ELEMENTS IN HAZELNUT
UME CRM1202

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Date
26/09/2016

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ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
α	Significance level
CRM	Certified reference material
ERM	European reference material
FAAS	Flame Atomic Absorption Spectrometry
GF-AAS	Graphite Furnance Atomic Absorption Spectrometry
GUM	Guide to the uncertainty in measurement
HR-ICP-MS	High resolution inductively coupled plasma mass spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ID-ICP-MS	Isotop dilution inductively coupled plasma mass spectrometry
LTS	Long term stability
ISO	International Organization for Standardization
$MS_{between}$	Mean square between-bottle from ANOVA
MS_{within}	Mean square within-bottle from ANOVA
n	Number of replicates per bottle
RSD	Relative standard deviation
s	Standard deviation
s_{bb}	Between-bottle standard deviation
SI	International System of Units
SGT	Single Grubbs' test
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_{rect}	Standard uncertainty related to possible between-bottle heterogeneity modelled as rectangular distribution
u_{char}	Standard uncertainty related to characterisation
u_{lts}	Standard uncertainty related to long term stability
$\bar{y}$	Average of all results of the homogeneity study

ABSTRACT

This report explains certification procedure of total concentration of B, Ca, Cd, Co, Cu, Fe, Mg, Mn, Ni, Sr and Zn elements in UME CRM1202 hazelnut matrix. This process involves sample preparation, homogeneity, short and long term stability, characterisation and value assignment steps. The studies are performed according to ISO Guide 34:2009 [1] and ISO Guide 35:2006 [2].

Certification 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].

TÜBİTAK UME organised and coordinated all the steps of this project including evaluation of data.

This reference material is intended to be a common reference point for method development and validation for analysis of a series of 11 elements in hazelnut matrix.

INTRODUCTION

Turkey have first rank in hazelnut export in the world making certified reference materials (CRM) in chemically detecting the quality is important. CRMs are used to ensure the quality of the measurement in terms of accuracy and traceability. Hazelnuts keep its vital role [4] in our life in terms of its fat and rich nutritional content. Elements like Zn, Fe, Ni, K, Mg can be described as nutritional in human metabolism, they may also show toxic effects in case of taking over a certain level. Although there are legal limitations for certain elements in food samples, there are no limits for nutritionals. Therefore detection of nutritional or toxic levels in food samples accurately and trustworthy is extremely important.

CRM use in chemical analyses plays an important role in quality and reliability of measurement results. The chemical properties certified in CRMs are agreed on and justified using scientific methods and published in accordance with certain criteria. The inquiries showed that there are no hazelnut CRMs (elements in hazelnut matrix) in the market resulting a big lack in the field. Therefore other CRMs are used in the analysis of elemental content in hazelnut samples instead which do not reflect hazelnut matrix entirely. However considering the matrix effect in chemical analyses, it is inevitable for laboratories to use matrix matched CRMs. During the production of this CRM remarkable effort has been performed to include all the elements in the certification which can be important to laboratories. High fat content typical to hazelnuts grown in Turkey is one of the parameters to be certified makes this CRM valuable and preferred.

It is essential to use correct CRM in realising traceability of measurement results and validation of methods. Within the scope of this project the most suitable material for production of CRM is the primary exportation hazelnut product amongst grown in Turkey. It has been aimed to certify concentration of B, Cd, Mg, P, Ca, Mn, Fe, Co, Ni, Cu, Zn, K elements. Besides it has been showed effort to certify further As, Ba, Sr, Se, Pb, Cr elements studied by others [5]. The values are stated as mass concentrations in micrograms or milligrams per kilogram (µg/kg or mg/kg). All elements' target values were kept at natural levels therefore no spiking done. For this reason some of the elements (Hg, Na, Sb, As, Cr, Pb, Se) whose concentrations are very low were taken out. During the certification period there has been requests from hazelnut producers to include total fat content based on dry mass. So it has been decided to certify above mentioned property with support from external accredited laboratories.

Certification process was fulfilled all the requirements of ISO Guide 34 and 35 Guides [1],[2]. UMECRM1202 is intended to be used as a quality assurance and quality control tool especially by the laboratories involved in the mandatory monitoring prescribed by relevant food and environmental legislations and regulations.

PARTICIPANTS

Laboratories contributing to the raw material procurement, processing, sterilization, homogeneity, stability and characterization studies are given in **Table** 1.

Table 1. Institutions and organizations involved in the production and certification process

Activity	Laboratory
Project Management and Data Evaluation	• TÜBİTAK Ulusal Metroloji Enstitüsü (UME), Gebze, Kocaeli, TURKEY
Sample Procurement	• Fiskobirlik Efit A.Ş., Gaziler Mahallesi Atatürk Bulvarı No:483 Merkez/Giresun, TURKEY
Processing	• TÜBİTAK Ulusal Metroloji Enstitüsü (UME), Gebze, Kocaeli, TURKEY
Sterilization	• Gamma Pak Sterilizasyon San. ve Tic. A.Ş Organize Sanayi Bölgesi Gazi Osman Paşa Mah. 2. Cad. No :6 59500 Çerkezköy-Tekirdağ, TURKEY
Homogenity study	• TÜBİTAK Ulusal Metroloji Enstitüsü (UME), Gebze, Kocaeli, TURKEY
Stability study	• TÜBİTAK Ulusal Metroloji Enstitüsü (UME), Gebze, Kocaeli, TURKEY (Short-term and long-term stability studies)
Characterization study	• TÜBİTAK Ulusal Metroloji Enstitüsü (UME), Gebze, Kocaeli, TURKEY
Total Fat Content Analysis (in alphabetical order)	• Bilim Sağ. ve Lab. Hiz. Tic. Ltd. Şti., Şehremini Mh. Kızılelma Cd. No: 6 Kat: 1-6 (Denizbank üstü) Fındıkzade, 34104 Fatih, İstanbul / TURKEY (ISO/IEC 17025 Türkak AB-0453) • Bursa Gıda ve Yem Kontrol Merkez Araştırma Enstitüsü Müdürlüğü Hürriyet Caddesi, No: 126, 16036, Osmangazi, Bursa / TURKEY (ISO/IEC 17025 Türkak AB-0030-T) • Çevre Gıda Analiz Laboratuvarı, Merkez Mah. Tatlıpınar Sok. Mart Plaza No:13 K:1-2, 34400 Kağıthane, İstanbul / TURKEY (ISO/IEC 17025 Türkak AB-0364-T) • DEPPO Özel Kontrol Laboratuvarı, Gıda Laboratuarı Üniversite Cad. No:71/B Ağaçlıyol, 35100 Bornova, İzmir / TURKEY (ISO/IEC 17025 Türkak AB-0635-T) • Gıda, Tarım ve Hayvancılık Bakanlığı, Ordu Gıda Kontrol Laboratuvar Müdürlüğü, Akyazı Mahallesi Kanuni Sultan Süleyman Cad. No:24/1, 52200 Altınordu, Ordu / TURKEY (ISO/IEC 17025 Türkak AB-0068-T) • Gözlem Gıda Kontrol ve Araştırma Laboratuvarı, Kozyatağı, Bayar Cad. No:78, 34736 Kadıköy, İstanbul / TURKEY (ISO/IEC 17025 Türkak AB-0529-T) • Intertek Test Hizmetleri A.Ş., Merkez Mahallesi Sanayi Cad. No.23 Altındağ Plaza 34197 Yenibosna, İstanbul / TURKEY (ISO/IEC 17025 Türkak AB-0532-T) • TÜBİTAK Bursa Test ve Analiz Laboratuvarı (BUTAL), TÜBİTAK BUTAL Gaziakdemir Mah. Merinos Cad. No: 11 16190 Osmangazi, Bursa / TURKEY (ISO/IEC 17025 Türkak AB-0494-T) • TÜBİTAK Marmara Araştırma Merkezi (MAM), TÜBİTAK Marmara Araştırma Merkezi Gıda Enstitüsü Barış Mah. Dr. Zeki Acar Cad. No:1 P.K. 21, 41470 Gebze-Kocaeli / TURKEY (ISO/IEC 17025 Türkak AB-0494-T) • TÜBİTAK Ulusal Metroloji Enstitüsü (UME), TÜBİTAK Gebze Yerleşkesi, Barış Mahallesi, Dr. Zeki Acar Cad. No.1, 41470 Gebze-Kocaeli/TURKEY

MATERIAL PROCESSING

Material Procurement

The source of the sample chosen as the candidate reference material is Fiskobirlik Entegre Tesisleri which is located in Giresun. This region is famous for their tombul type hazelnut (*Corylus avellana L.*) having high amount of fat content. The hazelnuts were bought from the company as inner nuts where they were roasted and blanched for further process (see details below). The humidity were maximum 5%. All these processes were done at Fiskobirlik facilities and brought to TÜBİTAK UME laboratories under vacuum packages.

Preparation steps, homogenising and bottling

Giresun tombul type hazelnuts (as bought from company) were dried in under HEPA filtered constant air flow drying oven at 50°C for 3 days. Dried hazelnuts were ground using two pair high roller mill (Food Grade, FERRELL ROSS, USA), and were sieved using vibrated sieving machine (LS24S54, SWECO, Belgium) equipped with nylon sieve under 2 mm particle size. Those separated under 2 mm particle (*ca* 30 kg) size were mixed with powder dry ice in non humid environment and homogenised using 3D mixer (3-D MegaMix, HKTM, Turkey). The homogenised materials were transferred to 1 kg containers under vacuum and kept at 4°C until bottling process. Before bottling homogeneity analyses were performed for a number of subsamples enough to represent entire batch.

The bottles and caps were cleaned with ~ %20 (v/v) concentrated nitric acid (Merck EMSURE ISO) and were rinsed with deionised water (Milli-Q, 18.2 $M\Omega \cdot cm^{-1}$). Following this step all cleaned bottles were dried at ISO 6 grade clean laboratory with laminar flow cabins (ISO 4 grade). 600 units with minimum 45 g each were bottled. These bottled materials were sent to gamma radiation for sterilisation (see p8). The irradiated samples were stored under -20°C as reference temperature.

Natural Levels

The concentrations of elements were measured semi quantitatively by HR-ICP-MS (Element 2, Thermo Finnigan, Bremen, Germany) before starting any further process. Results of this preliminary measurements are given in Table 2. As spiking to these kind of ground samples would greatly affect homogeneity, there wasn't any spiking at all and natural levels were targeted.

Table 2 Natural concentration levels for elements in hazelnut

Element	Natural Levels
B (mg/kg)	17.4
Ba (mg/kg)	5.89
Ca (mg/kg)	1500
Cd (µg/kg)	9
Co (mg/kg)	6.9
Cu (mg/kg)	16.3
Fe (mg/kg)	36.5
K (mg/kg)	5500
Mg (mg/kg)	1500
Mn (mg/kg)	95.7
Ni (mg/kg)	1.7
P (mg/kg)	3100
Sr (mg/kg)	6.9
Zn (mg/kg)	20.7

Sterilization

The bottles were sterilised by ^{60}Co γ-irradiation with minimum of 6 kGy dose. Following this step all the bottles were stored at -20 ºC in dark.

HOMOGENEITY

With the aim of checking the homogeneity of the material regarding the parameters to be certified, 10 units (another 10 were kept for safety) were selected using stratified sampling scheme, taking care that the complete batch was covered. The number of units is based on the produced batch size (approximately corresponding to the cubic root of the total number of units). The batch is divided into the same number of groups and one unit is randomly picked from each group. The samples were analysed in triplicate, for all elements. The measurements were performed under repeatability conditions, i.e. during one analytical run and using validated methods and according to a random sequence to permit distinction between possible trends in the analytical sequence and in the filling order. Candidate reference material (UME CRM1202), certified reference material (NIST SRM 2387) samples, spiked and blank samples were analysed within the sequence. All measurements were performed by HR-ICP-MS (Element 2, Thermo Finnigan, Bremen, Germany).

Total of 30 concentration values for each parameter obtained by the analysis of samples were evaluated by one-way analysis of variance (ANOVA). The unimodal distribution of data is an important prerequisite in order to apply the ANOVA statistical evaluation; therefore the distributions of sample averages as well as individual results were checked both for normality using normal probability plots and for unimodality using histograms. For all elements, the individual results and bottle averages showed an approximately normal and unimodal distribution, with the exception of the individual values for Co, Fe, K and Mg for

which bimodal distributions were observed. This minor deviation from unimodality does not significantly affect the estimation of the between-unit standard deviation. These results are given in Table 3.

Data were checked and statistically evaluated for the presence of any trend and/or outlier. Generally no trends were observed either in filling or in analytical sequence. Only for Mg, P and Sr there has been a trend for filling sequence. In this case the uncertainty originated from heterogeneity between bottle is calculated using rectangular distribution half band between highest and lowest results approach (see eqn 4.). One outlying result was found for iron, magnesium, phosphorus and for potassium (Grubbs' single test for maximum and minimum separately at α = 0.05). Since no technical reasons were identified for the outlying results, all data were retained for statistical analysis.

The ANOVA allowed the calculation of the within- (s_{wb}) and between-unit homogeneity (s_{bb}), estimated as standard deviations [6], according to the equations (1) and (2):

$$S_{wb} = \sqrt{MS_{within}} \quad (1)$$

where

MS_{within} : Mean squares within-bottle

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

$$S_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \quad (2)$$

where

$MS_{between}$: Mean squares between-bottle

n : Number of replicates per bottle

When $MS_{between}$ is smaller than MS_{within}, s_{bb} can not be calculated. Instead, u^*_{bb}, the heterogeneity that can be hidden by the method repeatability, is calculated, according to the following expression (3) [6]:

$$u^*_{bb} = \frac{S_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{v_{MS_{within}}}} \quad (3)$$

where

$v_{MS_{within}}$: Degrees of freedom of MS_{within}

For Mg and P, for which filling trend was observed (see above), an alternative estimate of heterogeneity was calculated. Between-bottle heterogeneity was modelled as rectangular distribution limited by the

outlying average [3] using equation (4). The standard uncertainty using this outlier (u_{rect}) was then estimated as;

$$u_{rect} = \frac{|maximum\ value - minimum\ value|}{2\sqrt{3}} \quad (4)$$

For the parameters for which ANOVA was applied, the larger value of s_{bb} or u^*_{bb} is taken as uncertainty contribution for homogeneity, u_{bb}. The results of homogeneity studies are given in Table 4.

Table 3. Shapiro-Wilk test results for within and between bottle normal distribution

Shapiro-Wilk test results for normal distribution*											
Analyte	Within bottle										Between bottle
	24	86	174	190	245	307	401	436	503	561	
B	+	+	+	+	+	+	+	+	+	+	+
Ba	+	+	+	+	+	+	+	-	+	+	+
Ca	+	+	+	+	+	+	+	+	+	+	+
Cd	+	+	+	+	+	+	+	+	+	+	+
Co	+	+	+	+	+	+	+	+	+	+	-
Cu	+	+	+	+	+	+	+	+	+	-	+
Fe	+	+	+	+	+	+	+	+	+	-	-
K	+	-	+	+	+	+	+	+	+	+	-
Mg	+	+	+	+	+	+	+	+	+	-	-
Mn	+	+	+	-	+	+	+	+	+	+	+
Ni	+	+	+	+	+	+	+	+	+	+	+
P	+	+	+	+	+	+	+	+	+	+	+
Sr	-	+	+	+	+	+	+	+	+	+	+
Zn	+	+	+	+	+	+	+	+	+	+	+

*Plus sign show normal distribution whereas minus show deviation from it.

Table 4. Results of the homogeneity study

Analyte	Mean Value	unit	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{rect,rel}$ [%]	$u_{bb,rel}$ [%]
B	17.41	mg/kg	4.36	2.20	1.42		2.20
Ba	5.89	mg/kg	3.12	0.63	1.01		1.01
Ca	1525	mg/kg	2.12	1.11	0.69		1.11
Cd	8.57	µg/kg	6.39	4.33	2.07		4.33
Co	282.9	µg/kg	3.41	1.04	1.11		1.11
Cu	16.40	mg/kg	1.69	0.92	0.55		0.92
Fe	36.58	mg/kg	2.88	1.51	0.94		1.51
K	5493	mg/kg	3.42	1.02	1.11		1.11
Mg	1509	mg/kg	1.37	1.25	0.45	3.97	3.97
Mn	95.64	mg/kg	2.11	0.59	0.69		0.69
Ni	1.73	mg/kg	3.07	1.57	1.00		1.57
P	3104	mg/kg	1.79	0.70	0.58	3.44	3.44
Sr	6.92	mg/kg	3.00	1.52	0.97	6.75	6.75
Zn	20.82	mg/kg	2.65	2.56	0.86		2.56

Even with the retention of outliers and inclusion to statistical evaluations for most of the elements between-unit variation is generally lower than maximum targeted value. The target values written in the beginning of the project (≤5%) were achieved.

The data used for the evaluation of homogeneity can be found in Annex 1.

Minimum sample intake

Minimum sample intake is defined as the minimum amount of material needed, so that the heterogeneity of the material does not affect the repeatability of the measurement method. For this purpose minimum sample intake studies were performed with 0.5 and 1.0 g. sample amounts. At least 3 parallel measurements using 10 bottles to represent entire batch were done. The result of this study, standard deviations between bottles is given in Table 5. There has been an immense decline in the heterogeneity between bottle for most of the elements. A final decision was made to suggest to use 1.0 g sample for this CRM.

Table 5. Results of minimum sample intake study. Values denote between bottle percent relative standard deviation.

Between bottle %RSD		
	0.5 g	***1.0 g***
B	3.2	3.2
Ba	2.3	1.8
Ca	2.8	1.6
Cd	2.5	5.5
Co	3.9	2.4
Cu	2.1	1.3
Fe	3.8	2.1
K	2.3	2.4
Mg	2.6	1.6
Mn	2.0	1.3
Ni	4.3	2.2
P	2.8	1.5
Sr	3.8	2.4
Zn	1.8	2.9

STABILITY

Stability studies are performed to simulate transportation conditions (short term stability) and storage conditions (long term stability).

26 bottles were kept for short term stability tests and 8 bottles were kept for long term stability tests where these bottles were selected in a random stratified manner.

For short term stability study, four different test temperatures was selected as +4 °C, +18 °C, +35 °C and +60 °C, with time periods of 1, 2 and 4 weeks. For each test period, 2 bottles were placed at designated temperatures. For the reference point, 2 bottles were put aside at -20 °C. Right after each period of test time, the 2 bottles were transferred to reference temperature +4 °C. At the end of duration, the analysis of all samples kept at different temperatures for different period of time was performed isochronously.

For long term stability, 8 bottles were kept at room temperature (18 ± 2) °C for 0, 3, 6, 12 and 18 months where 18 months time was the ultimate time period whilst this report is being written. Right after the storage time at room temperature, they were put at -20 °C stability cabinets. Again all the measurements were performed isochronously. Hazelnut material contains high level of fat (*ca* 70%) therefore this can affect the stability. For this reason another 2 temperature points were added (+4 °C and +35 °C) to usual lts setup. There is a high chance for fats to be melted and stick to the walls of bottles between +18 °C and +60 °C.

Results for short term stability study:

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 single outliers by applying the Grubbs' test at confidence levels of 95% and 99%. 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 α = 0.05 (95% confidence level). The graphs are given in Annex 2.

Some outliers for Ba (+4 °C), Ni (+4 °C, +18 °C and +35 °C), Fe (+4 °C), Ca (+4 °C) ve Mn (+4 °C) were identified in the statistical evaluation (Grubbs' test) of the data; nevertheless, 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 (except Zn at 18 °C). The data evaluation results for the short-term stability at +4 °C, 18 °C, 35 °C and +60 °C are summarised in Table 6.

Table 6. Data evaluation results of short term stability tests

Analyte	Is the slope significantly different from zero at test temperature 4 °C at a level of 95% and 99% confidence ?	Outliers*	Is the slope significantly different from zero at test temperature 18 °C at a level of 95% and 99% confidence ?	Outliers*	Is the slope significantly different from zero at test temperature 35 °C at a level of 95% and 99% confidence ?	Outliers*	Is the slope significantly different from zero at test temperature 60 °C at a level of 95% and 99% confidence ?	Outliers*
B	No	-	No	-	No	-	No	-
Ba	No	One (SGT, %95)	No	-	No	-	No	-
Ca	No	One (SGT, %95)	No	-	No	-	No	-
Cd	No	-	No	-	No	-	No	-
Co	No	-	No	-	No	-	No	-
Cu	No	-	No	-	No	-	No	-
Fe	No	One (SGT, %95, %99)	No	-	No	-	No	-
K	No	-	No	-	No	-	No	-
Mg	No	-	No	-	No	-	No	-
Mn	No	One (SGT, %95)	No	-	No	-	No	-
Ni	No	One (SGT, %95)	No	One (SGT, %95)	No	One (SGT, %95, %99)	No	-
P	No	-	No	-	No	-	No	-
Sr	No	-	No	-	No	-	No	-
Zn	Yes	-	No	-	No	-	No	-

*SGT: Single Grubbs Test

The material is stable at 4°C, 18 °C, 35°C and 60 °C for up to 4 weeks. Only Zn showed a trend at 18°C but no at other temperatures thus it has been decided not to take any precaution at all. Thus, the samples can be safely dispatched under conditions where the temperatures do not exceed 60 °C for up to 4 weeks, i.e. at ambient temperature.

Results for long term stability study:

Shelf life of the CRM has been determined through long term stability measurements. For the measurements, two bottles for each of the months of 0, 3, 6, 12 and 18 has been stored at 18 °C and transferred to reference temperature after each period of time to be measured isochronously afterwards. Two bottles, designated as reference bottles, of the week 0 was stored at -20 °C.

The data for each time point has been calculated by 2 replicate measurements for each of two bottles. Thus, the average of 4 measurements for each time point is given in Annex 3. The error bars on each time point is calculated as the standard deviation of 4 measurement results.

The outliers for elements of Ca (4 °C), Fe (4 °C), Mg (4 and 18 °C), Ni (18 °C) were detected by Grubbs' test. However no data were rejected since there is no clear indication of technical reason observed.

The graphs were plotted against time and the regression line calculated. The long term stability uncertainty, $u_{lts,}$ of the material is then calculated for the required shelf life with equation (5) [7] as:

$$u_{lts} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \times t \qquad (5)$$

where

RSD : the relative standard deviation of all results of the stability study

t_i : being the time point for each replicate

$\bar{t}$: being the average of all time points

t : being the proposed shelf life at 18 °C

The uncertainty contribution u_{lts} was established for 18 months (t) at 18 °C. This uncertainty was one of the three parameters of the overall uncertainty budget of the certified values. The results are given in Table 7. The graphs for long term stability are given in Annex 3.

Table 7. Long term stability results

Element	T (°C)	Time (Month)	$u_{lts,rel}$ (%)
B	4	12	4.8
		18	7.2
		35	14.5
		60	24.1
	18	12	3.6
		18	5.3
		35	10.7
		60	17.8
Ba	4	12	0.95
		18	1.42
		35	2.85
		60	4.74
	18	12	0.97
		18	1.46
		35	2.91
		60	4.85
Ca	4	12	0.99
		18	1.49
		35	2.98
		60	4.97
	18	12	1.69
		18	2.54
		35	5.08
		60	8.47

Table 7. Long term stability results (continued)

Element	T (°C)	Time (Month)	$u_{lts,rel}$ (%)
Co	4	12	1.33
		18	2.00
		36	3.99
		60	6.65
	18	12	2.22
		18	3.33
		36	6.66
		60	11.10
Cd	4	12	4.74
		18	7.11
		36	14.22
		60	23.71
	18	12	3.28
		18	4.92
		36	9.84
		60	16.41
Cu	4	12	1.21
		18	1.81
		36	3.63
		60	6.05
	18	12	0.89
		18	1.33
		36	2.66
		60	4.43
Fe	4	12	3.15
		18	4.72
		36	9.45
		60	15.75
	18	12	1.39
		18	2.09
		36	4.18
		60	6.96
K	4	12	0.74
		18	1.11
		36	2.22
		60	3.70
	18	12	0.89
		18	1.33
		36	2.66
		60	4.44
Mg	4	12	2.78
		18	4.18
		36	8.35
		60	13.92
	18	12	2.75
		18	4.12
		36	8.24
		60	13.74
Mn	4	12	0.77
		18	1.16
		36	2.31
		60	3.86
	18	12	0.87
		18	1.30
		36	2.60
		60	4.33

Table 7. Long term stability results (continued)

Ni	4	12	1.52
		18	2.28
		36	4.56
		60	7.61
	18	12	2.59
		18	3.89
		36	7.77
		60	12.96
P	4	12	12.81
		18	19.22
		36	38.44
		60	64.07
	18	12	8.21
		18	12.32
		36	24.63
		60	41.06
Sr	4	12	1.69
		18	2.54
		36	5.07
		60	8.46
	18	12	1.15
		18	1.72
		36	3.45
		60	5.75
Zn	4	12	2.98
		18	4.47
		36	8.95
		60	14.91
	18	12	1.70
		18	2.55
		36	5.10
		60	8.49

CHARACTERISATION

According to ISO Guide 34, characterization and value assignment can be undertaken in four different approaches [1]. Two of the four approaches applied in hazelnut certification study are i) *using a primary method of measurement* and ii) *application of two or more reference methods by a single laboratory*. Characterization measurements of B, Cd, Cu, Fe and Zn elements were accomplished by ID-ICP-MS, a method generally accepted as a primary method of measurement. The results of these measurements were validated by comparing the matrix matched HR-ICP-MS measurements. Although validated ID-ICP-MS results were sufficiently meet the value assignment requirements for the aforementioned elements, except Cd, the results were reported as the mean of both techniques. Characterization studies for the remaining elements, Ca, Co, Mg, Mn, Ni and Sr, were completed by employing more than one analytical technique. Thus, while HR-ICP-MS and GF-AAS were the methods used for the Co, Mn, Ni and Sr measurements, Ca and Mg characterization studies were completed using HR-ICP-MS and FAAS techniques. The elements and corresponding characterization technique(s) used are given in Table 8.

Table 8. Techniques used in characterization study

Technique / Element	HR-ICP-MS	GF-AAS	ID-ICP-MS	FAAS
B	+		+	
Ba*	+			
Ca	+			+
Cd			+	
Co	+	+		
Cu	+		+	
Fe	+		+	
K*	+			
Mg	+			+
Mn	+	+		
Ni	+	+		
P*	+			
Sr	+	+		
Zn	+		+	

*Results are given as informative values.

For each of HR-ICP-MS, GF-AAS and FAAS techniques, the measurements of two units, previously reserved for characterization study, were performed in two different days with independent sample and standard preparations. Three replicate measurements per unit in each day produced at least six independent measurement results in total. In ID-ICP-MS technique, B and Cd measurements were performed in the same day while Cu, Fe and Zn measurements were completed in two different days. In total, nine independent measurement results were produced for two bottles for all elements in ID-ICP-MS study. As mentioned before, the results of the ID-ICP-MS measurements were compared with independent HR-ICP-MS measurements performed in two different days.

PROPERTY VALUE AND UNCERTAINTY ASSIGNMENT

For each element, assignment of property values and uncertainty values were performed considering the characterization strategy followed, measurement results and associated uncertainties, and the uncertainty contributions from homogeneity and stability measurements.

Mass fractions of the elements B, Cu, Fe and Zn, characterized by ID-ICP-MS and HR-ICP-MS, were assigned as the mean of the results obtained by the two methods. Cd value, on the other hand, was assigned as the mean of ID-ICP-MS replicate measurements. The uncertainty contributions, u_{char}, were determined using *bottom-up* approach.

The value assignment of the elements, of which characterization studies were performed using the *application of two or more reference methods by a single laboratory* approach, were carried out by unweighed mean of the results obtained by each method. The uncertainty value, u_{char}, were determined by merging the uncertainty values obtained for each method.

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 equation (6).

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

For the conditions where the uncertainty contribution from the long-term stability study at reference temperature of 18 °C is larger than the u_{sts}, u_{sts} value is not included when merging the uncertainties. Thus, the u_{sts} values were not included in the uncertainty calculations. The results are given in Table 9.

Table 9. Certified values and their uncertainties

Element	mg/kg	U_{CRM} (k=2)
B	16.8	2.2
Ca	1550	110
Cu	16.4	1.0
Fe	36.1	2.9
Mg	1540	150
Mn	95.3	6.3
Ni	1.60	0.17
Sr	6.68	0.46
Zn	20.4	1.8
Element	**µg/kg**	U_{CRM} (k=2)
Cd	6.4	0.9
Co	278	28

INFORMATIVE VALUES

Elements

Since Ba, K and P were not measured by a second technique, they were given as informative values. These values are given in Table 10.

Table 10. Informative values for elements in hazelnut

Informative values		
Element	**mg/kg**	***U* (k=2)**
Ba	5.8	0.3
K	5890	550
P	3240	890

Total Fat Content

Preliminary processed (see page 7) raw materials are procured from Fiskobirlik/Efit A.Ş., Gaziler Mahallesi Atatürk Bulvarı No:483 Merkez/GİRESUN/TÜRKİYE. TÜBİTAK UME was asked to include total fat content based on dry mass certification by this company. The material where details of processing is given in page 7-8 were analysed for their fat content using collaborators. The laboratories selected for this purpose are all accredited where their information is given in page 6. Each laboratory sent the results of analysis performed according to their accredited methods and also to the measurement protocol that was sent before sending the materials. The laboratories were asked to treat two bottles as two separate samples. Each laboratory was asked to report 3 independent measurement results which were obtained on at least two different days. Therefore, each laboratory reported 6 independent measurement results for two sample bottle, together with their associated measurement uncertainty values and the approach used for the estimation of measurement uncertainty. As an alternative although TÜBİTAK UME is not accredited for the mentioned analyses, has given results according to standard method TS EN ISO 659 [8]. All laboratories measured water content of samples at 103±2 °C until constant weight again as mentioned in the protocol. Total fat content results are given based on dry mass in Table 11. Informative value and its uncertainty were assigned according to ISO Guide 35 [2].

Table 11. Total fat content based on dry mass, moisture content and their uncertainties

Lab Code	Total fat content, w/w (%)	Standard uncertainty u (*k=1*)	% u	Expanded uncertainty U (*k=2*)
1	66.85	0.46	0.69	
2	70.23	0.02	0.02	
3	70.98	0.08	0.11	
4	67.14	0.08	0.12	
5	69.43	0.03	0.04	
6	68.84	0.19	0.27	
7	67.42	0.18	0.27	
8	69.18	0.20	0.28	
9	69.99	0.24	0.35	
10	66.21	0.42	0.64	
Mean	68.6	0.5	0.75	1.0
	Moisture content, w/w (%)	Standard uncertainty u (*k=1*)	% u	Expanded uncertainty U (*k=2*)
Mean	1.4	0.1	7.1	0.2

These values were evaluated statistically and single Grubbs test were done for investigation of outliers. No outliers were detected. Shapiro-Wilk test were applied to investigate normal distribution of data, the test results showed normal distribution. Further studies on stability is required therefore values are given as informative.

TRACEABILITY

The metrological traceability of the certified reference material was ensured by using the SI traceable calibration standards. In the measurements carried out at TÜBİTAK UME laboratories, mono-elemental calibration standards purchased from NIST were used for all the elements measured. In order to check the accuracy of the measurements, matrix CRM, NIST SRM 2387 “Peanut Butter” was used for Ca, Cu, Fe, Mg, Mn, P, K and Zn analytes. The methods used during analyses are validated. Matrix matched calibration technique was used. The matching between results and certified values showed no important bias for homogeneity for all analytes. As there is no other matrix CRM for other analytes recoveries are investigated via standard addition to the samples.

INSTRUCTION FOR USE

Storage Conditions

The material must be stored at (18 ± 2) °C under dark conditions. It is recommended to store the material at 4 °C once the bottle is opened.

Safety Information

Material is produced for laboratory use only. Usual laboratory precautions apply. It is strongly recommended that the material must be handled and disposed according to the safety guidelines where applicable.

Intended Use

This material is intended to be used for method validation of the determination of element mass fractions and total fat content in hazelnut and quality control purposes.

Usage of the Material

The bottle must be shaken for one minute before opening for assurance of homogeneity. All precautions must be taken in order to prevent degradation or contamination with air intact.

Minimum Sample Intake

Minimum sample intake is 1 g for all elements (5 g if intended to be used for fat and moisture content analysis).

Use of Certified Value

For assessing the method performance, the measured values of the CRM are compared with the certified values [8]. The procedure can be described briefly as:

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

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

- 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 a of about 95% confidence level.

ACKNOWLEDGEMENTS

The authors would like to thank Leslie Coveney and Ruth Hearn for reviewing this project and for their valuable comments.

The effort shown by technical evaluation committee, Assoc. Dr. Ahmet C. Gören, Dr. İlker Ün, Dr. Fatma Akçadağ, and our technicians involved in cleaning labware, Zehra Çakılbahçe, in processing Hatice Altuntaş, again our researchers involved in hazelnut processing, Sema Akyürek, Dr. Burcu Binici, Seda Damla Hatipoğlu, Dr. Alper İşleyen is also acknowledged.

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[9] http://www.ermcrm.org/html/ERM_products/application_notes/application_note_1/application_note_1_english_en.pdf (22/03/2010).

REVISION HISTORY

Date	Explanation
31.08.2016	First Issue
26.09.2016	Homogeneity and stability results for As, Cr, Pb and Se are deleted from report
26.09.2016	Standard method name is added at page 20 with reference

Annex 1 Data for Homogeneity Study

Table A1. UME CRM 1202 homogeneity test results for B, Ba and Ca

Bottle no	**B** (mg/kg)			**Ba** (mg/kg)			**Ca** (mg/kg)		
	R1	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
24	17.75	16.43	16.89	5.76	6.37	6.04	1517	1611	1558
86	16.36	16.15	17.53	5.91	5.84	5.83	1516	1569	1513
174	15.52	17.51	16.58	5.66	5.75	5.92	1531	1549	1473
190	18.01	18.01	18.00	6.17	5.91	5.98	1511	1529	1550
245	18.21	17.30	18.39	5.68	6.09	5.96	1519	1523	1529
307	18.01	16.93	19.22	5.74	6.09	5.79	1507	1541	1550
401	18.31	17.74	16.90	6.02	6.05	5.88	1512	1533	1501
436	16.14	17.76	16.79	5.85	5.69	5.85	1546	1479	1504
503	18.46	17.84	17.28	5.71	6.09	5.96	1623	1529	1518
561	16.61	17.65	18.14	5.54	5.55	6.01	1470	1470	1477

Table A2. UME CRM 1202 homogeneity test results test results for Cd, Co and Cu

Bottle no	**Cd** (µg/kg)			**Co** (µg/kg)			**Cu** (mg/kg)		
	R1	**R1**	**R1**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
24	8.84	8.84	8.84	277.9	285.7	282.2	16.61	16.62	16.54
86	8.45	8.45	8.45	277.4	281.7	277.8	16.38	16.45	16.55
174	8.35	8.35	8.35	270.6	281.5	285.9	15.57	16.70	16.18
190	8.45	8.45	8.45	281.8	288.5	277.4	16.63	16.80	16.98
245	9.52	9.52	9.52	279.8	286.3	289.1	16.49	16.22	16.59
307	9.24	9.24	9.24	307.2	301.9	264.6	16.91	16.29	16.23
401	8.38	8.38	8.38	277.3	273.5	274.6	16.42	16.37	16.40
436	7.82	7.82	7.82	274.4	288.6	270.0	15.84	16.23	15.94
503	10.16	10.16	10.16	286.2	292.3	308.7	16.38	16.64	16.20
561	7.66	7.66	7.66	280.1	277.5	286.4	16.08	16.07	16.73

Table A3. UME CRM 1202 homogeneity test results for Fe, Mg, Mn and Ni

Bottle no	**Fe** (mg/kg)			**Mg** (mg/kg)			**Mn** (mg/kg)			**Ni** (mg/kg)		
	R1	**R1**	**R1**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
24	37.31	37.31	37.31	1496	1488	1478	95.73	96.04	98.37	1.73	1.78	1.83
86	35.41	35.41	35.41	1504	1480	1489	94.39	98.06	93.45	1.71	1.72	1.77
174	35.63	35.63	35.63	1513	1449	1506	97.92	93.00	91.41	1.61	1.72	1.73
190	35.96	35.96	35.96	1500	1508	1499	96.50	96.15	96.15	1.73	1.66	1.71
245	36.42	36.42	36.42	1512	1509	1481	97.97	96.07	94.27	1.74	1.74	1.73
307	36.72	36.72	36.72	1525	1511	1496	94.05	96.48	94.33	1.78	1.79	1.64
401	35.50	35.50	35.50	1516	1521	1512	94.83	96.02	94.05	1.71	1.74	1.78
436	37.54	37.54	37.54	1470	1506	1511	94.99	92.43	97.87	1.57	1.69	1.76
503	35.88	35.88	35.88	1598	1530	1542	96.98	97.91	100.56	1.85	1.81	1.75
561	36.02	36.02	36.02	1523	1559	1522	92.18	94.78	96.17	1.73	1.71	1.74

Table A4. UME CRM 1202 homogeneity test results for P, K, Sr and Zn

Bottle no	**P** (mg/kg)			**K** (mg/kg)			**Sr** (mg/kg)			**Zn** (mg/kg)		
	R1	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
24	3133	3107	3032	5497	5658	5337	6.77	7.31	7.32	21.37	20.74	20.46
86	3064	3077	3059	5431	5430	5334	7.22	6.97	6.77	20.83	20.18	20.72
174	3134	2947	3116	5557	5436	5580	6.96	6.75	6.68	20.41	20.89	20.60
190	3024	3108	3133	5315	5465	5292	7.24	7.23	6.97	20.91	21.62	22.15
245	3114	3010	3063	5528	5364	5460	6.67	6.89	6.93	21.45	20.63	21.00
307	3164	3113	3065	5485	5263	5424	6.82	7.12	6.78	22.82	21.32	21.94
401	3128	3164	3091	5798	5474	5410	6.95	7.01	6.92	20.07	20.53	20.19
436	3053	3140	3118	5377	5538	5418	6.90	6.87	7.05	20.27	20.87	19.81
503	3270	3158	3129	6309	5579	5470	6.97	7.07	6.50	21.50	20.30	21.19
561	3135	3126	3154	5361	5708	5486	6.57	6.35	6.91	19.80	19.16	20.91

Annex 2 Short Term Stability Plots

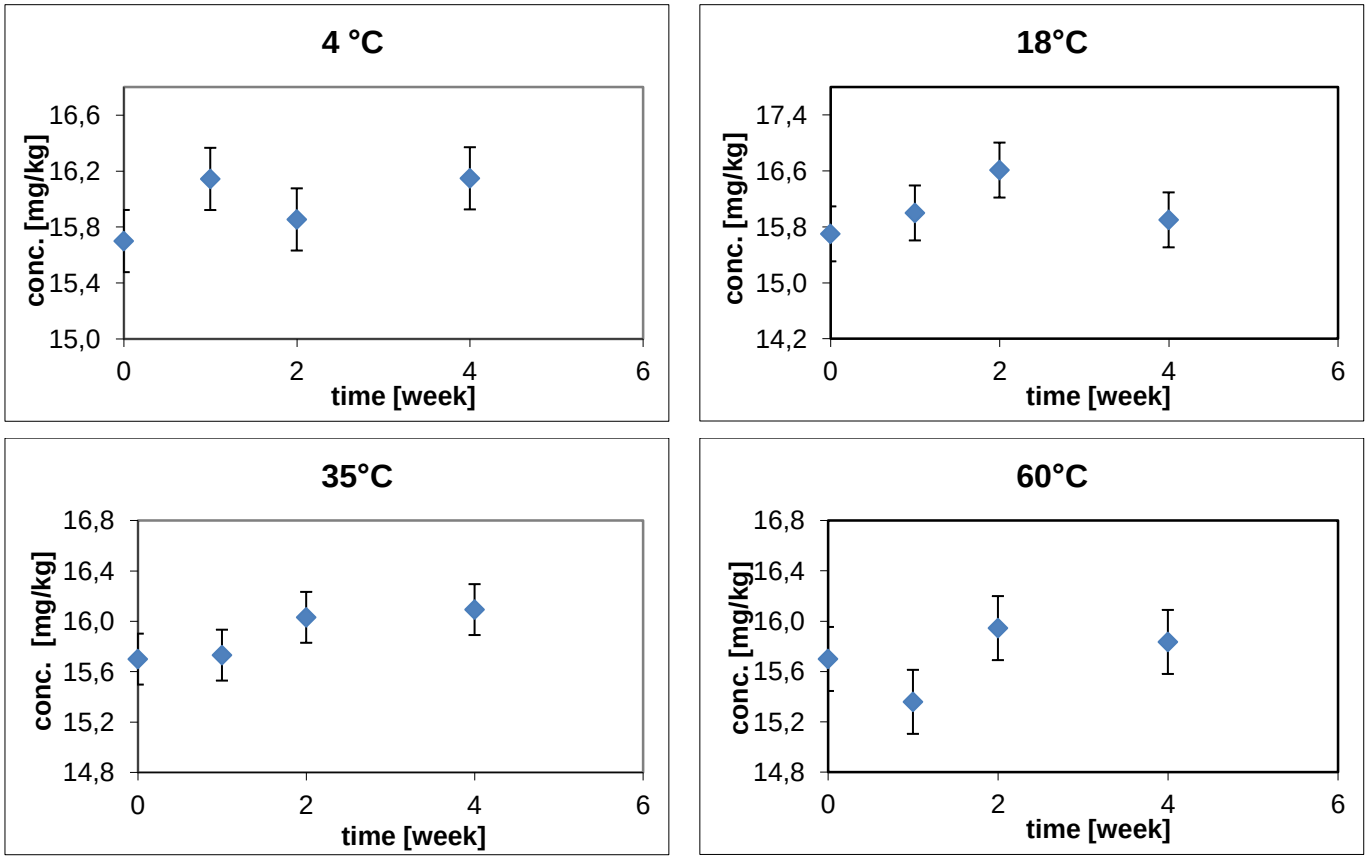

Figure 1. Short term stability plots for B

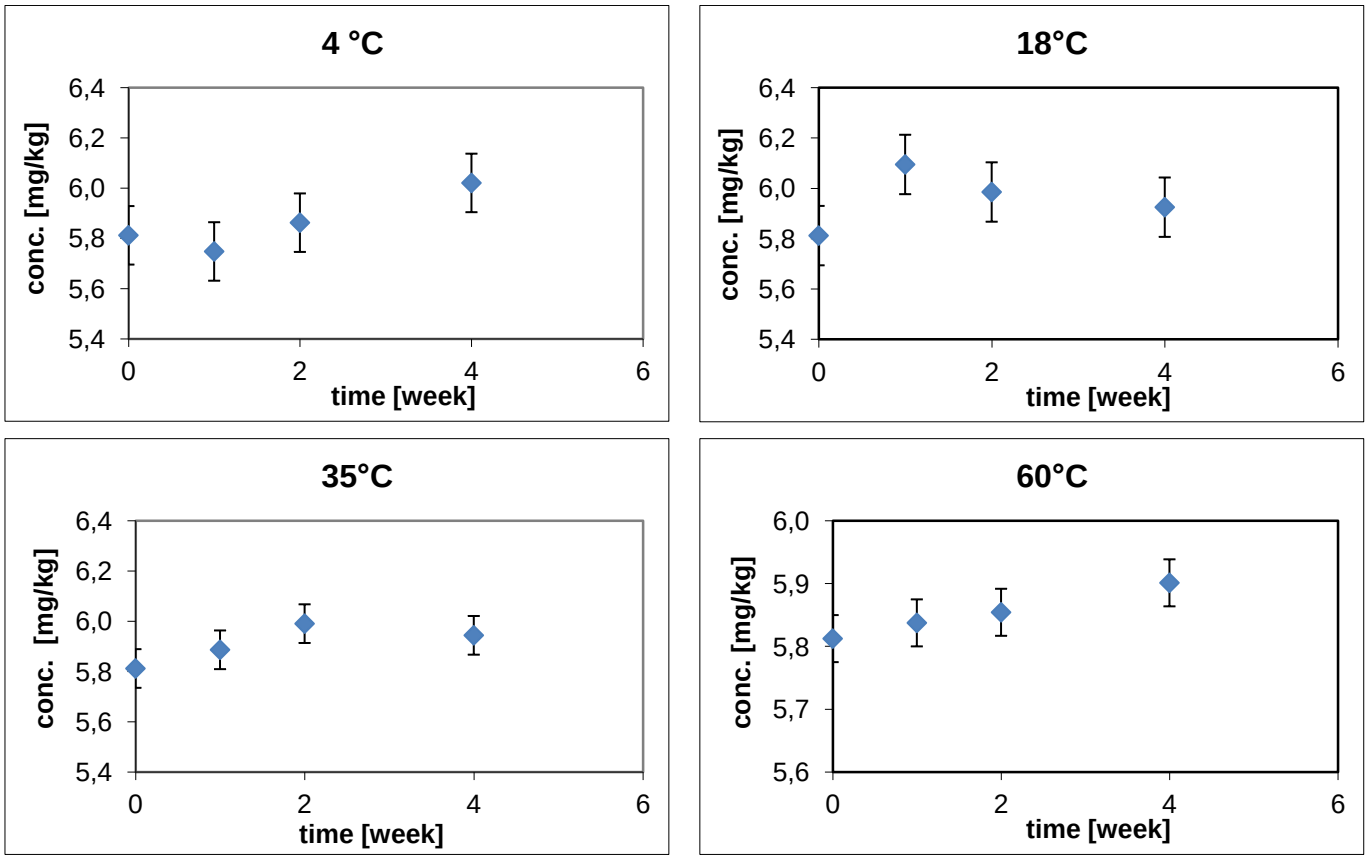

Figure 2. Short term stability plots for Ba

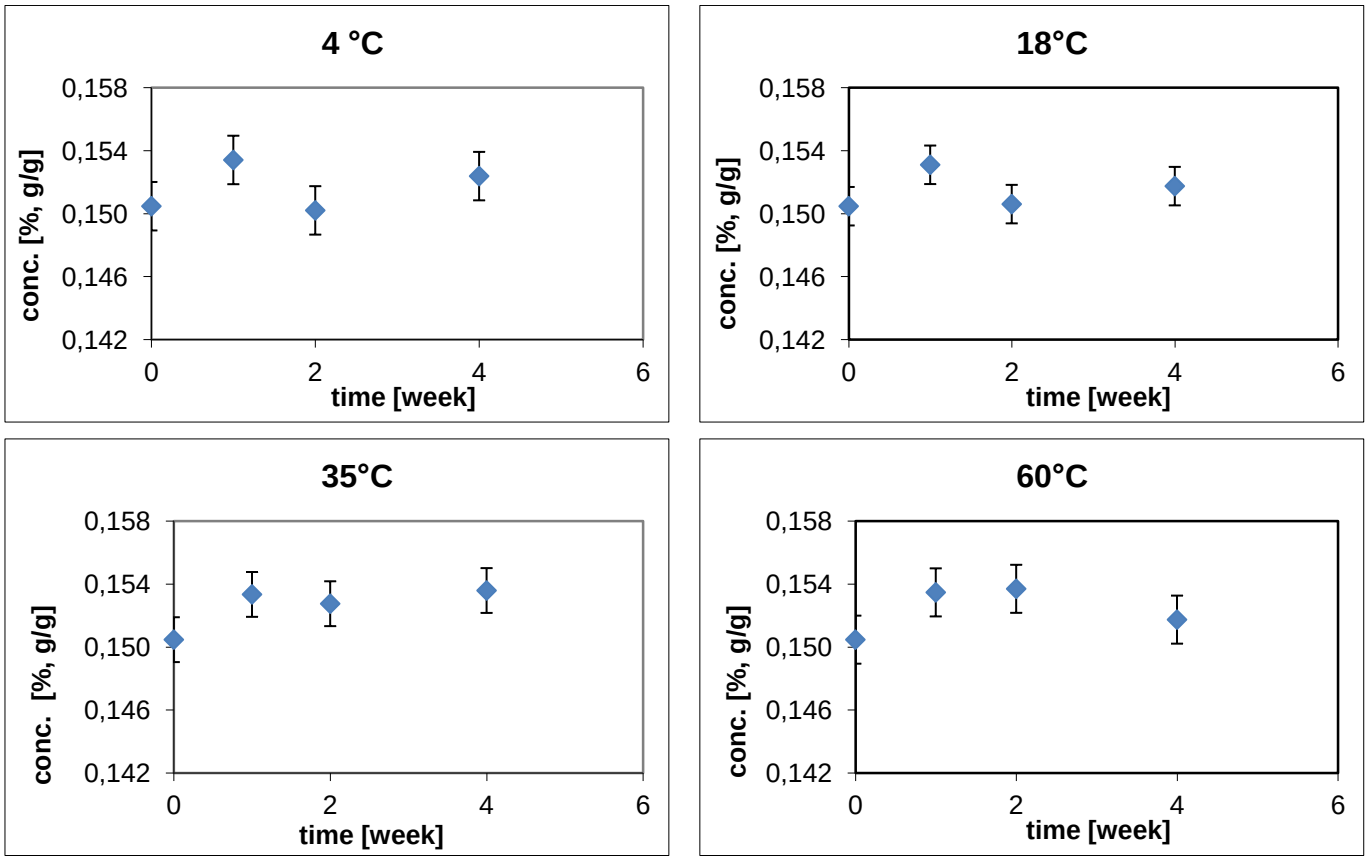

Figure 3. Short term stability plots for Ca

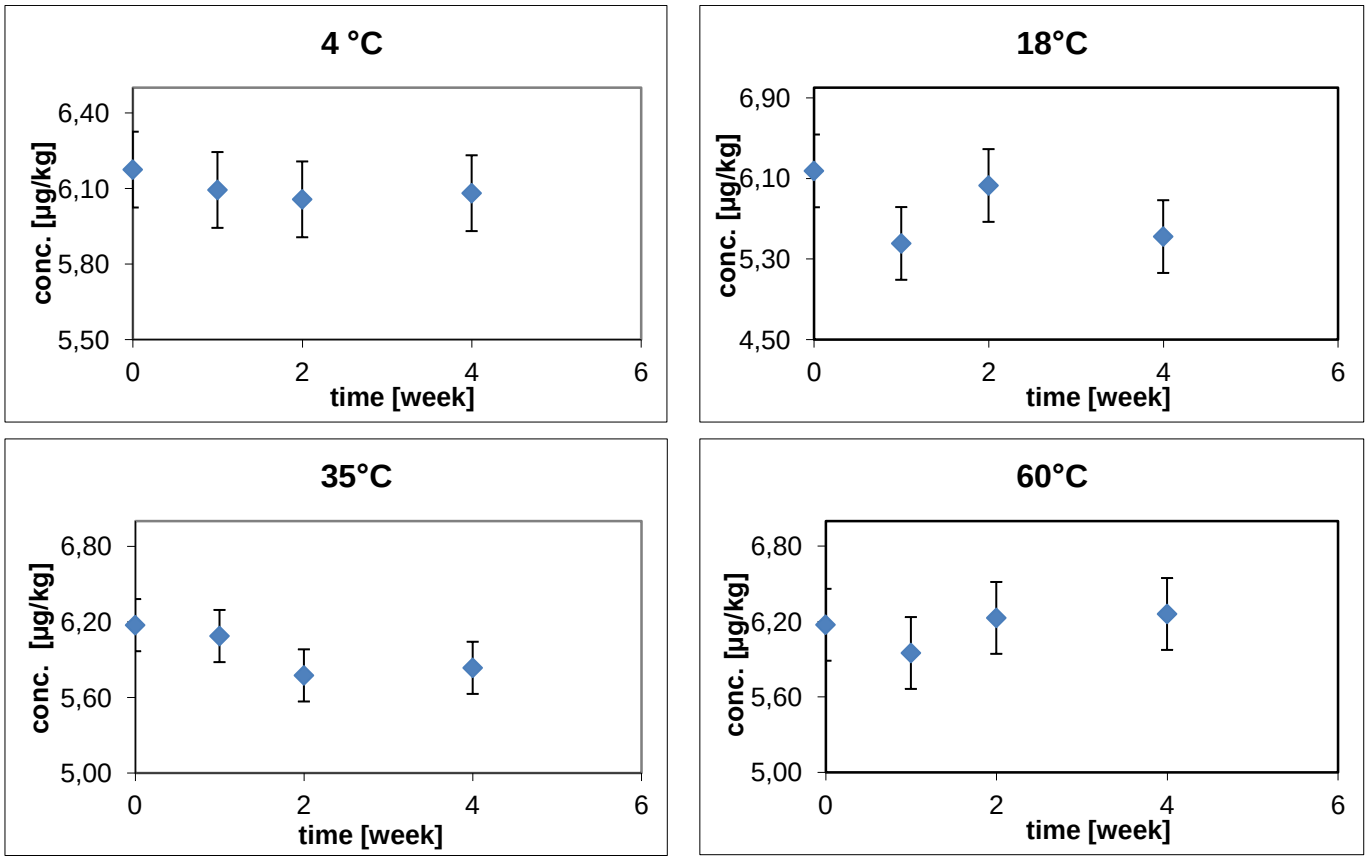

Figure 4. Short term stability plots for Cd

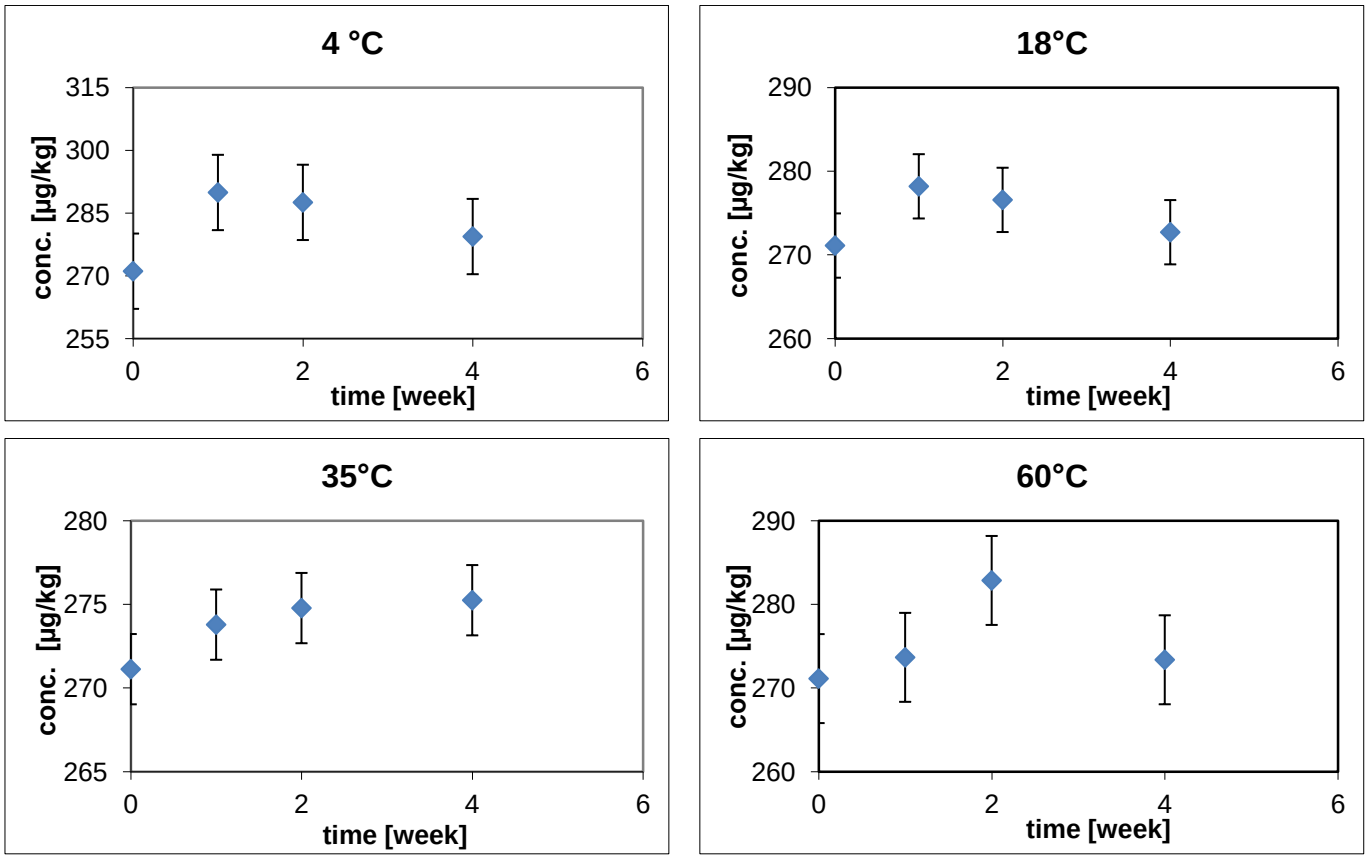

Figure 5. Short term stability plots for Co

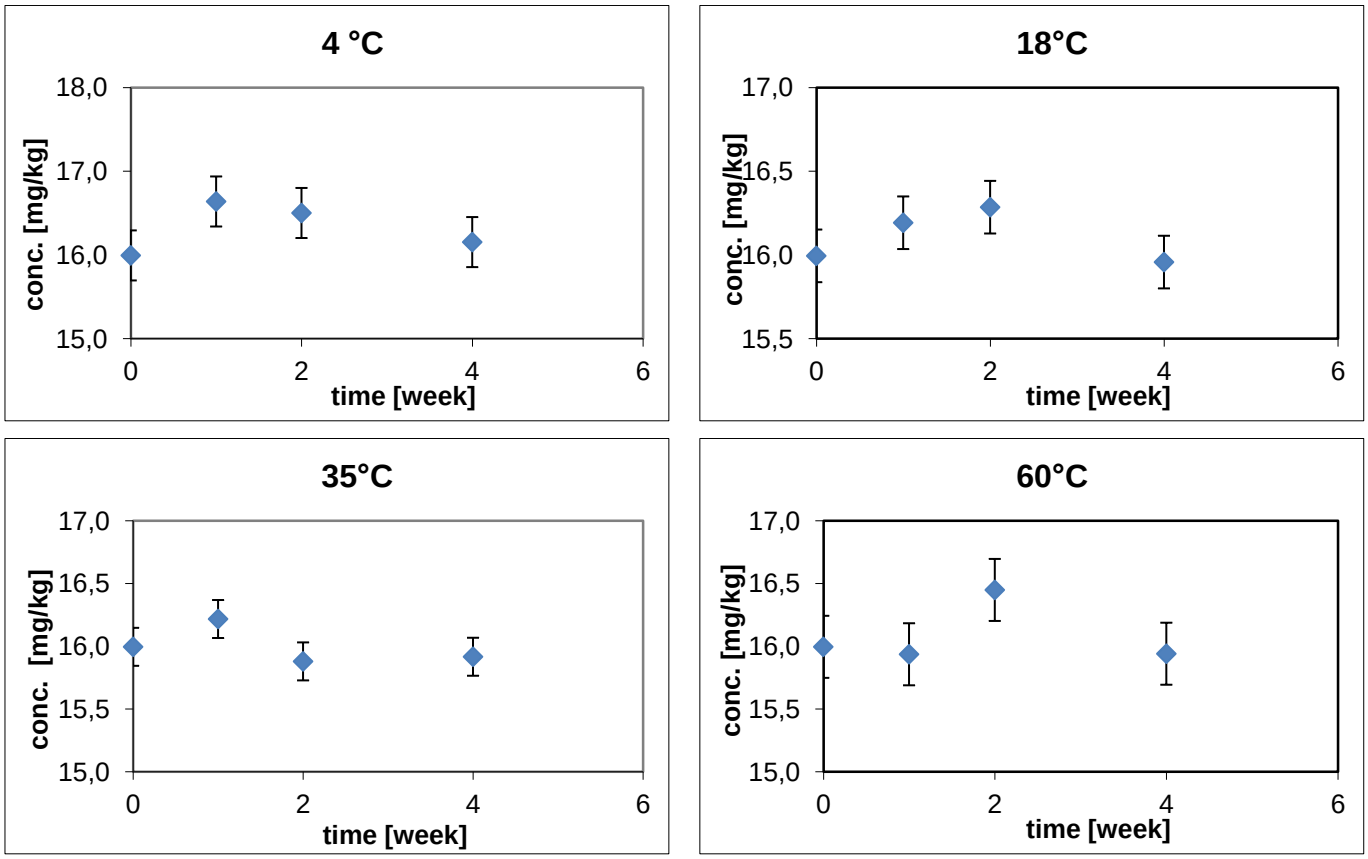

Figure 6. Short term stability plots for Cu

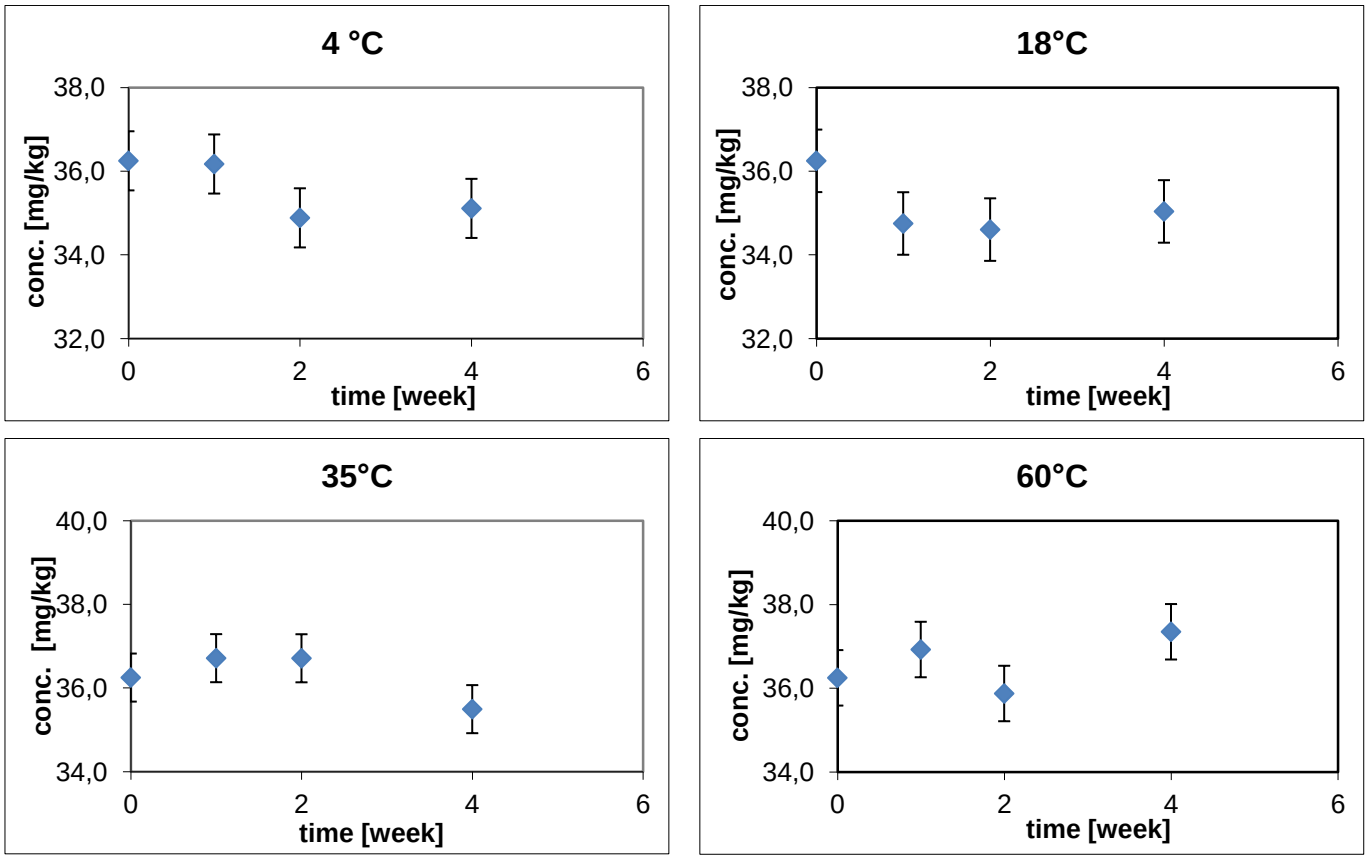

Figure 7. Short term stability plots for Fe

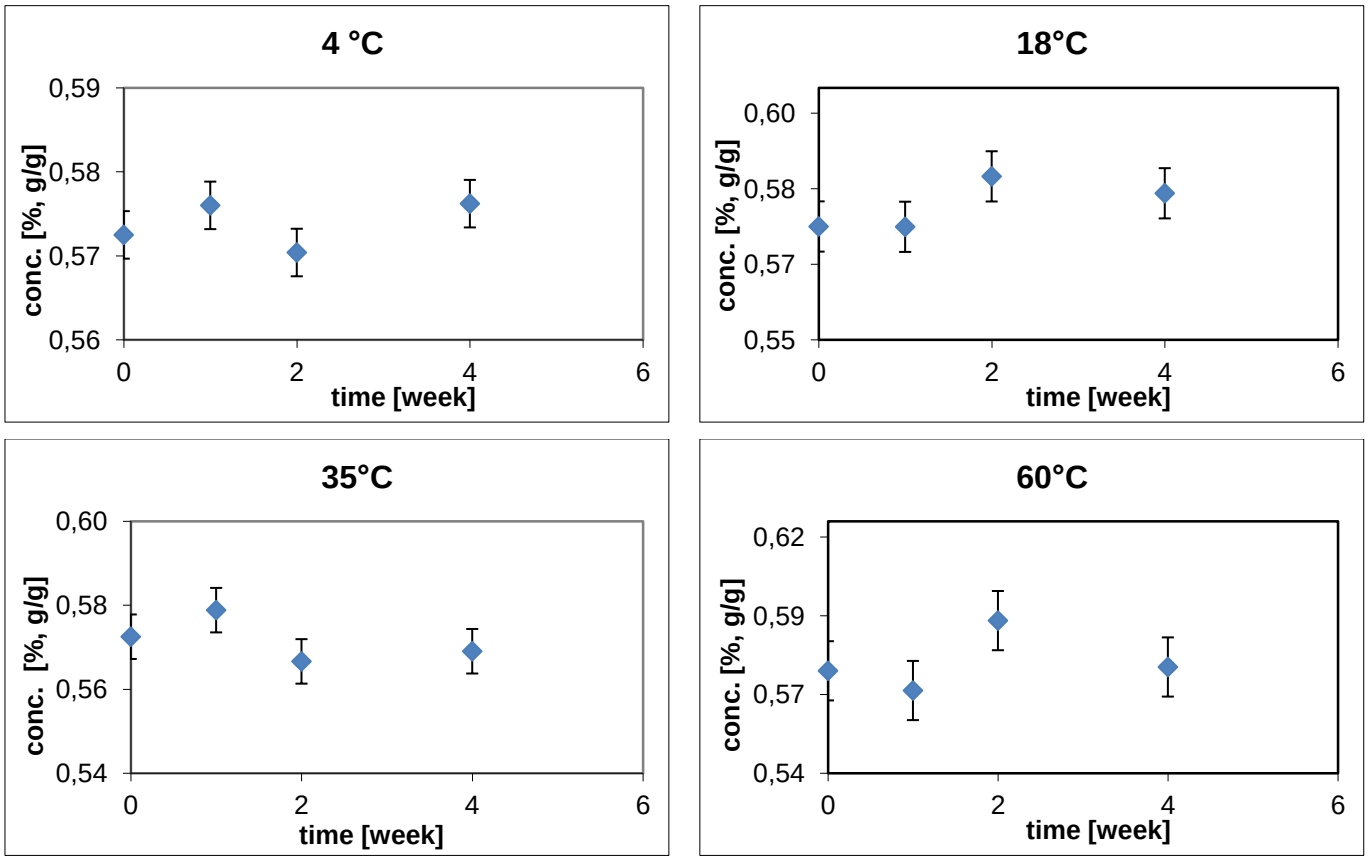

Figure 8. Short term stability plots for K

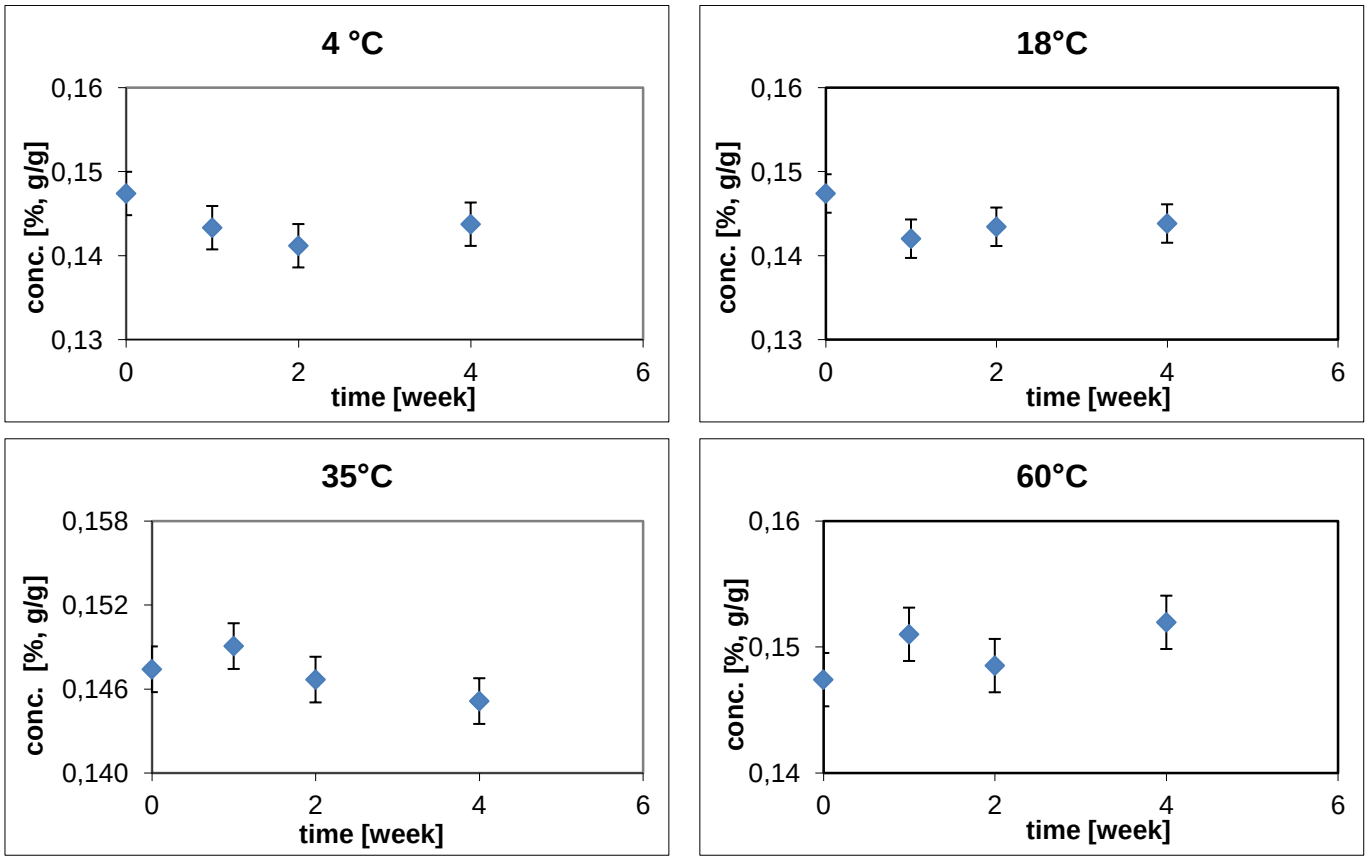

Figure 9. Short term stability plots for Mg

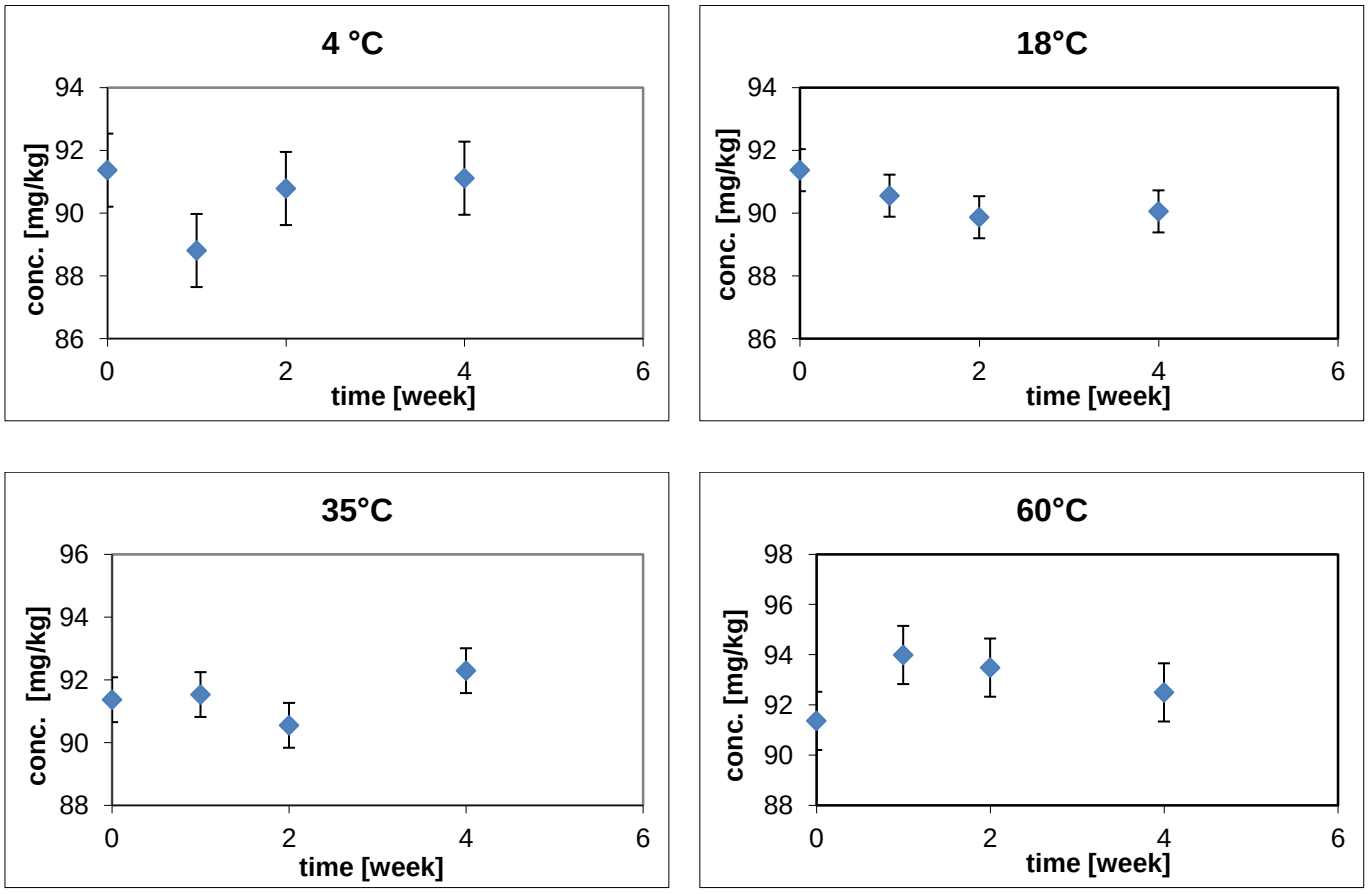

Figure 10. Short term stability plots for Mn

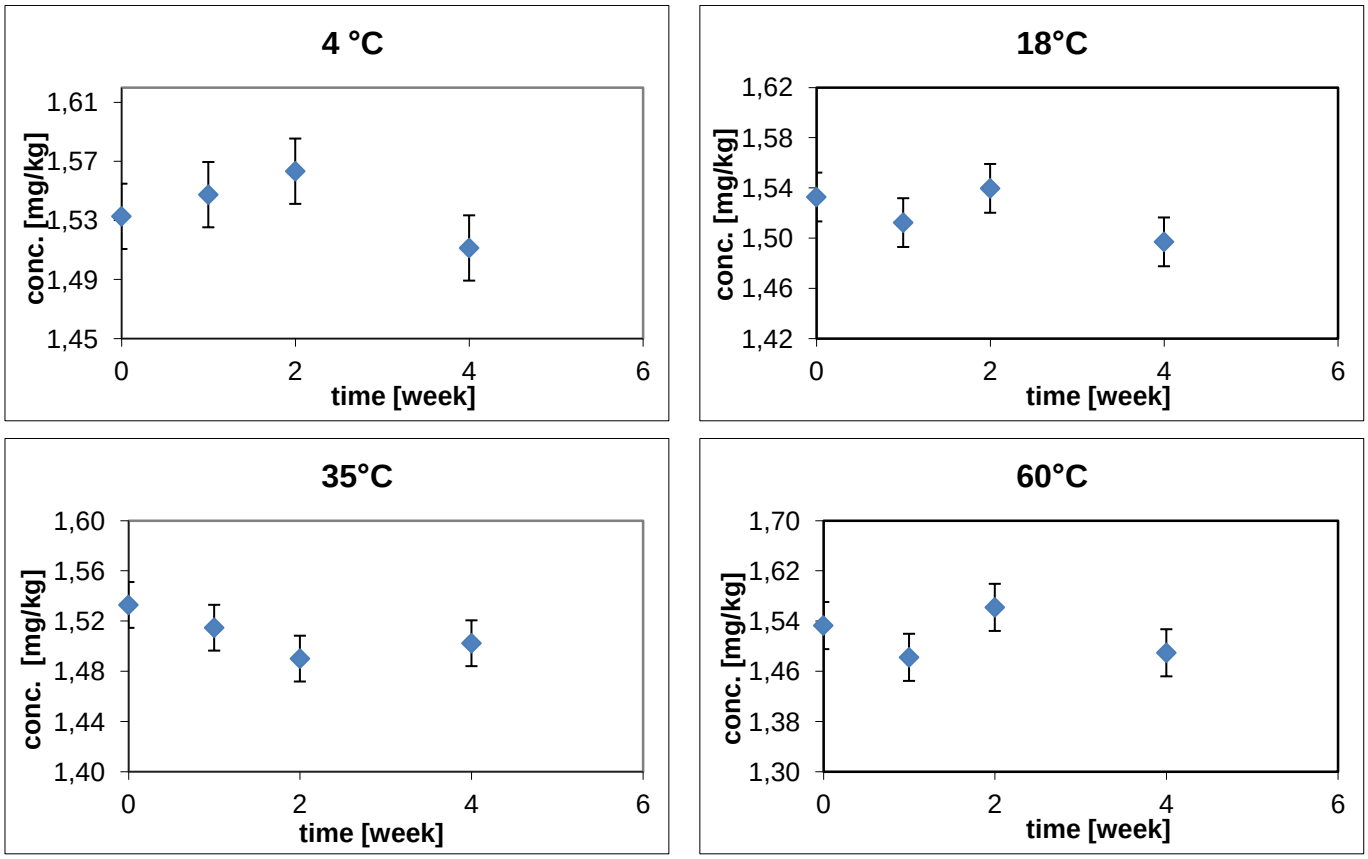

Figure 11. Short term stability plots for Ni

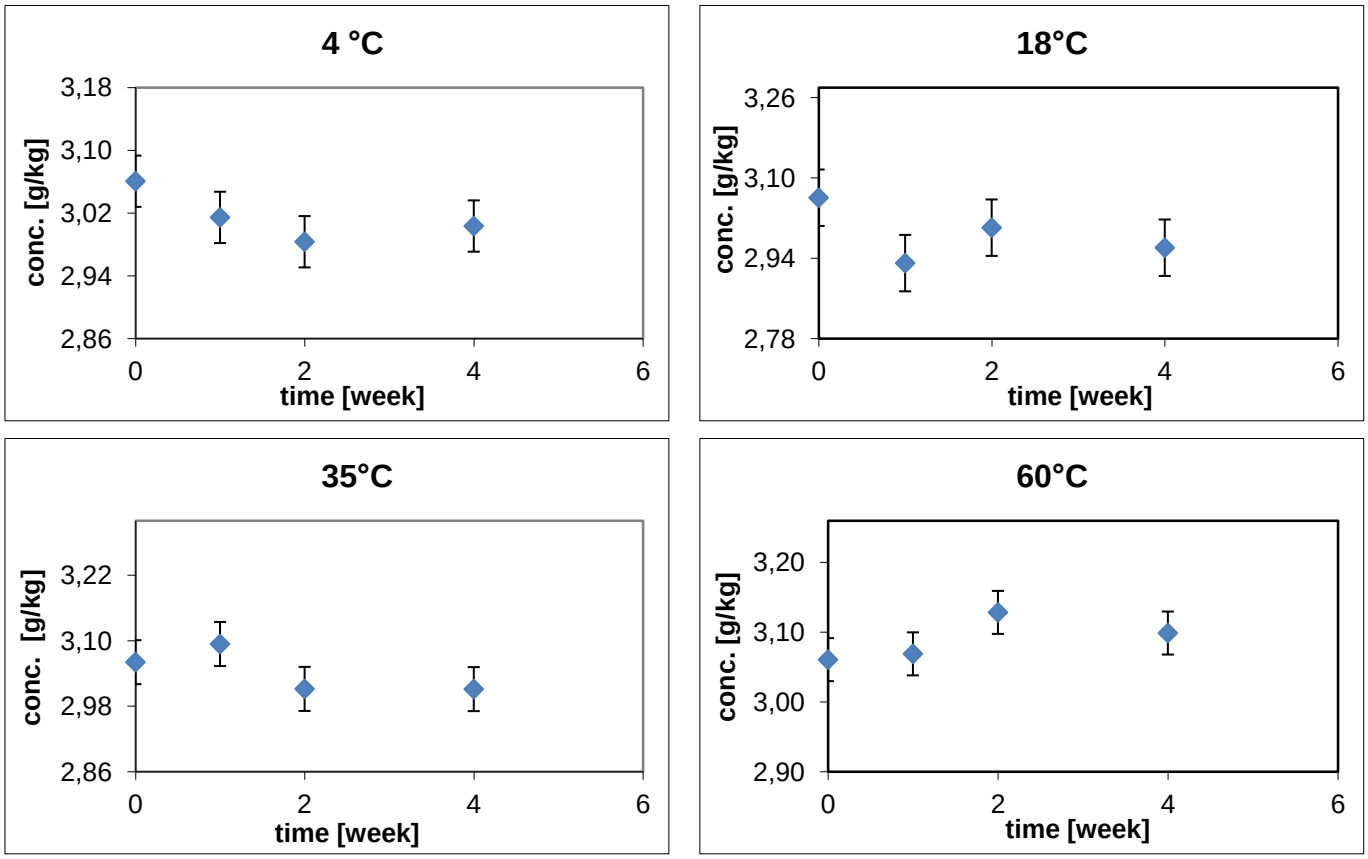

Figure 12. Short term stability plots for P

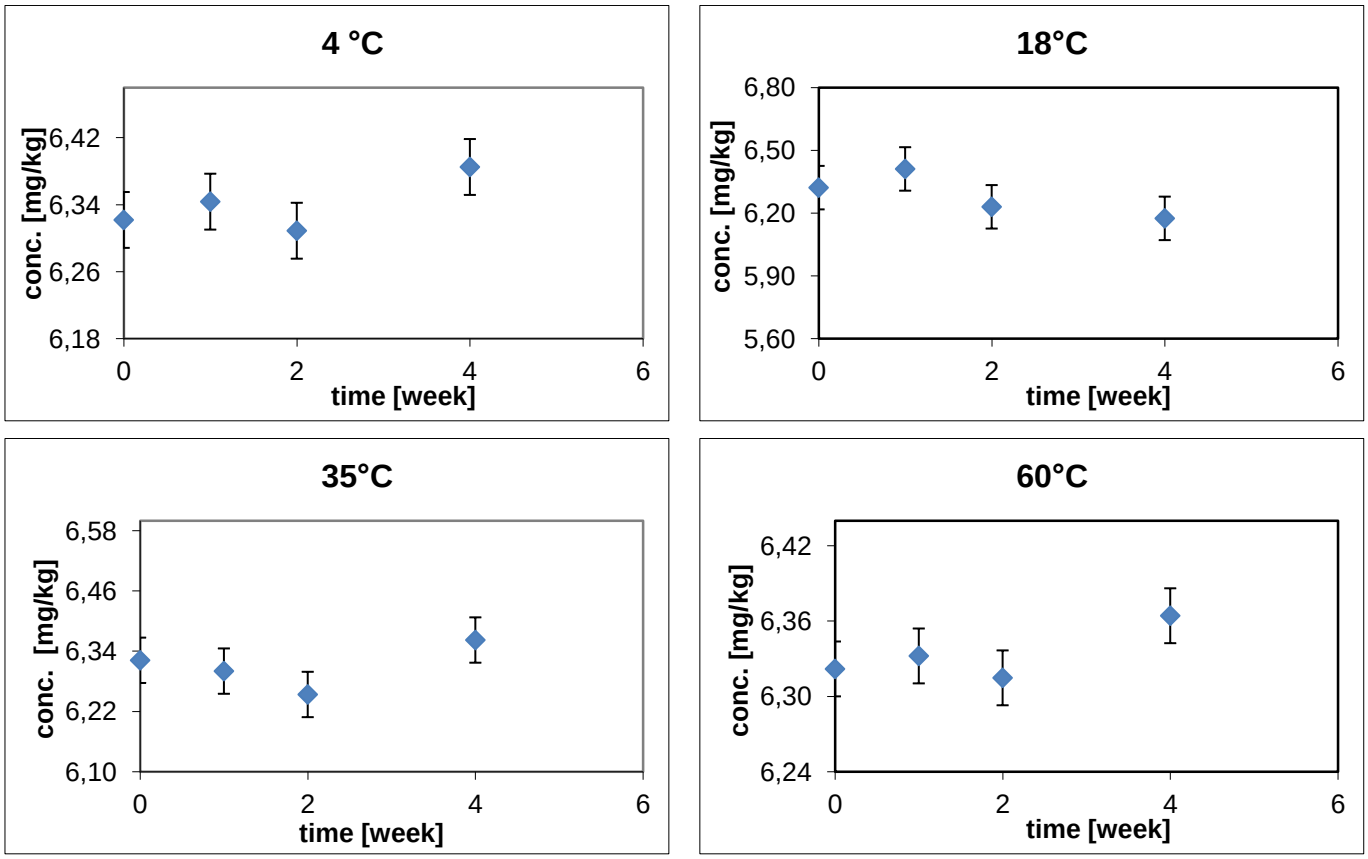

Figure 13. Short term stability plots for Sr

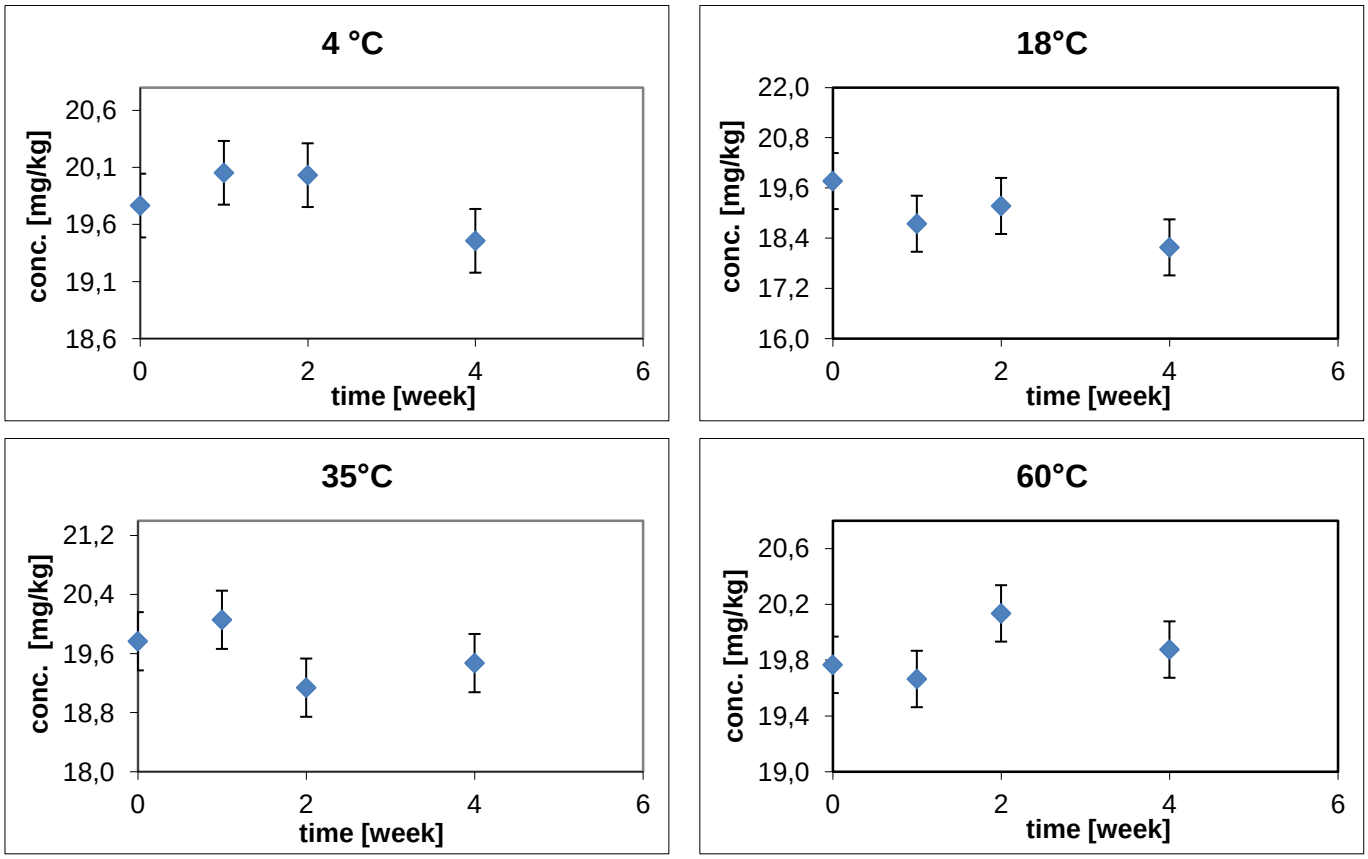

Figure 14. Short term stability plots for Zn

Annex 3. Long Term Stability Plots for 18 months at 18 °C

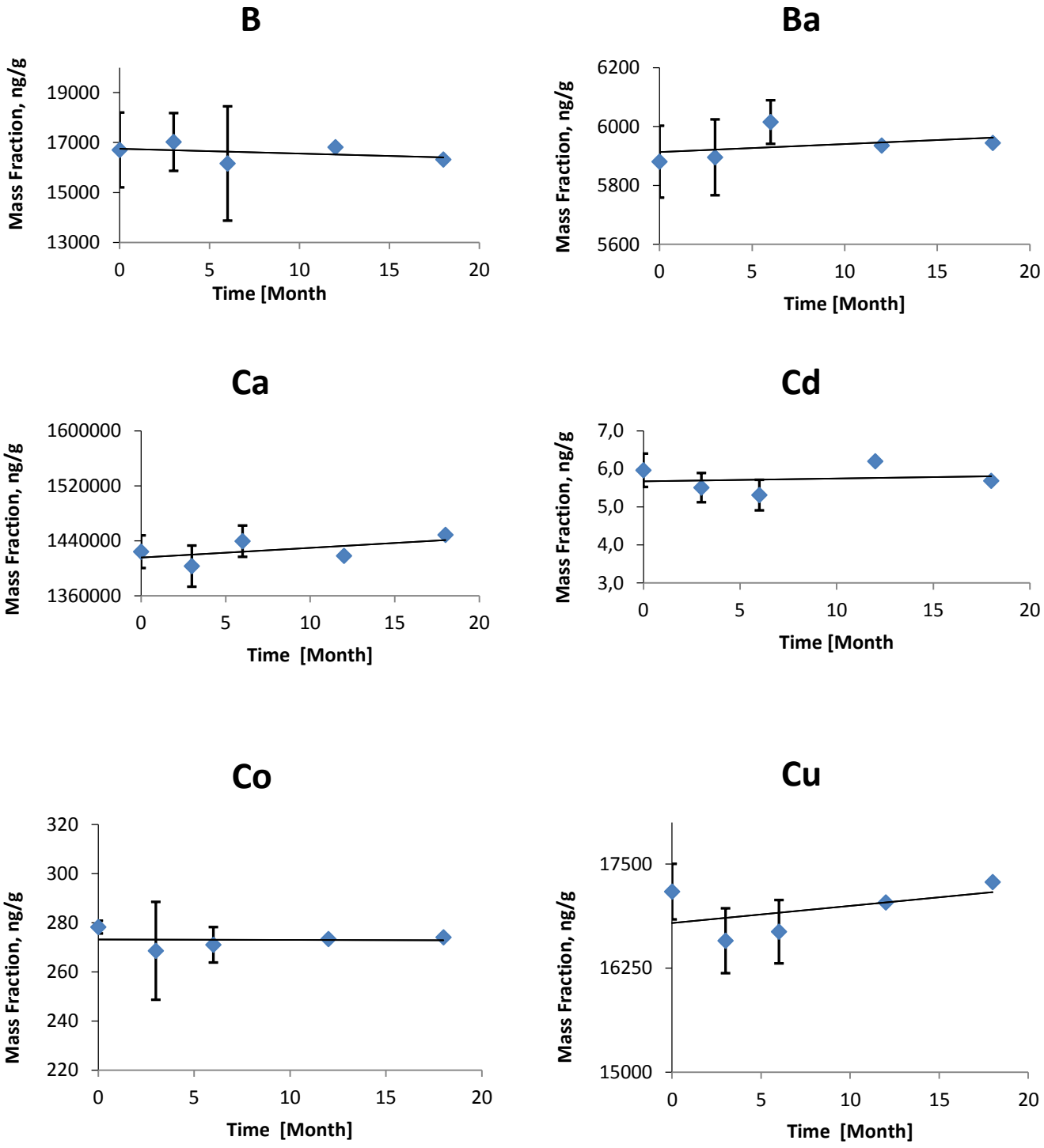

Figure 15. Long term stability plot for B, Ba, Ca, Cd, Co, Cu at 18 °C

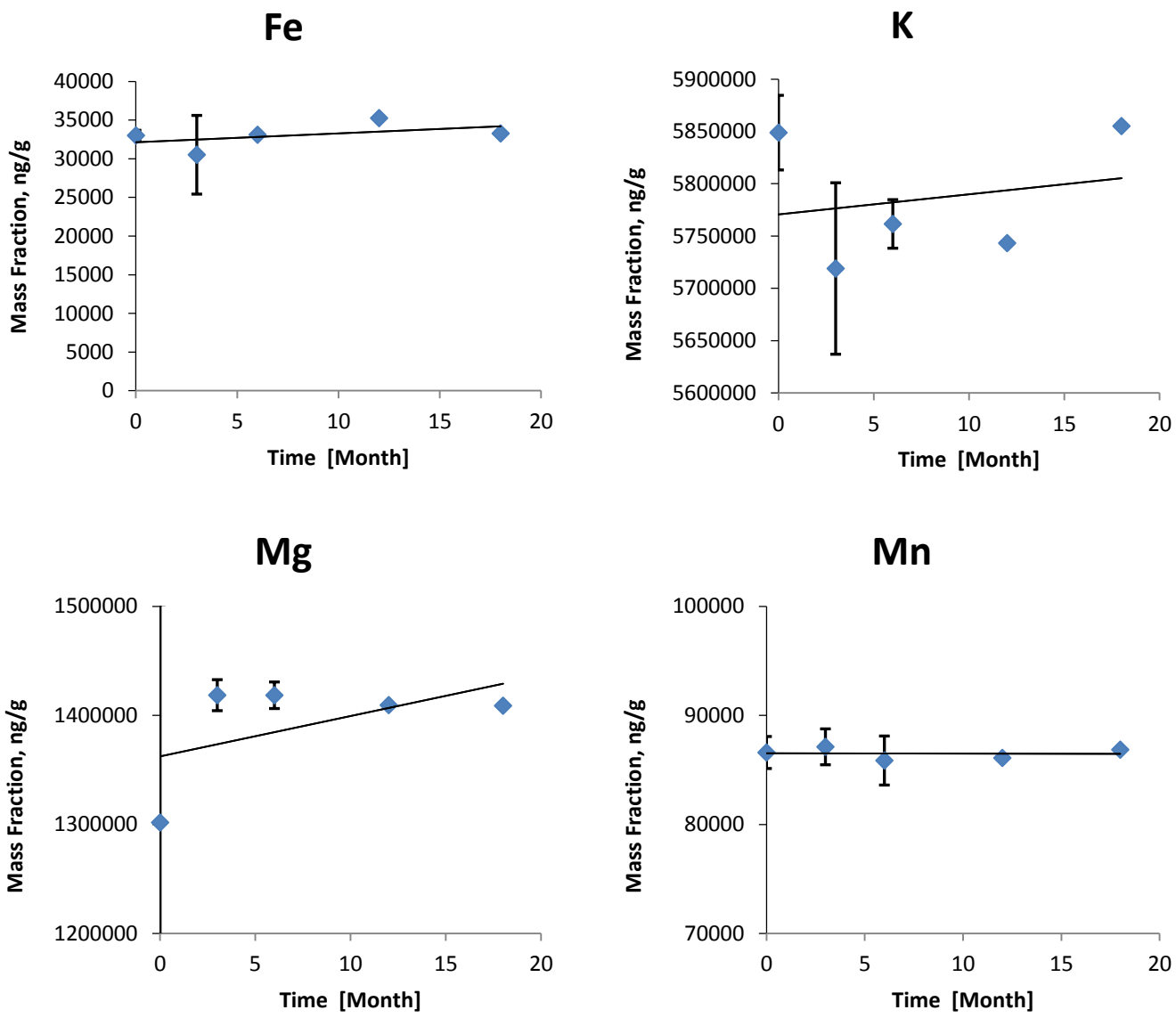

Figure 16. Long term stability plot for Fe, K, Mg, Mn at 18 °C

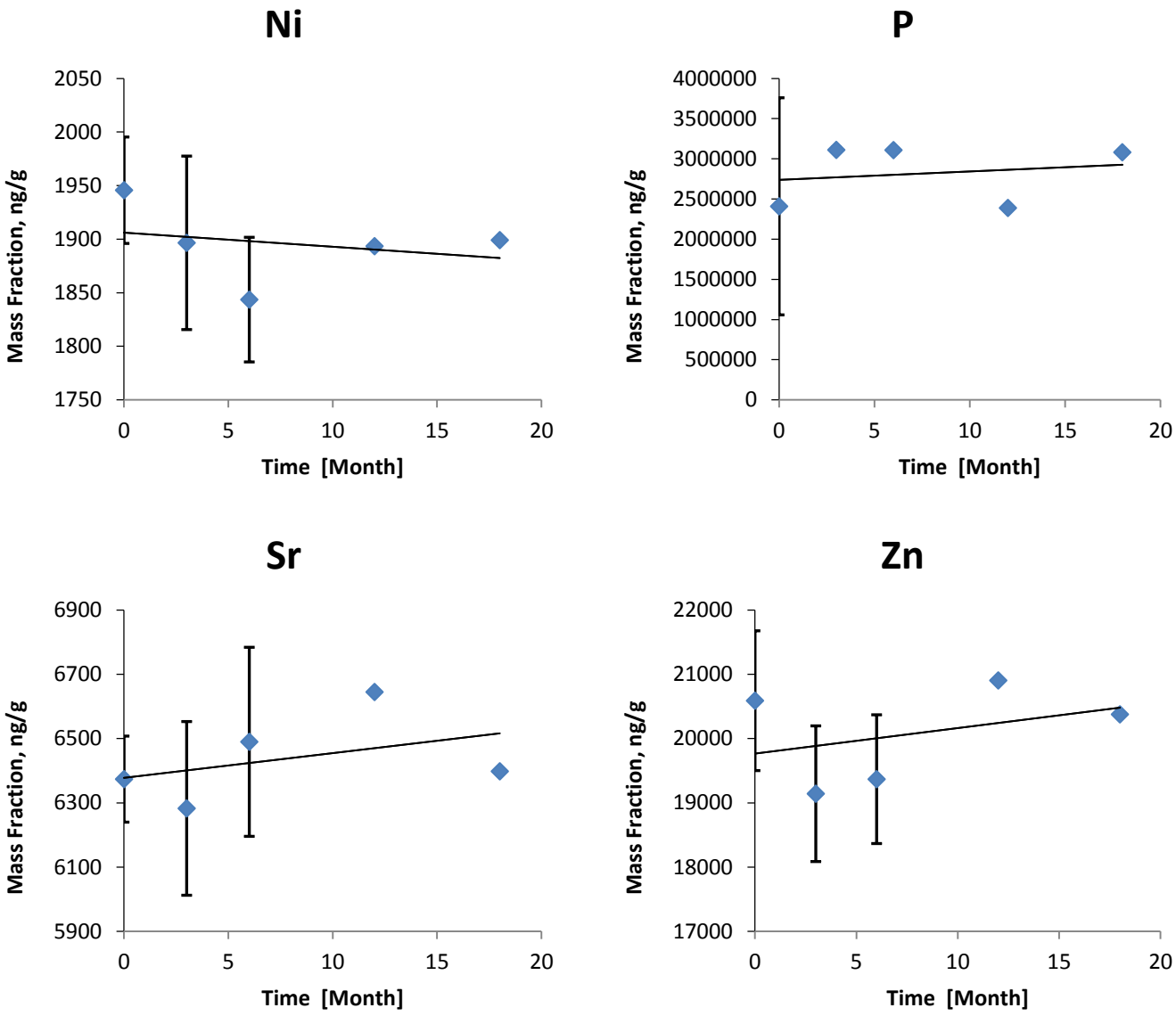

Figure 17. Long term stability plot for Ni, P, Sr, Zn at 18 °C