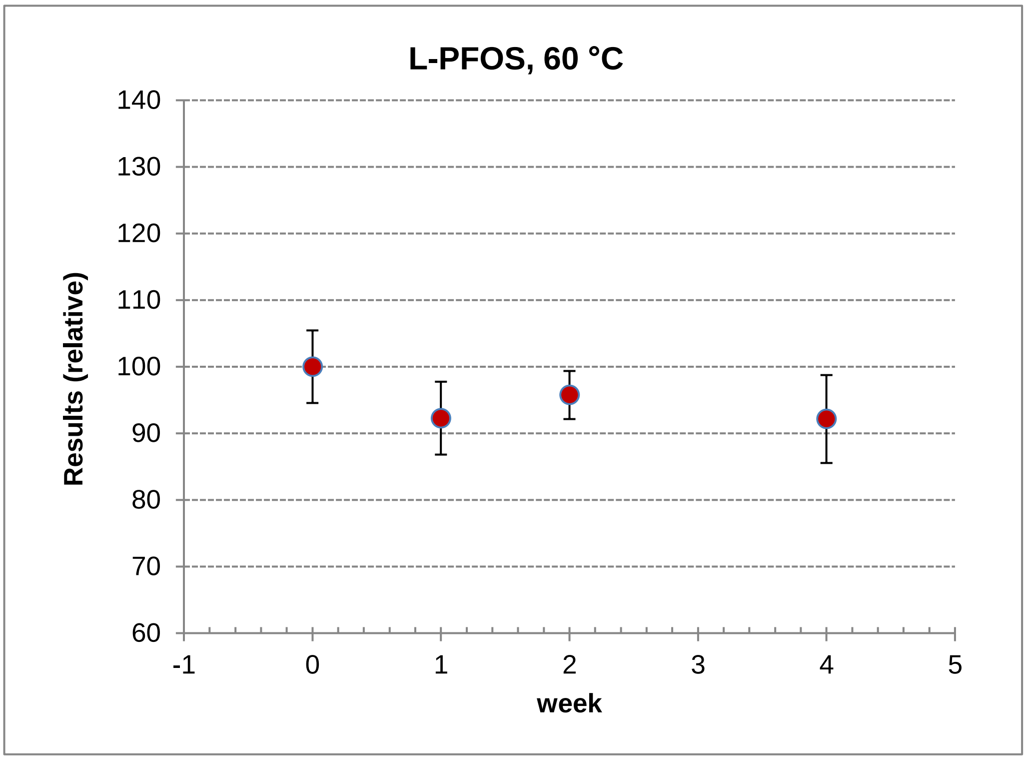


Figure A3.8. Short Term Stability Plot for L-PFOS at 60 °C by SYKE

ANNEX 4. Graphs for Long Term Stability Studies

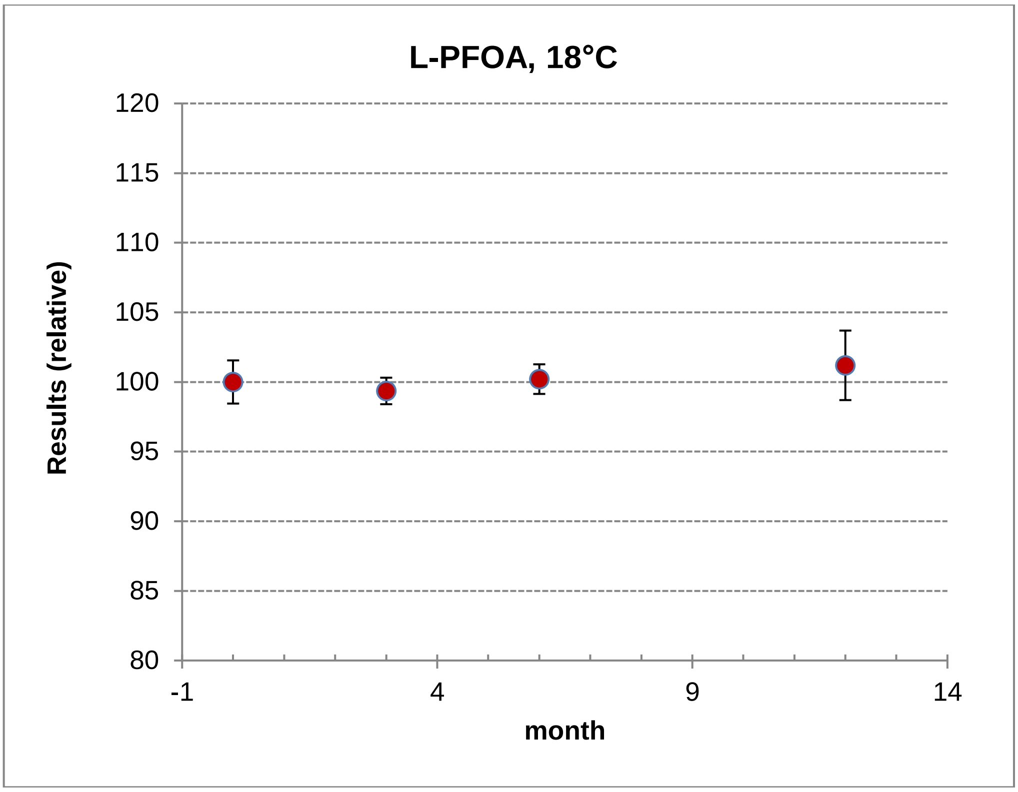


Figure A4.1. Long Term Stability Plot for L-PFOA at 18 °C by TÜBİTAK UME

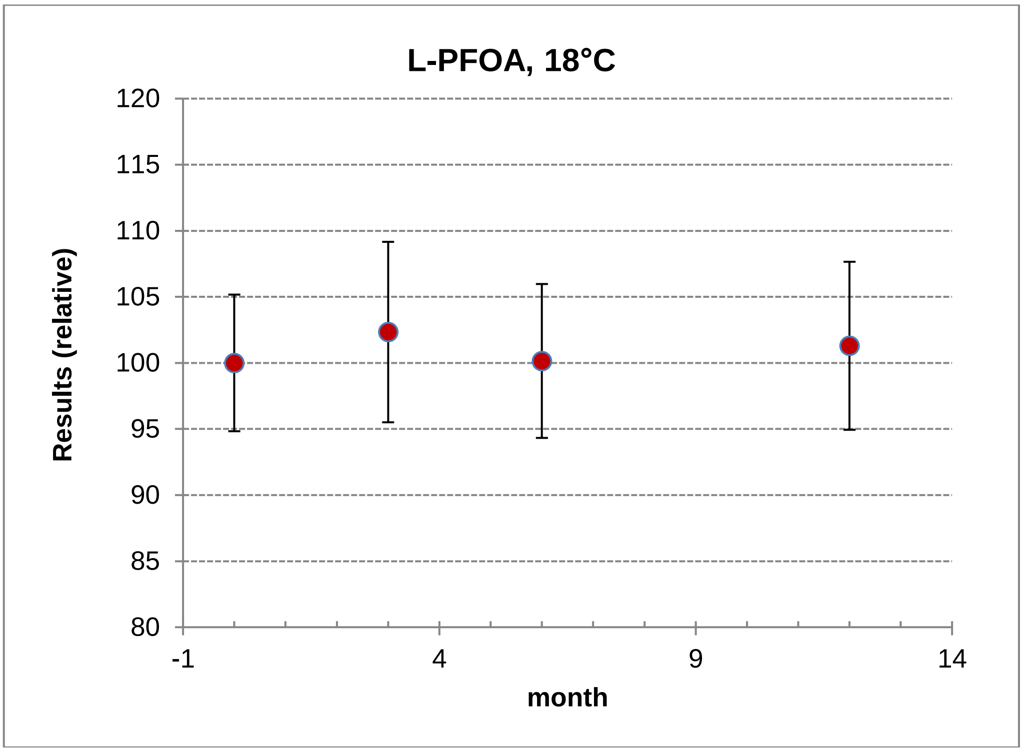


Figure A4.2. Long Term Stability Plot for L-PFOA at 18 °C by SYKE

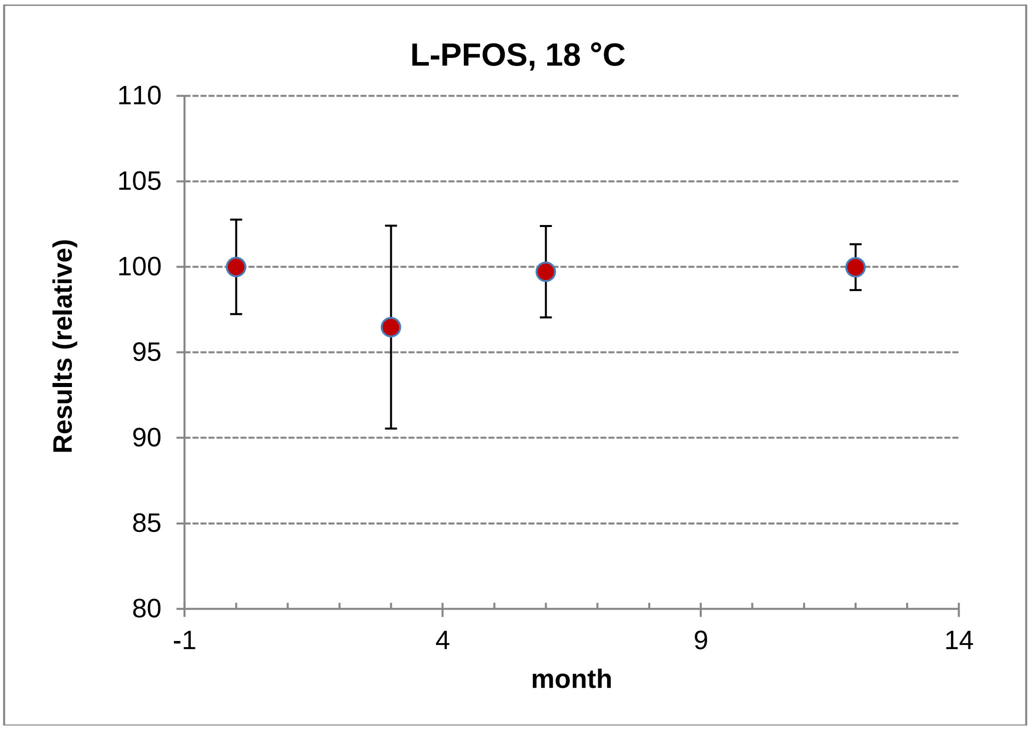


Figure A4.3. Long Term Stability Plot for L-PFOS at 18 °C by TÜBİTAK UME

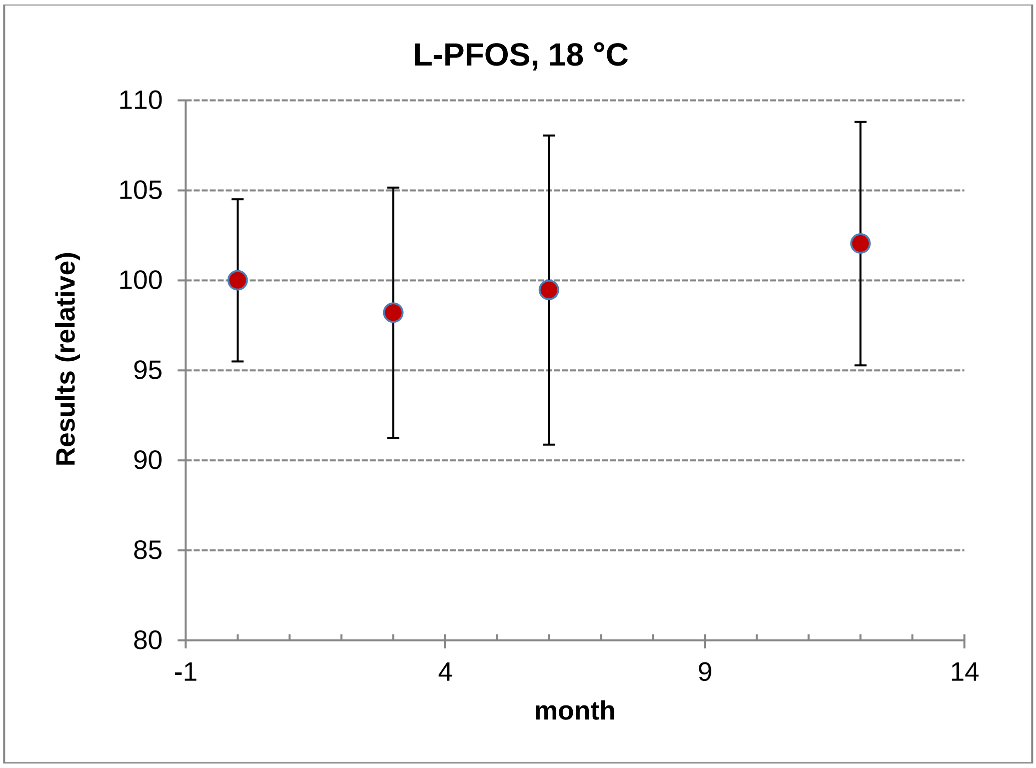


Figure A4.4. Long Term Stability Plot for L-PFOS at 18 °C by SYKE

ANNEX 5. Information about the Methods Used for the Characterization Study

Laboratory	Analytes	Sample Preparation	Calibration Strategy	Method/ Technique	CRM(s) used for Calibration and Quality Control
TÜBİTAK UME	L-PFOS, L-PFOA	IS spiked and bottle shaked by hand for 10 minutes and then waited for one hour at room temperature for equilibration. Extraction method from ISO 25101:2009 followed by using Waters OASIS Wax 150 mg 6 mL cartridge. Cartridge conditioned by 4 mL of ammonia/methanol solution, 4 mL of methanol and lastly 4 mL of water. Whole water sample eluted immediately after conditioning. Cartridge dried under vacuum. Washed with 4 mL acetate buffer solution. Bottle washed with 4 mL ammonia/methanol solution. Cartridge eluted first with 4 mL methanol and then with ammonia/methanol solution used for bottle wash. Concentrated under gentle stream of nitrogen at 40 °C till 200 µL.	5 point calibration IDMS	HR-LC-MS (THERMO Orbitrap Q Exactive**)**	NMIJ, NMIJ-4056a, 0.959 ± 0.005 kg/kg, SI traceable NMIJ, NMIJ-4056a, 9.93 ± 0.15 mg/kg (potassium salt value), SI traceable
SYKE	L-PFOS, L-PFOA	Spiking of internal standards and pH adjustment of pH 3 with formic acid. Solid-phase extraction according to ISO 25101:2009 with weak anion-exchange cartridges (Waters OASIS WAX 150 mg, 6 cc). Elution with 6 ml of basic methanol. Extract evaporated to dryness with a centrifugal evaporator, and reconstituted to 500 µL.	Six-point calibration curve $^{13}C_4$-PFOA; added in the beginning of the analysis (before pH adjustment and extraction) $^{13}C_4$-PFOS; added in the beginning of the analysis (before pH adjustment and extraction)	LC-MS/MS; Waters Acquity UPLC and Xevo TQ MS	Wellington PFC-MXB NMIJ CRM 4056-a Wellington PFC-MXB NMIJ CRM 4220-a Quality Control: IRMM-428

ANNEX 6. Data for Characterization Study

Laboratory	Analytes	Sample Name/ Unit No	Mass Fraction (ng/kg)	Mean Value (ng/kg)	Combined Standard Uncertainty (ng/kg)	Coverage Factor (k)	Expanded Uncertainty (ng/kg)
TÜBİTAK UME	L-PFOA	1018	4.80	4.72	0.19	2	0.38
		634	4.72				
		833	4.68				
		913	4.59				
		430	4.79				
	L-PFOS	1018	3.71	3.68	0.2	2	0.39
		634	3.72				
		833	3.63				
		913	3.66				
		430	3.68				
SYKE	L-PFOA	732	4.97	4.81	0.41	2	0.82
		143	4.59				
		581	4.56				
		1136	5.31				
		391	4.63				
	L-PFOS	732	3.99	3.93	0.33	2	0.67
		143	3.81				
		581	3.78				
		1136	4.41				
		391	3.64				

ANNEX 7. Graphs for CCQM K-156 Comparison Study

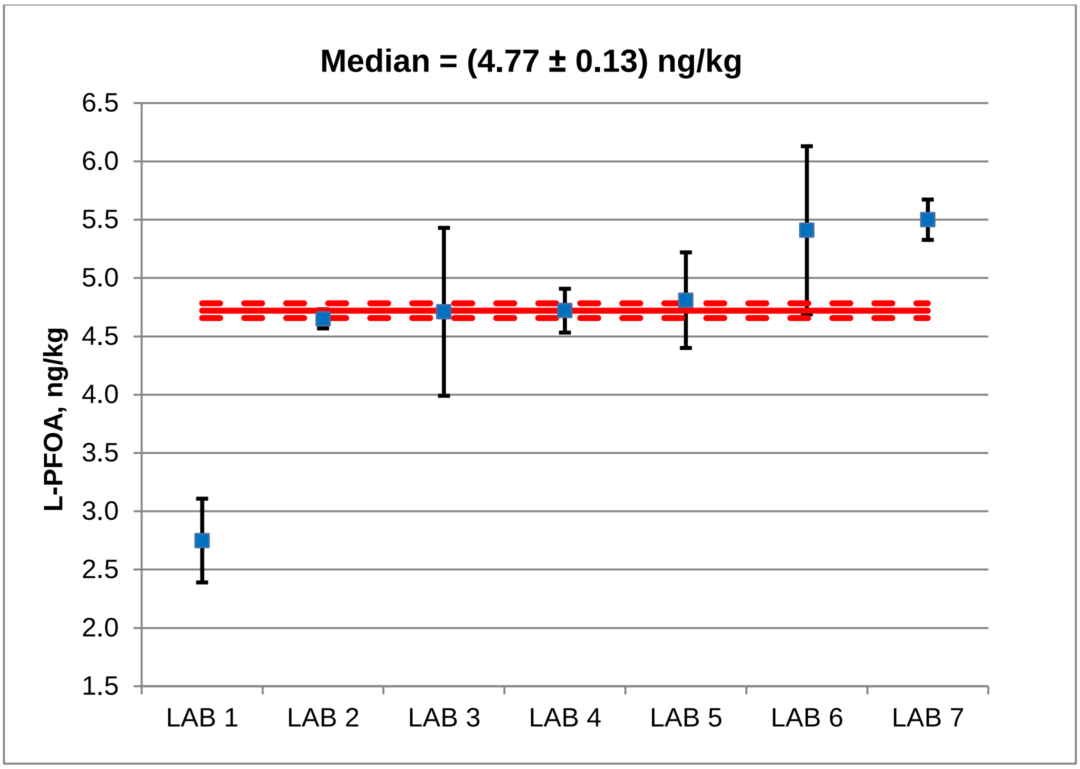


Figure A7.1. CCQM K-156 Comparison Study Plot for L-PFOA

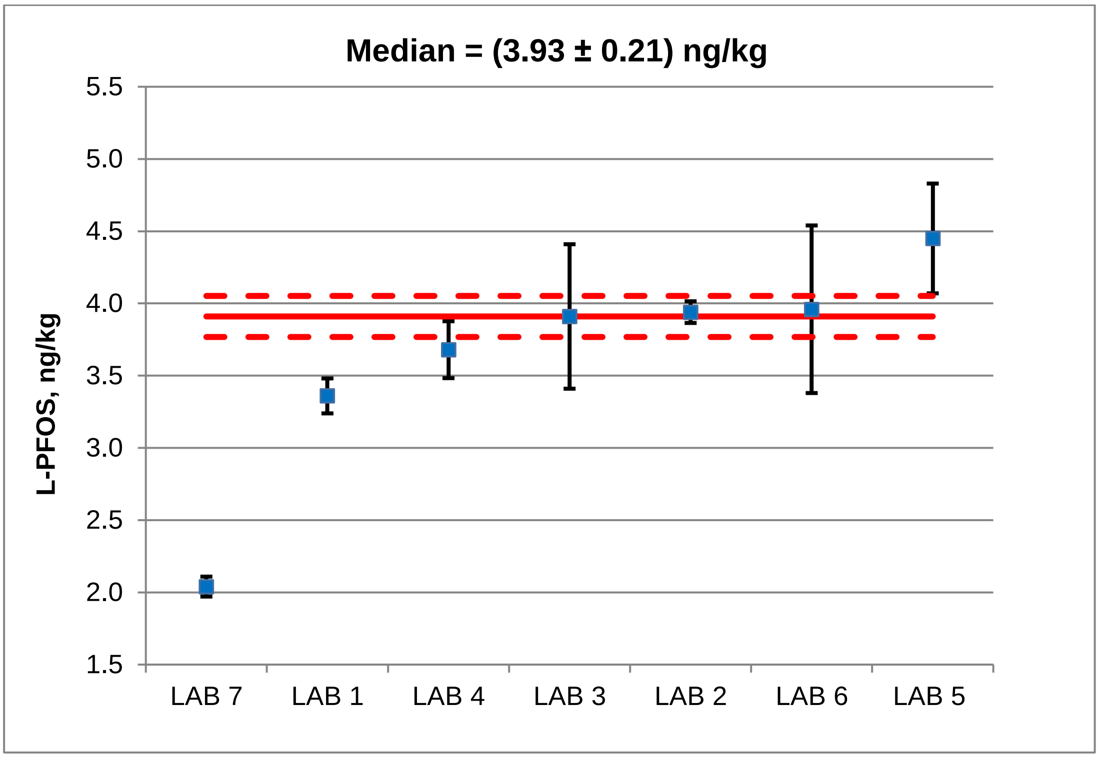


Figure A7.2. CCQM K-156 Comparison Study Plot for L-PFOS