# TÜBİTAK
# ULUSAL METROLOJİ ENSTİTÜSÜ

## Certification Report

# Elements in Seabass
# UME CRM 1209

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FRM-07-U-10-02/Rev.2/26.02.2020

# TABLE OF CONTENTS

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FRM-07-U-10-02/Rev.2/26.02.2020

## ABBREVIATIONS

| | |
|---|---|
| ANOVA | analysis of variance |
| $\alpha$ | significance level |
| CRM | certified reference material |
| ICP-MS | inductively coupled plasma mass spectrometry |
| ICP-OES | inductively coupled optical emission spectrometry |
| ID ICP-MS | isotope dilution ICP-MS |
| ISO | International Organization for Standardization |
| $MS_{between}$ | mean square between-unit from ANOVA |
| $MS_{within}$ | mean square within-unit from ANOVA |
| $RSD$ | 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 |
| $u_{bb}$ | standard uncertainty related to possible between-bottle heterogeneity |
| $u^{*}_{bb}$ | standard uncertainty of heterogeneity that can be hidden by method repeatability |
| $u_{char}$ | standard uncertainty related to characterization |
| $u_{lts}$ | standard uncertainty related to long term stability |

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

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FRM-07-U-10-02/Rev.2/26.02.2020

## ABSTRACT

This report explains certification procedure of total mass fractions of Cu, Fe, Hg, Se and Zn in UME CRM 1209 seabass matrix. The process involves sample preparation, homogeneity, short and long term stability, and characterization and value assignment steps. The studies are performed according to ISO 17034:2016 and ISO Guide 35:2017. TÜBİTAK UME organized and coordinated all the steps of this project including evaluation of the data.

Certified values and uncertainties fulfilled all requirements related to standards mentioned above and uncertainties are calculated according to Guide to the Expression of Uncertainty in Measurement (GUM).

The material is intended to be used for the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation for analysis of a series of elements in seabass matrix.

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FRM-07-U-10-02/Rev.2/26.02.2020

## INTRODUCTION

Reliable analysis of chemical indicators in fish samples for the purpose of environmental pollution assessment and monitoring, and food safety poses one of the greatest analytical challenges, due to the complexity of sample matrix. Elements (As, Cu, Ca, Fe, Hg, K, Mg, Na, P, Se and Zn) in seabass represent target parameters for the candidate certified reference material (CRM). 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 aim of this project is to produce CRM according to 17034:2016 [1] and ISO Guide 35:2017 [2] for enhancing the quality of measurements performed by laboratories as the matrix CRMs available in the market are starting points in the validation of analytical methods, ensuring accuracy and traceability of the measurement results. Certified values and uncertainties fulfilled all requirements related to standards mentioned above and uncertainties are calculated according to Guide to the Expression of Uncertainty in Measurement (GUM) [3].

It is essential to use the most appropriate CRM in realizing traceability of measurement results and validation of methods. Within the scope of this project the most suitable material for production of CRM was identified as the most consumed and having natural level of nutrients and/or contaminants. It has been aimed to certify concentration of As, Cd, Cu, Cr, Fe, Hg, Ni, Pb, Se, Zn and major analytes. All elements' target values were kept at natural levels therefore no spiking was done. For this reason, some of the elements (Cd, Cr, Ni, Pb) whose concentrations are so low that requires special sample preparation conditions were left out of the scope of the project.

## PARTICIPANTS

Sampling, processing the raw material, homogeneity, stability and characterization measurements were performed by experts of TÜBİTAK UME using existing measurement infrastructure.

**Table 1.** The laboratory/organizations and their contributions

| Laboratory/Organization | Work Package |
|---|---|
| TÜBİTAK UME<br>TÜBİTAK Gebze Yerleşkesi, Barış Mahallesi Dr. Zeki Acar Cad. No.1, 41470, Gebze, Kocaeli, Turkey | Project management, Material processing and Certification |

## MATERIAL PROCESSING

Three seabasses, which were 9.5 kg in total, were purchased from a local market, and stored at -20 °C until processing. After the material thermally equilibrate to room temperature, the fillet parts of the fishes were cut into approximately 1 cm$^3$ pieces with a stainless steel knife. The batch, then, was subjected to lyophilization (MillRock, Piatkowski, USA) and transferred to tightly closed containers

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FRM-07-U-10-02/Rev.2/26.02.2020

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# UME CRM
# 1209

stored at -20 °C. Total weight of fillet parts subjected to lyophilization process was 4.4 kg, and the total weight remaining after lyophilization was 1.3 kg.

Before the milling process, the milling device, vibrating titanium grinding mill (Palla VM-KT, MBE Coal & Minerals Technology GmbH, Germany), was prepared for processing by cleaning and drying. For this purpose, some ice was added to the milling chamber, and was milled resembling the material to be processed. The mill, then was let dry until all the liquid gone off the surfaces. Afterwards, fish tissue samples previously subjected to lyophilization process were frozen in liquid nitrogen and, then grinded with the mill. Grinding was carried out at temperatures between -190 °C and -100 °C. The collected powder fish tissue was placed in tightly closed containers and stored at -20 °C.

The powdered fish tissue transferred to a container, and this container was placed into a larger container containing dry ice. The larger container was placed into 3D mixer (Mega Mixer, HKTM, Turkey) in cold environment to be homogenized for 3 hours. Homogenized powder fish tissue was placed into tightly closed plastic containers and stored at -20 °C.

Due to the oily nature of the sample, no sieving process could be applied before filling into the bottles. Lumps observed in the samples were crumbled by hand before filling. Filling and capping was carried out manually by weighing 10 g of sample into amber glass bottles, which were previously cleaned and dried under a HEPA filtered laminar flow cabinet (AQUARIA, Bio Activa 180, Italy). The filled bottles were labeled in the following filling order. A total of 84 units of bottles were filled.

## HOMOGENEITY

Homogeneity study between the units is performed to show that the assigned values are valid for all units within the stated uncertainty. The study is performed with a number of samples representing the whole batch. In this project, nine units were selected by using random stratified sampling strategy. Homogeneity tests were carried out by measuring three sub-samples under repeatability conditions. The samples to be analyzed were introduced to the instruments by random order to find out any trend arising from analytical and/or filling sequences.

The data were evaluated statistically by regression analysis for the presence of any trend in analytical and filling sequence at 95% confidence level. Analytical sequence trends were found only for Cu, K, Mg, P, and the data were reprocessed to correct for these trends. Grubbs test (one sided) was applied to all data for the presence of outlier at 95% confidence level. All the outliers except one were kept in the data set of homogeneity. One outlier in Fe data were clearly result of cross-contamination of that sample. The result was approximately 10 mg/kg higher than the all individual results that obtained not only for homogeneity but also for short term, long term and characterization measurement results. Therefore, that outlier was rejected as the second one was kept for the statistical evaluation. Data were visually checked whether all individual data follow a unimodal distribution using histograms and normal probability plots. It was found that the distribution was normal and unimodal. 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 presented in Table 2.

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**UME CRM 1209**

**Table 2.** Statistical evaluation of homogeneity assessment results for seabass

| Parameter | Is there a Trend? | | Is there an Outlier? | | Distribution |
| --- | --- | --- | --- | --- | --- |
| | Analytical sequence | Filling sequence | All data | Unit averages | All data |
| As | No | No | No | No | Normal/unimodal |
| Ca | No | No | No | No | Normal/unimodal |
| Cu | Yes | No | Yes,1 | No | Normal/unimodal |
| Fe | No | No | Yes, 2 | No | Normal/unimodal |
| Hg | No | No | Yes, 1 | No | Normal/unimodal |
| K | Yes | No | No | No | Normal/unimodal |
| Mg | Yes | No | No | No | Normal/unimodal |
| Na | Yes | No | No | No | Normal/unimodal |
| P | Yes | No | No | No | Normal/unimodal |
| Se | No | No | No | No | Normal/unimodal |
| Zn | No | No | No | No | Normal/unimodal |

The ANOVA allowed the calculation of the within- ($s_{wb}$) and between-unit homogeneity ($s_{bb}$), estimated as standard deviations, according to the following equations:

$$s_{wb} = \sqrt{MS_{within}} \tag{1}$$

In this equation, $MS_{within}$ represents the mean squares within-unit.

$s_{wb}$ is equivalent to the $s$ of the method, provided that subsamples are representative for the whole unit.

$$s_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \tag{2}$$

Where;

$MS_{between}$ : Mean squares between-unit,

$n$ : Number of replicates per unit.

When $MS_{between}$ is smaller than $MS_{within}$, $s_{bb}$ cannot be calculated. Instead, $u^*_{bb}$, the heterogeneity that can be hidden by the method repeatability, is calculated according to the following equation [5]:

$$u^*_{bb} = \frac{s_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{\nu_{MSwithin}}} \tag{3}$$

In this equation, $\nu_{MSwithin}$ represents degrees of freedom of $MS_{within}$.

The occurrence of $MS_{between} < MS_{within}$ can be seen, if material heterogeneity is smaller than that can be detected by the analytical methodology used.

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FRM-07-U-10-02/Rev.2/26.02.2020

For the parameters for which ANOVA was applied, the larger value of $s_{bb}$ or $u^*_{bb}$ was taken as uncertainty contribution for homogeneity, $u_{bb}$ (expressed in bold characters in Table 3).

**Table 3.** Results of the homogeneity study

| Element | $s_{wb,rel}$, % | $s_{bb,rel}$, % | $u^*_{bb,rel}$, % | $u_{bb,rel}$, % |
|:---:|:---:|:---:|:---:|:---:|
| As | 1.84 | 1.91 | 0.54 | **1.91** |
| Ca | 3.48 | 1.32 | 1.03 | **1.32** |
| Cu | 1.87 | 0.38 | 0.55 | **0.55** |
| Fe | 2.58 | 1.12 | 0.76 | **1.12** |
| Hg | 3.23 | $MS_{between} < MS_{within}$ | 0.95 | **0.95** |
| K | 1.94 | 1.46 | 0.57 | **1.46** |
| Mg | 1.81 | 1.08 | 0.53 | **1.08** |
| Na | 1.57 | 1.25 | 0.46 | **1.25** |
| P | 1.79 | 1.16 | 0.53 | **1.16** |
| Se | 3.66 | 2.66 | 1.08 | **2.66** |
| Zn | 2.04 | 1.21 | 0.60 | **1.21** |

The plotted data used for the evaluation of homogeneity are presented in Annex 1.

## STABILITY

The stability of the material which are exposed to different environmental conditions that may occur during shipment, and shelf life should be evaluated at defined storage conditions by reference material producers. The measurement design of the stability studies followed an isochronous scheme as described below [4] and the uncertainty contribution of stability of material has been calculated as described by Linsinger et. al. [5]. In this 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"), effectively "freezing" the degradation status of the materials. Test samples were moved to -80 °C (reference temperature) after completion of the test time. All samples were analyzed at the same time. Three replicate measurements from each unit were performed under the repeatability conditions.

### Short Term Stability Results:

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. 14 samples were randomly selected and 12 samples were subjected to the test temperatures for the specified time intervals.

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 results were screened for outliers by applying the single Grubbs' test at confidence levels of 95% and 99%. Number of detected outliers are given in the Table 4.

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The measured concentration values were plotted against time and the regression lines were calculated to check for significant trends indicating possible changes in the concentrations of the analytes by time. The calculated slope values were tested for significance using a $t$-test, with $t_{\alpha,df}$ being the critical $t$-value (two-tailed) for a significance level $\alpha = 0.05$ (95% confidence level). The graphs are presented in Annex 2.

Outliers (shown in Table 4) were identified in the statistical evaluation (Grubbs' test) of the data. With the exception for two outlier belong to data set of Se, as there was no technical reason to exclude them from evaluation, they remained in the data set. The data points were plotted against storage time at the test temperature and the regression line was calculated. In all cases, the slope of the regression line was not found to be significantly different from zero. The data evaluation results for the STS at 18 °C and 60 °C are summarized in Table 4.

Uncertainty calculations are performed using Equation (4). Maximum time for transfer is chosen as two weeks.

$$u_{sts,rel} = \frac{RSD}{\sqrt{\Sigma(t_i - \bar{t})^2}} \times t \qquad (4)$$

where;

$RSD$ : the relative standard deviation of the all values obtained in the stability study,
$t_i$  : the time point for each replicate,
$\bar{t}$  : the mean of the all time points,
$t$  : the maximum time suggested for the transfer (two week).

**Table 4.** Results of short term stability tests

| Element | 18 °C $u_{sts,rel}$ (%) | 60 °C $u_{sts,rel}$ (%) | 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? | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 18 °C | 60 °C | 18 °C | 60 °C | 18 °C | 60 °C | 18 °C | 60 °C |
| As | 1.07 | 1.13 | - | - | - | - | No | No | No | No |
| Ca | 2.10 | 1.00 | - | 1 | - | - | No | No | No | No |
| Cu | 0.58 | 0.98 | - | - | - | - | No | No | No | No |
| Fe | 1.09 | 1.11 | 1 | - | - | - | No | No | No | No |
| Hg | 1.21 | 1.68 | - | 3 | - | 3 | No | No | No | No |
| K | 1.76 | 2.43 | - | 3 | - | 1 | No | No | No | No |
| Mg | 1.66 | 2.13 | - | 2 | - | 2 | No | No | No | No |
| Na | 2.10 | 1.41 | 1 | 1 | - | 1 | No | No | No | No |
| P | 2.17 | 1.47 | 1 | 1 | - | 1 | No | No | No | No |
| Se | 1.54 | 1.92 | 1 | 2 | 1 | 1 | No | No | No | No |
| Zn | 0.99 | 1.79 | - | 1 | - | - | No | No | No | No |

*SGT: Single Grubbs' Test

The material is found to be stable at 60 °C for up to 4 weeks. The uncertainty related to short term stability of material was calculated for two weeks at 60 °C for all analytes. Thus, the samples can be safely dispatched under conditions where the temperatures do not exceed 60 °C for up to 2 weeks, i.e. at ambient temperature without applying any cooling elements.

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FRM-07-U-10-02/Rev.2/26.02.2020

## Long Term Stability Results:

Shelf life of the CRM has been determined through long-term stability (LTS) measurements. For the measurements, four units for each of the months of 0, 2, 4 and 6 have been stored at -20 °C and transferred to reference temperature (-80 °C) after each period of time to be measured isochronously afterwards. Four units, designated as reference units, of the 'month 0' was stored at -80 °C.

The data for each time point has been calculated by three replicate measurements for each of two units. Thus, the average of six measurements for each time point is given in Annex 3. The error bars on each time point are calculated as the standard deviation of six measurement results.

The results were screened for outliers by applying the single Grubbs' test at confidence levels of 95% and 99%. Since no technical reason can be found to reject these data, all outliers were included in the LTS calculations.

The graphs were plotted against time and the regression lines were calculated. The relative LTS uncertainty, $u_{lts,rel}$ for each parameter is then calculated using Equation (5) for the required shelf life as [6]:

$$u_{lts,rel} = \frac{RSD}{\sqrt{\Sigma(t_i - \bar{t})^2}} \times t \tag{5}$$

where,

$RSD$ : the relative standard deviation of all the values obtained in the stability study,

$t_i$ : the time point for each replicate,

$\bar{t}$ : the mean of the all-time points,

$t$ : the suggested shelf life at -20 °C (12 months).

The uncertainty contribution $u_{lts}$ was calculated for 12 months (t) at -20 °C. This uncertainty was one of the four parameters contributing to the overall uncertainty of the certified values. The results are given in Table 5. The graphs for long term stability are presented in Annex 3.

**Table 5.** Results of the long-term stability tests for 12 months

| Element | The slope significantly different from zero at -20 °C?* | $u_{lts,rel}$ (%) for shelf-life of 12 months at -20 °C | Number of individual outlying result at 95% confidence level | Number of individual outlying result at 99% confidence level |
|---------|---------|---------|---------|---------|
| As | No | 7.15 | 1 | 1 |
| Ca | No | 2.01 | - | - |
| Cu | No | 3.13 | - | - |
| Fe | No | 5.84 | 1 | 1 |
| Hg | No | 3.38 | - | - |
| K | No | 0.71 | - | - |
| Mg | No | 2.37 | - | - |
| Na | No | 0.76 | - | - |
| P | No | 0.65 | - | - |
| Se | No | 5.94 | - | - |
| Zn | No | 3.50 | - | - |

*Data are evaluated at confidence level of 95%.

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FRM-07-U-10-02/Rev.2/26.02.2020

Based on the results obtained, -20 °C was found to be suitable to keep the samples for up to 12 months. In addition, to ensure stability beyond the initially determined shelf life, stability will be re-evaluated in certain periods, based on the results of post-certification monitoring.


## CHARACTERIZATION

According to ISO 17034 standard, the characterization and the value assignment can be carried out in different ways. In this project, the characterization of the material was carried out using of 'a reference method by a single laboratory'. TÜBİTAK UME used validated a potentially primary method, ID-ICP-MS, for characterization of certified parameters.

Characterization study were performed by using two bottles. The samples were selected randomly from the set of samples by the random stratified sampling technique, and at least six independent measurement results for two sample units, together with their associated measurement uncertainty values and the approach used for the estimation of measurement uncertainty were used in the assignment of certified value. The summary of characterization results is given in Table 6.

**Table 6.** Summary of characterization measurements

| Element | Value | $U_{char}$ (k=2) |
|:---:|:---:|:---:|
| Cu | 1.20 | 0.03 |
| Fe | 18.6 | 0.4 |
| Hg | 0.715 | 0.023 |
| Se | 1.00 | 0.01 |
| Zn | 17.9 | 0.5 |


## PROPERTY VALUE AND UNCERTAINTY ASSIGNMENT

The uncertainty component of the certified value is composed of the uncertainty contributions from the characterization study ($u_{char}$), the homogeneity study ($u_{bb}$), the short-term stability study ($u_{sts}$) and the long-term stability study ($u_{lts}$). The uncertainty of the CRM were determined by combining the components affecting value of the assigned uncertainty are calculated using the following equation:

$$U_{CRM} = k\sqrt{u_{char}^2 + u_{bb}^2 + u_{sts}^2 + u_{lts}^2} \tag{6}$$

The expanded uncertainty of the certified value $U_{CRM}$ is calculated with a coverage factor of $k = 2$.

The certified values and uncertainties are summarized in Table 7, and uncertainty contribution percentages are given in Table 8.

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FRM-07-U-10-02/Rev.2/26.02.2020

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**UME CRM 1209**

**Table 7.** Summary of certified values and their uncertainties

| Element | Certified Value (mg/kg) | $U_{CRM}$ (mg/kg, $k = 2$) | $U_{CRM,rel}$ (%, $k = 2$) | $u_{char,rel}$ (%) | $u_{bb,rel}$ (%) | $u_{lts,rel}$ (%) | $u_{sts,rel}$ (%) |
|---|---|---|---|---|---|---|---|
| Cu | 1.20 | 0.09 | 7.5 | 1.50 | 0.55 | 3.13 | 0.98 |
| Fe | 18.6 | 2.3 | 12.4 | 0.99 | 1.12 | 5.84 | 1.09 |
| Hg | 0.715 | 0.060 | 8.4 | 1.60 | 0.95 | 3.38 | 1.68 |
| Se | 1.00 | 0.14 | 14.0 | 0.35 | 2.66 | 5.94 | 1.92 |
| Zn | 17.9 | 1.6 | 8.9 | 1.30 | 1.21 | 3.50 | 1.79 |

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

| Element | $u_{bb}$ (%) | $u_{lts}$ (%) | $u_{sts}$ (%) | $u_{char}$ (%) |
|---|---|---|---|---|
| Cu | 2.3 | 73.6 | 7.2 | 16.9 |
| Fe | 3.3 | 90.9 | 3.2 | 2.6 |
| Hg | 5.1 | 64.5 | 15.9 | 14.5 |
| Se | 15.3 | 76.4 | 8.0 | 0.3 |
| Zn | 7.9 | 65.8 | 17.2 | 9.1 |

**INFORMATIVE VALUES**

Although the homogeneity and stability studies were performed for the values given in the Table 9, they have been provided as informative value as the characterization study has not been performed. Thus, the values provided are the mean of the values obtained in homogeneity and reference points of stability measurements. The uncertainty estimation was carried out similar to ones for the certified values using Equation (6).

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FRM-07-U-10-02/Rev.2/26.02.2020

Page 13 / 31

**TÜBİTAK**
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UME CRM
1209

**Table 9.** Informative values

| Element | Value[3], g/kg | Uncertainty[3,4], g/kg |
|---|---|---|
| As [1] | 0.0017 | 0.0003 |
| Ca [2] | 0.67 | 0.05 |
| K [2] | 13.1 | 1.1 |
| Mg [2] | 0.91 | 0.08 |
| Na [2] | 2.35 | 0.17 |
| P [2] | 6.55 | 0.76 |

[1] The value has been assigned from homogeneity, short term and long term stability measurements performed in three different days by HR-ICP-MS method using matrix matched external calibration technique.

[2] The value has been assigned from homogeneity, short term and long term stability measurements performed in three different days by ICP-OES method using matrix matched external calibration technique.

[3] The informative values and the uncertainties are traceable to the International System of Units (SI). Assigned values are corrected for dry mass as described in the section ""Instruction for use".

[4] The expanded uncertainty of the informative value has been composed of homogeneity, stability and measurement uncertainty, and is stated as the standard uncertainty of measurement multiplied by the coverage factor $k = 2$, which for a normal distribution corresponds to a coverage probability of approximately 95%. The standard uncertainty of measurement has been determined in accordance with GUM "Guide to the Expression of Uncertainty in Measurement.

**TRACEABILITY**

In this study, all measurements including the homogeneity and stability studies were performed by validated methods. All sample preparations for the measurements were performed gravimetrically using calibrated balances and weights which are traceable to SI via TÜBİTAK UME. Metrological traceability of measurements were also ensured by using the calibration standards of NIST SRM 3100 series traceable to SI. Isotopically enriched certified reference materials traceable to SI were used in ID-ICP-MS measurements in the characterization of Cu, Hg, Fe and Zn. The source of traceability of standards used in the measurements were given in Table 10. The certified or IUPAC values of isotopic ratios were used in ID-ICP-MS measurements.

**Table 10.** The standards used for traceability of the assigned values

| No | Name of Standard | Standard No | Traceability |
|---|---|---|---|
| 1 | As Standard Solution | SRM 3103a | NIST |
| 2 | Ca Standard Solution | SRM 3109a | NIST |
| 3 | Cu Standard Solution | SRM 3114 | NIST |
| 4 | Fe Standard Solution | SRM 3126a | NIST |
| 5 | Hg Standard Solution | SRM 3133 | NIST |
| 6 | K Standard Solution | SRM 3141a | NIST |
| 7 | Mg Standard Solution | SRM 3131a | NIST |
| 8 | Na Standard Solution | SRM 3152a | NIST |
| 9 | P Standard Solution | SRM 3139a | NIST |
| 10 | Se Standard Solution | SRM 3149 | NIST |

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FRM-07-U-10-02/Rev.2/26.02.2020

Page 14 / 31

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# ULUSAL METROLOJİ ENSTİTÜSÜ
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# UME CRM
# 1209

| No | Name of Standard | Standard No | Traceability to SI via |
|----|------------------|-------------|------------------------|
| 11 | Zn Standard Solution | SRM 3168a | NIST |
| 12 | Cu Isotopic Reference Material | IRMM 632 | IRMM |
| 13 | Fe Isotopic Reference Material | IRMM 620 | IRMM |
| 14 | Hg Isotopic Reference Material | ERM-AE640 | IRMM |
| 15 | Hg Isotopic Reference Material | ERM-AE639 | IRMM |
| 16 | Zn Isotopic Reference Material | IRMM 654 | IRMM |

## INSTRUCTIONS FOR USE

### Storage conditions

This material should be stored at (-20 ± 2) °C in a dark environment. This material can be safely dispatched under conditions where the temperatures do not exceed 60 °C for up to two weeks, i.e. at ambient temperature without applying any cooling elements. 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

The usual laboratory safety measures apply as in the case of similar materials. It is highly recommended that the material must be handled and disposed according to the safety guidelines where applicable.

### Minimum sample intake

The homogeneity of the material has been proved according to the homogeneity tests performed with a sample of approximately 0.75 g and no significant problem of heterogeneity have been observed. Therefore, this can be considered as the minimum sample amount to be used in the analysis. Before opening and taking sample, bottle should be shaken to re-homogenize the content. After use, the bottle should be immediately and tightly recapped.

### Use of Certified Value

All results should be corrected for dry mass content of the material. Moisture content of the material should be determined by drying approximately 0.5 g of sample at (102 ± 2) °C and atmospheric pressure for 6 hours. It is recommended to perform the moisture content determination of the material simultaneously with each measurement.

For assessing the method performance, the measured values of the CRM are compared with the certified values [6]. The procedure can be described briefly as follows:

- Calculate the absolute difference between mean measured value and the certified value ($\Delta_m$).
- Combine measurement uncertainty ($u_{meas}$) with the standard uncertainty of the certified value ($u_{CRM}$) using following equation:

$$u_\Delta = \sqrt{u_{meas}^2 + u_{CRM}^2} \qquad (7)$$

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FRM-07-U-10-02/Rev.2/26.02.2020

- Calculate the expanded uncertainty ($U_\Delta$) from the combined uncertainty ($u_\Delta$) 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 about 95% confidence level.

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/

## REFERENCES

[1]     ISO 17034:2016 - General requirements for the competence of reference material producers, International Orgnization for Standardization, Geneva

[2]     ISO Guide 35:2017 Reference Materials - Guidance for characterization and assessment of homogeneity and stability, International Orgnization for Standardization, Geneva

[3]     JCGM 100:2008, Evaluation of measurement data — Guide to the expression of uncertainty in measurement, BIPM

[4]     A. Lamberty, H. Schimmel, J. Pauwels, The study of the stability of reference materials by isochronous measurements, in: Fresenius. J. Anal. Chem., Springer Verlag, 1998: pp. 359–361. doi:10.1007/s002160050711

[5]     T.P.J. Linsinger, J. Pauwels, A. Lamberty, H.G. Schimmel, A.M.H. Van Der Veen, L. Siekmann, Estimating the uncertainty of stability for matrix CRMs, Fresenius. J. Anal. Chem. 370 (2001) 183–188

[6]     ERM Application Note 1: Comparison of a measurement result with the certified value, (2010) https://ec.europa.eu/jrc/sites/jrcsh/files/erm_application_note_1_en.pdf

## REVISION HISTORY

| Date | Remarks |
|---|---|
| 12.02.2021 | First issue |

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FRM-07-U-10-02/Rev.2/26.02.2020

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## ANNEXES

### Annex 1. Graphs for Homogeneity Studies
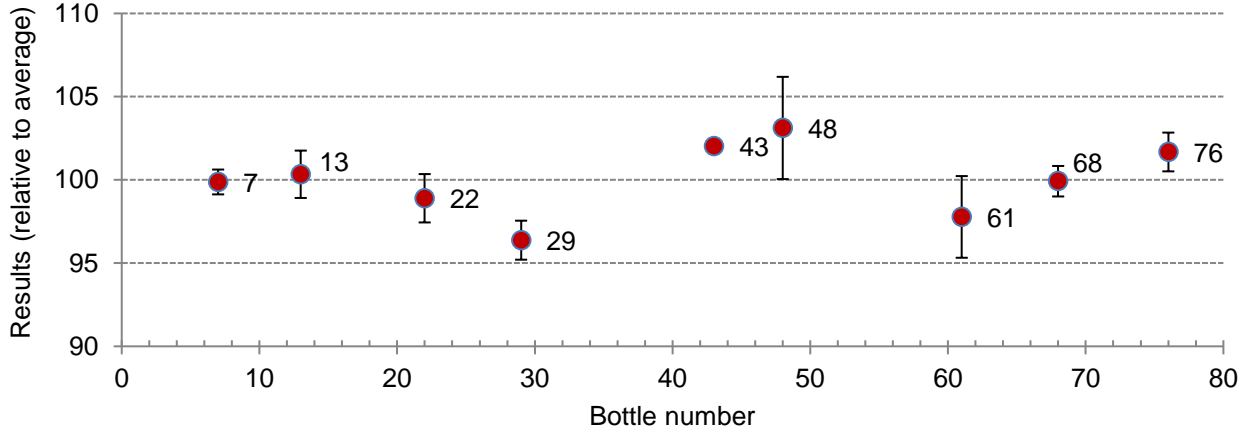


**Figure A1.1.** Homogeneity plot for As
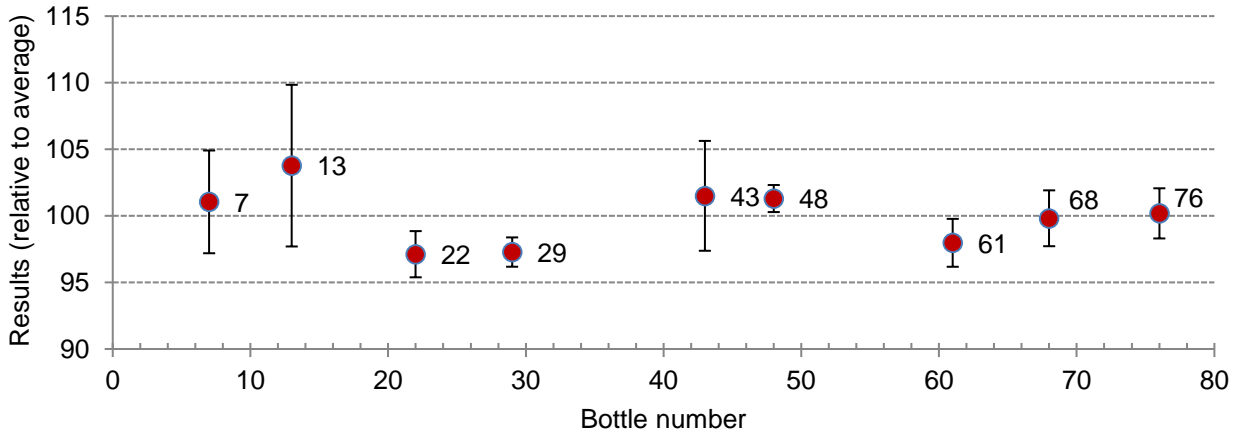


**Figure A1.2.** Homogeneity plot for Ca
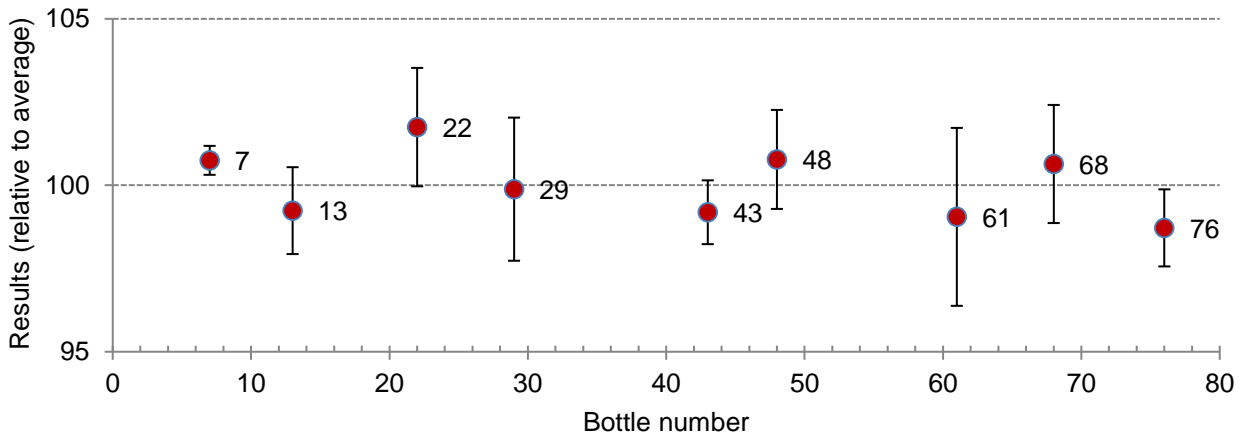


**Figure A1.3.** Homogeneity plot for Cu

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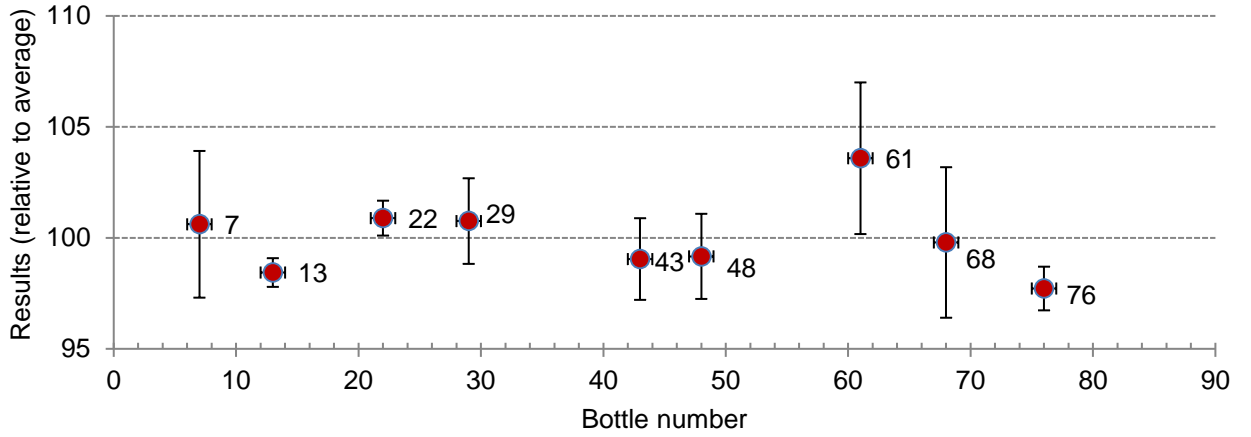


**Figure A1.4.** Homogeneity plot for Fe
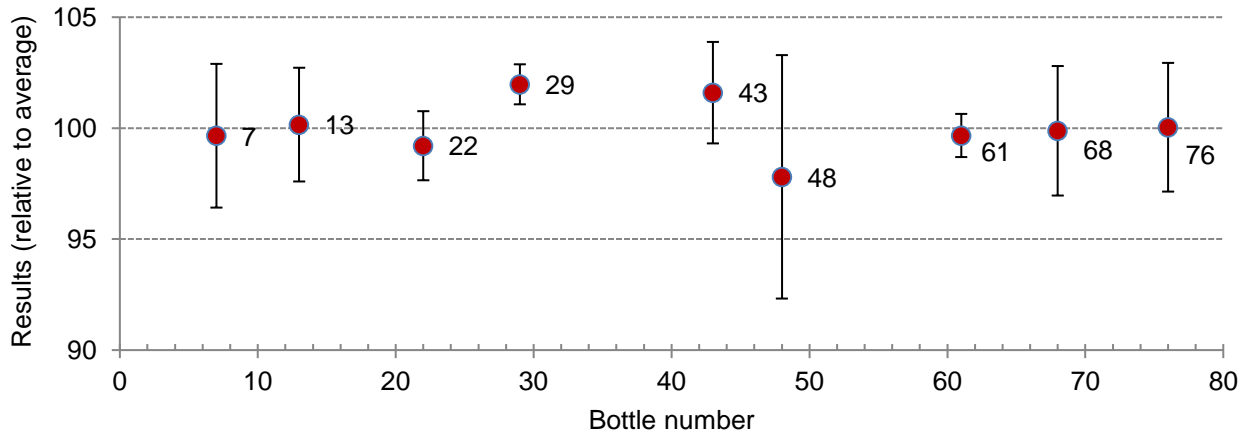


**Figure A1.5.** Homogeneity plot for Hg
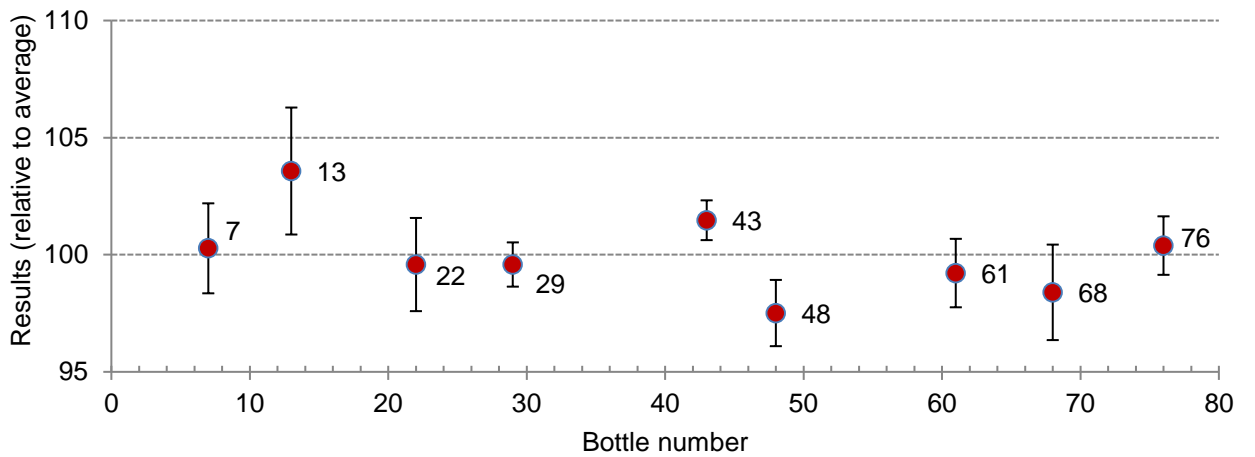


**Figure A1.6.** Homogeneity plot for K

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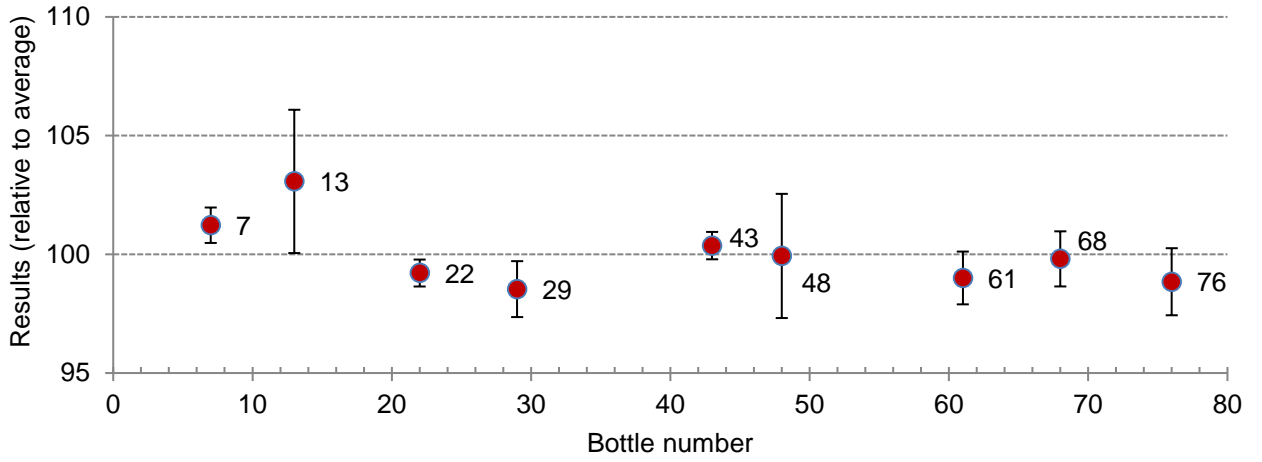


**Figure A1.7.** Homogeneity plot for Mg
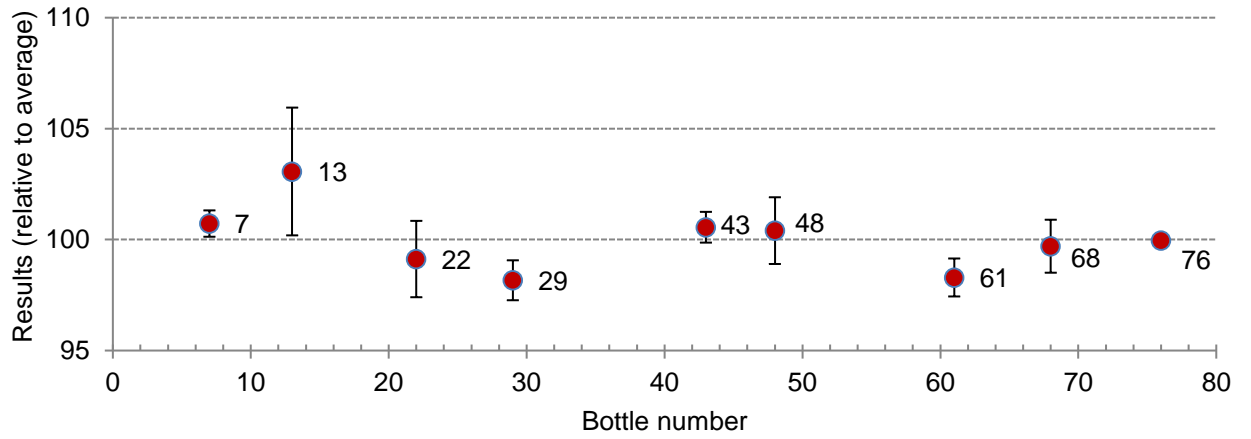


**Figure A1.8.** Homogeneity plot for Na
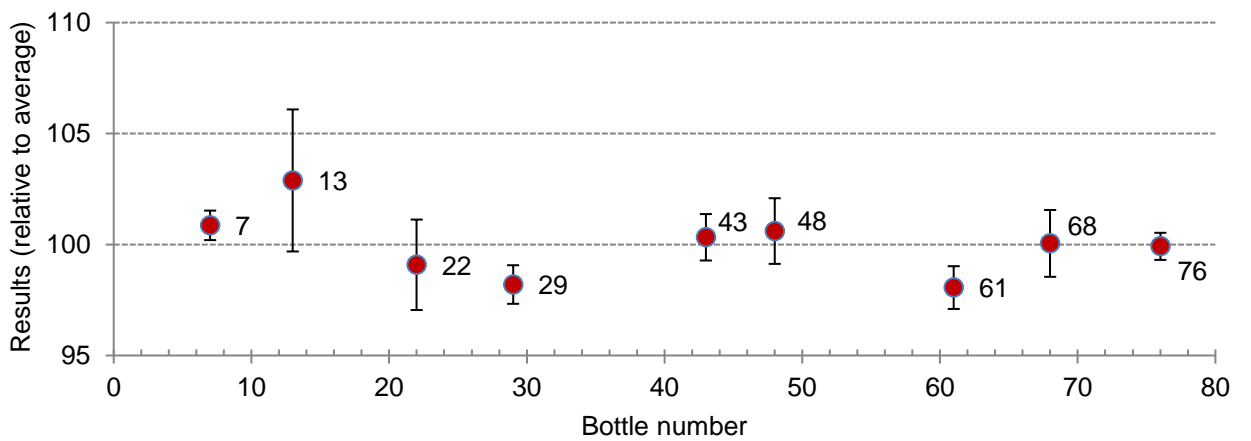


**Figure A1.9.** Homogeneity plot for P

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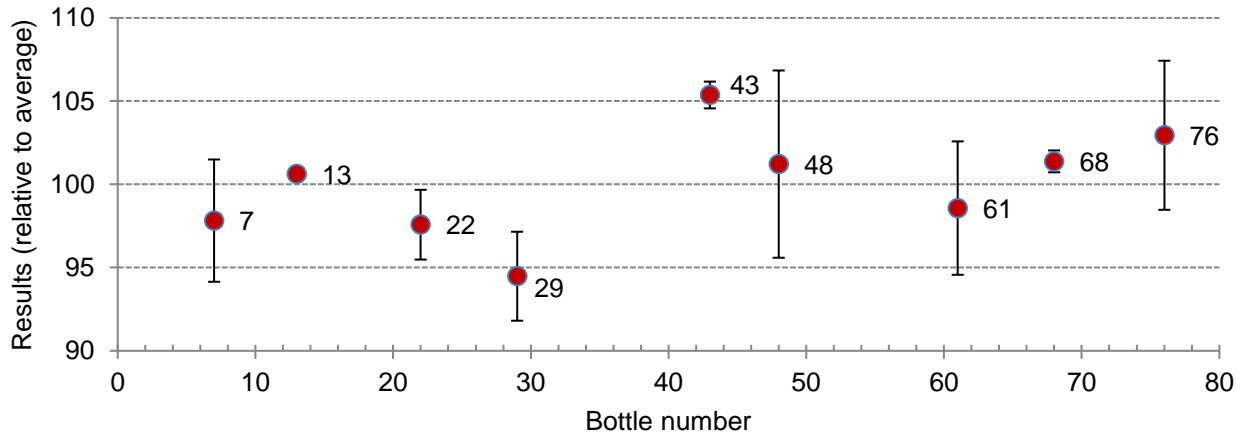


**Figure A1.10.** Homogeneity plot for Se
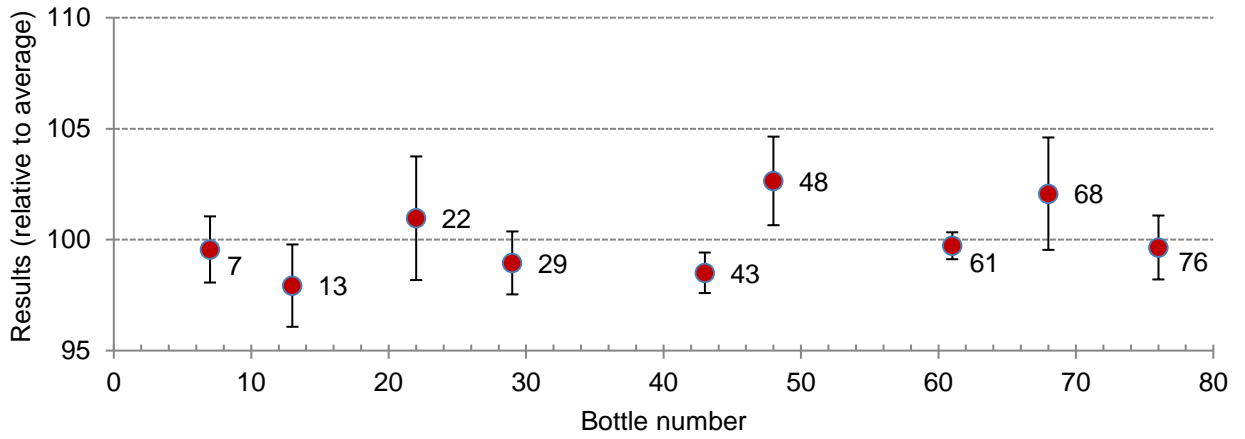


**Figure A1.11.** Homogeneity plot for Zn

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## Annex 2. Graphs for Short Term Stability Studies
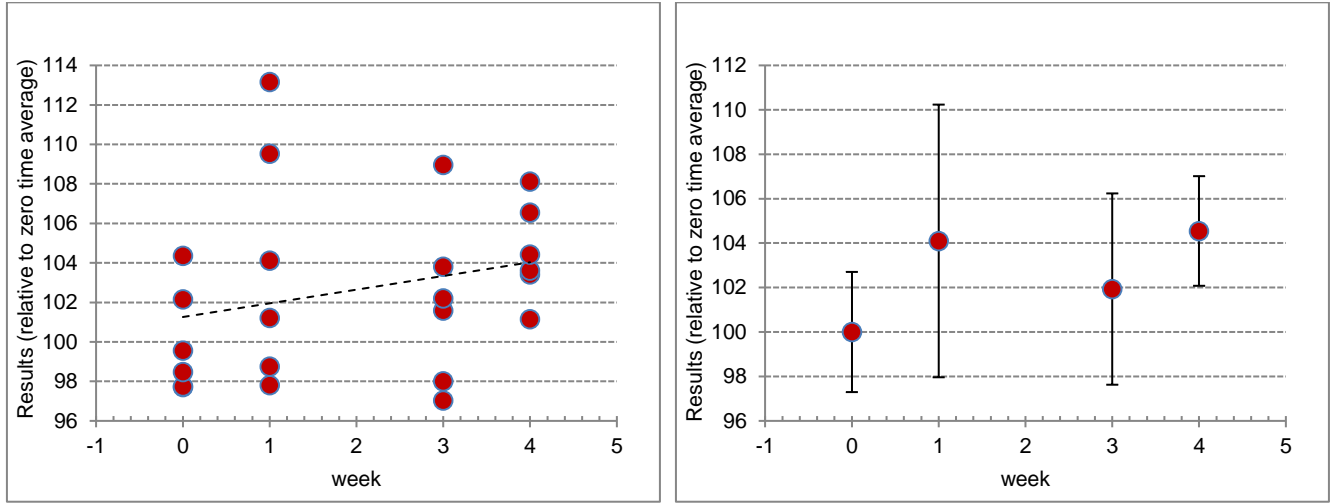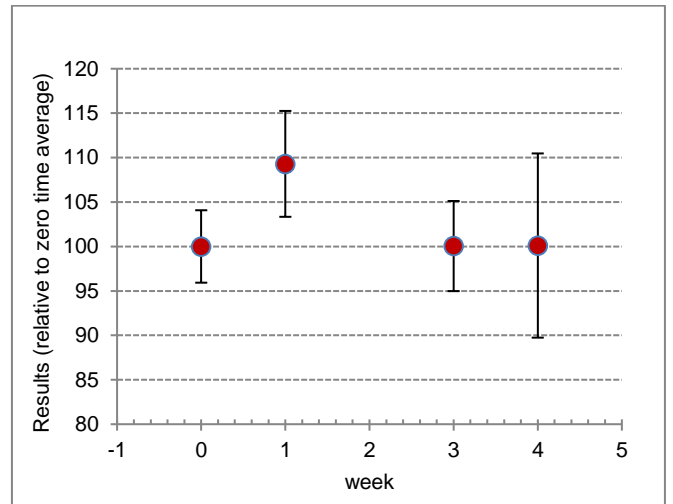


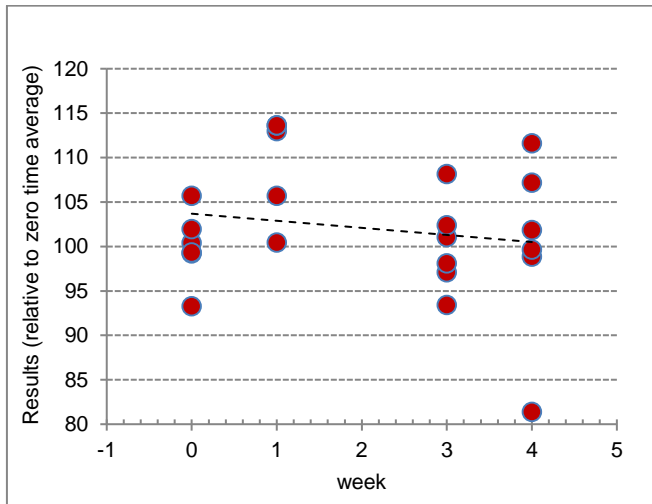**Figure A2.1.** STS plot for As at 18 °C
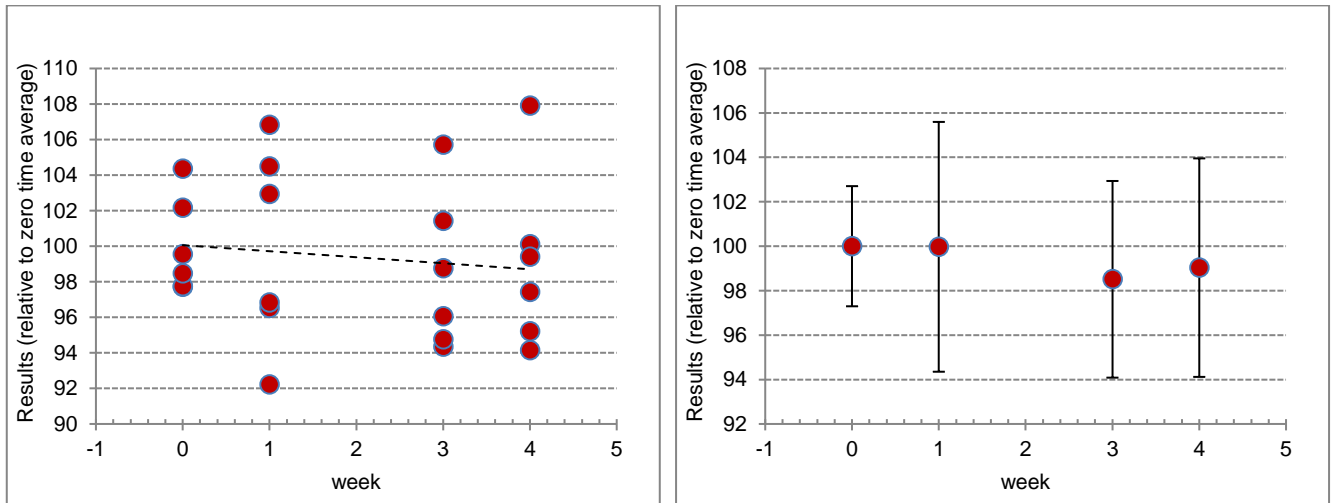

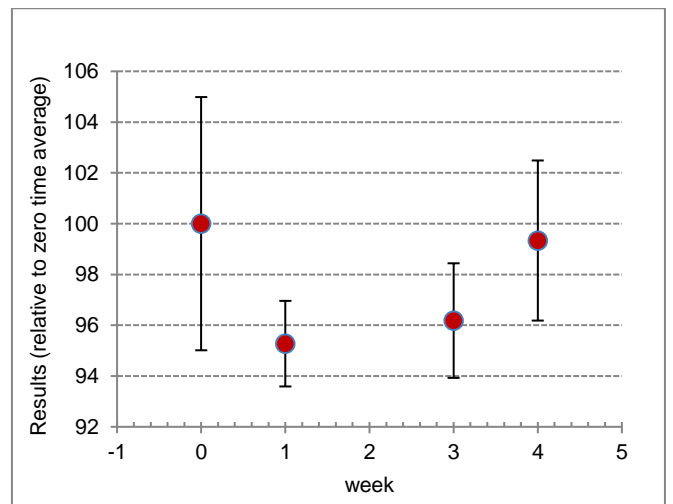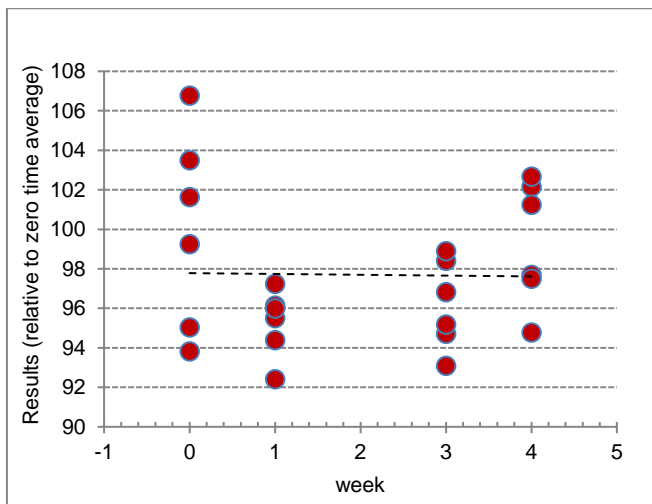
**Figure A2.2.** STS plot for As at 60 °C
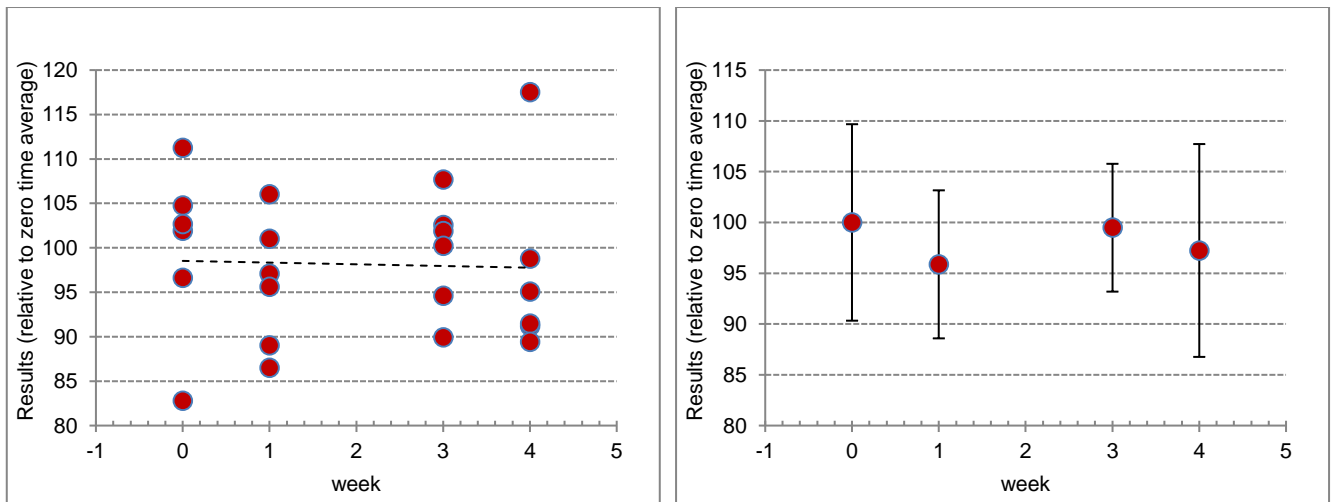


**Figure A2.3.** STS plot for Ca at 18 °C

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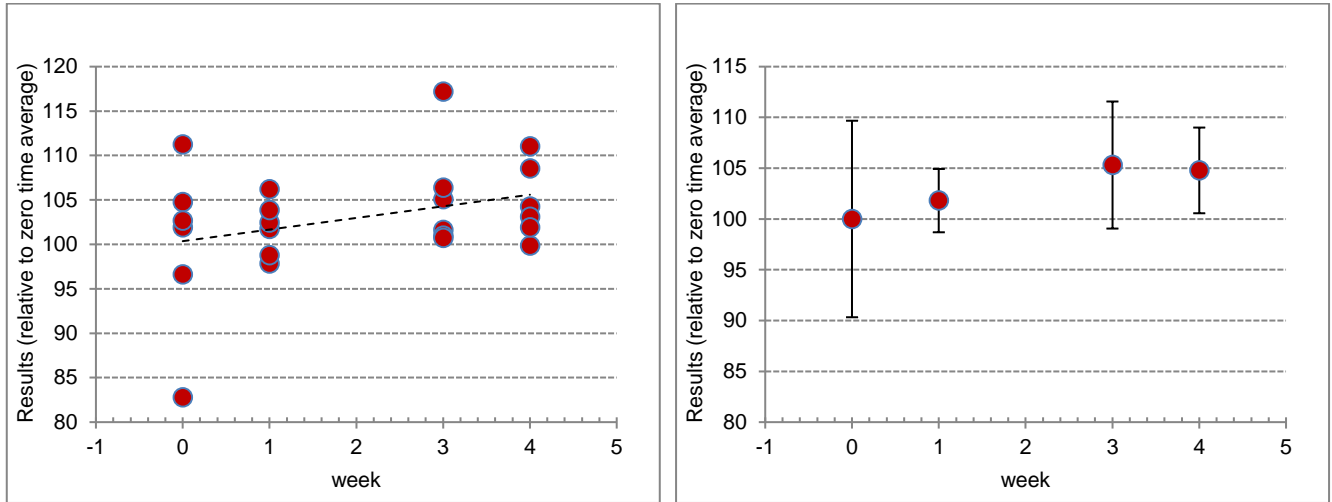


**Figure A2.4.** STS plot for Ca at 60 °C
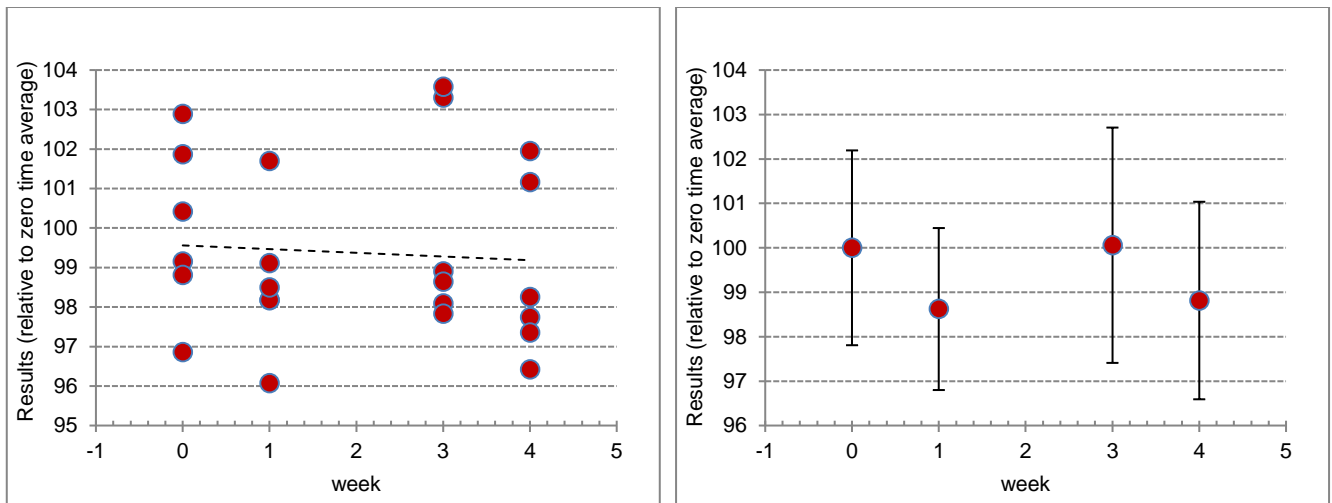


**Figure A2.5.** STS plot for Cu at 18 °C
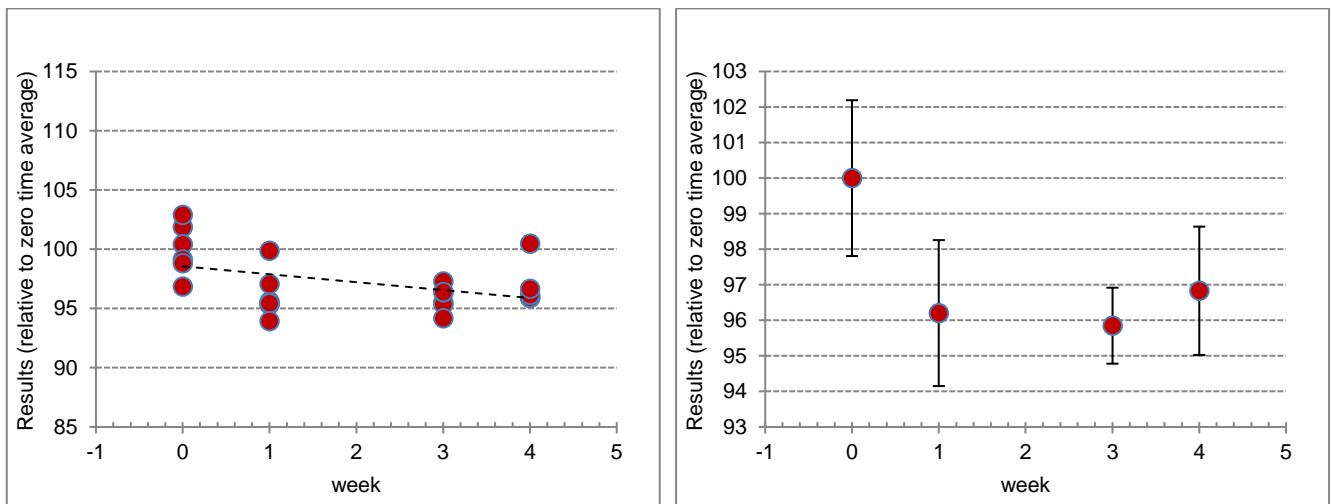


**Figure A2.6.** STS plot for Cu at 60 °C

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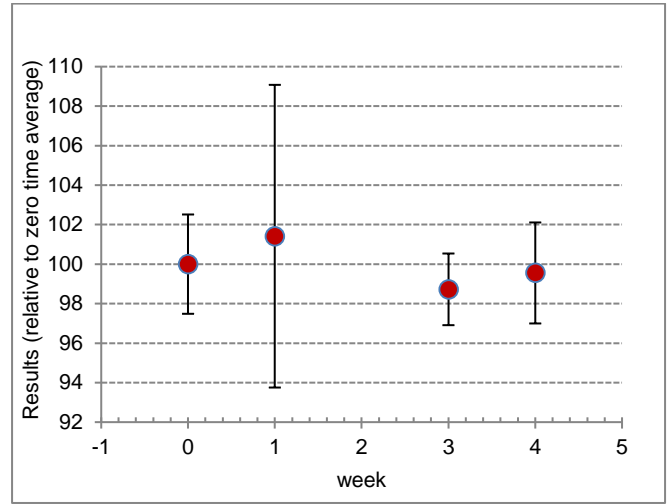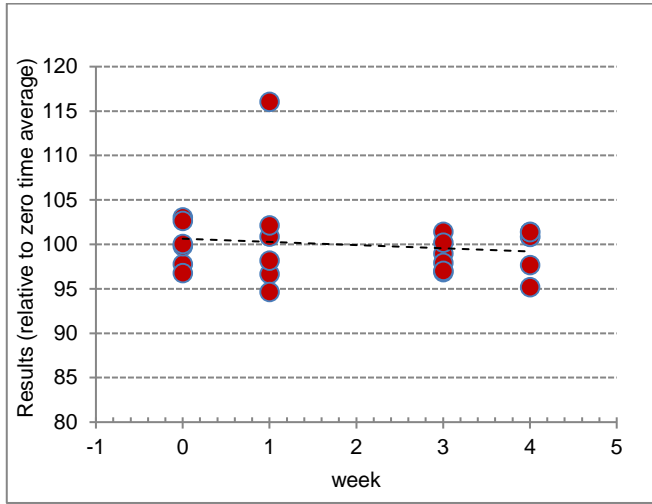


**Figure A2.7.** STS plot for Fe at 18 °C
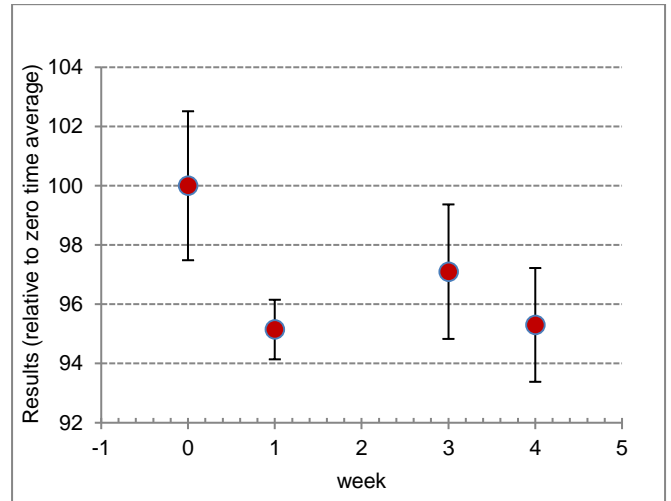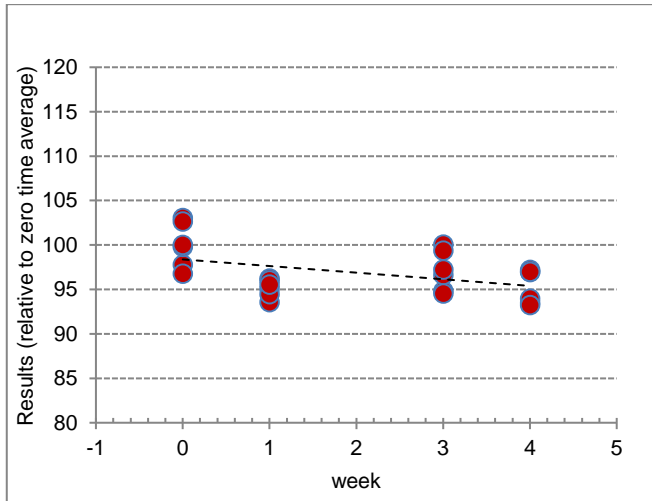


**Figure A2.8.** STS plot for Fe at 60 °C
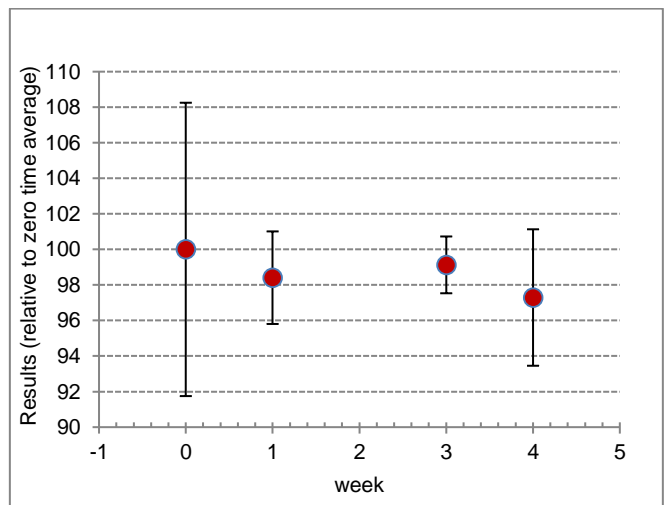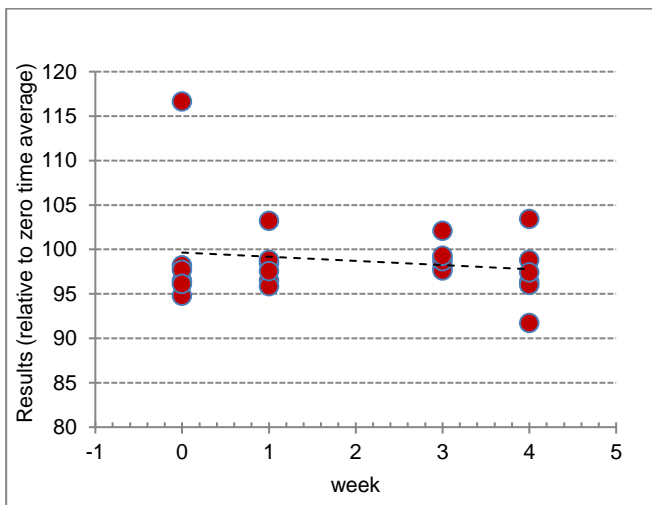


**Figure A2.9.** STS plot for Hg at 18 °C

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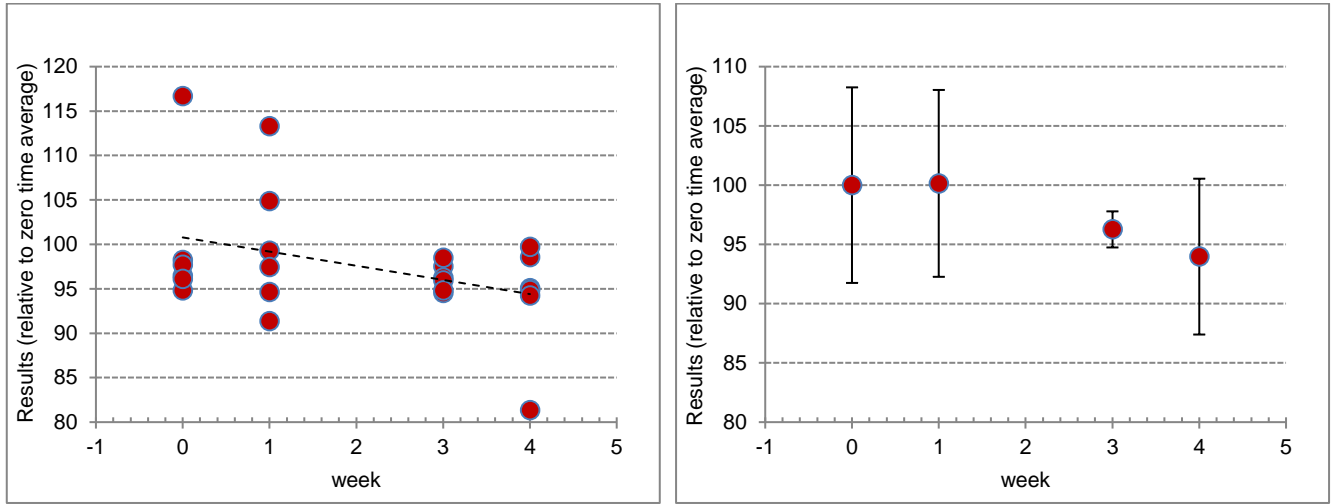


**Figure A2.10.** STS plot for Hg at 60 °C
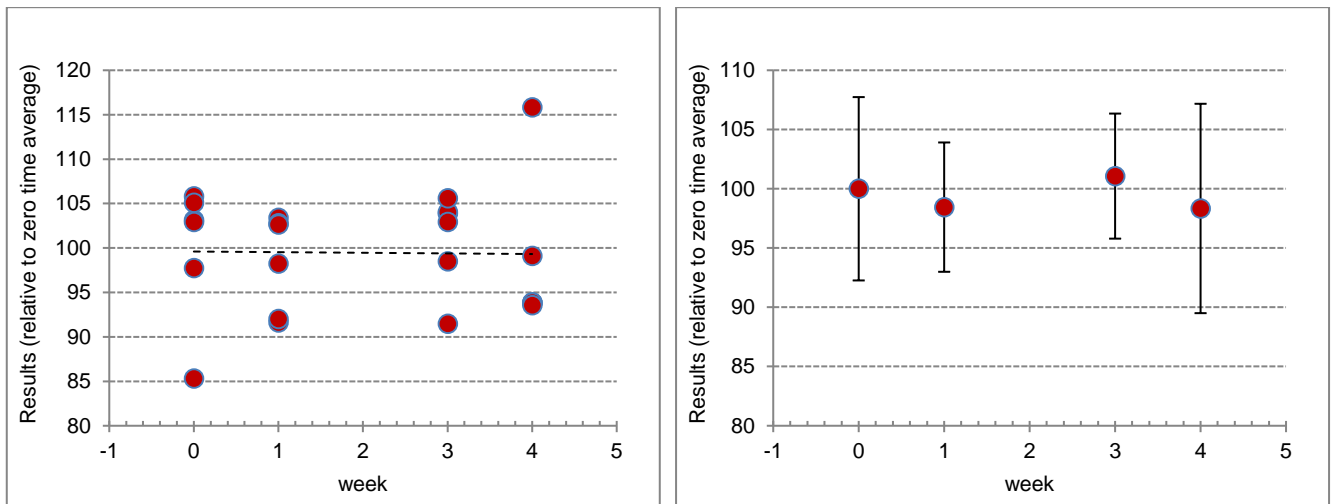


**Figure A2.11.** STS plot for K at 18 °C
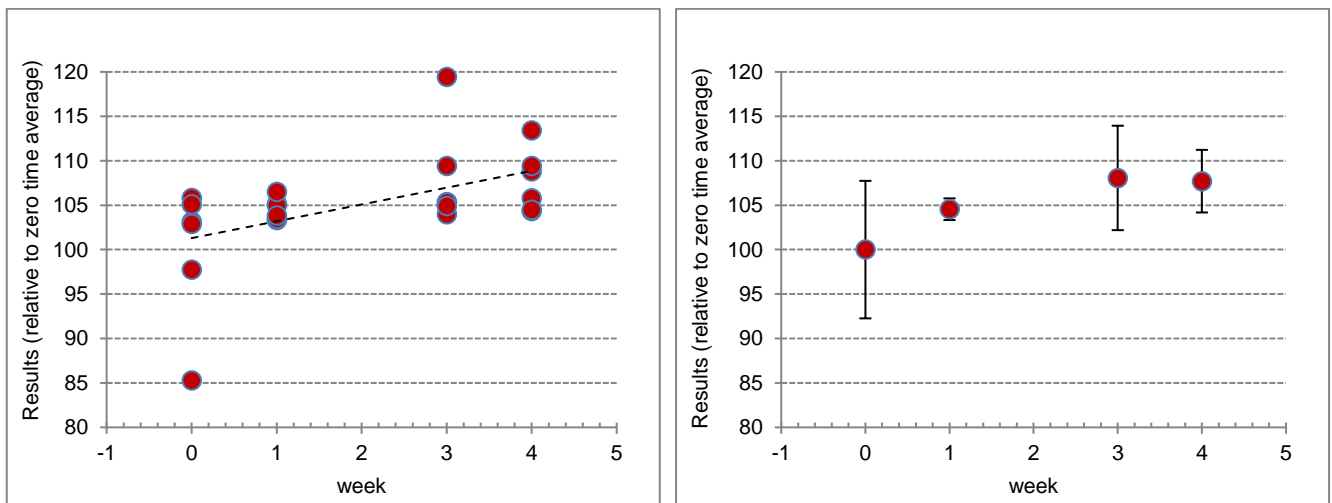


**Figure A2.12.** STS plot for K at 60 °C

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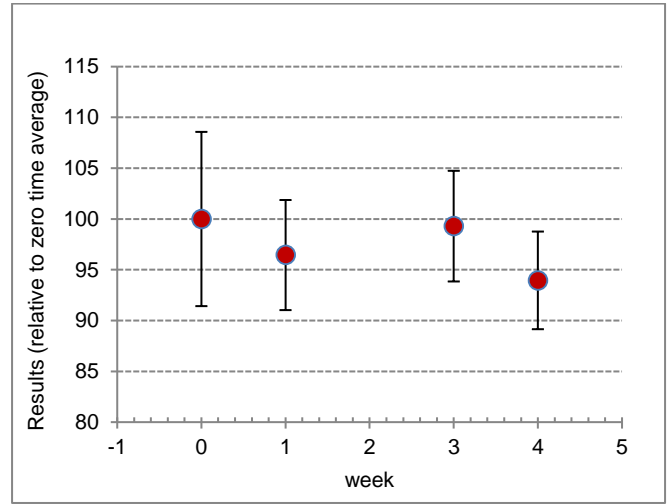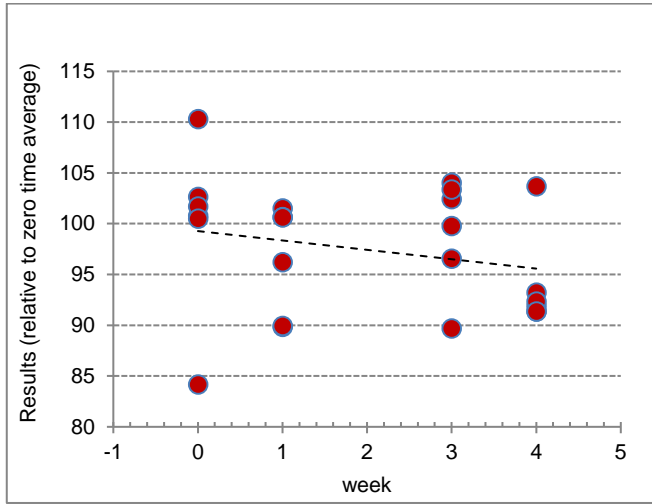


**Figure A2.13.** STS plot for Mg at 18 °C
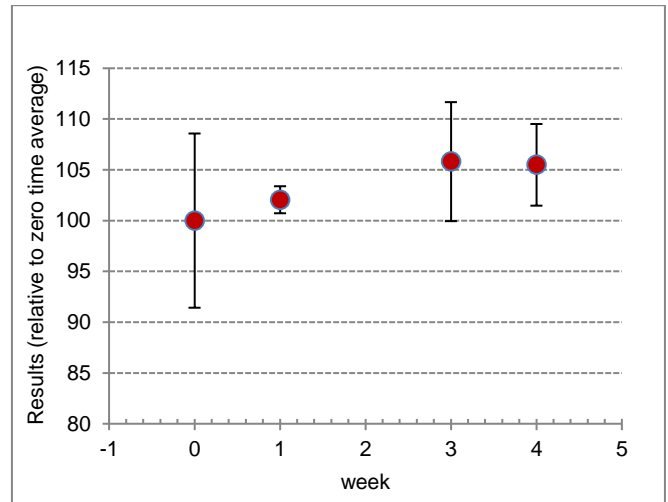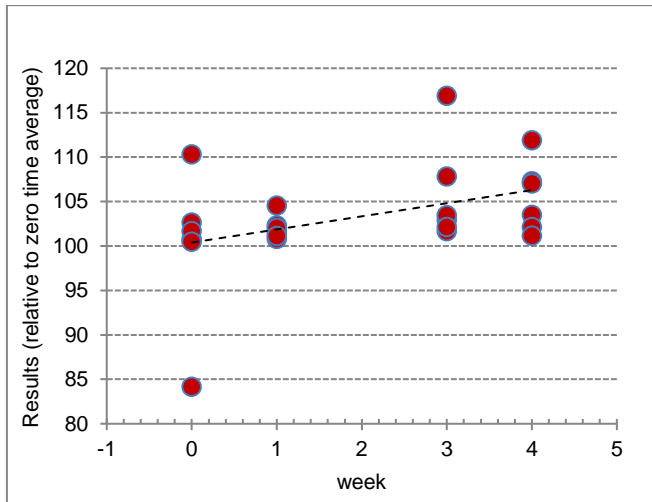


**Figure A2.14.** STS plot for Mg at 60 °C
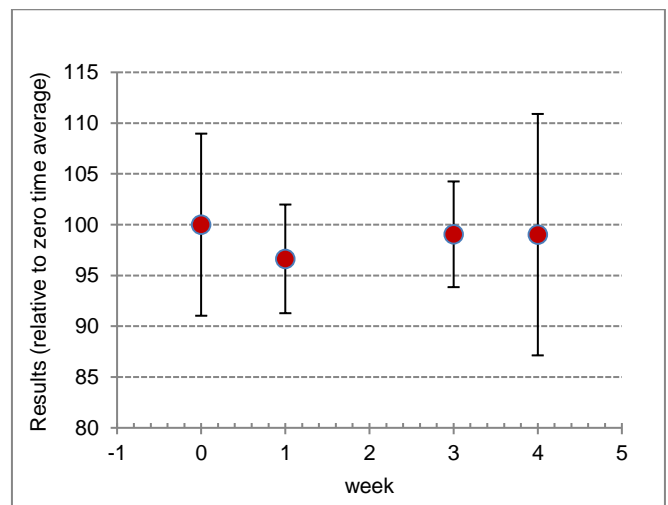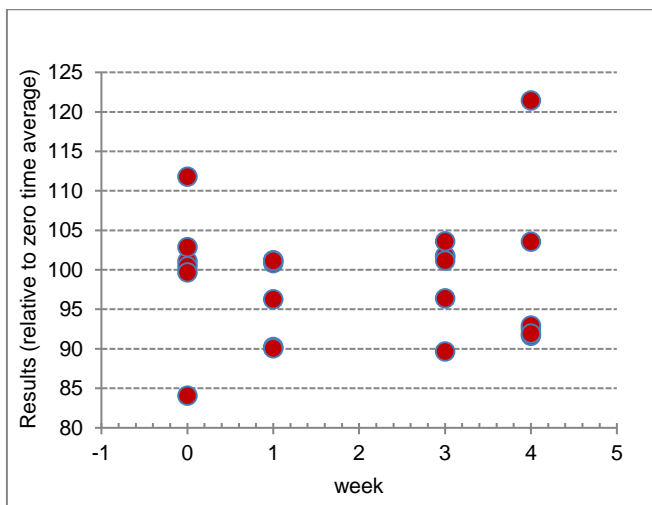


**Figure A2.15.** STS plot for Na at 18 °C

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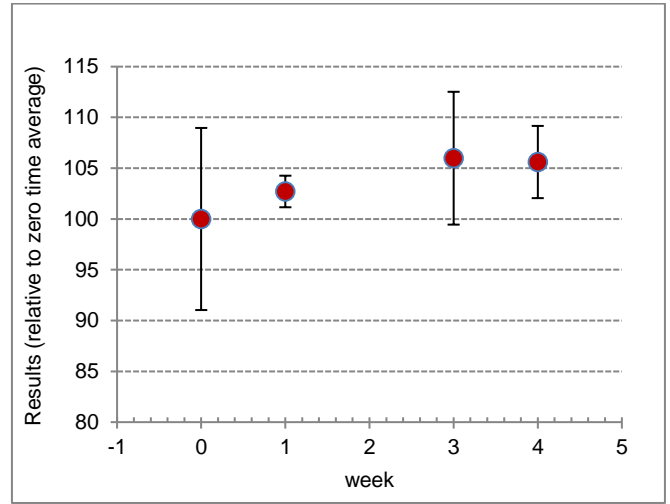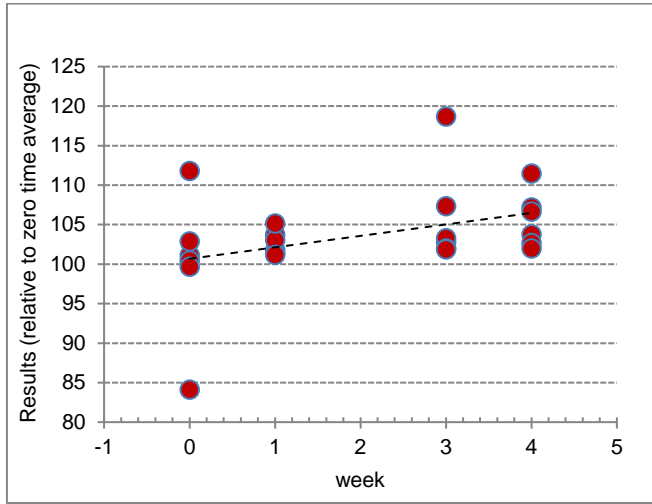


**Figure A2.16.** STS plot for Na at 60 °C
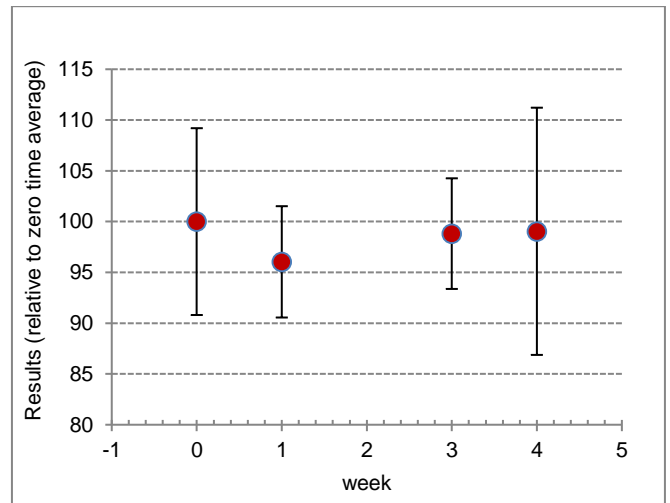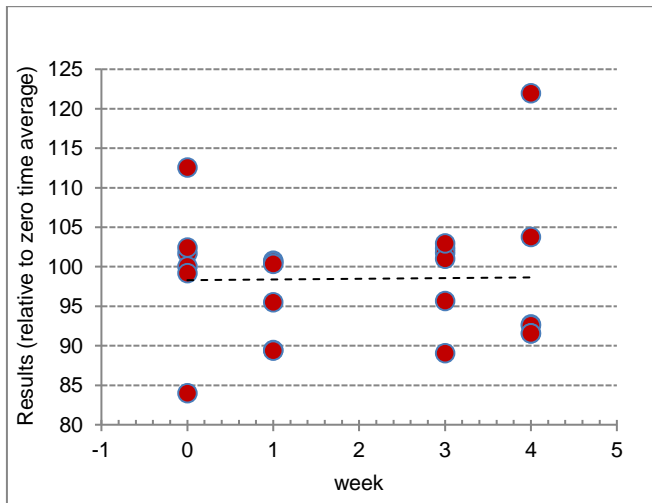


**Figure A2.17.** STS plot for P at 18 °C
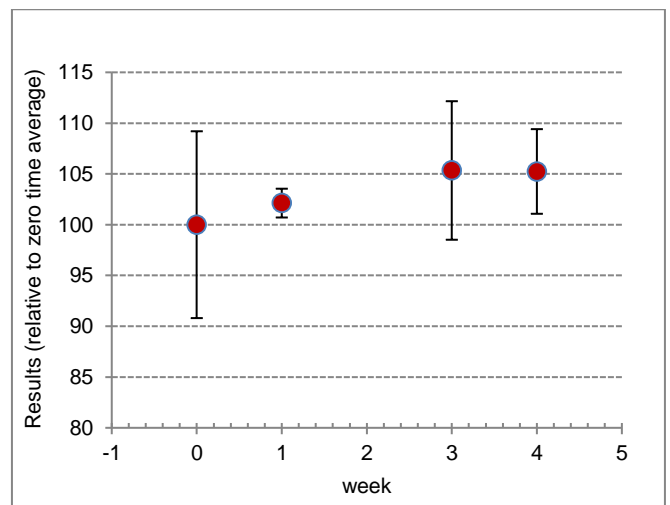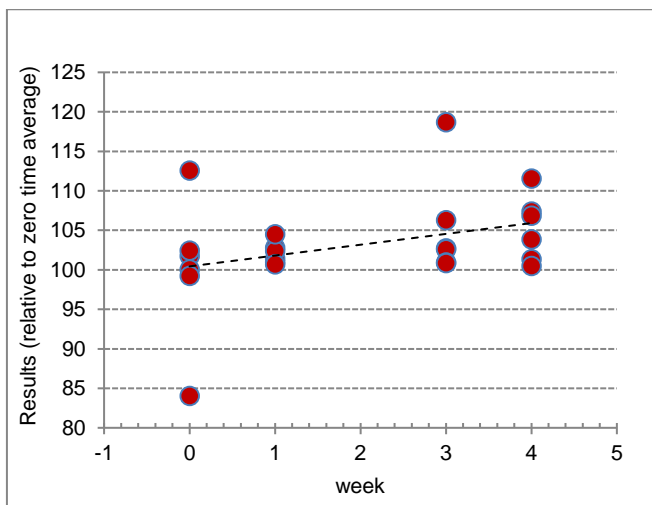


**Figure A2.18.** STS plot for P at 60 °C

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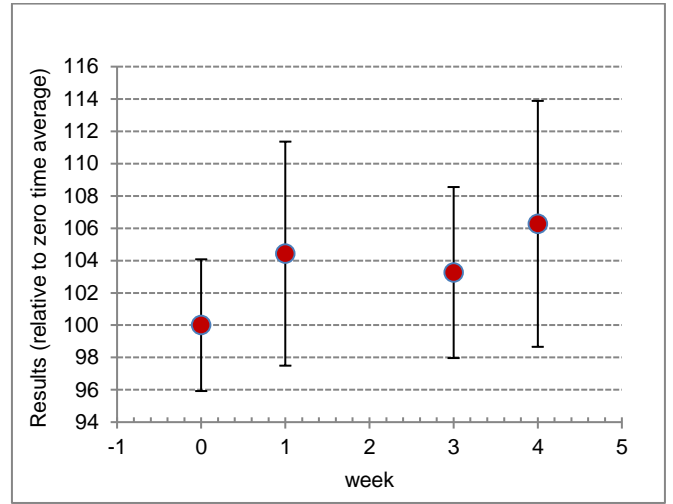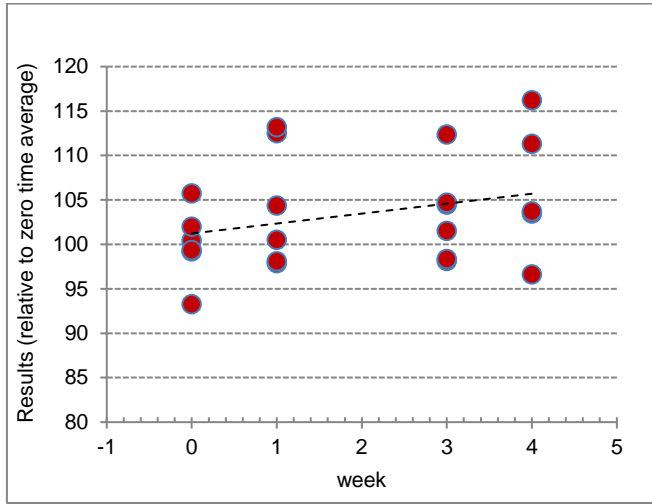


**Figure A2.19.** STS plot for Se at 18 °C



**Figure A2.20.** STS plot for Se at 60 °C



**Figure A2.21.** STS plot for Zn at 18 °C

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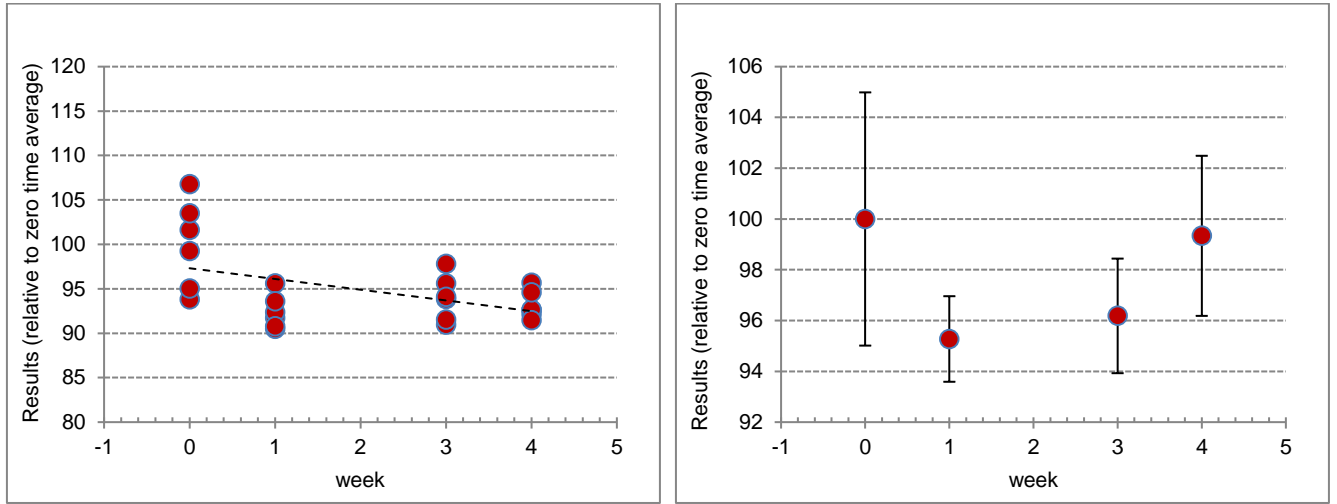


**Figure A2.22.** STS plot for Zn at 60 °C

Page 28 / 31

**TÜBİTAK**
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NATIONAL METROLOGY INSTITUTE

UME CRM
1209

### Annex 3. Graphs for Long Term Stability Studies
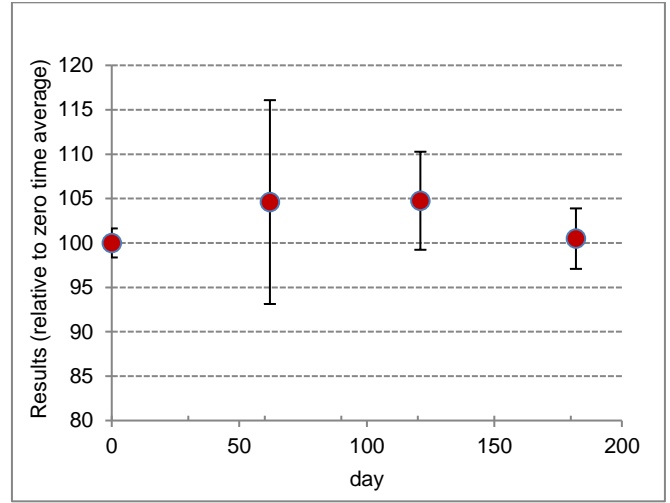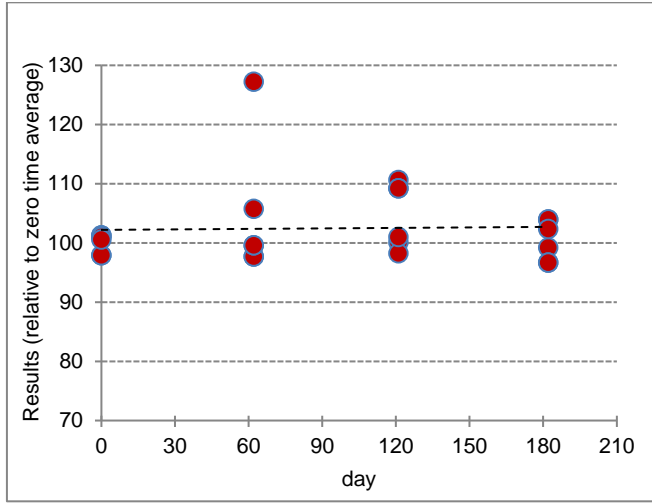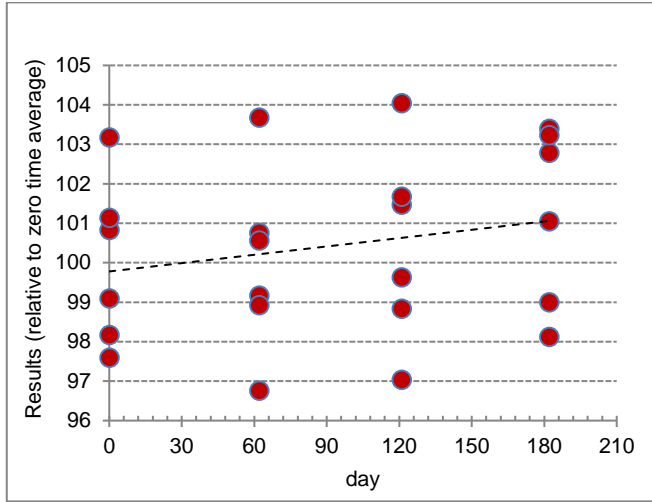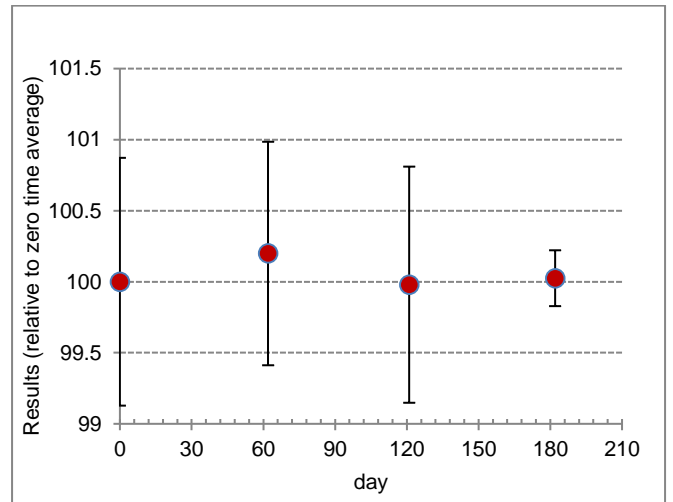


**Figure A3.1.** LTS plot for As at -20 °C
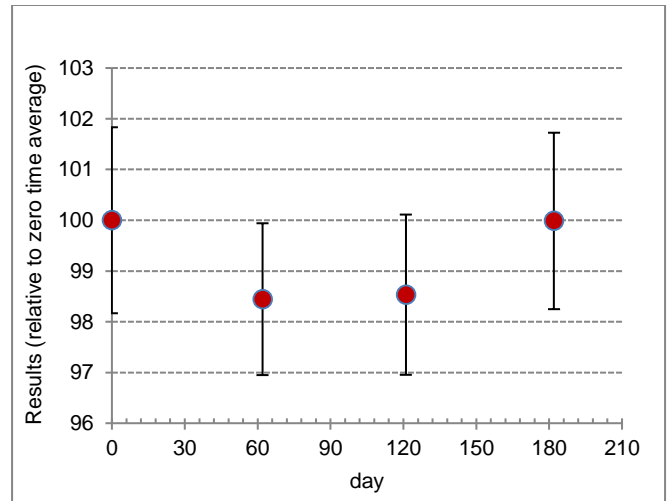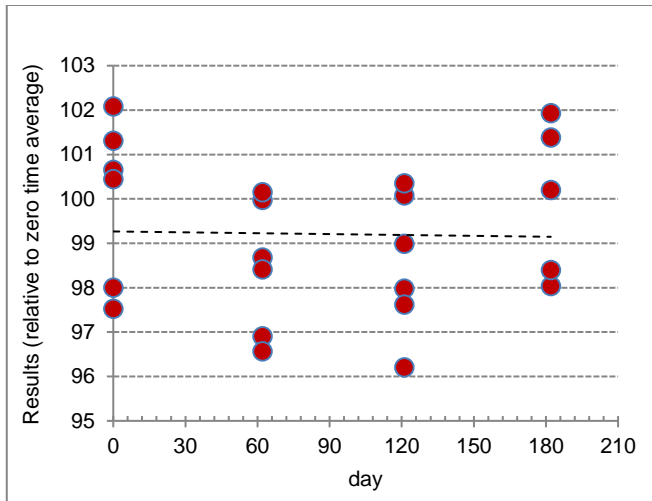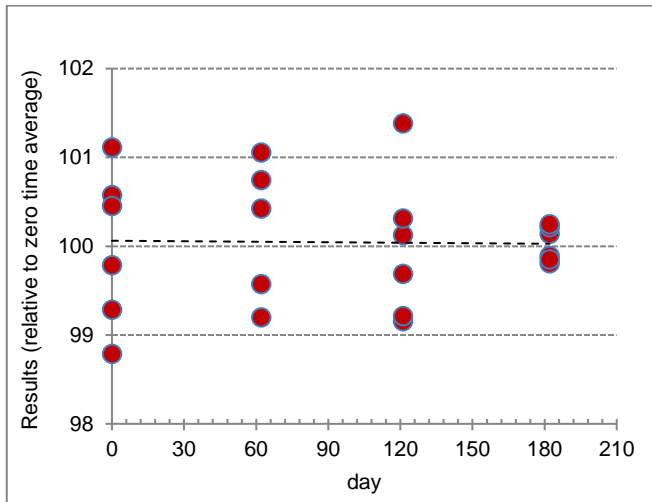


**Figure A3.2.** LTS plot for Ca at -20 °C
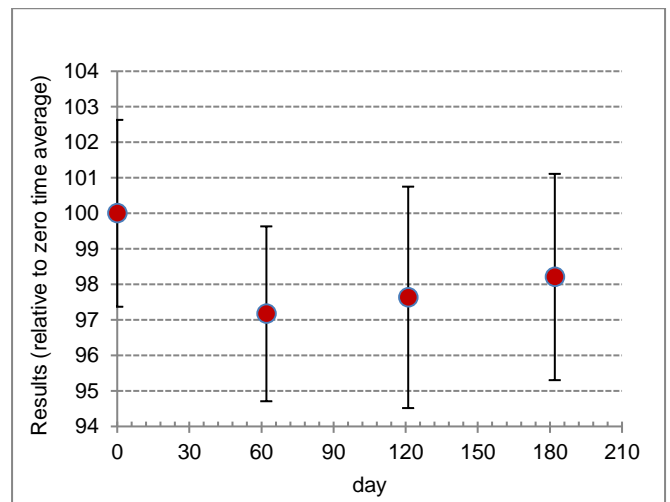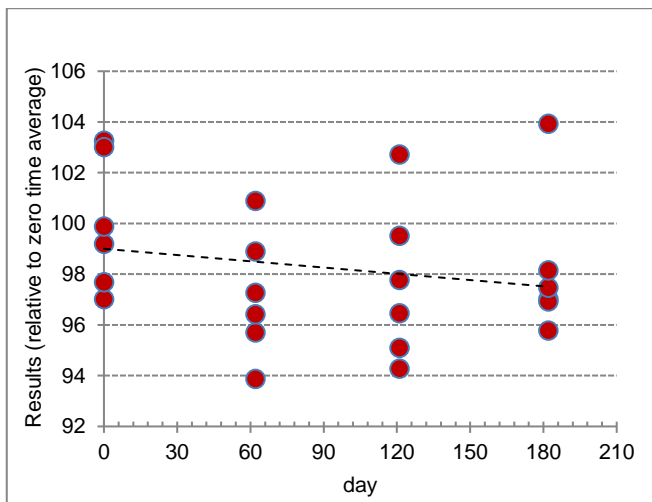


**Figure A3.3.** LTS plot for Cu at -20 °C

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**UME CRM 1209**
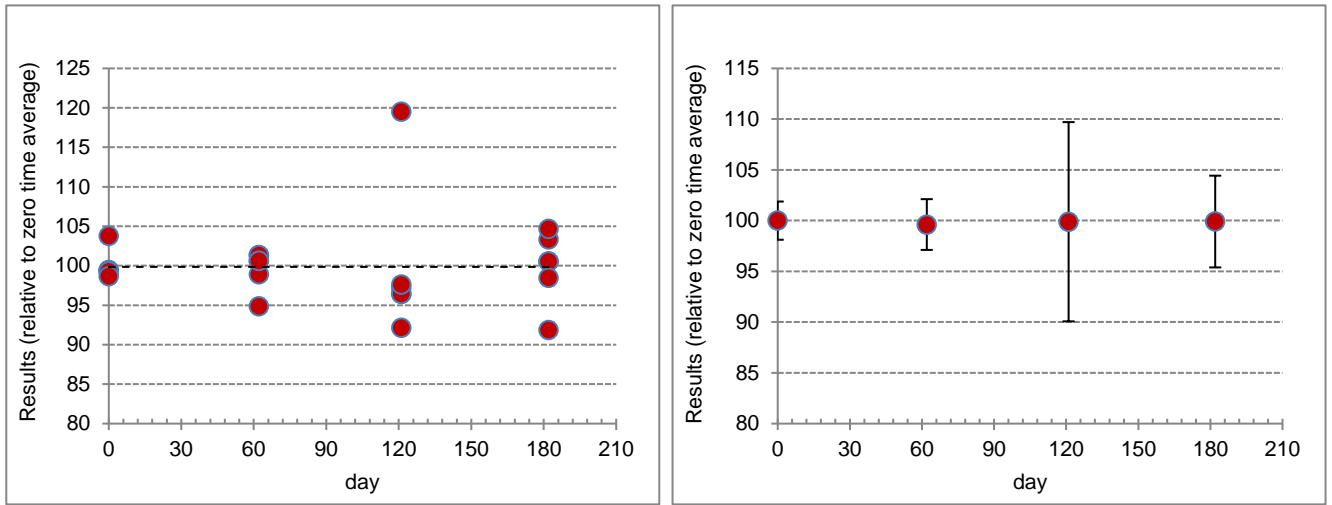


**Figure A3.4.** LTS plot for Fe at -20 °C
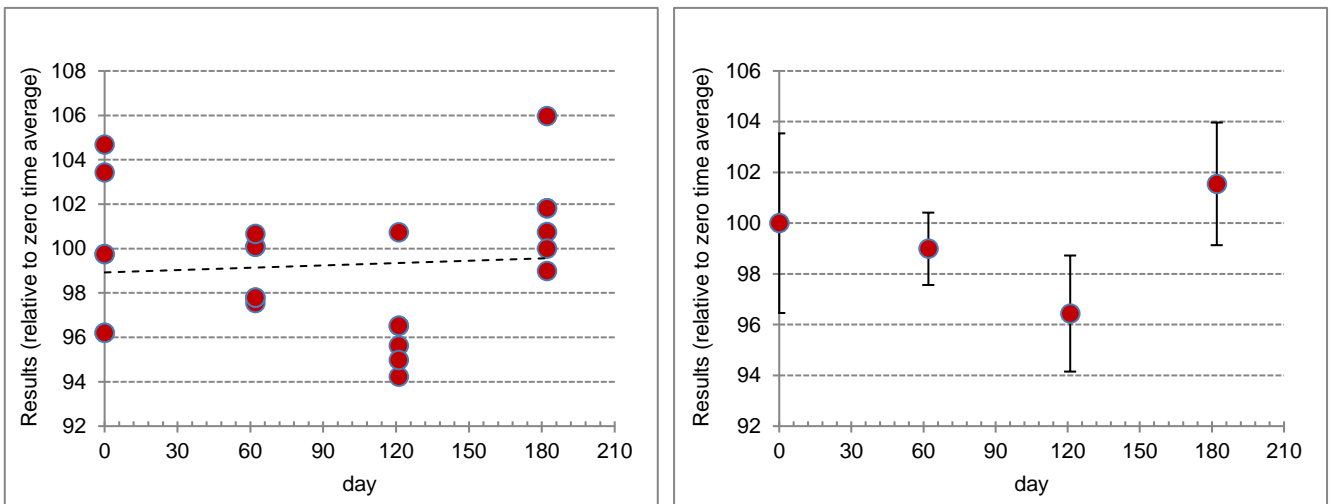


**Figure A3.5.** LTS plot for Hg at -20 °C
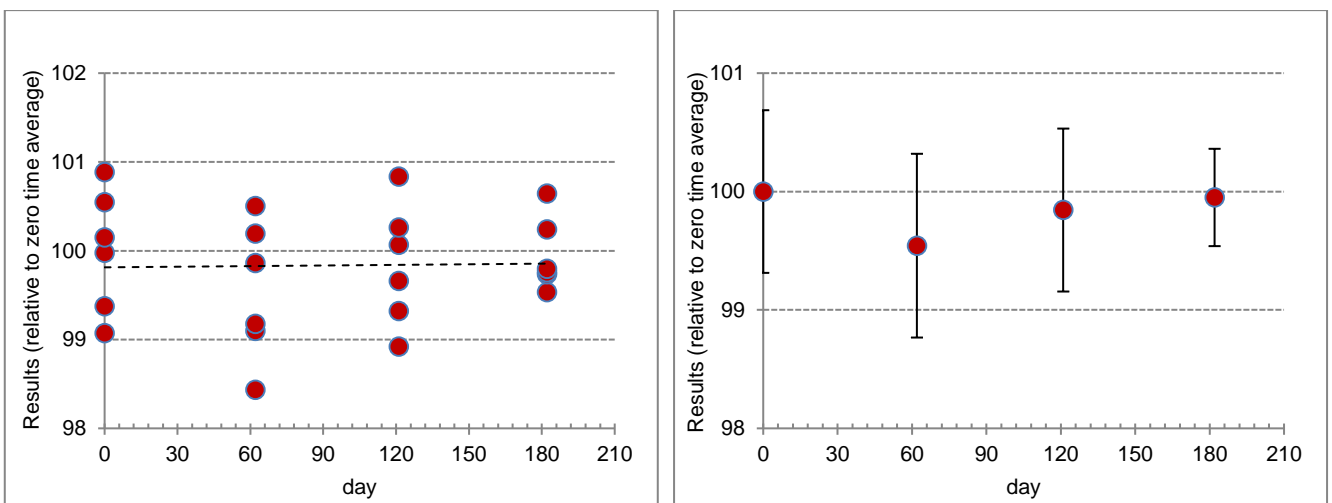


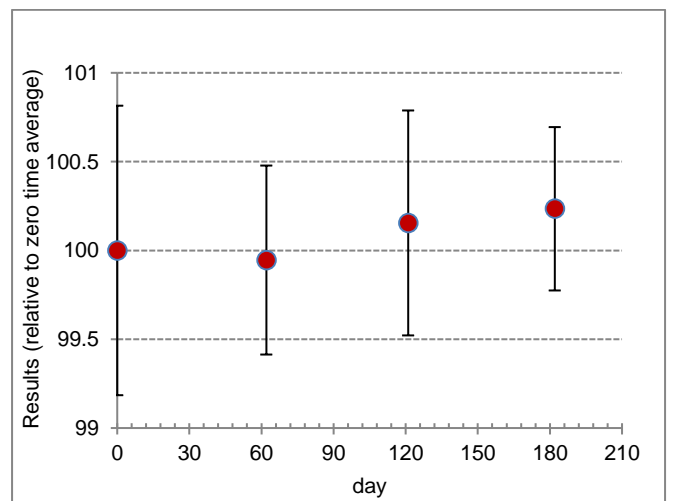**Figure A3.6.** LTS plot for K at -20 °C

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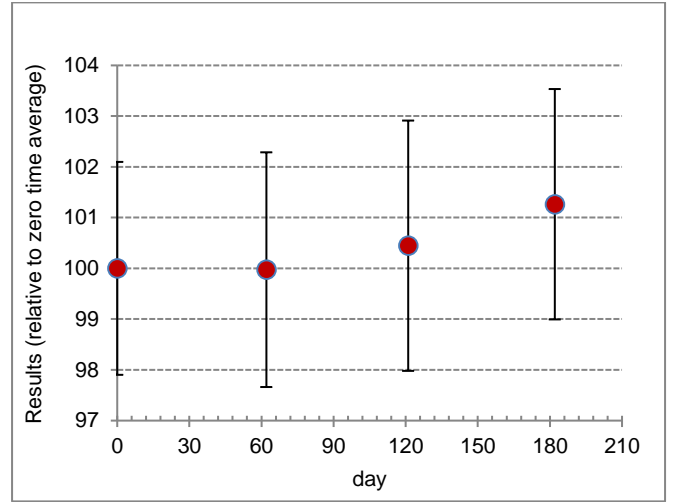


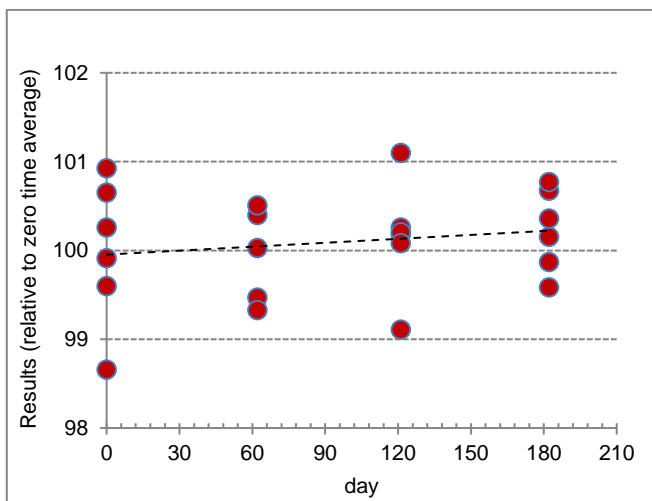**Figure A3.7.** LTS plot for Mg at -20 °C



**Figure A3.8.** LTS plot for Na at -20 °C



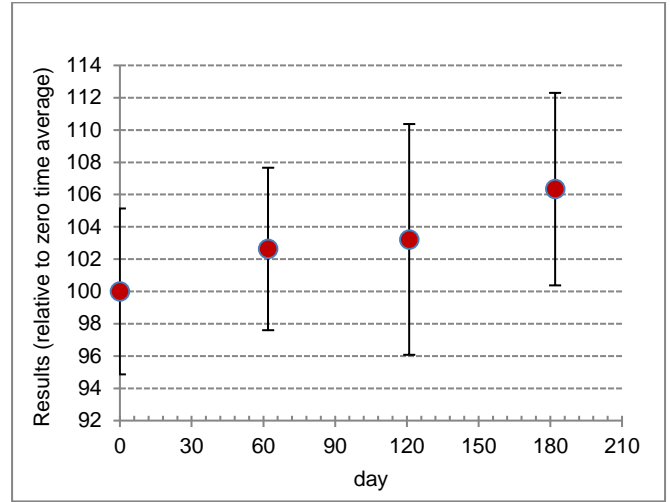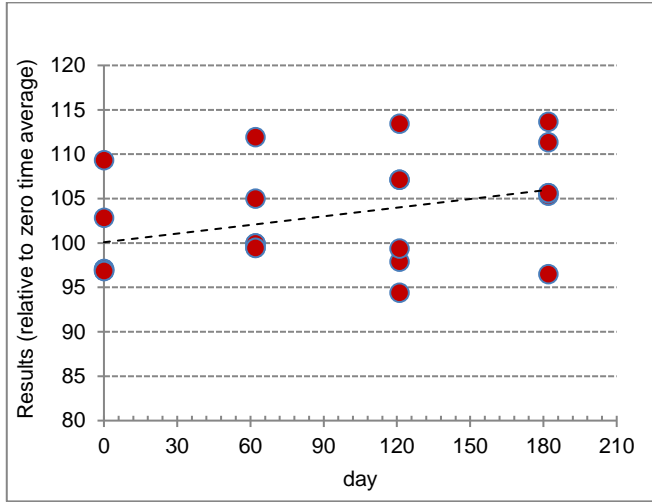**Figure A3.9.** LTS plot for P at -20 °C

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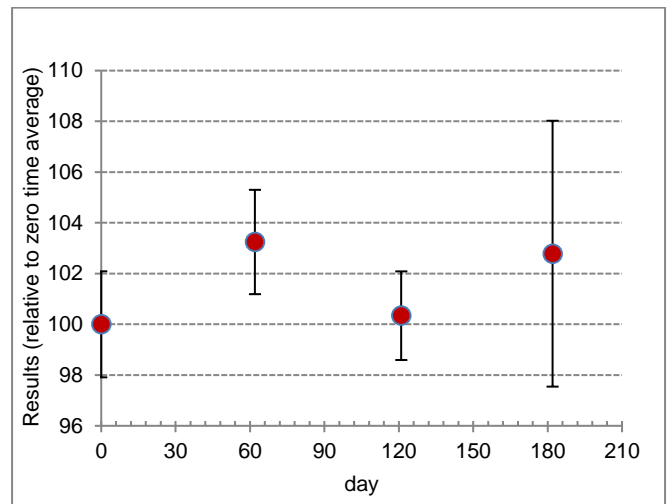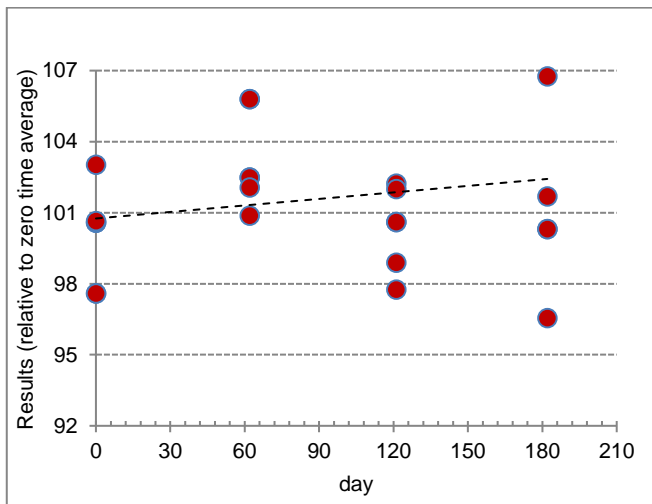
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**1209**



**Figure A3.10.** LTS plot for Se at -20 °C



**Figure A3.11.** LTS plot for Zn at -20 °C

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