Elements in Sea Water
UME CRM 1206

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ABBREVIATIONS

ANOVA	analysis of variance
α	significance level
CRM	certified reference material
GUM	Guide to the expression of uncertainty in measurement
HDPE	High density polyethylene
HR ICP-MS	High resolution inductively coupled plasma mass spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-MS/MS	Inductively coupled plasma mass spectrometry
ID-ICP-MS	Isotope dilution ICP-MS
ISO	International Organization for Standardization
k	Coverage factor
LDPE	Low density polyethylene
LTS	Long term stability
$MS_{between}$	Mean square between-bottle from ANOVA
MS_{within}	Mean square within-bottle from ANOVA
n	Number of replicates per unit
NIST	National Institute of Standards and Technology
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene floride
QQQ-ICP-MS	Triple quadrupole ICP-MS
RSD	Relative standard deviation
RSS	Random stratified sampling
s	Standard deviation
s_{bb}	Between-bottle standard deviation
SI	International System of Units
SRM	Certified reference material
STS	Short term stability
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
UME	TÜBİTAK National Metrology Institute of Turkey, Turkey
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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ABSTRACT

This report describes the production of UME CRM 1206, a sea water material certified for the mass fraction of Cd, Cu, Cr, Fe, Ni, Pb and Zn. The material was produced following the requirements of ISO 17034:2016 standard. The starting material was 400 L of seawater collected from the two locations in Marmara Sea (40° 46.200' N; 29° 12.956' E and 40° 31.423 N; 27° 11.333' E). The sample was acidified, spiked and filtered before filling into 250 mL low density polyethylene (LDPE) bottles which were placed into an aluminized PET/M (metallized polyethylene terephthalate) sachets. The material was sterilized by 25 kGy γ-radiation via ^{60}Co source. Between unit-homogeneity was quantified, and stability during dispatch and storage conditions were assessed in accordance with ISO Guide 35:2017. The material was characterized by using a primary measurement method (ID-ICP-MS) applied by one laboratory. Uncertainties of the characterization measurements were estimated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM). Certified values include uncertainties related to possible inhomogeneity, and instability and characterization measurements. 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.

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INTRODUCTION

Measurement capabilities have become important as water matrices need to be evaluated for compliance with the countries' own quality standards. It is well known that systematically monitoring useful parameters such as contaminants, and keeping them under control directly affect the quality of life. Besides, the importance of air and soil quality, water quality is also quite important for Earth. Understanding the dynamics of ocean life has become crucial for over the last decade as oceans occupy about two-thirds of Earth (70%) and they are the main reservoir of CO_2 and have a notable role in the global carbon cycle [4,5]. Countries having coasts have to follow well established regulations to help those mechanisms and keep mostly environmental pollution under control by performing routine analyses. However, as the necessity of monitoring trace elements in seawater is well established, the challenges of these measurements have raised the topic for the last few decades. Thus, laboratories performing sampling and tests in this field regulated by respective regulations, need matrix certified reference materials (CRMs) for appropriate quality controls.

The production and certification of UME CRM 1206 in accordance with ISO 17034 [1] and ISO Guide 35:2017 [2] was performed using TÜBİTAK UME infrastructure. Uncertainties of the characterization measurements were estimated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [3]. Target concentration level of each analyte was decided to meet field laboratories' needs, and based on the absence of similar certified reference materials in the market. This CRM was produced to be used by laboratories who monitor parameters defined by legislations and by research laboratories for method development and validation and quality control.

PARTICIPANTS

Sampling, processing the raw material (except sterilization), homogeneity, stability and characterization measurements were performed by experts in TÜBİTAK UME using existing infrastructure. The participants involved and their contributions to the project are presented in Table 1.

Table 1. The laboratory/organisations and their contributions

Laboratory/Organization	Work Package
TÜBİTAK UME TÜBİTAK Gebze Yerleşkesi, Barış Mahallesi Dr. Zeki Acar Cad. No.1, 41470, Gebze, Kocaeli, Turkey	Project management, Material processing and Certification
GAMMA PAK Sterilizasyon Sanayi ve Ticaret A.Ş. Organize Sanayi Bölgesi Gazi Osman Paşa Mahallesi 2. Cad. No. 6, 59500, Çerkezköy, Tekirdağ, Turkey	Material Processing (Sterilization)

MATERIAL PROCESSING

The sampling of the raw material for sea water reference material was performed by TÜBİTAK Marmara Research Center - Environment and Cleaner Production Institute Research Vessel on August 09, 2018 and August 15, 2018 in the Marmara Sea by marine scientists accompanied by UME researchers. The samples are pumped using immersible pump into 10 L precleaned high density polyethylene drums. Raw material for candidate certified reference materials were collected from two different location of the Marmara Sea (40° 46.200' N; 29° 12.956' E and 40° 31.423 N; 27° 11.333' E). Approximately 100 L sea water from each location was sampled in to precleaned drums and all the drums were acidified to pH 1.6 with sub boiled HNO_3 and stored at 4 °C until the further processing steps take place.

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In order to determine natural levels of these two different batch, subsampling was performed from each drums (20 sampling) into precleaned falcon tubes in the amount of 40 mL. Analysis of these samples were performed by ICP-MS/MS for all target elements. Raw material was below the target levels of candidate reference material. However as natural level of zinc was lower in the second batch, this one was preferred to be used in the CRM production and appropriate mass of target elements ware spiked using series of NIST 3100 into raw materials.

Whole processing of reference materials including spiking, homogenization and filling had been performed in ISO 6 Clean Chemical Laboratory at TÜBİTAK UME. Two 114 L HDPE drums, the PTFE/PVC tubes and air acid pump (PVDF) used for homogenization were washed with series of different steps. First step was filling the drums with distilled water and run the homogenization system for several times. Drums were left for 5 days with full of this water. In the second step, the drums were filled with an in-house prepared solution ~5.0% (v/v) of concentrated HNO_3 (Emsure grade, Merck) and the same procedure was applied as described in the first step. In the third step, after the drums were flashed with distilled water filled by 1.0% sub-boiled HNO_3 containing 100 ng/mL Au solution and left for three days after running the homogenization system and subsequently rinsed with extensive amount of de-ionized water (PURELAB Flex, 18.2 $M\Omega\cdot cm^{-1}$). Prior to pumping of seawater into drums, the line of the pump rinsed with several liters of seawater.

Cleanness of 250 mL low density polyethylene (LDPE) of regarding 10 target elements were checked in 62 bottles which were chosen randomly one bottle from each packets. The bottles were filled fully with 2.0% (v/v) sub-boiled HNO_3 containing 100 ng/L Au solution and left for two days. The measurements of these leaching solutions were performed by HR-ICP-MS (Thermo Finnigan, Element 2, Germany). The results showed that the levels of trace elements in the leaching solutions were significantly high regarding the target levels of reference material which may lead to inhomogeneity for Zn and relatively high homogeneity uncertainty for some certain elements. Therefore, a cleaning procedure was applied on a small group of bottle and re-measured by HR-ICP-MS to be sure that the background levels minimized. After this step, developed cleaning procedure was applied to the whole batch.

Cleaning procedure includes following stages:

1) Rinsed by ultrapure water three times and filled by 2.0% (v/v) sub-boiled HNO_3 containing 100 ng/L Au solution.

2) Left for one week.

3) Rinsed by ultrapure de-ionized water and filled by it.

4) Left for one week.

5) Dried in ISO 4 laminar flow cabins at ISO 6 clean laboratory.

Approximately 100 L raw material was transferred into 114 L HDPE drum and the material was homogenized for four hours after spiking and finally whole water was filtered from one drum to another via 0.8/0.2 μm (Pall Corp, Supor® Membrane, AcroPackTM 1000, PN 12992) which also used for removing bacterial retention. Filling of bottles were performed manually in ISO 6 Clean laboratory. A total subsequently labeled 400 bottles were filled (250 mL for each) and dispatched for gamma irradiation at 25 kGy. All the bottles were placed into aluminized PET sachets after gamma irradiation and stored at + 4 °C.

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HOMOGENEITY

Establishment the equivalence between the various units is a key requirement for a certified reference material according to ISO 17034 standard. Although the uncertainty of within-unit inhomogeneity is not taken into account in the calculation of value assignment, it is essential to establish it in order to represent the whole unit. Therefore, within-unit inhomogeneity has to be determined to define the minimum sample intake from a unit.

The homogeneity of a batch should be determined by number of selected units corresponds to approximately cubic root of total number of the produced batch. This number should not be less than ten. Based on this, 10 bottles were selected using random stratified sampling scheme (RSS) covering whole batch for the between unit homogeneity study (Unit No: 20, 78, 117, 149, 168, 221, 267, 298, 339, and 381). This was done by dividing whole batch into equal fragments, and a representative unit was randomly selected from each one so that the whole batch was covered. Three independent sub sampling were taken from each unit. Triethylamine assisted $Mg(OH)_2$ coprecipitation combined by isotope dilution mass spectrometry (TEA/$Mg(OH)_2$-ID^{3}MS) method [6,7] was used for the between unit homogeneity measurement by ICP-MS/MS (8800 QQQ-ICP-MS, Agilent Technologies, Japan) for Cd, Cr, Cu, Fe, Ni, Pb and Zn. Cold vapor-double IDMS by HR-ICP-MS and matrix matched external calibration hydride generation by ICP-MS/MS was used for the analysis of Hg and As, respectively. Therefore, the measurements were performed under the high precision conditions and all sub-samples were introduced to the ICP-MS such a randomized order so that is possible to differentiate the analytical drift from a trend in production filling sequence.

The data set for all parameters were evaluated statistically in the following order:

a) Regression analyses to evaluate potential trends in each analytical run at 95% and 99% confidence level. It is observed that there was significant analytical trend at 95% confidence level during the measurements of As, Pb and Zn.

As the analytical sequence and the unit numbers were not correlated, mathematical correction of the dataset for the significant analytical trend of the measurements was performed using the Equation (1) where trends significant at least 95% confidence level:

$$C_{Corrected} = C_{Measured} - b \cdot i \qquad (1)$$

where;

b : slope of the linear regression,

i : position of the result in the analytical sequence.

b) Regression analyses to evaluate potential trends in filling sequence order at 95% and 99% confidence level.

c) Datasets were checked for individual results and unit outliers at 95% and 99% confidence level using Grubbs outlier test.

d) As the unimodal distribution of data is a prerequisite in order to apply the statistical evaluation one-way analysis of variance (ANOVA), the distribution of individual results were checked for both normal distribution via normal probability plot and unimodality with histogram.

For all analytes, the individual results followed an approximately normal and unimodal distribution.

Analysis of Variance is a statistical tool used to estimate uncertainty contribution from homogeneity of the materials. Uncertainties of homogeneity between units were evaluated with one way ANOVA.

The Equation (2) is used for repeatability of method (s_{wb}) and Equation (3) is used for the calculation of standard deviation between units (s_{bb}).

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$$s_{wb} = \sqrt{MS_{within}} \tag{2}$$

where;

MS_{within}: mean of square of variance within the unit,

s_{wb} : "s" of the method as long as sub samples represent the whole unit.

$$s_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \tag{3}$$

where;

$MS_{between}$: mean of square of variance between units,

n : number of replicates per unit.

The occurrence of $MS_{between} < MS_{within}$ for some elements demonstrates that material heterogeneity is smaller than that can be detected by the analytical methodology used. In these cases, since s_{bb} cannot be calculated, u_{bb}^* is calculated as heterogeneity contributing to uncertainty including method repeatability using Equation (4).

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

$\nu_{MSwithin}$: degree of freedom of MS_{within}.

With the exception of iron and chromium, method repeatability (s_{wb}), between-unit standard deviation (s_{bb}) and u_{bb}^* were evaluated using the Equations (2), (3) and (4) as described above. In the cases for presence of outlying bottle mean, an alternative data evaluation was necessary in able to handle more proper homogeneity assessment. Since any similar outlying values were observed in the data set consisting of short term stability studies (14 unit) and long term stability studies (14 unit) for Fe and Cr, the use of homogeneity bottles more than once for analysis of other analytes might be the reason of cross-contamination of the outlying bottle. However, as there was no certain evidence for this probability, between unit homogeneity was modeled as a rectangular distribution and following equation was applied for rectangular standard uncertainty (u_{rect}) of homogeneity.

$$u_{rect} = \frac{|Outlier\ value - Average\ value|}{\sqrt{3}} \tag{5}$$

The data for evaluation of the homogeneity study is presented in Table 2.

The results of evaluation of the between-unit variation are given in Table 3 and the highest value between s_{bb}, u_{bb}^* and u_{rect} was assigned as u_{bb}, uncertainty of homogeneity.

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Table 2. Summary of technically evaluated data set of homogeneity study

Element	Significance of the trend on a 95% confidence level		Outlier (95% confidence level)		Distribution
	Analytical Sequence	Filling Sequence	Individual Results	Unit	Individual results
As	+	-	1	-	Normal/Unimodal
Cd	-	-	-	-	Normal/Unimodal
Cr	+	-	1	1	Normal/Unimodal
Cu	-	-	2	-	Normal/Unimodal
Fe	-	-	-	1	Normal/Unimodal
Hg	-	-	1	-	Normal/Unimodal
Ni	-	-	1	-	Normal/Unimodal
Pb	+	-	-	-	Normal/Unimodal
Zn	+	-	-	-	Normal/Unimodal

Table 3. Results of homogeneity study

Element	$s_{wb,rel}$, %	$s_{bb,rel}$, %	$u^*_{bb,rel}$, %	$u_{rec,rel}$, %	$u_{bb,rel}$, %
As	1.93	$MS_{between} < MS_{within}$	0.56	-	0.56
Cd	1.17	$MS_{between} < MS_{within}$	0.34	-	0.34
Cr	0.95	2.00	0.28	2.87	2.87
Cu	2.21	0.78	0.64	-	0.78
Fe	1.59	3.46	0.46	5.27	5.27
Hg	5.22	$MS_{between} < MS_{within}$	1.52	-	1.52
Ni	0.33	0.18	0.09	-	0.18
Pb	0.27	$MS_{between} < MS_{within}$	0.08	-	0.08
Zn	1.62	1.62	0.47	-	1.62

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

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Establishment of within unit homogeneity was necessary to demonstrate that the mass fraction of analyte in each individual aliquots of sample represents the same mass fraction of analyte in whole unit. In this respect, the within-unit heterogeneity is closely correlated to the minimum sample intake which is the minimum amount of sample that is representative for the whole unit, and should be taken as the amount in the analysis. The certified value within its stated uncertainty is guaranteed when the sample sizes equal to or above the minimum sample intake used for analysis. This material is not expected to have any significant within-unit heterogeneity as it is a solution. Nevertheless, minimum sample intake can be taken as the amount that homogeneity study has been performed which was 5 mL for Cd, Cr, Cu, Fe, Ni, Pb, Zn and 1 mL for As.

STABILITY

The stability of the units 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 [8], and the uncertainty contribution of stability of material has been calculated as described by Linsinger et al. [9]. The bottles used for stability analysis were selected using RSS. Stability measurements were performed by TEA/Mg(OH)$_2$-ID^{3}MS method using ICP-MS/MS for Cd, Cr, Cu, Fe, Ni, Pb and Zn, cold vapor-double IDMS by HR-ICP-MS for Hg and matrix match external calibration hydride generation by ICP-MS/MS for As. In order to obtain adequate uncertainty related to stability of the material, three replicates for each unit were analyzed. All the analysis were performed in a randomized unit/replicate order in order to be able distinguish any analytical trend from the material degradation that may occur within storage period.

I. Short Term Stability

For the short term stability (STS) measurements, according to the designed test temperatures and time points, 14 units were selected by RSS from the whole batch produced. The tests were performed for one, two and four weeks at pre-defined test temperatures, +18 °C and +60 °C. Two units for each time period were used. The bottles kept at test temperatures for defined time periods were transferred to reference temperature, +4 °C where "reference" units were already kept. For Zn, 30 °C and 40 °C temperatures were also studied as the slopes of regression lines was significantly different from zero at 60 °C.

The evaluation of stability measurements were carried out for each temperatures, and Grubbs' test at confidence levels of 95% and 99% were applied, separately. One outlier for each of chromium, mercury and lead was detected at 95% confidence level for the data set of 18 °C. All the outliers were retained in statistical analysis as any technical reason could be detected.

In the evaluation of dataset, the mass fractions versus time were plotted and the regression lines were calculated to check for significant trends indicating possible changes in the concentrations of the analytes by time (*regression analysis*). The calculated slopes of the regression line were tested using two-tailed *t*-test using $t_{\alpha,df}$ as the critical *t* value at $\alpha = 0.05$ (95% confidence level). The results obtained for STS measurements are summarized in Table 4, and graphical representations of data are shown in Annex 2. Mercury showed a degradation at 18 °C. On the other hand, as mercury was also found to be unstable for long term, further investigation was not conducted for transporting conditions for mercury. Except Zn, analytes were found stable in exposure to 60 °C for four weeks. Thus, additional test temperatures were investigated only for Zn. It was found that degradation of Zn was statistically

observed at 40 °C after two weeks as seen in Figure Therefore, uncertainty related to STS of material was calculated for two weeks at 40 °C Zn and 60 °C for all the remaining analytes. Uncertainty contribution resulting from STS was calculated by applying Equation (6). Uncertainties was calculated for two week exposure period.

$$u_{sts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \times t \qquad (6)$$

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 (2 week).

Table 4. Summary of results for short term stability test

Analyte	$u_{sts,rel}$* (%)				Significance of the trend on a 95% confidence level				Number of individual outlying results at 95% confidence level			
	18 °C	30 °C	40 °C	60 °C	18 °C	30 °C	40 °C	60 °C	18 °C	30 °C	40 °C	60 °C
As	0.74	-	-	0.66	No	N.A	N.A	No	-	N.A	N.A	-
Cd	0.30	-	-	0.29	No	N.A	N.A	No	-	N.A	N.A	-
Cr	0.17	-	-	0.21	No	N.A	N.A	No	1	N.A	N.A	-
Cu	0.15	-	-	0.24	No	N.A	N.A	No	-	N.A	N.A	-
Fe	0.46	-	-	0.28	No	N.A	N.A	No	-	N.A	N.A	-
Hg	2.21	-	-	4.20	Yes	N.A	N.A	Yes	1	N.A	N.A	1
Ni	0.09	-	-	0.08	No	N.A	N.A	No	-	N.A	N.A	-
Pb	0.12	-	-	0.09	No	N.A	N.A	No	1	N.A	N.A	-
Zn	0.45	0.51	1.13	14.6	No	No	Yes	Yes	-	1	-	-

II. Long Term Stability

The uncertainty related to the degradation during the storage of candidate CRM at 18 °C has been studied by performing the long term stability (LTS) measurements. Two units for each storage time period (0, 2, 4, 6, 9, 12 and 15 months) and three replicates from each unit were measured for LTS analysis. The reference temperature has been set to 4 °C, and each unit was transferred to reference temperature at the end of the period spent at 18 °C.

All the data obtained in LTS study was screened for outlier values using single Grubbs test at 95% and 99% confidence levels. Two outlying individual results for Cr, one outlying individual result was found for Cu, Fe and Zn. The outliers for Cu and Fe were belong to same replicate of the same unit and as the samples were prepared in same vial, it was decided that cross contamination took place in sample

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preparation as other two replicates of the unit were compatible with the rest of the data. Therefore, these outliers were removed in statistical evaluation. The outlier detected in the data set of Zn was also removed since the value was 1.5 higher than the average of all the data obtained in the LTS and also such a high value was not observed in the data set of homogeneity, short term stability and characterization.

On the other hand, one of the outliers belongs to chromium data was removed with other two replicates of unit no 142 as the second outlier retained for the calculation of uncertainty. The detailed investigation showed that even the outlier replicate was removed, the average mass fraction for unit no 142 was actually significantly higher than the rest of the bottle averages. As the uncertainty resulting from homogeneity for chromium was estimated in a way of covering bottle outliers, this data set for unit no 142 was removed completely in order to eliminate the overestimation of uncertainty.

The slopes of regression line calculated on the graphs plotted against time and mass fractions were evaluated to determine if any significant trend by time exists using t-test at 95% confidence level. For all elements except Hg, the slopes of the regression lines were not significant at laboratory temperature (18 ± 2) °C. LTS measurements showed that the storage conditions and/or packaging was not proper for the stability of mercury. Therefore, the certification of mercury was cancelled.

Uncertainty contribution of long term stability, u_{lts}, is calculated using Equation (7). The shelf life of UME CRM 1206 was determined as 12 months after sales date at 18 °C, and the uncertainty contribution of LTS was calculated based on this period (

Table 5). Graphical representation of the data related to each analyte is given in Annex 3. Additionally, post-certification monitoring of the stability is going to be done in certain periods.

$$u_{lts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \times t \tag{7}$$

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 suggested shelf life at 18 °C (12 months).

Table 5. Summary of results for long term stability study

Analyte	$u_{lts,rel}$* (%)	Significance of the trend on a 95% confidence level	Number of individual outlying result at 95% confidence level	Number of individual outlying result at 99% confidence level
As	1.38	No	-	-
Cd	0.56	No	-	-
Cr	2.64	No	2	-
Cu	0.51	No	1	-
Fe	0.90	No	1	-
Ni	0.12	No	-	-
Pb	0.28	No	-	-
Zn	1.34	No	1	-

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CHARACTERIZATION

The use of a reference method by a single laboratory is one of the options for the characterization of a candidate CRM according to ISO 17034 [1]. This approach was applied throughout this study by employing a primary reference method for certified values. For this purpose, combination of triethylamine assisted $Mg(OH)_2$ co-precipitation and triple isotope dilution mass spectrometry (TEA/$Mg(OH)_2$-ID³MS) were developed and validated as described by Ari et al. [6,7]. Characterization measurements were conducted by using three randomly selected bottles with four independent sub-samples per bottle. Measurement uncertainties for each analyte was calculated by bottom up approach using Gum Workbench® software [10]. The calculation of the results was also performed using MS Excel to cross validate the calculations. The summary of characterization results is presented in Table 6.

Table 6. Summary of characterization measurements (n = 12)

Element	Value	U_{char} (k=2)
Cd	0.4327	0.0071
Cu	1.018	0.012
Cr	2.442	0.033
Fe	12.732	0.062
Ni	4.568	0.037
Pb	1.068	0.016
Zn	8.521	0.075

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 between units 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 and calculated using the following equation:

$$U_{CRM} = k\sqrt{u_{char}^2 + u_{bb}^2 + u_{sts}^2 + u_{lts}^2} \tag{8}$$

The uncertainty of the certified value was expanded by a coverage factor of $k = 2$ for a confidence level 95%. Certified values and associated uncertainties are given in Table 7, and the relative uncertainty contributions to the combined uncertainty are given in Table 8.

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Table 7. Certified value and uncertainty components

Element	Certified Value (µg/kg)	U_{CRM} (µg/kg, $k = 2$)	$U_{CRM,rel}$ (%, $k = 2$)	$u_{char,rel}$ (%)	$u_{bb,rel}$ (%)	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)
Cd	0.433	0.010	2.3	0.82	0.34	0.29	0.56
Cr	2.44	0.20	8.2	0.68	2.87	0.21	2.64
Cu	1.019	0.023	2.3	0.57	0.78	0.24	0.51
Fe	12.7	1.4	11	0.24	5.27	0.28	0.90
Ni	4.568	0.043	0.94	0.41	0.18	0.08	0.12
Pb	1.068	0.017	1.6	0.74	0.08	0.09	0.28
Zn	8.52	0.42	4.9	0.44	1.62	1.13	1.34

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

Element	u_{char} (%)	u_{bb} (%)	u_{sts} (%)	u_{lts} (%)
Cd	56.6	9.7	7.1	26.4
Cr	2.9	52.4	0.3	44.3
Cu	25.9	48.5	4.6	20.7
Fe	0.2	97.0	0.3	2.8
Ni	76.1	14.7	2.9	6.5
Pb	85.5	1.0	1.3	12.2
Zn	3.3	44.6	21.7	30.5

INFORMATIVE VALUE

Although the homogeneity and stability studies were performed for arsenic, due to the lack of alternative analytical method to ICP-MS that can be used for characterization of total arsenic in seawater, it has been provided as informative value (Table 9). The characterization of As has been performed by using in house validated method, matrix matched external calibration by ICP-MS/MS. Characterization measurements were performed using three units (No 69, No 167 and No 332) with two independent sub-sample per each. Three completely independent measurements (independent calibration plots and samples) were performed in three different days. Accuracy of the method has been confirmed by two different matrix certified reference material.

Table 9. Percent contribution of each uncertainty component to U_{CRM} for As

Element	Informative Value (µg/kg)	U_{CRM} (µg/kg, $k = 2$)	$U_{CRM,rel}$ (%, $k = 2$)	$u_{char,rel}$ (%)	$u_{bb,rel}$ (%)	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)
As	2.52	0.10	4.0	0.86	0.56	0.74	1.38

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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. The source of traceability of standards used in the measurements are presented in Table . IUPAC values of isotopic ratios were used in ID-ICP-MS calculations.

Table 10. The standards used for traceability of the certified values

No	Name of Standard	Standard No	Traceability
1	As Standard Solution	NIST SRM 3103a	NIST
2	Cd Standard Solution	NIST SRM 3108	NIST
3	Cr Standard Solution	NIST SRM 3112a	NIST
4	Cu Standard Solution	NIST SRM 3114	NIST
5	Fe Standard Solution	NIST SRM 3126a	NIST
6	Ni Standard Solution	NIST SRM 3136	NIST
7	Pb Standard Solution	NIST SRM 3128	NIST
8	Sb Standard Solution	NIST SRM 3102a	NIST
9	Zn Standard Solution	NIST SRM 3168a	NIST
10	Pb Isotopic Reference Material	SRM 981	NIST
11	Pb Isotopic Reference Material	SRM 982	NIST
12	Pb Isotopic Reference Material	SRM 991	NIST

INSTRUCTIONS FOR USE

Before use, the bottle should be kept in the laboratory environment to equilibrate with room temperature and should be shaken before opening the cap to avoid a bias due to condensed water at the bottleneck. To avoid contamination, it is highly recommended that the bottle should be kept and opened in a clean environment and pipette should not be inserted into the bottle. After use, the bottle should be immediately and tightly recapped.

This material can be safely dispatched under conditions where the temperature do not exceed 40 °C for up to 2 weeks without applying any cooling elements.

Storage conditions

This material should be stored at (18 ± 4) °C in a dark and clean environment.

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 in the certificate.

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Safety precautions

The usual laboratory safety measures apply as in the case of similar powders. It is strongly recommended that the material must be handled and disposed according to the safety guidelines where applicable. Please refer to the Safety datasheet (SDS) before any use of the material.

Minimum sample intake

Minimum sample intake is 5 mL for Cd, Cr, Cu, Fe, Ni, Pb, Zn and 1 mL for As.

Use of Certified Value

For assessing the method performance, the measured values of the CRM are compared with the certified values [11]. The procedure can be described briefly as follows:

- 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 following equation:

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

- 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 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/

ACKNOWLEDGMENTS

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Authors would like to thank Prof. Dr. Sezgin Bakırdere for his support as a consultant during the project.

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REVISION HISTORY

Date	Remarks
12.02.2021	First Issue

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Annex 1. Graphs of Homogeneity Study
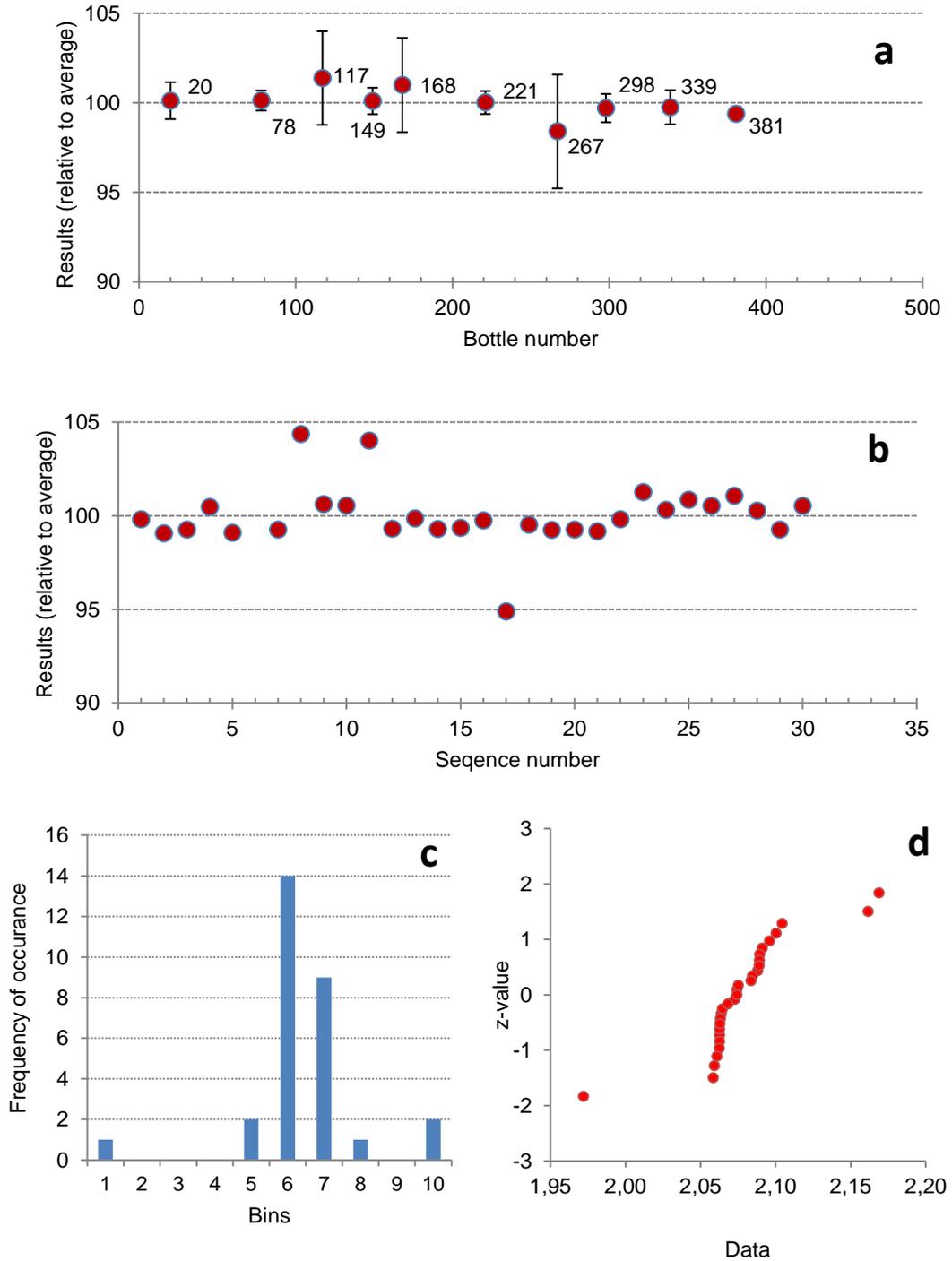
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b. Graph of analytical sequence order

c. Histogram
d. Normal Probability Plot

Figure A1.1. Homogenity graphs for As
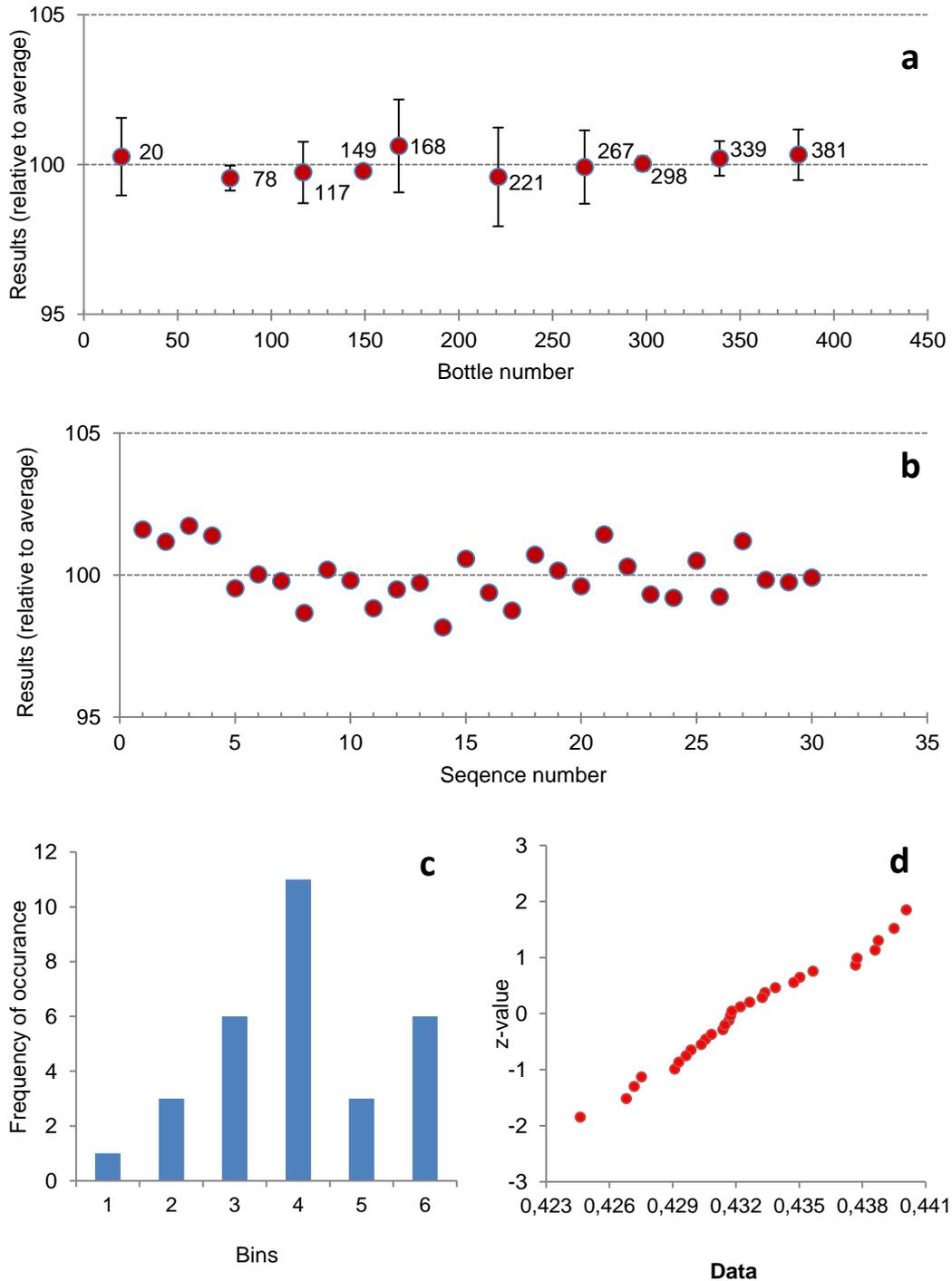
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a. Graph of filling sequence order
b. Graph of analytical sequence order
c. Histogram
d. Normal Probability Plot

Figure A1.2. Homogeneity graphs for Cd
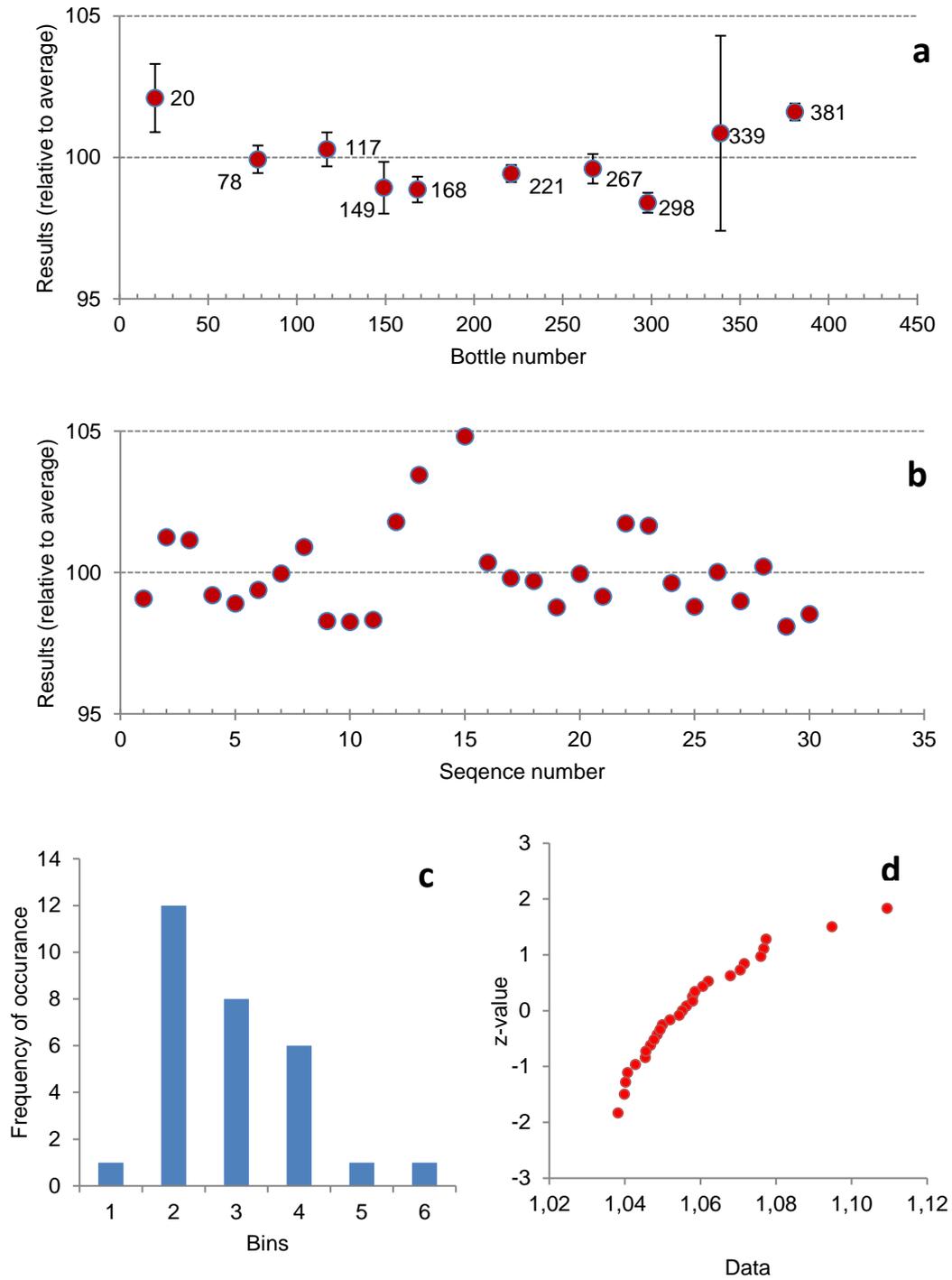
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a. Graph of filling sequence order   c. Histogram

b. Graph of analytical sequence order   d. Normal Probability Plot

Figure A1.3. Homogeneity graphs for Cu
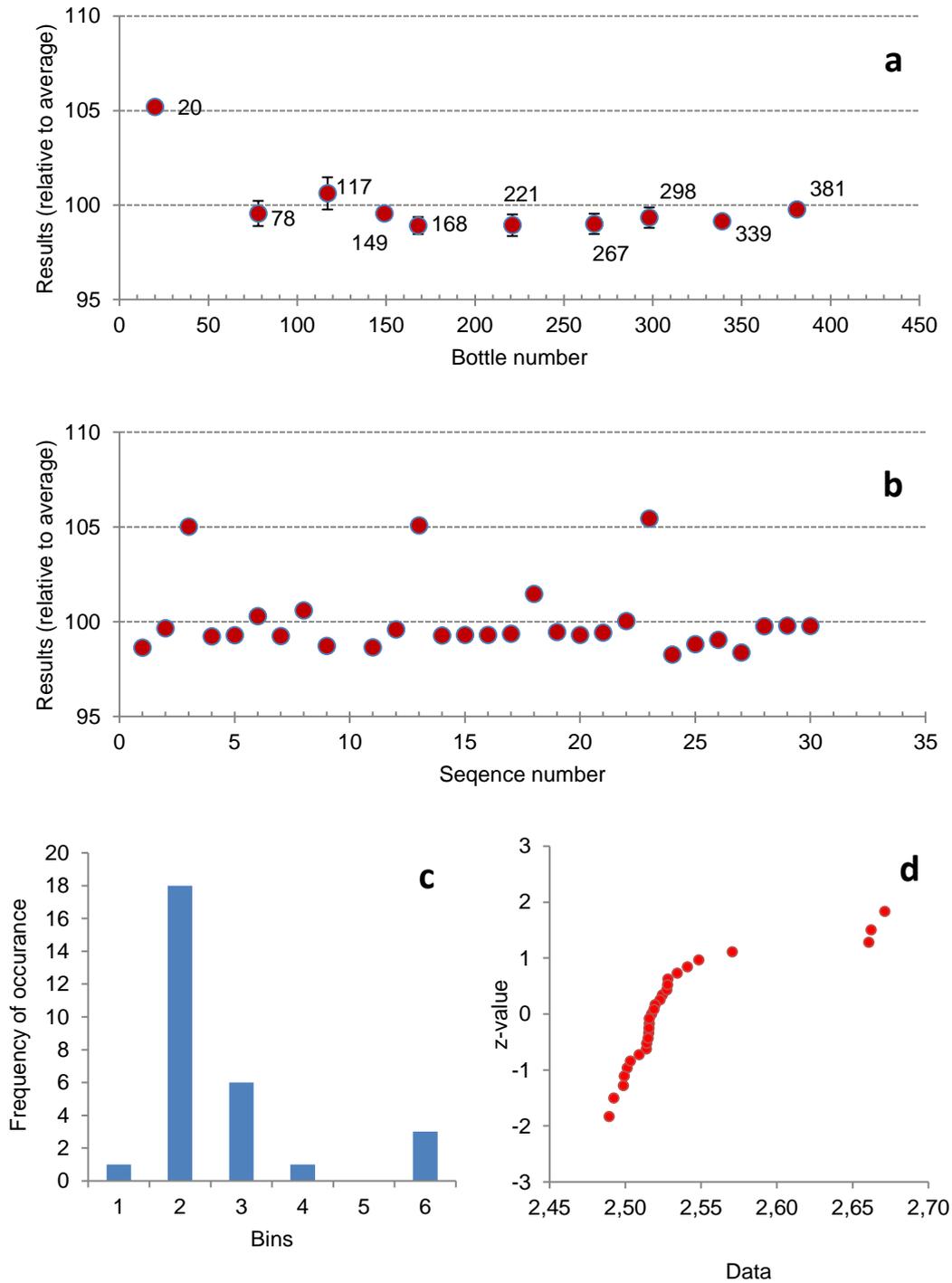
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a. Graph of filling sequence order   c. Histogram
b. Graph of analytical sequence order   d. Normal Probability Plot

Figure A1.4. Homogeneity graphs for Cr
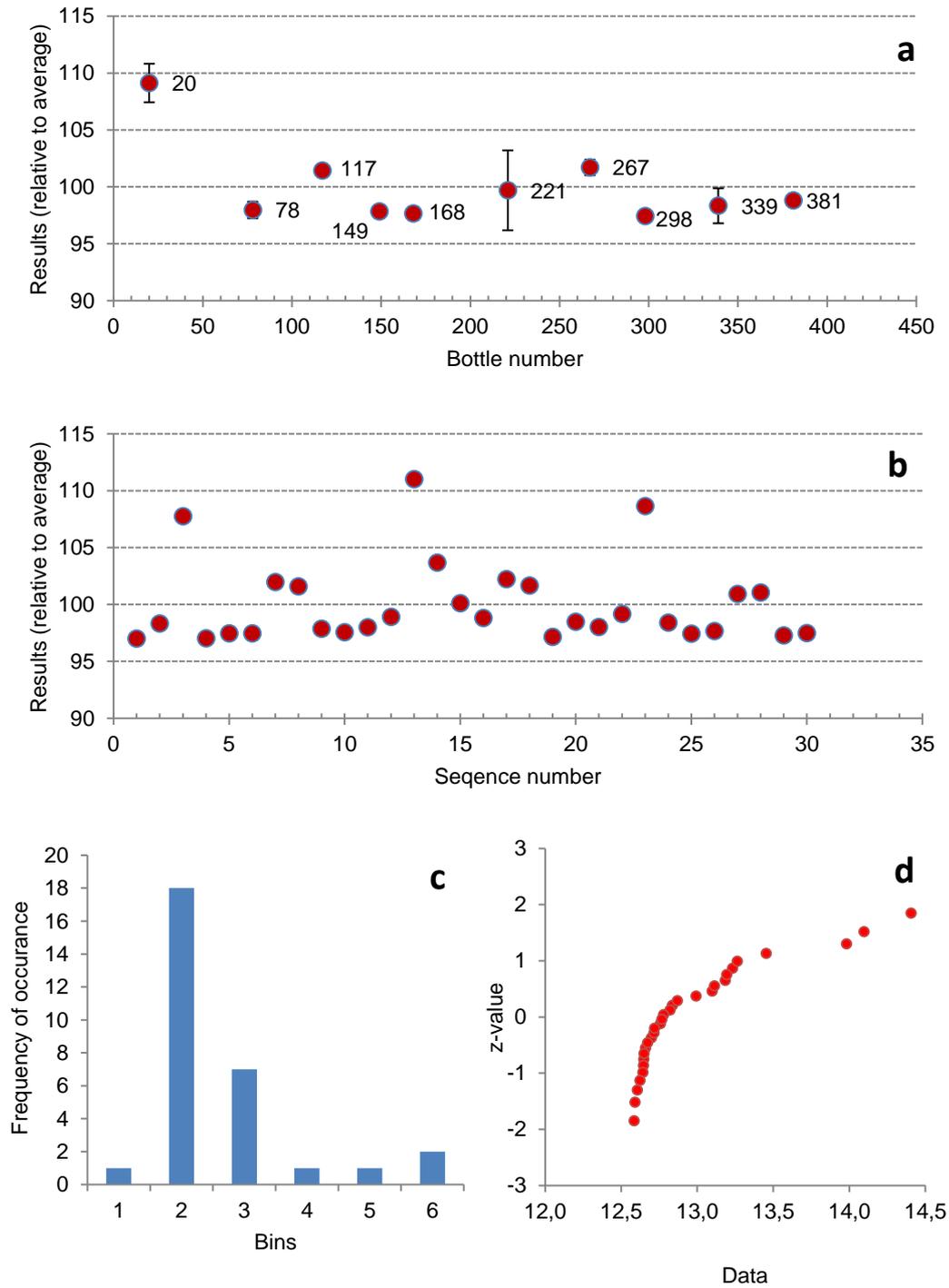
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a. Graph of filling sequence order
b. Graph of analytical sequence order
c. Histogram
d. Normal Probability Plot

Figure A1.5. Homogeneity graphs for Fe
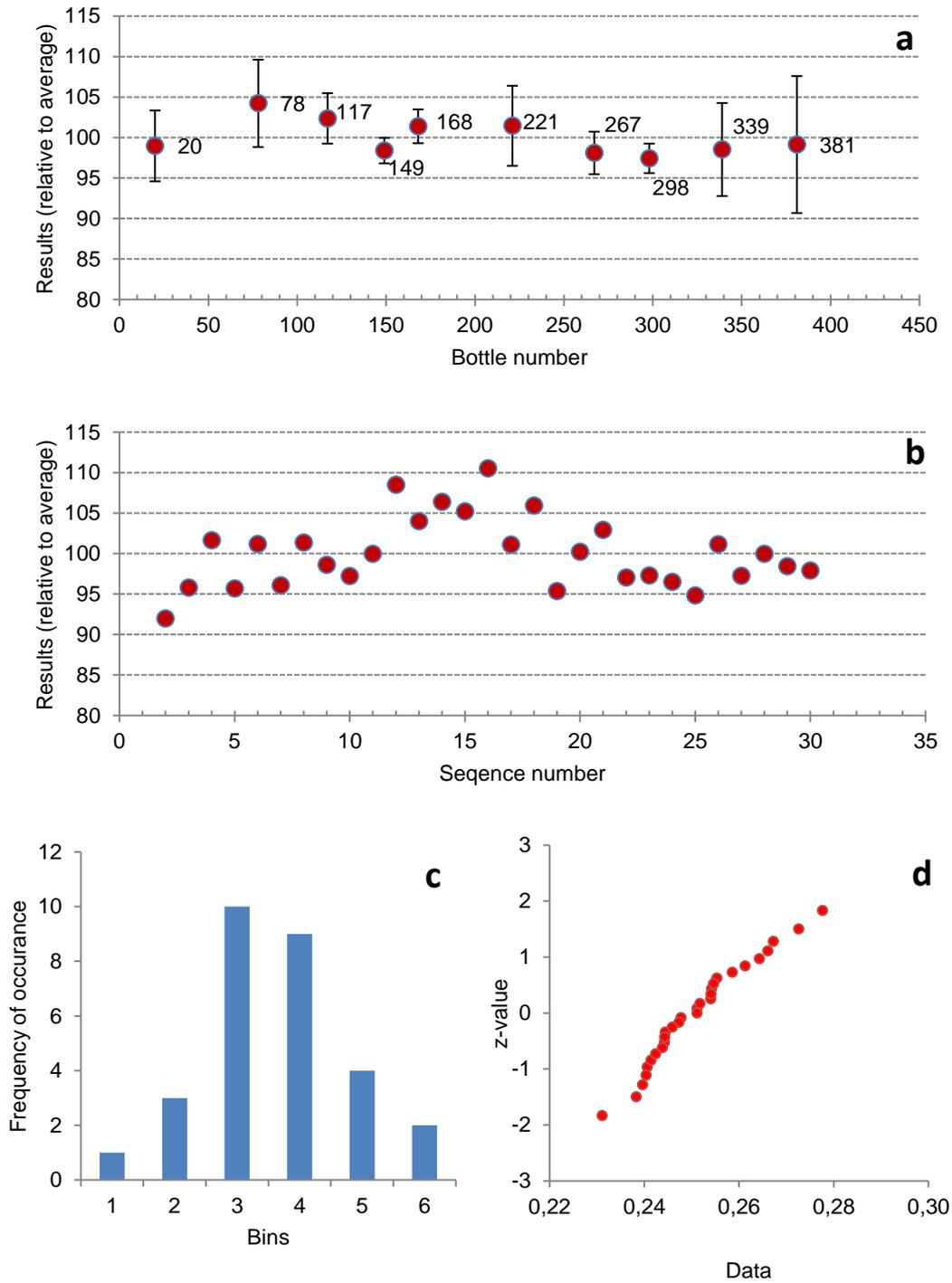
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a. Graph of filling sequence order
b. Graph of analytical sequence order
c. Histogram
d. Normal Probability Plot

Figure A1.6. Homogeneity graphs for Hg
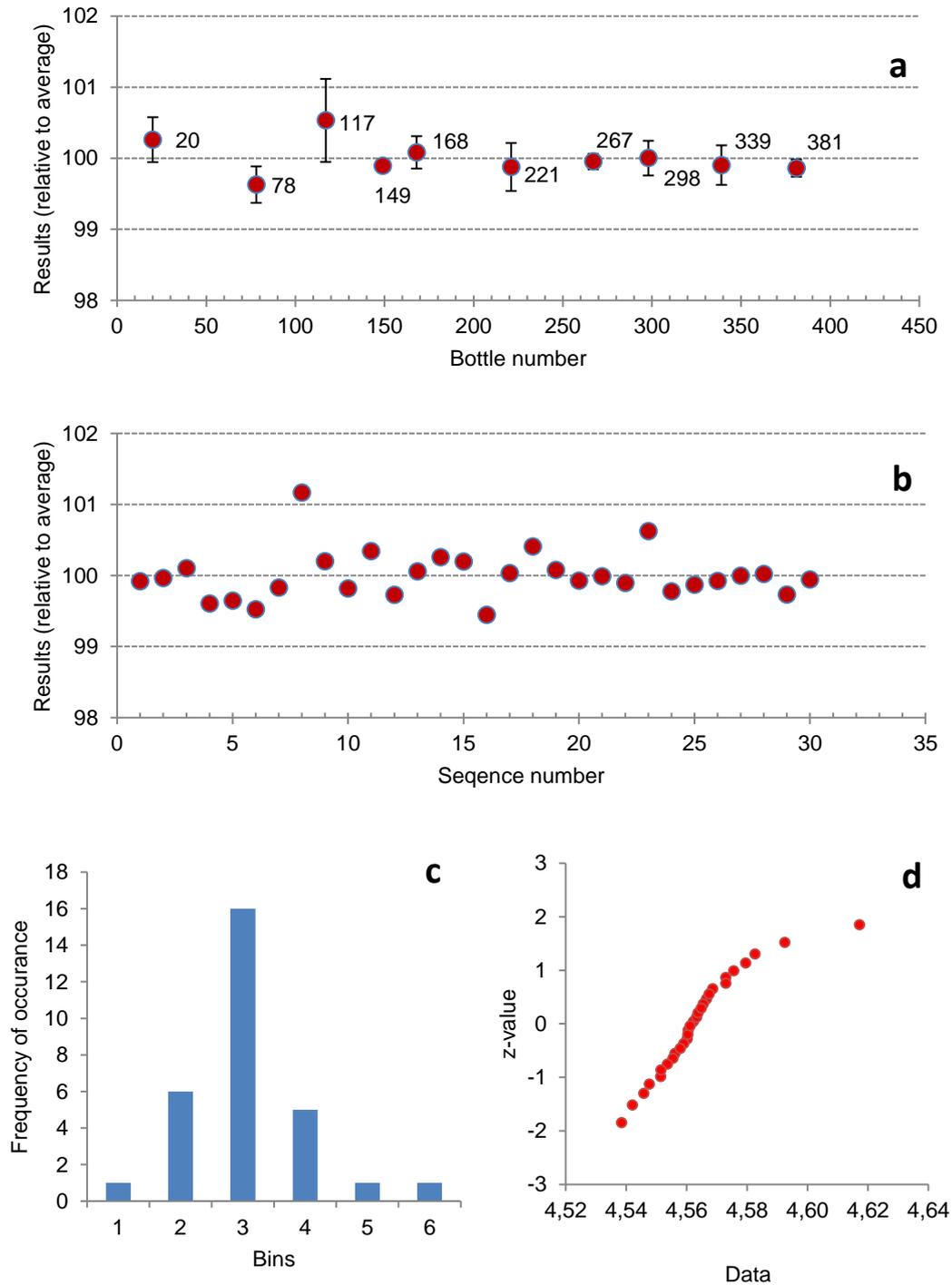
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a. Graph of filling sequence order
b. Graph of analytical sequence order
c. Histogram
d. Normal Probability Plot

Figure A1.7. Homogeneity graphs for Ni
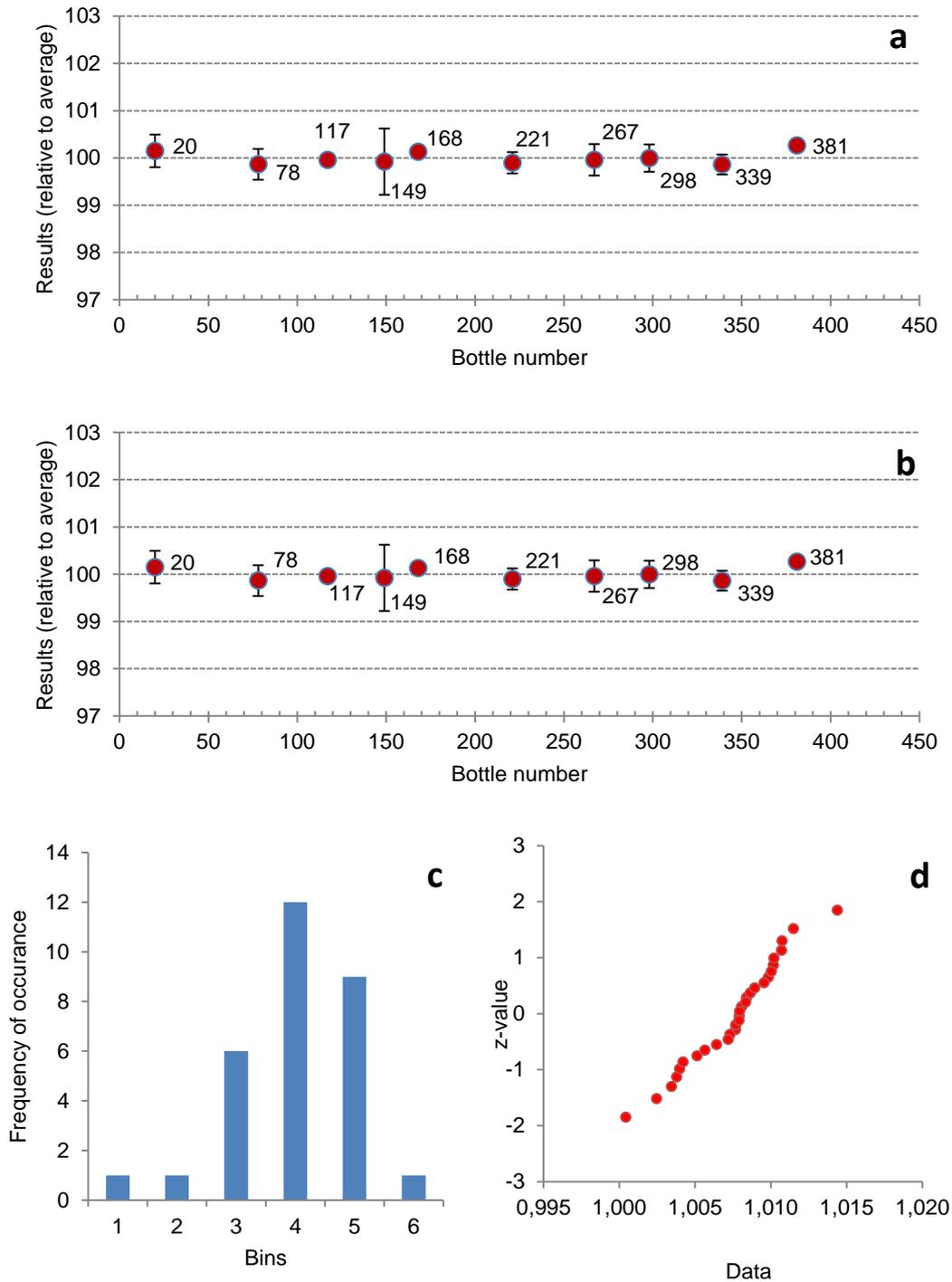
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a. Graph of filling sequence order
b. Graph of analytical sequence order
c. Histogram
d. Normal Probability Plot

Figure A1.8. Homogeneity graphs for Pb
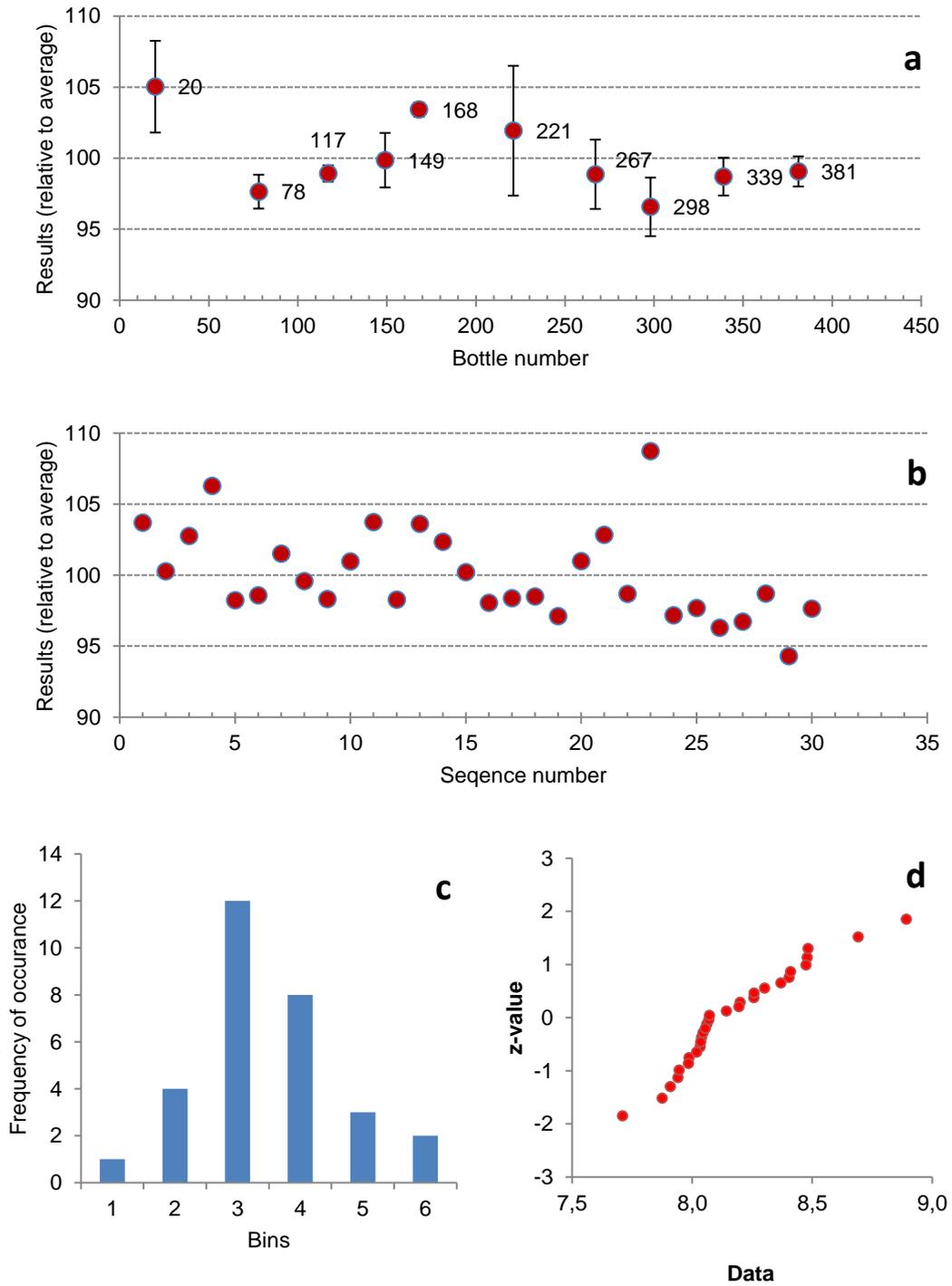
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a. Graph of filling sequence order
b. Graph of analytical sequence order
c. Histogram
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Figure A1.9. Homogeneity graphs for Zn

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Annex 2. Graphs of Short Term Stability study
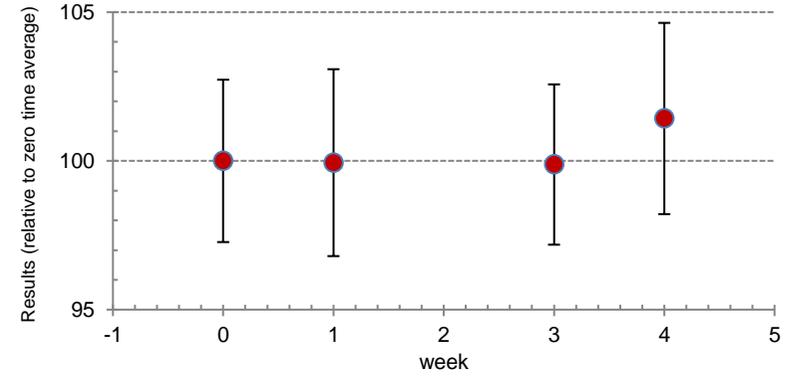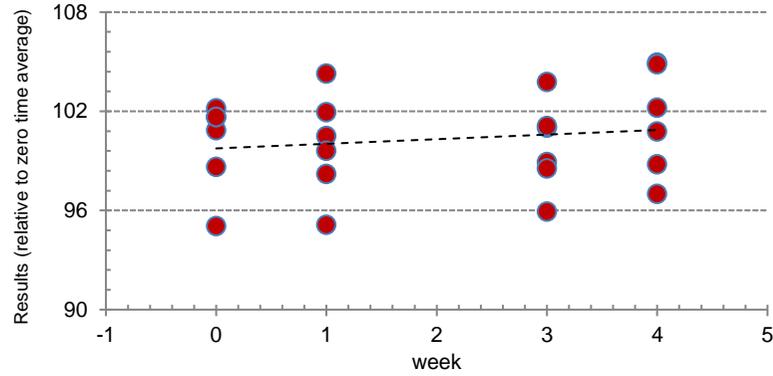
Figure A2.10. Short Term Stability at 18 °C for As
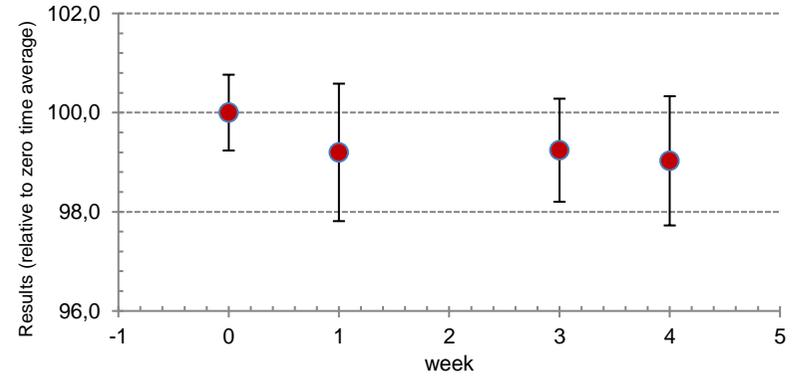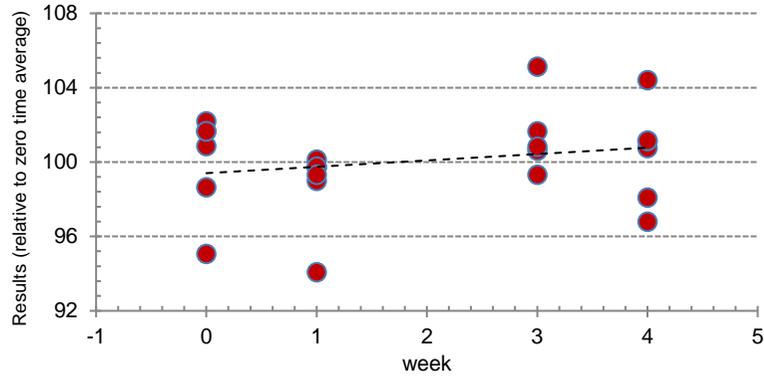
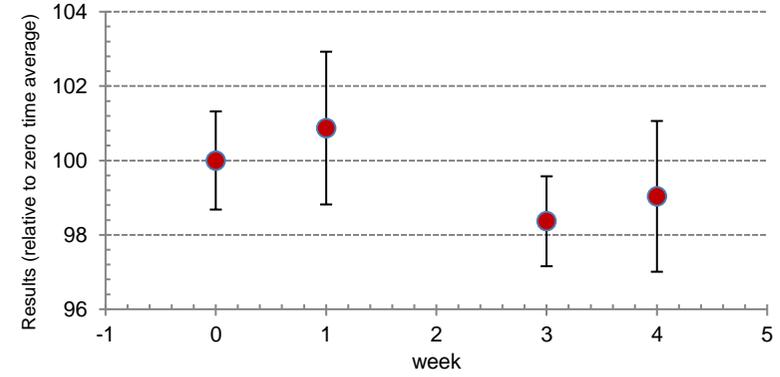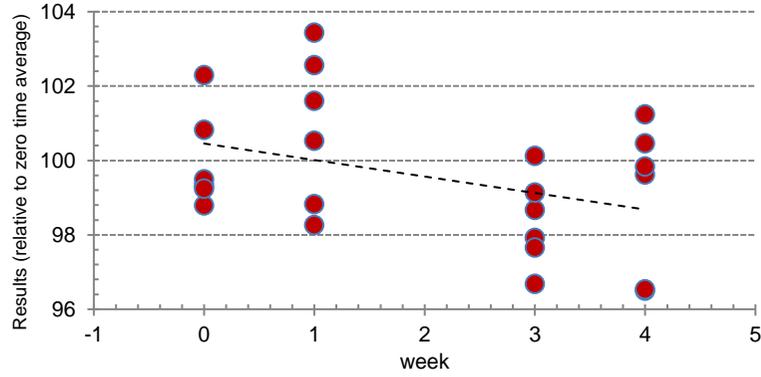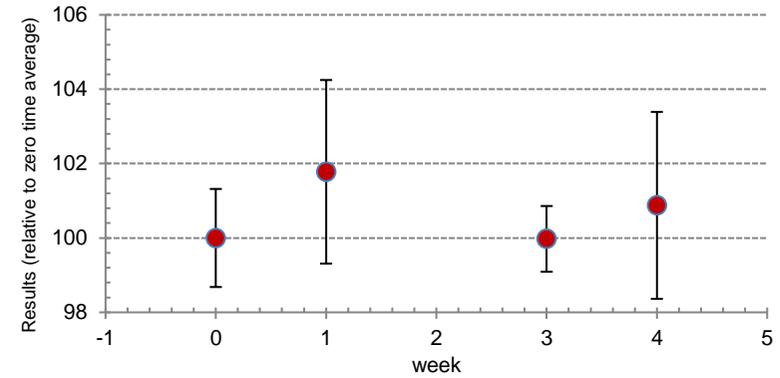**Figure A2.2.** Short Term Stability at 60 °C for As

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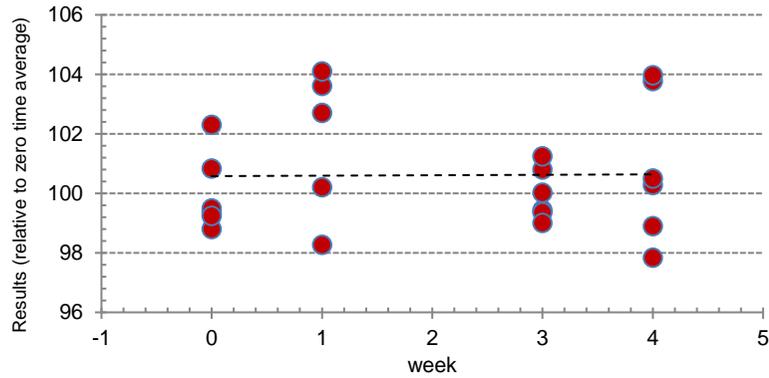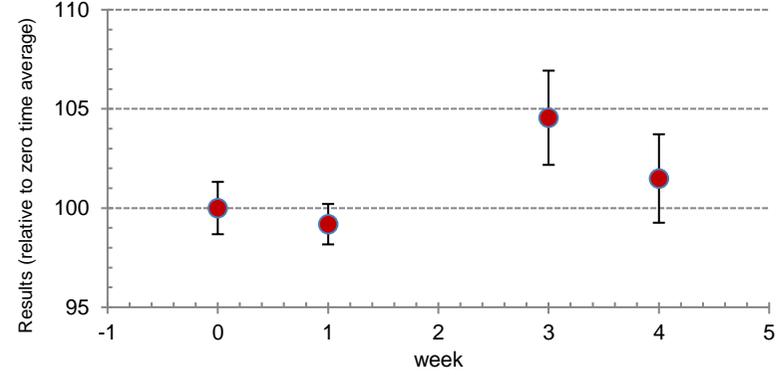
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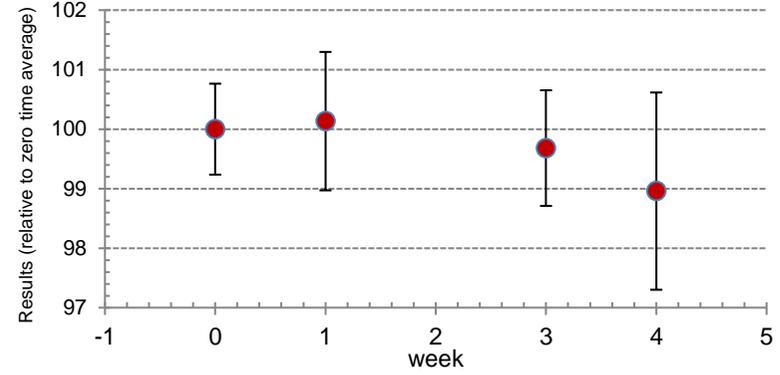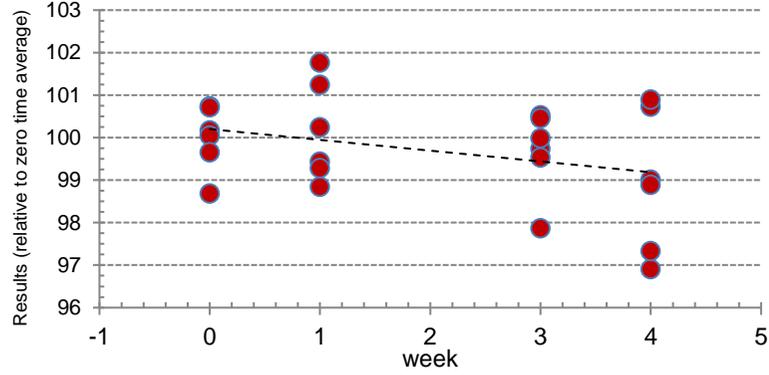
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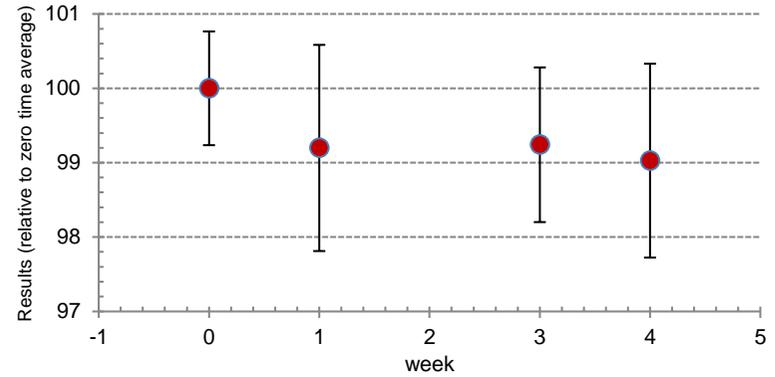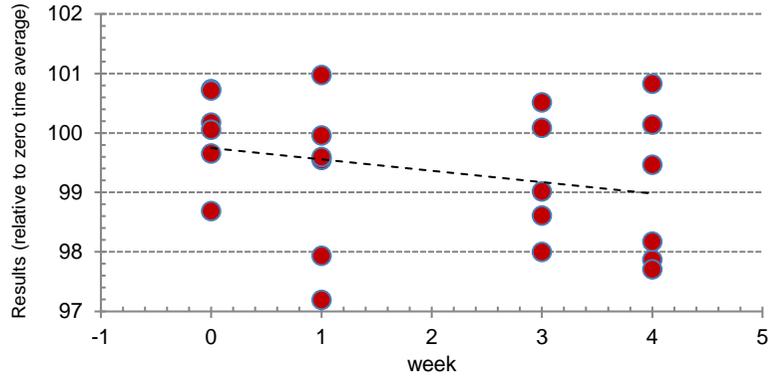
Figure A2.4. Short Term Stability at 60 °C for Cd

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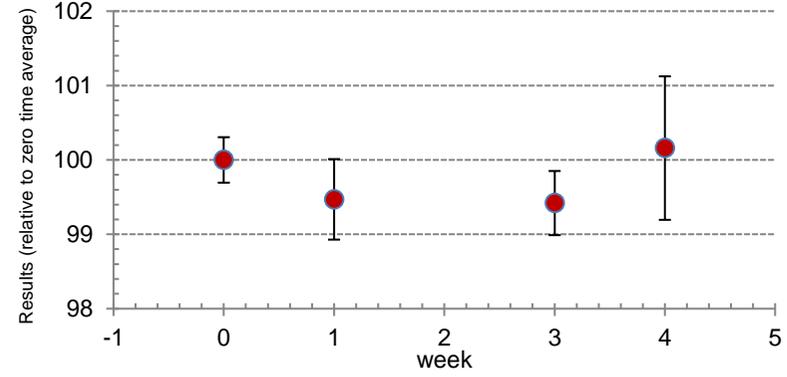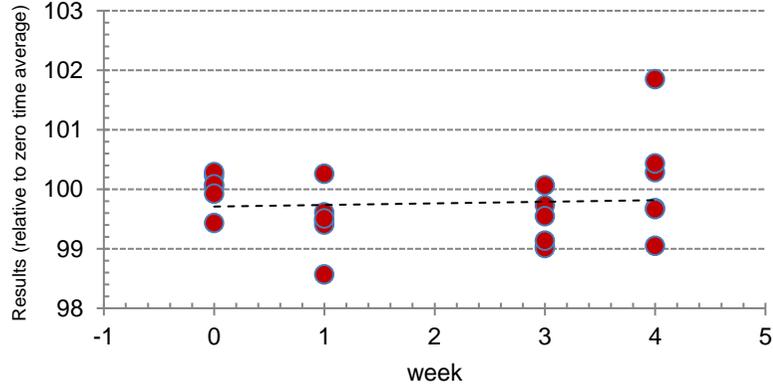
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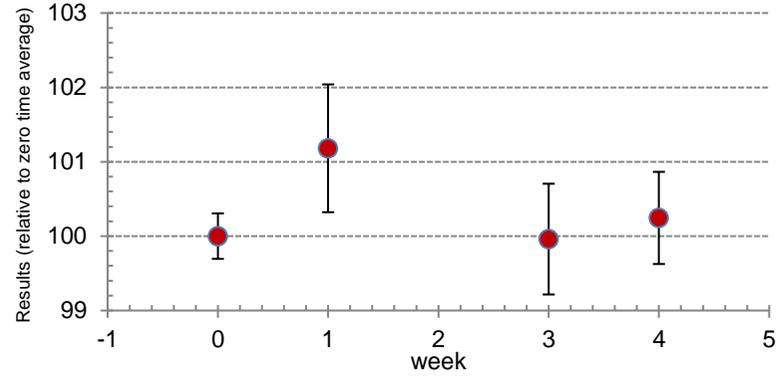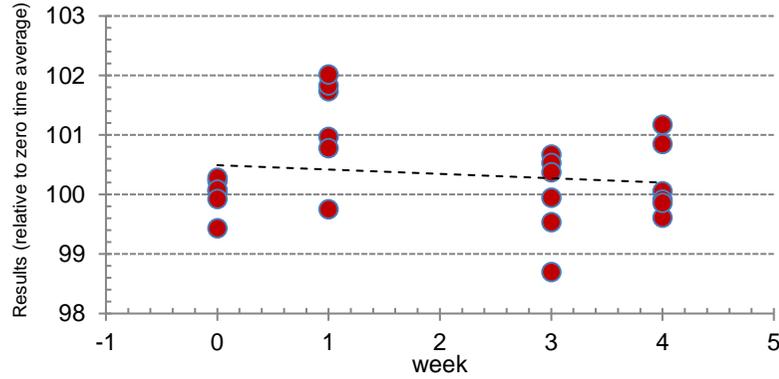
Figure A2.6. Short Term Stability at 60 °C for Cr

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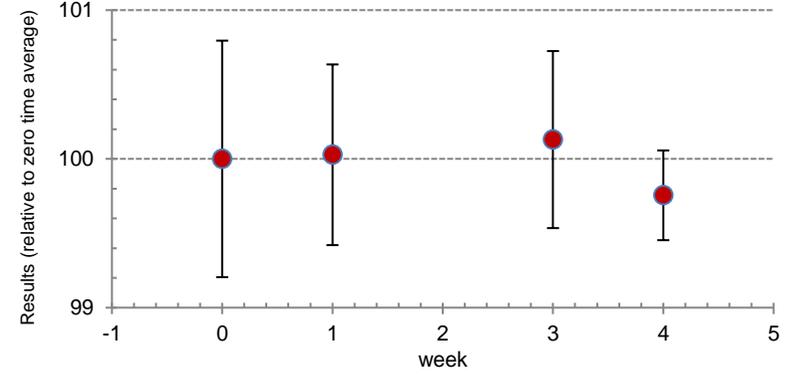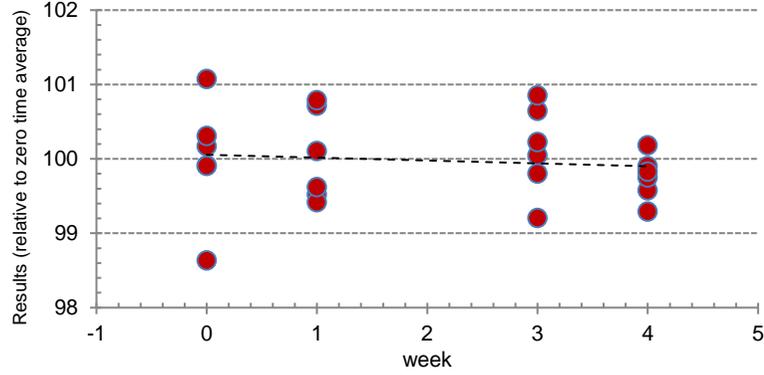
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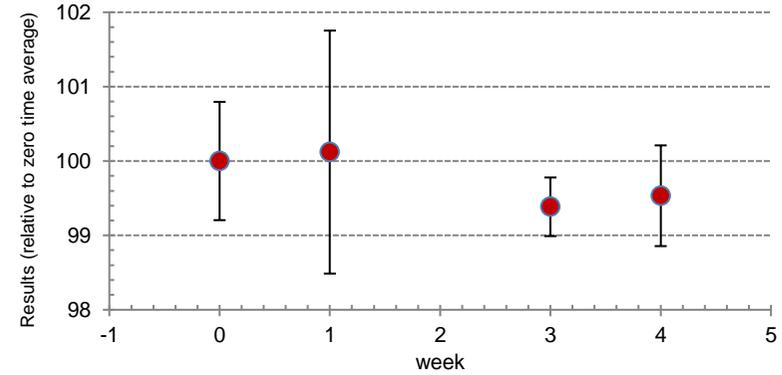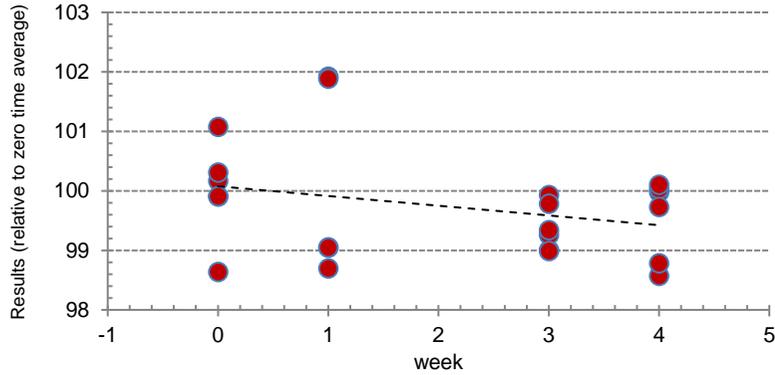
Figure A2.8. Short Term Stability at 60 °C for Cu
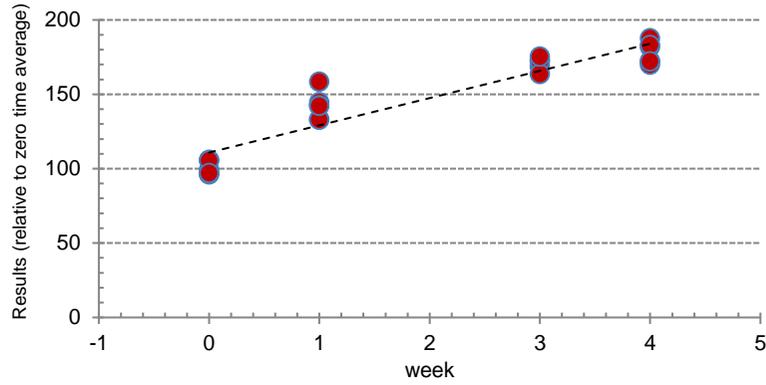
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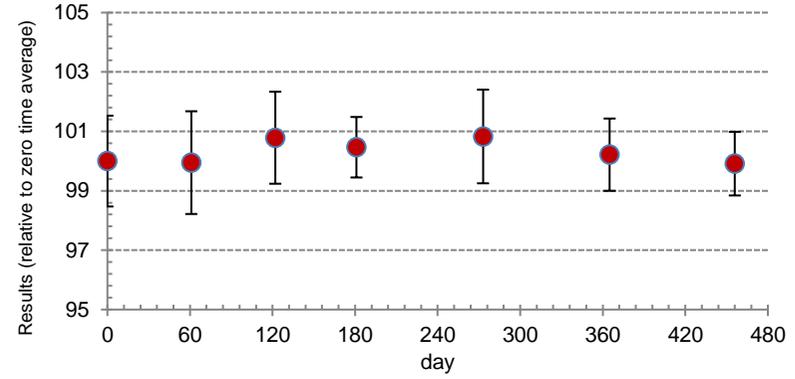
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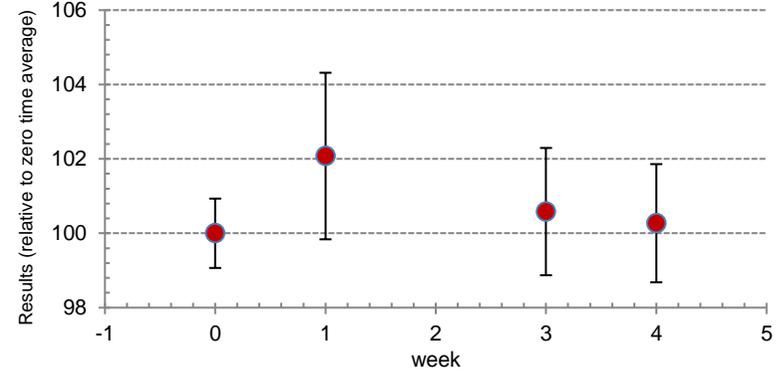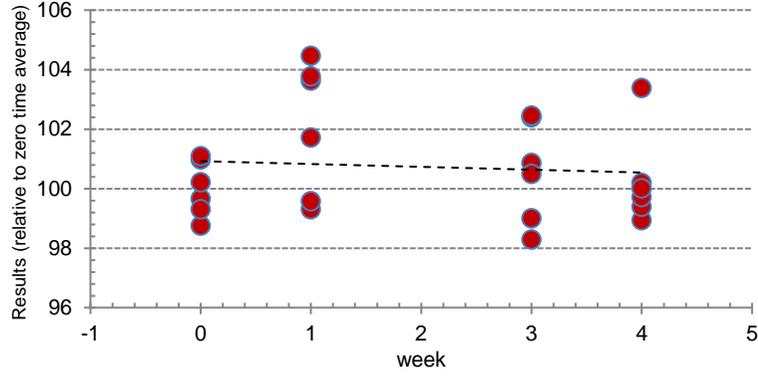
Figure A2.9. Short Term Stability at 18 °C for Fe

Figure A2.10. Short Term Stability at 60 °C for Fe

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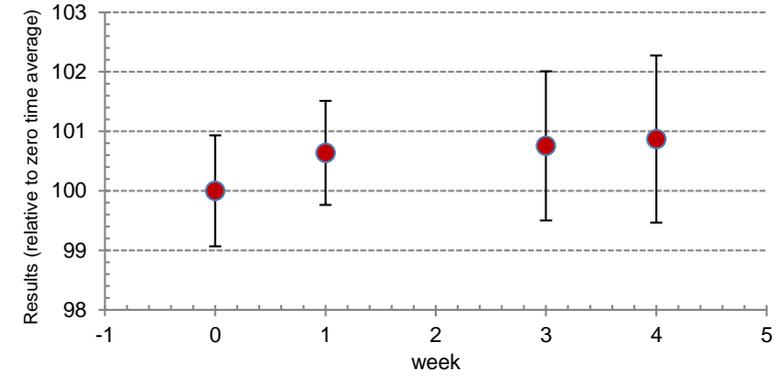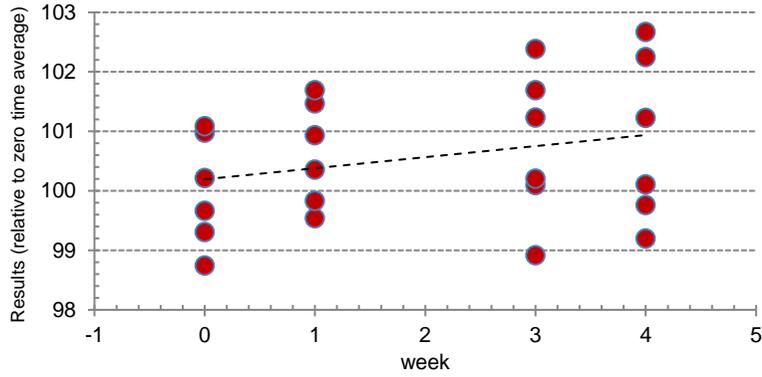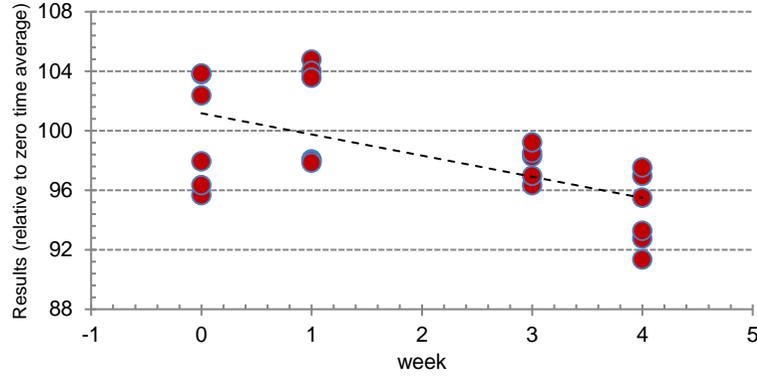
Figure A2.11. Short Term Stability at 18 °C for Hg
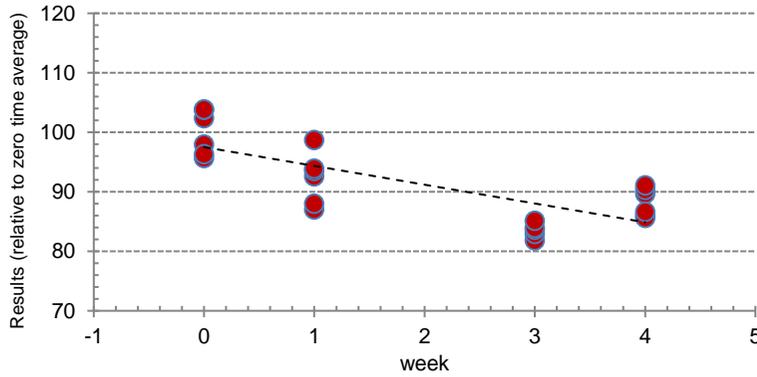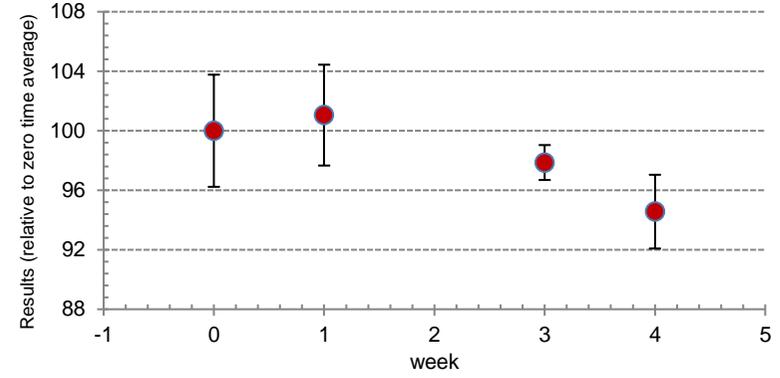
Figure A2.12. Short Term Stability at 60 °C for Hg

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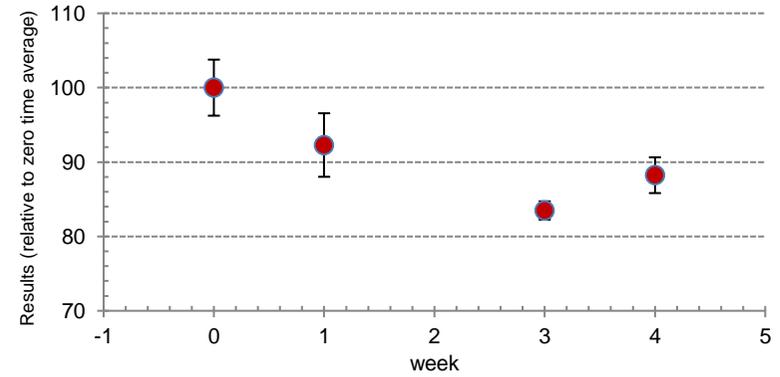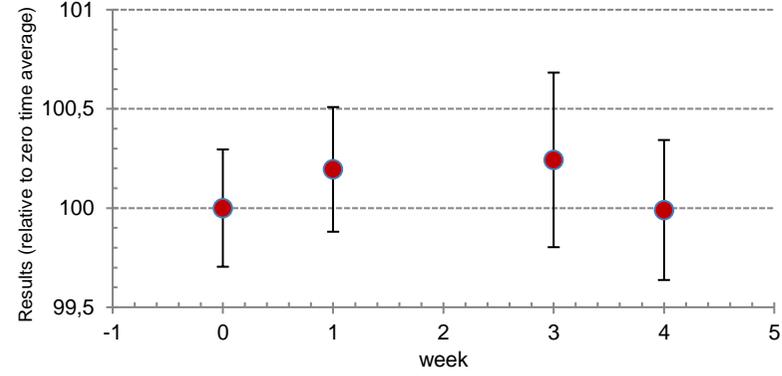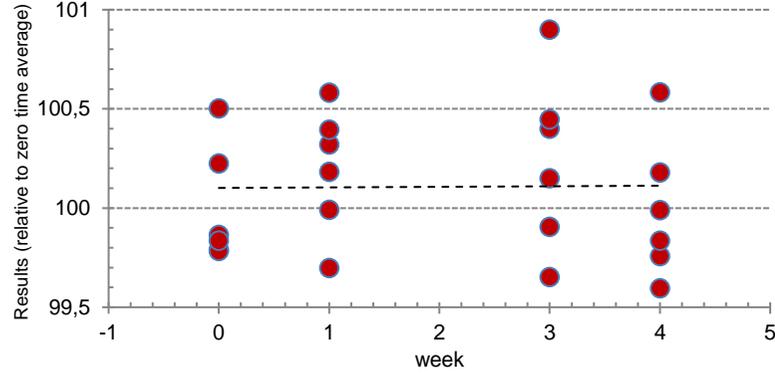
Figure A2.13. Short Term Stability at 18 °C for Ni
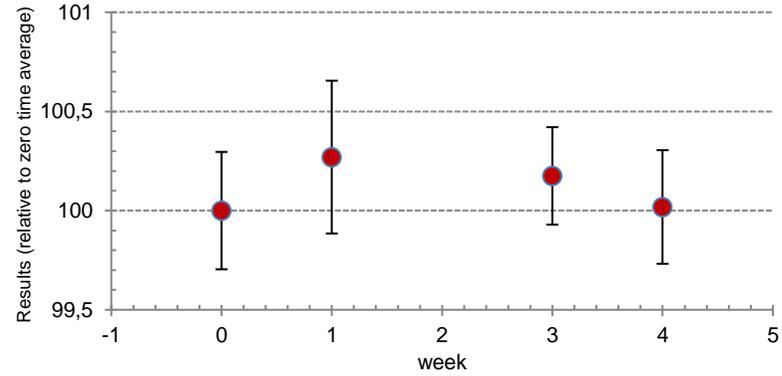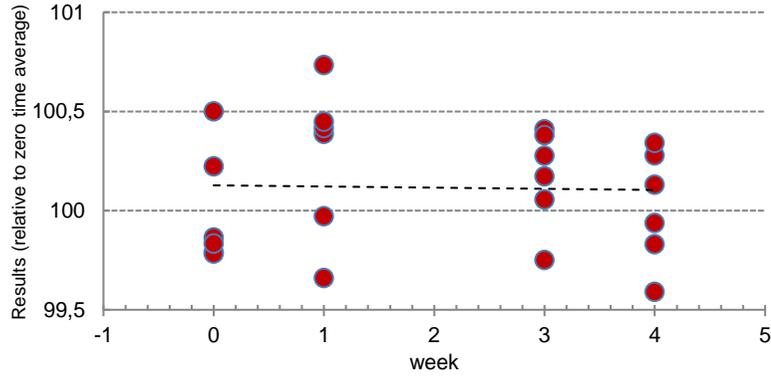
Figure A2.14. Short Term Stability at 60 °C for Ni

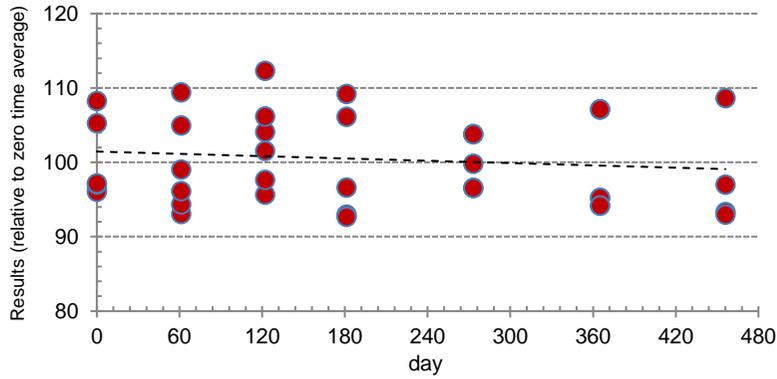TÜBİTAK Gebze Yerleşkesi PK 54 41470 Gebze-Kocaeli /TÜRKİYE   T +90 262 679 50 00   F +90 262 679 50 01   www.ume.tubitak.gov.tr

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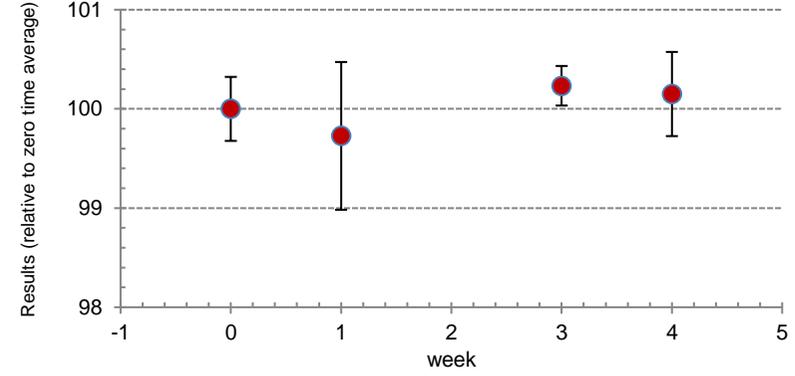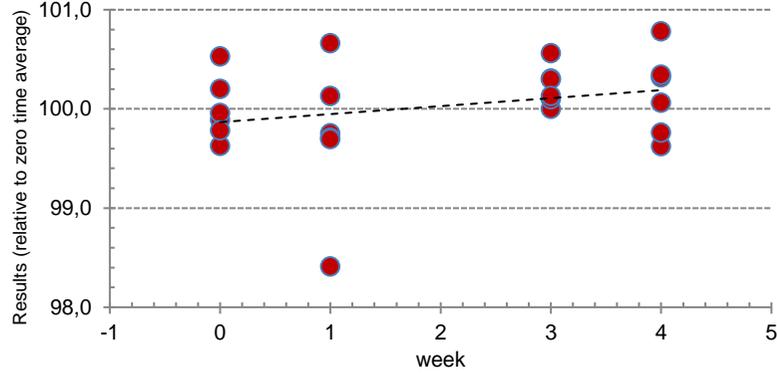
Figure A2.15. Short term stability at 18 °C for Pb
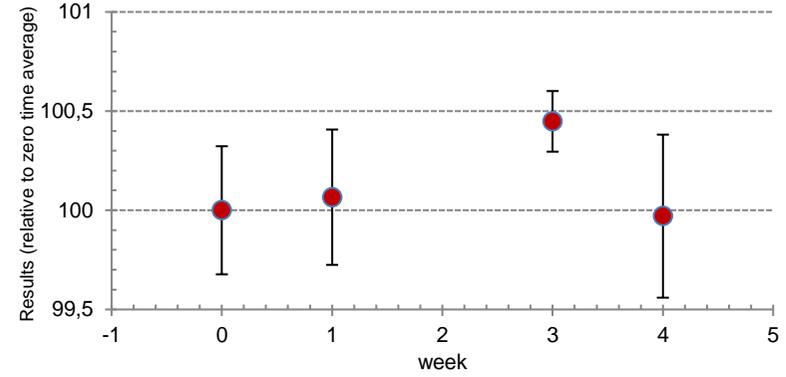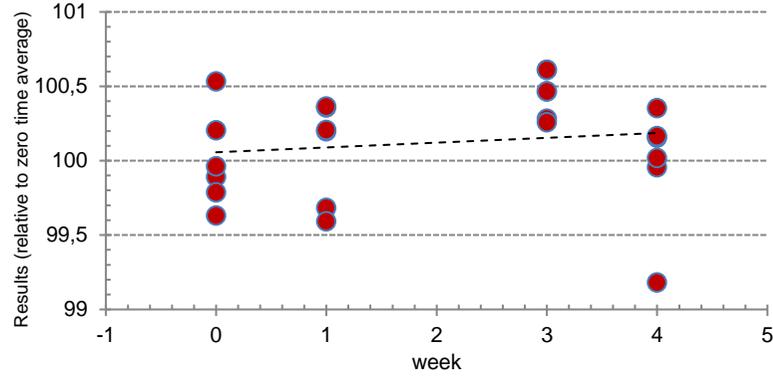
Figure A2.16. Short term stability at 60 °C for Pb

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Figure A2.17. Short term stability at 18 °C for Zn

Figure A2.18. Short term stability at 30 °C for Zn

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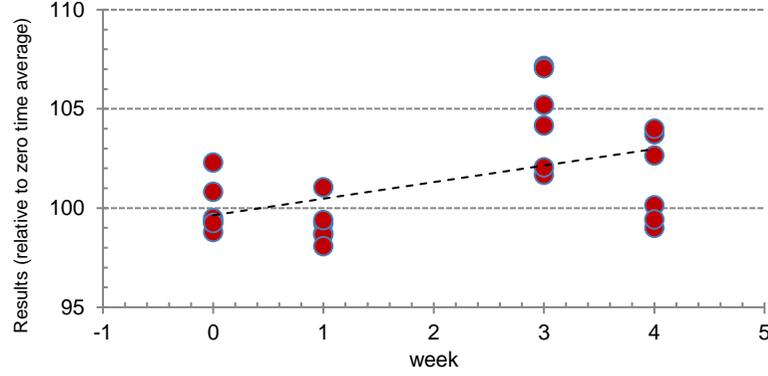
Figure A2.19. Short term stability at 40 °C for Zn
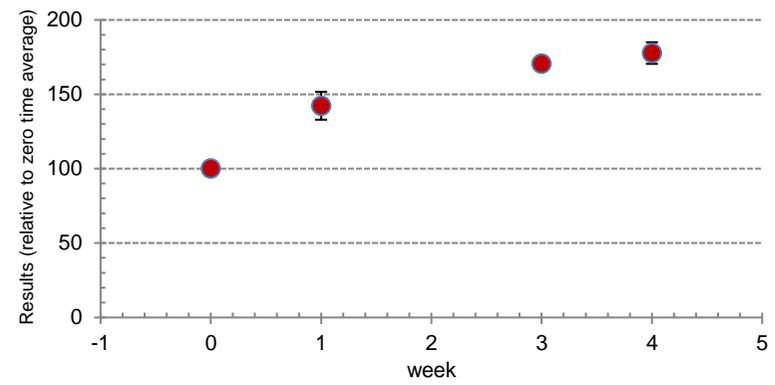
Figure A2.20. Short term stability at 60 °C for Zn

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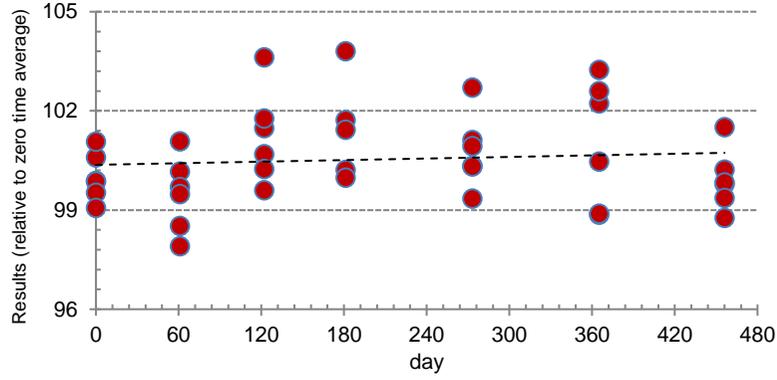FRM-07-U-10-02/Rev.2/26.02.2020

Annex 3. Graphs of Long Term Stability Study
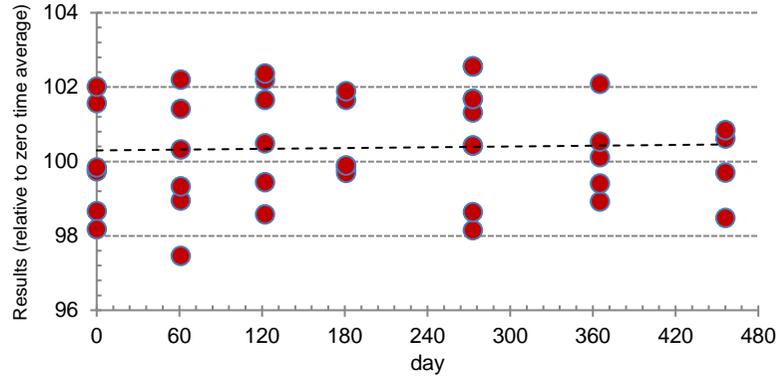
Figure A11. Long term stability for Cd
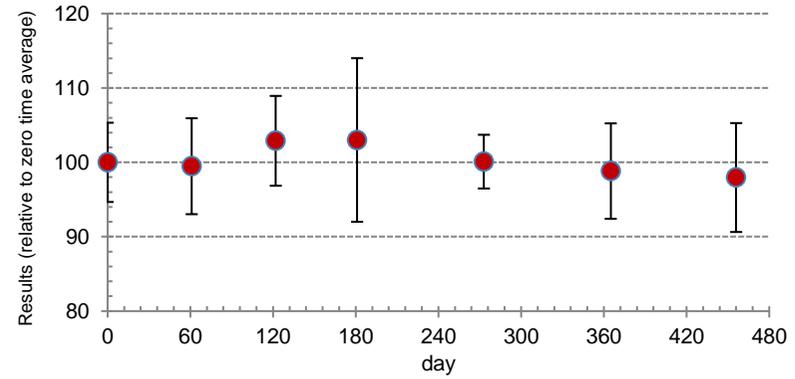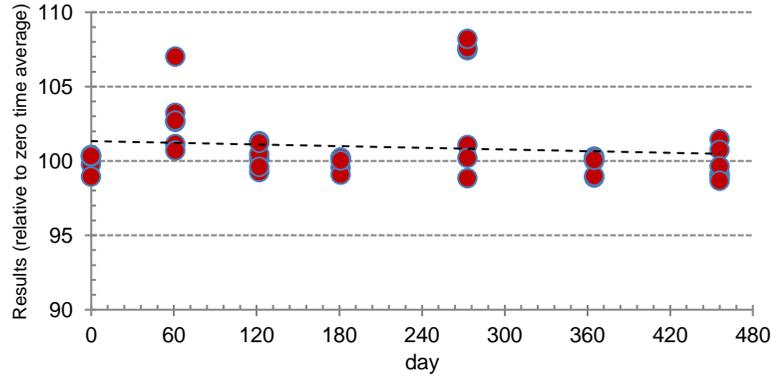

Figure A3.2. Long term stability for Cr

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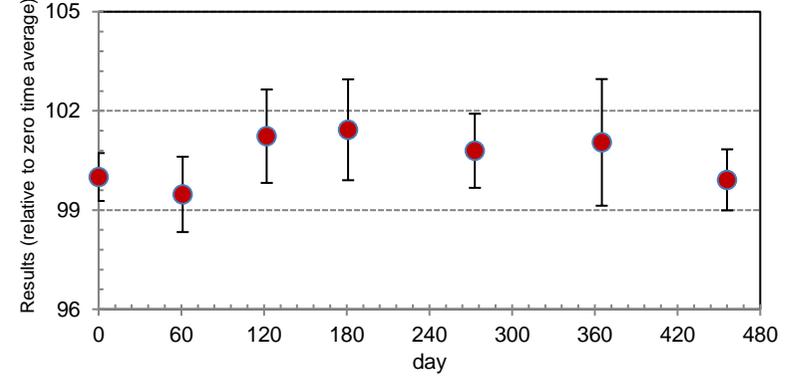
Figure A123. Long term stability for Cu
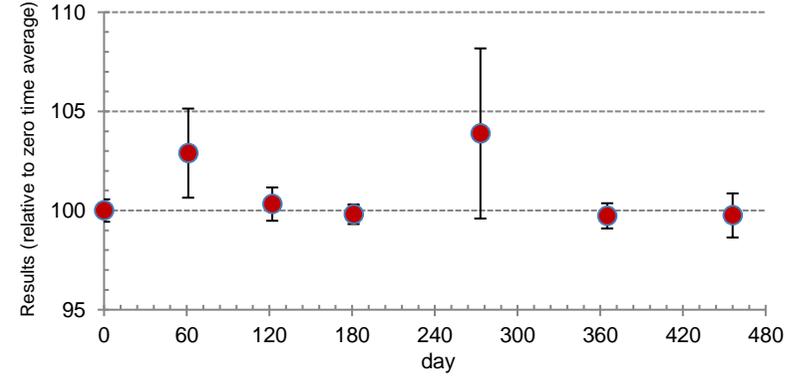
Figure A13 Long term stability for Fe
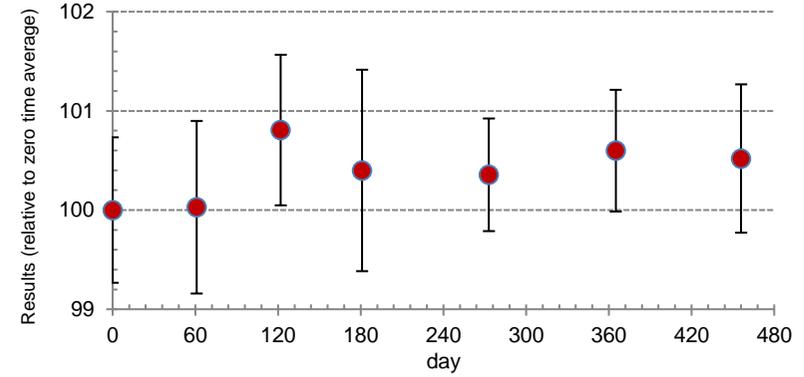
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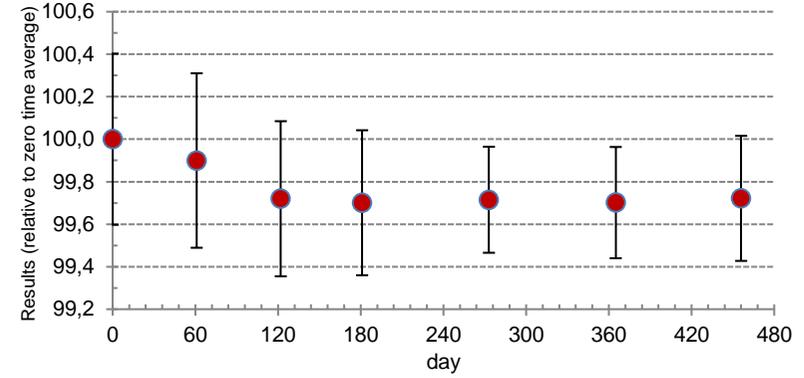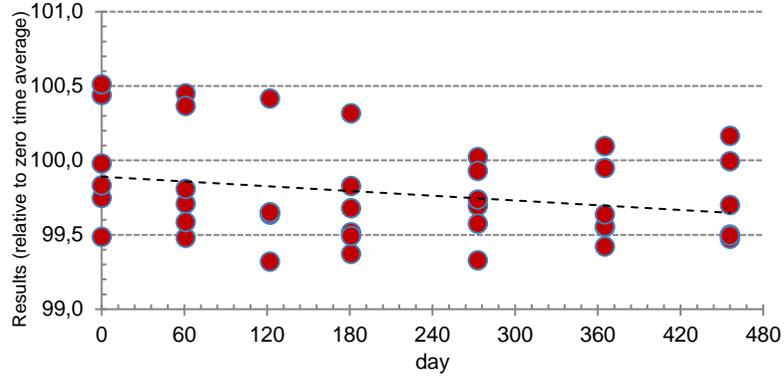
Figure A14.5. Long term stability for Ni
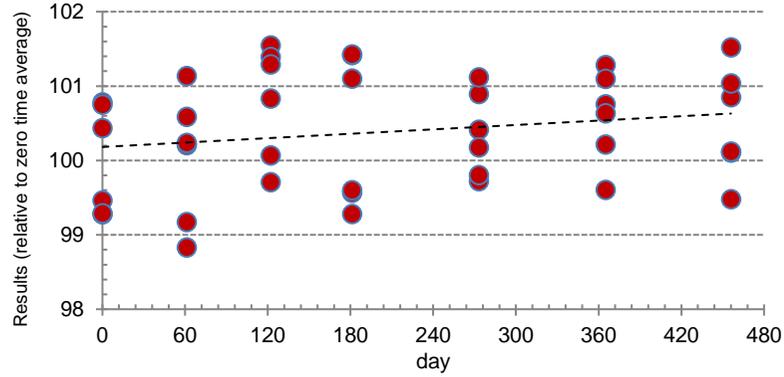
Figure A156. Long term stability for Pb

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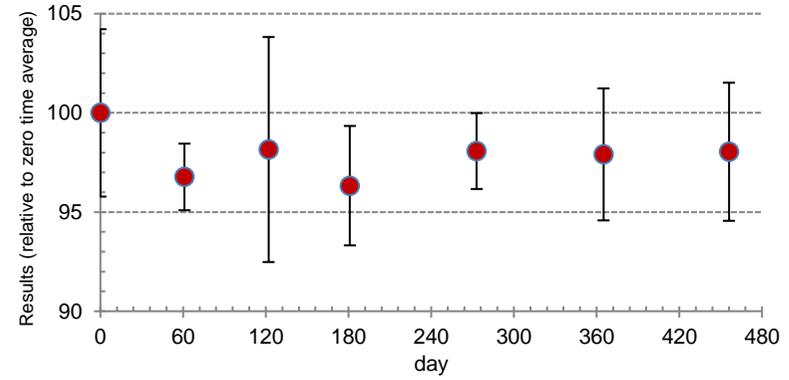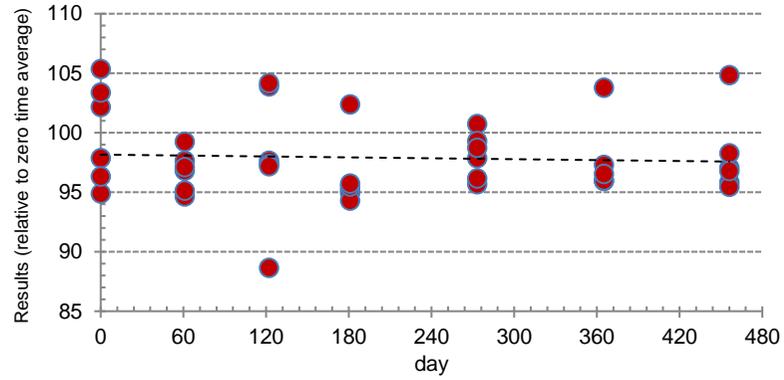
Figure A16.7. Long term stability for Zn

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