REPUBLIC OF TURKEY
YILDIZ TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

APPLICATIONS OF MASS SPECTROMETRY: CERTIFICATION OF A REFERENCE MATERIAL, INVESTIGATION OF PLANT METABOLISM AND PROVENANCE STUDIES

Betül ARI ENGİN

DOCTOR OF PHILOSOPHY THESIS

Department of Chemistry

Analytical Chemistry Program

Supervisor

Prof. Dr. Sezgin BAKIRDERE

Co-Supervisor

Dr. Süleyman Z. CAN

January, 2022

REPUBLIC OF TURKEY

YILDIZ TECHNICAL UNIVERSITY

GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

APPLICATIONS OF MASS SPECTROMETRY: CERTIFICATION OF A REFERENCE MATERIAL, INVESTIGATION OF PLANT METABOLISM AND PROVENANCE STUDIES

A thesis submitted by Betül ARI ENGİN in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY is approved by the committee on 10.01.2022 in Department of Chemistry, Analytical Chemistry Program.

Prof. Dr. Sezgin BAKIRDERE Yıldız Technical University Supervisor	Dr. Süleyman Z. CAN TÜBİTAK National Metrology Institute Co-Supervisor

Approved By the Examining Committee

Prof. Dr. Sezgin BAKIRDERE, Supervisor
Yıldız Technical University _____

Dr. Süleyman Z. CAN, Co-Supervisor
TÜBİTAK UME _____

Prof. Dr. Yücel ŞAHİN, Member
Yıldız Technical University _____

Prof. Dr. Yusuf DİLGİN, Member
Çanakkale On Sekiz Mart University _____

Prof. Dr. Güleda ENGİN, Member
Yıldız Technical University _____

Prof. Dr. Fatma Bedia ERİM BERKER, Member
Istanbul Technical University _____

I hereby declare that I have obtained the required legal permissions during data collection and exploitation procedures, that I have made the in-text citations and cited the references properly, that I haven't falsified and/or fabricated research data and results of the study and that I have abided by the principles of the scientific research and ethics during my Thesis Study under the title of Applications of Mass Spectrometry: Certification of A Reference Material, Investigation of Plant Metabolism and Provenance Studies supervised by my supervisor, Prof. Dr. Sezgin BAKIRDERE. In the case of a discovery of false statement, I am to acknowledge any legal consequence.

Betül ARI ENGİN

Dedicated to my family

ACKNOWLEDGEMENTS

I would like to express my deep appreciation and respect to my supervisor Prof. Dr. Sezgin Bakırdere and Dr. Süleyman Z. Can for their endless guidance, support, encouragement, understanding, patience and suggestions throughout this study. This thesis would never have taken shape without their support. Special thanks to Professor Bakırdere for instilling endless motivation on science to me and all his students with great patience.

I would also like to thank my thesis committee; Prof. Dr. Yücel Şahin and Prof. Dr. Yusuf Dilgin for their insightful comments and encouragement.

My endless thank to Prof. Dr. Osman Yavuz Ataman for meeting me with analytical chemistry during my bachelor and Master of Science.

My deepest thank is to my husband Erinç Engin for encouraging me to deal with unsocial life and endless problems in the thesis.

I would also like to thank to my mother and all family members for their endless support and trust.

I owe thanks to Inorganic Chemistry Group member of TÜBİTAK UME, for their understanding and support during this thesis. Special thanks to Zehra Çakılbahçe Haliloğlu for her help whenever I need and for her valueable friendship.

I would like to thanks to Bakırdere Research Group members, especially to Çağdaş Büyükpınar, Aylin Kasa, and Buse Zaman, for enjoyable time and friendships.

Betül ARI ENGİN

TABLE OF CONTENTS

LIST OF SYMBOLS vii
LIST OF ABBREVIATIONS ix
LIST OF FIGURES xii
LIST OF TABLES xv
ABSTRACT xx
ÖZET xxii
1 INTRODUCTION 1
1.1 Literature Review ... 1
1.1.1 General Information ... 2
1.1.2 Certified Reference Materials in Inorganic Analysis ... 8
1.1.3 Speciation Analysis ... 18
1.1.4 Traceability and Authentication of Food ... 33
1.2 Objective of the Thesis ... 37
1.3 Hypothesis ... 38
2 MATERIALS AND METHODS 40
2.1 Instrumentation ... 40
2.1.1 Instrumentation for Certification of Trace Elements in Seawater Certified Reference Material ... 40
2.1.2 Instrumentation for Analysis of Leek Samples ... 41
2.1.3 Instrumentation for Authentication Study ... 41
2.2 Chemicals and Materials ... 42
2.2.1 Chemicals and Materials for the Certification of Trace Elements in Seawater Certified Reference Material ... 42
2.2.2 Chemical and Materials for Analysis of Leek Samples ... 43
2.2.3 Chemical and Materials for Provenance Study ... 45
2.3 Experimental ... 46
2.3.1 Certification of Trace Elements in Seawater Certified Reference Material ... 46
2.3.2 Experimental for Analysis of Leek Samples ... 66
2.3.3 Experimental for Provenance Study of Walnuts ... 80
3 RESULTS AND DISCUSSION 87
3.1 Production of Trace Elements in Seawater Certified Reference Material ... 87

3.1.1 Development of Reference Method for the Elements Interested 87
3.1.2 Validation of Matrix Matched External Calibration - ICP-MS/MS Method for of Total As in Sea Water 99
3.1.3 Between and Within Unit Homogeneity 104
3.1.4 Assessment of Short Term and Long Term Stability..... 116
3.1.5 Characterization 149
3.1.6 Property Value and Uncertainty Assignment..... 153
3.2 Metabolization of Inorganic Selenium by Leek..... 156
3.2.1 Cultivation of Leek 156
3.2.2 Investigation of Selenium Uptake 161
3.2.3 Selenium Bioaccessibility in Leek 164
3.2.4 Selenium Bioavailability form Leek 177
3.3 Multi-element Composition in Walnuts and Provenance Soils for Geographical Traceability 181
3.3.1 Method Validation for Determination of Total Elemental Mass Fraction in Walnut..... 181
3.3.2 Evaluating the Performances of Two Digestion Program for Walnut Mineralization..... 186
3.3.3 Total Elemental Mass Fractions of Walnut Sample 188
3.3.4 Evaluation of Digestion Procedures for Mineralization of Soil 195
3.3.5 Method Validation for Determination of Total Elemental Mass Fraction in Soil 205
3.3.6 Total Elemental Mass Fractions of Soil Samples 210
3.3.7 Evaluating Elemental Composition Relation of Walnuts 218
3.3.8 The Multi-element Composition Relation Between Walnuts and Soils 220
3.4 Conclusion 222
REFERENCES **226**
PUBLICATIONS FROM THE THESIS **249**

LIST OF SYMBOLS

s(b)	Between Day Standard Deviation
u_b	Between Day Uncertainty
s_{bb}	Between-Bottle Standard Deviation
xy	Blend of X and Y
yz	Blend of Y and Z
C	Concentration
k	Coverage Factor
°C	Degree Celcius
df	Degrees of Freedom
Δ	Difference
U	Expanded Uncertainty
g	gram
HR	High Resolution
R_X, R_y, R_Z	Isotope Ratio in Sample, iCRM and PSRM
Y	Isotopically Enriched Standard, iCRM
kg	kilogram
LOD	Limit of Detection
LOQ	Limit of Quantification
L	Liter
LR	Low Resolution
$K_{xy,}K_{zy}$	Mass Bias Correction Factor
C_X, C_Y, C_Z	Mass Fraction of Sample, iCRM and PSRM
m_y, m_{y2}, m_{y3},	Mass of Isotopically Enriched Standard
m_{z2}, m_{z3}	Mass of PSRM
m_x	Mass of Sample
$MS_{between}$	Mean Square Between-Bottle from ANOVA
MS_{within}	Mean Square Within-Bottle from ANOVA
r_{Xy}, r_{zy2}, R_{zy3}	Measured Isotope Ratio in Sample-iCRM (sample blend), iCRM-PSRM (calibration blend)
MR	Medium Resolution
μg	microgram
mg	milligram
mL	Milliliter
min	Minute
ng	Nanogram
N	Number of Between Day Replicates
n	Number of Replicates per Unit
P	Number of Within Day Replicates
%	Percent
i	Position of The Result in The Analytical Sequence
Z	Primary Standard Reference Material with Natural Isotopic Composition, PSRM
R_{EE}	Recovery Rate in Enzymatically Extract
RGIE	Recovery Rate in Gastrointestinal Digest

u_{rec}	Rectangular Distributed Uncertainty
R^2	Regression Coefficient
RSD	Relative Standard Deviation
X	Sample
b	Slope of Linear Regression
s	Standard Deviation
s.u	Standard Uncertainty
u^*_{bb}	Standard Uncertainty of Heterogeneity that can be hidden by method repeatability
u_{bb}	Standard Uncertainty Related to Between-Bottle Heterogeneity
u_{char}	Standard Uncertainty Related to Characterization
u_{lts}	Standard Uncertainty Related to Long Term Stability
u_{sts}	Standard Uncertainty Related to Short Term Stability
ΣR_X, ΣR_Y	Sum of All Isotope Amount Ratios of the Same Denominator
C_{SSE}	Sum of Four Se Species in Extract
T	Temperature
t_i	The Time for Each Replicate
t	Time
C_T	Total Concentration of Se in Dried Sample
C_{TE}	Total Concentration of Se in Extract
C_{TGI}	Total Concentration of Se in Gastrointestinal Digest
C_{SSGI}	Total Concentration of Sum of Four Se Species in Gastrointestinal Digest
TF	Translocation Factor
λ	Wavelength
s(w)	Within Day Standard Deviation
u_w	Within Day Uncertainty
s_{wb}	Within-Bottle Standard Deviation

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
AFS	Atomic Fluorescence Spectrometry
ANOVA	Analysis of Variance
APS	Adenosine Phosphoselenate
ATP	Adenosine Triphosphate
BAM	Bundesanstalt für Material for schung und -prüfung (Federal Institute for Materials Research and Testing)
BEC	Background Equivalence Concentration
BIPM	Bureau international des poids et mesures (International Bureau of Weights and Measures)
CASS-6	Nearshore Seawater Certified Reference Material for Trace Metals and other Constituents
CCQM	Comité Consultatif pour la Quantité de Matière (Consultative Committee for Amount of Substance)
CE	Capillary Electrophoresis
CITAC	Cooperation on International Traceability in Analytical Chemistry
CRC	Collision/Reaction Cell
CRM	Certified Reference Material
CV-AAS	Cold Vapor Atomic Absorption Spectrometry
CVG-AFS	Chemical Vapor Generation-Atomic Fluorescence Spectrometry
CVS	Cathodic Stripping Voltammetry
DAD	Diod Array Detector
DIN	Deutsches Institut für Normung (German Institute for Standardization)
DNA	Deoxyribonucleic Acid
EA	Elemental Analyzer
EN	European Norm
ERM	European Reference Materials
ETAAS	Electrothermal Atomization Atomic Absorption Spectrometry
EU	European Union
EURACHEM	Network of Organizations in Europe
FAAS	Flame Atomic Absorption Spectrometry
FID	Flame Ionisation Detector
GC	Gas Chromatography
GF-AAS	Graphite Furnace Atomic Absorption Spectrometry
GLHK	Government Laboratory of Hong Kong
GSH	Glutathione
GUM	Guide to the Expression of Uncertainty in Measurement
HFBA	Heptafluorobutyric acid
HG-AAS	Hydride Generation Atomic Absorption Spectrometry
HMDE	Hanging Mercury Drop Electrode
HMI	High Matrix Introduction

HR-ICP-MS	High Resolution-Inductively Coupled Plasma-Mass Spectrometry
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectrometry
ICP-MS/MS	Inductively Coupled Plasma-Mass Spectrometry
ICP-QMS	Inductively Coupled Plasma-Quadrupole Mass Spectrometry
ICP-QQQ	Triple Quadrupole Inductively Coupled Plasma
ID^3MS	Triple Isotope Dilution Mass Spectrometry
IDMS	Isotope Dilution Mass Spectrometry
IEC	International Electrotechnical Commission
IRM	Isotope Ratio Measurements
IRMM	Institute for Reference Materials and Measurements
IRMS	Isotope Ratio Mass Spectrometry
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
iCRM	Isotopically Certified Reference Material
JCGM	Joint Committee for Guides in Metrology
LC	Liquid Chromatography
LDPE	Low Density Polyethylene
LGC	Laboratory of the Government Chemist
LOD	Limit of Detection
LOQ	Limit of Quantification
LTS	Long Term Stability
MAM	Marmara Araştırma Merkezi
MC-ICP-MS	Multi Collector Inductively Couple Plasma Mass Spectrometry
MP	Mobile Phase
MX014	Trace Elements in Sea Water
NA	Not Applicable
ND	Not Detected
NIST	National Institute of Standards and Technology
NMI	National Metrology Institute
NMIA	National Measurement Institute of Australia
OES	Optical Emission Spectrometry
PCR	Polymerase Chain Reaction
PDO	Protected Designation of Origin
PEEK	Polyether ether ketone
PGI	Protected Geographical Indication
PSRM	Primary Standard Reference Material
PTFE	Polytetrafluoroethylene (Teflon™)
PVC	Polyvinylchloride
PVDF	Polyvinylidene difluoride
RM	Reference Materials
RP-IP-HPLC	Reverse Phase – Ion Pairing High Performance Liquid Chromatography
RSD	Relative Standard Deviation
RSS	Randomly Selected Samples
SAX	Strong Anion Exchange Chromatography
SD	Standard Deviation
SF-ICP-MS	Sector Field Inductively Coupled Plasma

SI	International System
SQ	Single Quad
SRM	Standard Reference Materials
STS	Short Term Stability
TAGs	Triacylglycerol
TEA	Triethylamine
TF	Translocation Factors
TIMS	Thermal Ionization Mass Spectrometry
TMMS	Treated Matrix Matched Seawater
TSG	Traditional Specialties Guaranteed
TÜBİTAK	Türkiye Bilimsel ve Teknolojik Araştırma Kurumu
UK	United Kingdom
UME	Ulusal Metroloji Enstitüsü
US	United States
WHO	World Health Organization

LIST OF FIGURES

Figure 1.1 Schematic representation of atomic emission spectrometry.....4

Figure 1.2 Schematic representation of ICP-MS/MS and operating principles in MS/MS mode 7

Figure 1.3 Examples of operating modes of ICP-MS/MS for arsenic determination.....8

Figure 1.4 Metabolic pathway of inorganic selenium into SeMet and its derivatives in plants 26

Figure 1.5 Metabolic pathway of inorganic selenium into MeSeCys and its further derivatives in plants.....27

Figure 2.1 Total salinity of the supenatant solutions after applying single step co-precipitation 51

Figure 2.2 Total salinity of the supernatant solutions after applying two step co-precipitation 52

Figure 2.3 TEA assisted $Mg(OH)_2$ co-precipitation recovery studies 53

Figure 2.4 Performance of cell gases on Cd isotopes in SQ mode of ICP-MS/MS 60

Figure 2.5 Cell gases performance on Cr isotopes 61

Figure 2.6 Cell gases performance on Fe isotopes 62

Figure 2.7 Cultivation of leek samples in climatic chamber 68

Figure 2.8 An example for growing period of a leek supplemented by Se(IV) 68

Figure 2.9 Calibration graphs of ^{76}Se, ^{78}Se and ^{80}Se obtained for the analysis of NIST SRM 1573a.....71

Figure 2.10 Representative calibration graphs of ^{76}Se, ^{78}Se and ^{8}0Se obtained for total Se in the analysis of nutritional solutions.....71

Figure 2.11 Representative calibration graphs of ^{76}Se, ^{78}Se and ^{80}Se obtained for total Se in the analysis of enzymatically extracted samples.72

Figure 2.12 Linear calibration plot of Se(IV) obtained by SAX-HPLC-ICP-MS/MS 74

Figure 2.13 Linear calibration plot of Se(VI) obtained by SAX-HPLC-ICP-MS/MS 75

Figure 2.14 Chromatogram obtained by SAX-HPLC-ICP-MS/MS for 20 ng/mL mix standard of selenium species, enzymatically extracted sample and spiked enzymatically extracted sample 76

Figure 2.15 Chromatogram obtained by SAX-HPLC-ICP-MS/MS for 20 ng/mL mix standard of selenium species, gastrointestinal digested sample and spiked gastrointestinal digested sample 76
Figure 2.16 Linear calibration plot of SeMet obtained by IP-HPLC-ICP-MS/MS 77
Figure 2.17 Linear calibration plot of MeSeCys obtained by IP-HPLC-ICP-MS/MS 78
Figure 2.18 Linear calibration plot of $SeCys_2$ obtained by IP-HPLC-ICP-MS/MS 78
Figure 2.19 Chromatogram obtained by IP-HPLC-ICP-MS/MS for 20 ng/mL mix standard of selenium species, enzymatically extracted sample and spiked enzymatically extracted sample 79
Figure 2.20 Chromatogram obtained by IP-HPLC-ICP-MS/MS for 20 ng/mL mix standard of selenium species, gastrointestinal digested sample and spiked gastrointestinal digested sample. 79
Figure 2.21 Representative photos of sieved soil 82
Figure 2.22 Representative photos of grinded walnut kernel samples. 83
Figure 3.1 Comparison of uncertainty budget of IDMS, ID^2MS, ID^3MS 97
Figure 3.2 The results Fe in UME CRM 1206 obtained by different calibration approaches 98
Figure 3.3 Calibration plot obtained for UME CRM 1206 100
Figure 3.4 Calibration plot obtained for NMIA MX014 100
Figure 3.5 Calibration plot obtained for CASS 6 101
Figure 3.6 Homogeneity graph of As 107
Figure 3.7 Homogeneity graph of Cd 108
Figure 3.8 Homogeneity graph of Cu 109
Figure 3.9 Homogeneity graph of Cr 110
Figure 3.10 Homogeneity graph of Fe 111
Figure 3.11 Homogeneity graph of Hg 112
Figure 3.12 Homogeneity graph of Ni 113
Figure 3.13 Homogeneity graph of Pb 114
Figure 3.14 Homogeneity graph of Zn 115
Figure 3.15 Short term stability at 18 °C for As 119
Figure 3.16 Short term stability at 60 °C for As 120
Figure 3.17 Short term stability at 18 °C for Cd 121
Figure 3.18 Short term stability at 60 °C for Cd 122
Figure 3.19 Short term stability at 18 °C for Cr 123

Figure 3.20 Short term stability at 60 °C for Cr 124
Figure 3.21 Short term stability at 18 °C for Cu 125
Figure 3.22 Short term stability at 60 °C for Cu 126
Figure 3.23 Short term stability at 18 °C for Fe 127
Figure 3.24 Short term stability at 60 °C for Fe 128
Figure 3.25 Short term stability at 18 °C for Hg 129
Figure 3.26 Short term stability at 60 °C for Hg 130
Figure 3.27 Short term stability at 18 °C for Ni 131
Figure 3.28 Short term stability at 60 °C for Ni 132
Figure 3.29 Short term stability at 18 °C for Pb 133
Figure 3.30 Short term stability at 60 °C for Pb 134
Figure 3.31 Short term stability at 18 °C for Zn 135
Figure 3.32 Short term stability at 30 °C for Zn 136
Figure 3.33 Short term stability at 40 °C for Zn 137
Figure 3.34 Short term stability at 60 °C for Zn 138
Figure 3.35 Long term stability for As 141
Figure 3.36 Long term stability for Cd 142
Figure 3.37 Long term stability for Cr 143
Figure 3.38 Long term stability for Cu 144
Figure 3.39 Long term stability for Fe 145
Figure 3.40 Long term stability for Ni 146
Figure 3.41 Long term stability for Pb 147
Figure 3.42 Long term stability for Zn 148
Figure 3.43 Summary of measurements uncertainty budgets for Cd, Cr and Cu 151
Figure 3.44 Summary of measurements uncertainty budgets for Ni, Pb and Zn 152
Figure 3.45 Growth effect of selenium species on leek 158
Figure 3.46 Selenite uptake rate by leeks sample 163
Figure 3.47 Selenate uptake rate by leeks sample 163
Figure 3.48 Selenium accumulation capacity of leek samples 164
Figure 3.49 Graphical representation of translocation of inorganic selenium in leek 168
Figure 3.50 Certificate of UME CRM 1206 Elements in Sea Water 223

LIST OF TABLES

Table 1.1 Some of examples for certified reference materials available for environmental analysis 10

Table 1.2 Literature review on species specific analysis for As and Cr 20

Table 1.3 Natural isotopes of selenium 21

Table 1.4 Typical Se amount in some common rock types 22

Table 1.5 Some of the most interested selenium species in speciation analysis 30

Table 1.6 Literature review on selenium speciation analysis in various matrixes 31

Table 2.1 The list of the standards used in the certification of trace elements in seawater certified reference material 45

Table 2.2 The list of chemicals used in analysis of leek samples 45

Table 2.3 Background and target levels in seawater used in processing ... 47

Table 2.4 Theoretical spiked levels of investigated analytes in TMMS 50

Table 2.5 The most probable polyatomic interferences on target analytes 56

Table 2.6 Investigation of cell gases performances for Cd and Cu based on BEC and Isotope ratio measurements 57

Table 2.7 Investigation of cell gases performances for chromium based on BEC and isotope ratio measurements 58

Table 2.8 Investigation of cell gases performances for iron based on BEC and isotope ratio measurements 59

Table 2.9 Optimum ICP-MS/MS parameters for As measurements in seawater 64

Table 2.10 Preparation of calibration solution for matrix matched external calibration 65

Table 2.11 Microwave digestion program for leek samples 69

Table 2.12 ICP-MS/MS parameters for total and speciation analysis of selenium 70

Table 2.13 Optimum parameters for SAX-HPLC 74

Table 2.14 Detection and quantification limits of SAX-ICP-MS/MS system 75

Table 2.15 Detection and quantification limits of IP-HPLC-ICP-MS/MS system 78

Table 2.16 Origin of walnut samples 81

Table 2.17 First Step of Microwave Digestion Program for walnut samples 84

Table 2.18 Second Step of Microwave Digestion Program for walnut samples 84

Table 2.19 Summary of tested digestion procedures for mineralization of soil 85

Table 2.20 Microwave digestion program A.....85

Table 2.21 Microwave digestion program B.....85

Table 2.22 Microwave digestion program C.....85

Table 3.1 Comparison of most commonly used analytical methods in terms of blank levels 89

Table 3.2 Trueness of three IDMS calibration strategies based on Fe measurements 93

Table 3.3 Sensitivity and precision of TEA/$Mg(OH)_2$-ID^3MS for target analytes.....94

Table 3.4 Trueness of TEA/$Mg(OH)_2$-ID^3MS for target analytes – NMX014.....94

Table 3.5 Trueness performance of TEA/$Mg(OH)_2$-ID^3MS for target analytes-CASS 6 95

Table 3.6 Sensitivity and precision of matrix matched external calibration – ICP-MS/MS for total As determination in seawater 99

Table 3.7 Evaluation of method precision for determination of total As in seawater.....102

Table 3.8 Trueness of matrix matched external calibration –ICP-MS/MS for total As determination in seawater.....103

Table 3.9 Summary of technically evaluated data set of homogeneity study 106

Table 3.10 Results of homogeneity study.....106

Table 3.11 Summary of results for short term stability test.....118

Table 3.12 Summary of results for long term stability test.....140

Table 3.13 Summary of UME CRM 1206 characterization measurements 149

Table 3.14 Certified value and uncertainty components 154

Table 3.15 Percent contribution of each uncertainty parameter to U_{CRM}...155

Table 3.16 Fortification type and amount of leek samples 156

Table 3.17 Dried mass of root, stem and leaf of a leek samples belongs to control group.....157

Table 3.18 Dried mass of root, stem and leaf of a leek samples cultivated by selenite.....157

Table 3.19 Dried mass of root, stem and leaf of a leek samples cultivated by selenate 157

Table 3.20 Dried mass of whole leek sample fortified by selenium species 158

Table 3.21 Descriptive statistics on masses for Se(IV) and Se(VI) at level-1 fortification 159

Table 3.22 Results of test statistics on masses for selenite at level-1 fortification 159

Table 3.23 Results of test statistics on masses for selenite at level-2 fortification 159

Table 3.24 Descriptive statistics on masses for selenite and selenate at level-2 fortification 160

Table 3.25 Results of test statistics on masses for selenite at level-2 fortification 160

Table 3.26 Results of test statistics on masses for selenate at level-2 fortification 160

Table 3.27 Descriptive statistics on masses for selenite and selenate at level-4 and level- 6 fortification 161

Table 3.28 Results of test statistics on masses for selenite at level-4 fortification 161

Table 3.29 Results of test statistics on masses for selenate at level-6 fortification 161

Table 3.30 Total Se amount in edible parts of leek samples cultivated by inorganic selenium species 165

Table 3.31 Descriptive statistics on total Se (mg/kg) in edible parts for Se(IV) and Se(VI) at level-1 and level-2 fortification.....166

Table 3.32 Results of test statistics total Se in edible parts for selenite and selenate at level-1 fortification 166

Table 3.33 Results of test statistics total Se in edible parts for selenite and selenate at level-2 fortification 167

Table 3.34 Descriptive statistics on total Se (mg/kg) in edible parts for selenite and selenate at higher level fortification.....167

Table 3.35 Results of test statistics on total Se in edible parts for selenite and selenate at high level fortification.....167

Table 3.36 Translocation factor of Se species in leek at different exposure concentration 168

Table 3.37 Descriptive statistics on translocation of selenium in edible parts 169

Table 3.38 Results of test statistics on translocation of selenium in edible parts 169

Table 3.39 Extraction efficiencies determined for Level-1 fortification by Se(IV) and Se(VI) 170

Table 3.40 Extraction efficiencies determined for Level-2 fortification by Se(IV) and Se(VI) 171

Table 3.41 Extraction efficiencies determined for Level-3 and Level-5 fortification 171

Table 3.42 Extraction efficiencies determined for Level-4 and Level-6 fortification 172

Table 3.43 Biotransformation of inorganic species uptaken by leek 173

Table 3.44 Summarized measurement results for selenium species determined in the extracts of leaves 175

Table 3.45 Summarized measurement results for selenium species determined in the extracts of stems 176

Table 3.46 Total amount of selenium species in edible parts of leek 177

Table 3.47 Bioavailability of selenium from leaves and stems of Se enriched leeks 178

Table 3.48 Summarized measurement results for selenium species determined in the gastrointestinal digested leaf of leeks 180

Table 3.49 Summarized measurement results for selenium species determined in the gastrointestinal digested stem of leeks....180

Table 3.50 Analytes and working range of method in peanut 182

Table 3.51 Sensitivity and precision of the method for multi elemental analysis in walnuts by HR-ICP-MS 183

Table 3.52 Trueness of the method for multi elemental analysis in walnuts by HR-ICP-MS using NIST SRM 2387 Peanut Butter 184

Table 3.53 Trueness of the method for multi elemental analysis in walnuts by HR-ICP-MS using UME CRM 1202 Elements in Hazelnut ..184

Table 3.54 Trueness of the method for multi elemental analysis in walnuts by HR-ICP-MS obtained by gravimetrically spiking before digestion....185

Table 3.55 Estimated measurement uncertainty values for determination of total elemental content in walnut....185

Table 3.56 Evaluation of digestion techniques for walnut mineralization 187

Table 3.57 Total elemental mass fractions of trace groups in walnut samples 189

Table 3.58 Total elemental mass fractions of minor groups in walnut samples 191

Table 3.59 Total elemental mass fractions of major groups in walnut samples 193

Table 3.60 Measurement results of NIST 2711a using different digestion procedures.....196

Table 3.61 Performances of digestion procedures based on NIST SRM 2711a 198

Table 3.62 Measurement results of sample No 5-İstanbul using different digestion procedures 201

Table 3.63 Measurement results of sample No 6-Bursa using different digestion procedures 202

Table 3.64 Comparison of digestion performances of procedures on Sample No 5 203

Table 3.65 Comparison of digestion performances of procedures on Sample No 6 204

Table 3.66 Analytes and working range of the method for multi elemental determination in soil by HR-ICP-MS 206

Table 3.67 Sensitivity and precision of the method for multi elemental analysis in soil by HR-ICP-MS.....206

Table 3.68 Trueness of the method for multi elemental analysis in soil by HR-ICP-MS 207

Table 3.69 Estimated measurement uncertainty values for the determination of total elemental content in soil by HR-ICP-MS.....208

Table 3.70 Analytes and working range of the method for multi elemental analysis in soil by ICP-OES 209

Table 3.71 Sensitivity and precision of the method for multi elemental determination in soil by ICP-OES 209

Table 3.72 Trueness of the method for multi elemental analysis in soil by ICP-OES 210

Table 3.73 Estimated measurement uncertainty values for the determination of total elemental content in soil by ICP-OES 210

Table 3.74 Total elemental mass fractions of target analytes in soil samples 212

Table 3.75 Correlation matrix for element concentration in walnuts 219

Table 3.76 Correlation coefficients of elemental composition between walnuts and soil 221

ABSTRACT

Applications of Mass Spectrometry: Certification of a Reference Material, Investigation of Plant Metabolism and Provenance Studies

Betül ARI ENGİN

Department of Chemistry

Doctor of Philosophy Thesis

Supervisor: Prof. Dr. Sezgin BAKIRDERE

Co-supervisor: Dr. Süleyman Z. CAN

Mass spectrometry has played a key role in inorganic chemical metrology for more than 25 years. Applications of inductively coupled plasma mass spectrometry (ICP-MS) allow wide variety of challenging measurements using different calibration strategies and methodologies with developing technologies. This thesis demonstrates some of the advance applications of ICP-MS in different research areas.

In the first chapter, ICP-MS/MS was used in the certification measurements of a candidate seawater reference material. Chemistry Laboratories of TÜBİTAK National Metrology Institute (UME) leaded to process and perform the certification measurements for UME CRM 1206. To be used in certification measurements, isotope dilution mass spectrometry (IDMS) which is a potentially primary technique and triethylamine assisted $Mg(OH)_2$ co-precipitation strategy (TEA/$Mg(OH)_2$) were combined and validated for target analytes.

In the second chapter, the investigation of plant metabolization performing total elemental and speciation analyses were conducted by ICP-MS/MS. Vegetables from the *Allium genus* are of particular importance due to being potential sources

of selenium. In this thesis, the metabolization of inorganic selenium fortification of *Allium porrum* (Leek) at different levels in hydroponic medium was investigated. Growth effect of selenium fortification, uptake and biotransformation rate of inorganic selenium species, translocations, bioavailability and bioaccessibility of selenium were investigated.

In the last chapter, pre-investigation for provenance of walnuts were performed by multi element profiling on the walnuts samples and their provenance soils. In the multi element profiling study, walnut samples were analyzed using a high resolution ICP-MS (HR-ICP-MS), while soil analyses were done by using HR-ICP-MS and ICP-OES. Optimization of digestion procedures for walnuts and soils were performed and precision, trueness and measurement uncertainties of the proposed methods were evaluated during the method developments. The relationship of elements in walnut and also between walnuts and their soils of origin were evaluated according to the one-to-one correlation coefficient of the elements and the correlation coefficient of different elements pairs.

Keywords: Inductively coupled plasma mass spectrometry, certification of a reference material, seawater, isotope dilution mass spectrometry, plant metabolization, selenium, multi element profiling, walnut

ÖZET

Kütle Spektrometresi Uygulamaları: Referans Malzemenin Sertifikalandırılması, Bitki Metabolizmasının İncelenmesi ve Köken Tayini Çalışmaları

Betül ARI ENGİN

Kimya Bölümü

Doktora Tezi

Danışman: Prof. Dr. Sezgin BAKIRDERE

Eş-Danışman: Dr. Süleyman Z. CAN

Kütle spektrometresi, 25 yılı aşkın süredir inorganik kimyasal metrolojide önemli bir rol oynamıştır. Endüktif eşleşmiş plazma kütle spektrometresi (ICP-MS) uygulamaları, farklı kalibrasyon stratejileri, metodolojileri ve çok çeşitli enstrümantasyonları kullanarak çeşitli ölçümler sağlar. Bu tez, farklı araştırma alanlarında ICP-MS'nin bazı ileri düzey uygulamalarını içermektedir.

Birinci bölümde, aday bir deniz suyu referans malzemesinin sertifikasyon ölçümlerinde ICP-MS/MS kullanılmıştır. Deniz suyunun işlenmesi ve sertifikasyon ölçümlerinin gerçekleştirilmesine TÜBİTAK Ulusal Metroloji Enstitüsü (UME) Kimya laboratuvarları öncülük etmiştir. Sertifikasyon ölçümlerinde kullanılmak üzere potansiyel bir birincil teknik olan izotop seyreltme kütle spektrometresi (IDMS) ve trietilamin destekli $Mg(OH)_2$ ile birlikte çöktürme (TEA/$Mg(OH)_2$) kombinasyonu geliştirilmiş ve hedef analitler için geçerli kılınmıştır.

Tezin ikinci bölümünde, ICP-MS/MS ile toplam element ve tür analizleri yapılarak bitki metabolizmasının araştırılması yapılmıştır. *Allium* cinsi sebzeler, selenyum

için potansiyel kaynaklar olduklarından özel ilgi görmektedir. Bu tezde, topraksız tarım ile yetiştirilen *Allium porrum* (pırasa) bitkisinde düşük ve yüksek seviyelerde selenit ve selenat takviyesinin metabolizasyonu araştırılmıştır. Selenyum takviyesinin büyüme etkisi, inorganik selenyum türlerinin alım ve biotransformasyon oranları, selenyumun translokasyonu, biyoyararlanımı ve biyoerişebilirliği araştırılmıştır.

Son bölümde, ceviz örnekleri ve menşe toprakları üzerinde çok elementli profilleme yapılarak cevizlerin kimlik doğrulaması için ön inceleme yapılmıştır. Çok elementli profilleme çalışmasında, ceviz analizleri yüksek çözünürlüklü endüktif eşleşmiş kütle spektrometresi (HR-ICP-MS) ile gerçekleştirilirken, toprak analizleri ise HR-ICP-MS ve ICP-OES kullanılarak yapılmıştır. Ceviz ve topraklar için çözünürleştirme prosedürlerinin optimizasyonunu yapılmış ve metot geçerli kılma aşamasında ise önerilen metotların kesinliği, doğruluğu ve ölçüm belirsizlikleri değerlendirilmiştir. Cevizdeki elementlerin ve aynı zamanda ceviz ve menşe toprakları arasındaki ilişkiler, elementlerin birebir korelasyon katsayısına ve farklı element çiftlerinin korelasyon katsayısına göre değerlendirilmiştir.

Anahtar Kelimeler: Endüktif eşleşmiş plazma kütle spektrometresi, referans malzeme sertifikasyonu, deniz suyu, izotop seyreltmeli kütle spektrometrisi, bitki metabolizması, selenium, çok elementli profil oluşturma, ceviz

1
INTRODUCTION

1.1 Literature Review

Science of measurement, metrology has a great importance in most aspects of industrial, environmental and health life. It helps to make some critical decisions in these fields based on the measurement results. Especially environmental and food analysis have been mainly focused research topics for many years as they are directly related to quality of human life. To date, many kinds of analytes which can be categorized as toxic and essential have been investigated in many different matrixes.

Over 99% (m/m) of the Earth's crust consist of only 10 elements (Si, O, Al, Fe, K, Ca, Mg, Na, Ti and H) and rest of these elements can be considered as trace elements as they form less than 0.5% (m/m). While they do not play a fundamental role in the makeup of the Earth's crust, they have great importance in the economy, ecology, agriculture, medicine, toxicology, and variety of other fields. While these elements are present in water, soil, sediments and air at trace levels, their base concentration levels are increasing due to several artificial activities like mining, industrial activities and sludge dumping. Soils and sediments are the main source of trace elements for biota and humans. Whilst the mobility, bioavailability and bioaccessibility of trace elements depend on their chemical reactivity, their levels and species can increase in plants grown up on polluted soil or water as a result of bioaccumulation and biomagnification effects [1].

Aside from the importance of soil quality, the quality of water on Earth is equally critical. Researches on the dynamics of ocean life has become more important in the recent decades, as oceans cover almost two-thirds of the Earth's surface area, serve as a major CO_2 reservoir, and play a significant role in the global carbon cycle [2], [3]. Despite the fact that total phytoplankton biomass in the oceans accounts for just 1-2 % of total world plant carbon, they are in charge of the majority of the Earth's carbon cycle [4]. Along with nutritional elements, trace

elements like Cd, Cu, Fe, Co, Ni, Mn and Zn are also are essential in ocean life by regulating the growth of phytoplankton which are taking role in carbon cycle [5]–[8]. Moreover, several elements and their isotopes can also reveal information on fundamental changes in the seas, biological productivity, and ocean circulation and more [6], [9].

The variety of investigated materials and analytes, as well as the diversity in their physical and chemical form(s), necessitates specific and unique techniques using all available analytical chemistry equipment and methodologies. Moreover, in developing world life, these techniques need to be also improved considering detection limits, time of analysis and quality of measurements. Therefore, metrology for trace elements analysis has always been one of the trending topics.

1.1.1 General Information

1.1.1.1 Elements

Several physical characteristics distinguish metals from nonmetals [10]. On the other hand, the chemical properties of elements can be classified based on their position in the periodic table: more metallic as goes towards the lower left corner of the periodic table and nonmetallic goes towards the upper right corner [11]. Moreover, there is obviously a need for subdivision of metals considering their individual properties and the terms that are most commonly used are provided in an IUPAC technical report prepared by Duffus [12]. These commonly used terms are listed as light metal, metalloid/semimetal, essential metal, heavy metal, beneficial metal, available metal, toxic metal, abundant metal, micronutrient and trace metal. While most of limitation of these terms are arbitrary and imprecise, the term “heavy metal” which is widely used in literature has been questioned for many years by several authors in different aspects like density of elements, toxicity or physicochemical properties [11], [13]–[15] and there is no clear definition provided by any authoritative body such as IUPAC [12].

1.1.1.2 Importance of Elements in Life Science

A set of biologically necessary elements is required for life. Oxygen, carbon, nitrogen, hydrogen, phosphorus, and sulfur, as well as many trace elements like

Cu, Co, Mn, Fe, Se and Zn are essential for human being [16]–[20]. These trace elements can be toxic depending on the amount of exposed [18]–[20]. Beside these main elements present in living organism, some elements such as Cr, As, Hg, Cd, Sn, Pb can lead toxic effects [21]–[23].For example, although the exact mechanism of lead absorption is unknown, lead builds up in the bones, particularly in the bone marrow. It's a neurotoxic that causes behavioral issues as well as intellectual and mental retardation [24]. Furthermore, it is thought that lead binds to oxo-groups in enzymes, influencing almost every stage of heme production and cause anemia by interfering with calcium and vitamin D metabolism [25], [26]. Acethylcholinesterase, acid phosphatase, ATPase, and other enzymes are all inhibited by this element.

Living organisms can be exposed to these elements through food chain which is directly connected to environmental conditions. Therefore, one of the most common problem all over the world is to keep to three main components of environment which are water sources, soil and air under control in terms of contamination which requires significant attention not only because of its environmental hazardous effects but also involving the risks to human health's as well as negative economic outcomes [27]. Most of the country all over the world apply certain regulations to protect the air [28], [29], water [30] and soil quality [31] and also to provide safe and healthy foods [32], [33]. In order to be successful in this target in long term, developing measurement capabilities is gaining great importance as regular high quality measurements for certain parameters are needed to evaluate situation and taking necessary actions based on related regulations.

1.1.1.3 Determination Strategies

Many analytical techniques are available for the determination of heavy metals and can be summarized as:

- Classical techniques
- Spectroscopic techniques
- Electrochemical techniques

Among these, spectroscopic techniques has been focused in the subject of the thesis.

i. Atomic Absorption and Emission spectrometry

Atomic absorption spectrometry (AAS) is one the most commonly preferred techniques due to its cost and robustness for routine analysis and has many application in the analysis of food, environmental, pharmaceutical, petroleum, coal [34]. Flame atomic absorption (FAAS) was introduced by Alan Walsh in 1955 [35] and it was the simplest and the most widely used approach. However, it has poor sensitivity originating from both short residence time of the free atoms in the flame and low nebulization efficiency. Therefore, more efficient sample introduction systems and atomization processes have been developed to improve the sensitivity of AAS. Electrothermal atomization atomic absorption spectrometry (ETAAS), cold vapor AAS (CV-AAS) and hydride generation atomic absorption spectrometry (HG-AAS) have been developed as sample introduction techniques which provides 100-1000 fold enhancement in sensitivity compared to FAAS [36] with advantages of minimizing possible matrix effects and interferences resulting from sample matrixes. There are many developments in sample introduction system and source for excitation to use the emitted spectra for the determination of elements in many different matrices and schematic representation of the most common system for emission spectrometer is provided in Figure 1.1 [37].

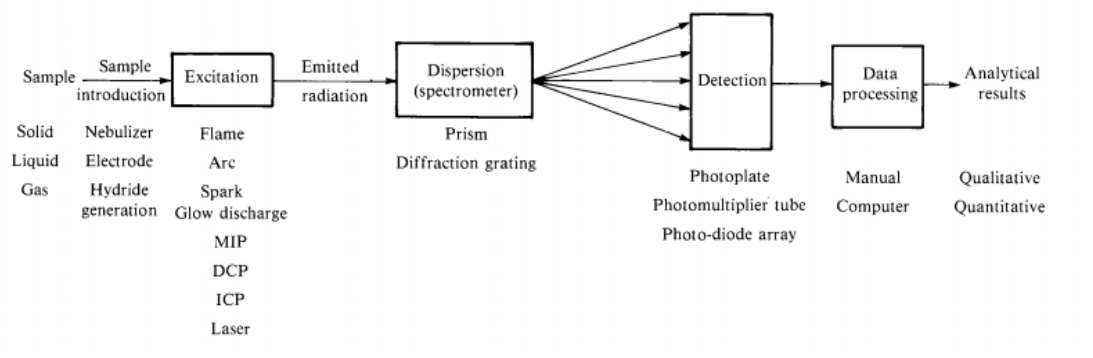


Figure 1.1 Schematic representation of atomic emission spectrometry

Based on the literature knowledge, the most widely preferred technique is inductively coupled plasma atomic emission spectroscopy (ICP-AES) by considering the sensitivity and cost effectiveness.

ii. Atomic Florescence Spectrometry

Another most commonly used atomic spectrometry technique is atomic florescence spectroscopy, AFS, which uses the emitted photons after excitation of free atoms from ground states by a specific light beam of wavelength for quantification of elements. As it is known as one of the most common atomic spectrometric techniques with simple instrumentation, high sensitivity, and low acquisition/running costs, AFS has been widely used in many different fields for trace element determination. Chemical vapor generation-AFS (CVG-AFS) for the hydride-forming elements (Sb, As, Pb, B, Cd, Sn, Ge, Se, Te, Hg, Zn) [38], [39] and photochemical vapor generation-AFS (photo-CVG-AFS) [40]–[42] for cobalt, iron and nickel are mainstream techniques for last decades.

iii. Inductively Coupled Plasma Mass Spectrometry

In the 1980s, the move from FAAS to ICP-AES as a workhorse in environmental laboratories was prompted by a commercial need for larger sample throughputs and more automation. Many water laboratories switched to inductively coupled plasma mass spectrometry (ICP-MS) in the early 1990s because the technique promised to combine the throughput of ICP-AES with the sensitivity of ETAAS [43]. Improvement in instrumental design were used to increase sensitivity [43] and ICP-MS has become one of the key techniques in inorganic chemical analysis since 1993 [44].

Spectral interference is the most intrinsic weakness as it results in inaccurate determinations. After introducing ICP-MS in 1981 by Date *et al.* [45], both the technological developments in ICP-MS design and academic studies made help in eliminating or at least minimizing the spectral interferences [43]. Many approaches for dealing with spectral interference have been proposed and applied such as cold plasma conditions, mathematical correction [46], matrix separation, alternate sample introduction methods, or aerosol desolvation [47]. In the literature, several sample introduction systems such as chemical vapor generation,

electrothermal vaporization, membrane desolvation and different sample preparation approaches to isolate target analyte from matrix have been developed [48]–[51]. Even though a lot of effort is needed to perform these approaches, measurement uncertainty is relatively high besides not allowing to study multi-element. On the other hand, one of the attempt to overcome specific kinds of spectroscopic interferences for quadrupole-based instruments was introduction of cool plasma (650 – 800 W) which helps to reduce ionization of an element with high first ionization energy like Ar (1st IE 15.76 eV) and decrease ArAr signals as resulting in sensitivity loss due to poorer ionization efficiency of target element (e.g Se) and rising oxide formations which may also cause spectral overlap [52].

Double focusing (sector field-SF) ICP-MS or multicollector ICP-MS for especially isotope ratio measurements can be preferred to resolve some polyatomic spectral interferences by operating at medium (R∼4000) or high resolution modes (R∼10000). Most interferences produced by spectrum overlap of an analyte ion's signal with that of a polyatomic ion even at the same nominal mass could be resolved by ICP-SF-MS. Although most of the spectral interferences can be overcome by increasing the resolution, sensitivity loss become the issue for accuracy of the ultra-trace analysis since ion transmission efficiency decrease as resolution increase [50], [53]. Despite this, the disadvantages of SF-ICP-MS such as its higher purchase price, more technical complexity, and lower robustness with respect to ICP-QMS, as well as its inability to resolve isobaric overlap have motivated scientists to continue working on quadrupole-based ICP-MS [54].

Another instrumental development in the mass spectrometer was multipole reaction or collision cell, and Turner *et al.*[55] reported the first application of hexapole collision cell with He. Different working principles of collision/reaction cell gas system (CRC) are applied depending on the interfering molecules and target analyte. The gases (O_2, NH_3, CH_4, He and H_2) used in cell can either react with analyte (mass shift) or interfering molecules to convert them to other masses which has not interference effect on the selected mass of analyte [56]. Although using the cell gases seems to be well established techniques in eliminating the spectral interferences, detection limits get higher due to sensitivity loss as a result

of dilution of target analyte with reaction/collision cell gases and makes the measurements at trace levels challenging.

In 2012, the ICP-tandem mass spectrometer (MS/MS) was introduced with an additional quadrupole in front of the CRC [57]. This technological development provided a better understanding of the reaction processes and the origin of the reaction product ions seen, as well as a better control over the reactions taking place in the cell. Working principle of MS/MS is represented in Figure 1.2 [58] and also possible operating modes of the ICP-MS/MS instrument were shown on detection of As in Figure 1.3 [57].

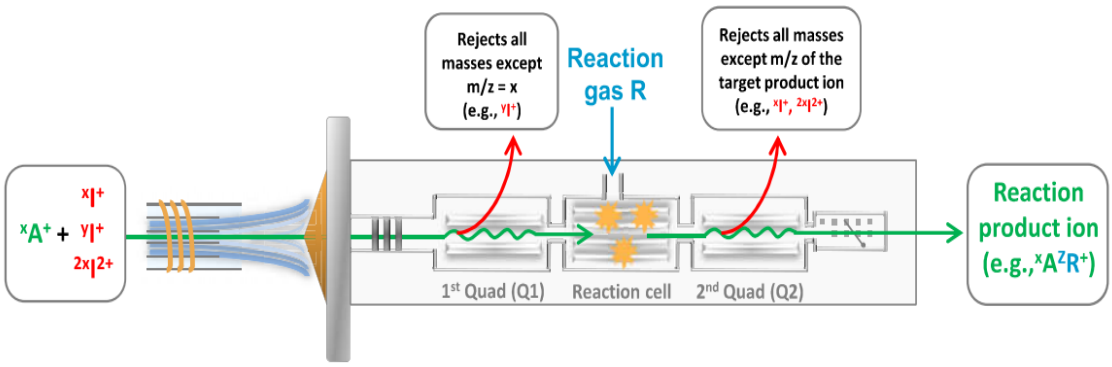


Figure 1.2 Schematic representation of ICP-MS/MS and operating principles in MS/MS mode[58]

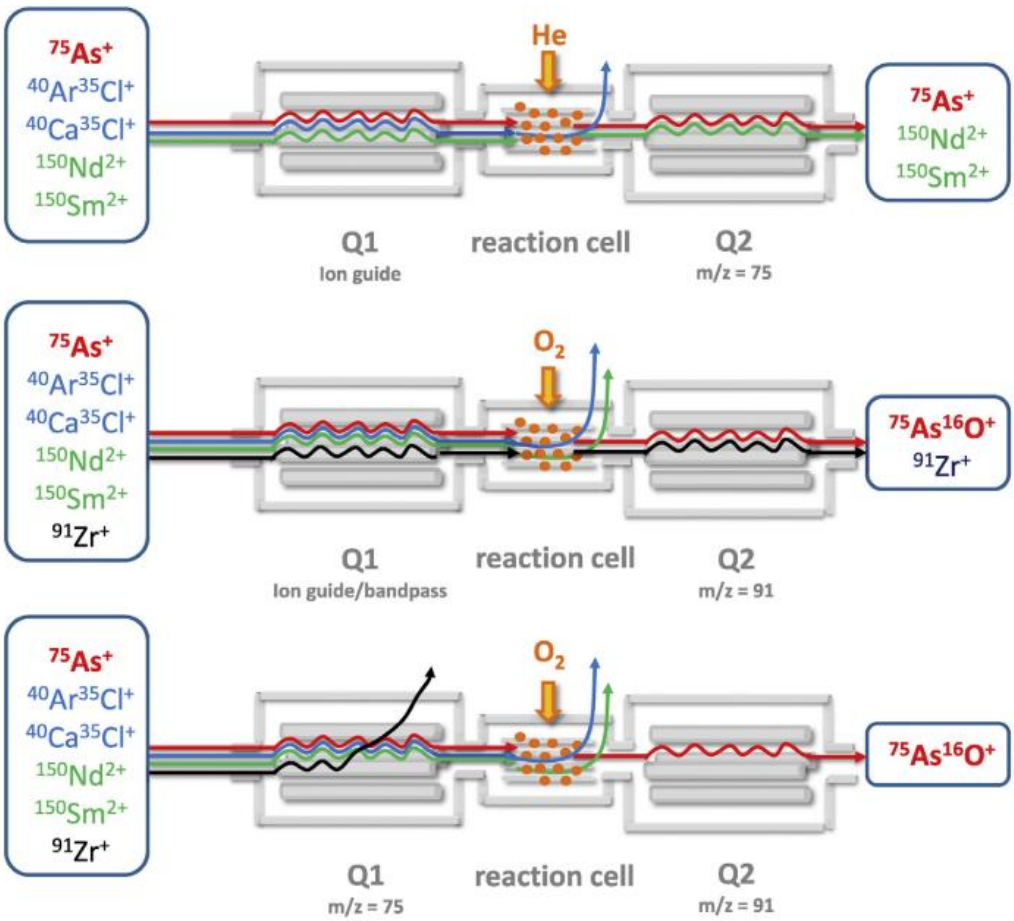


Figure 1.3 Examples of operating modes of ICP-MS/MS for arsenic determination [57]

The application of ICP-MS includes a wide range of measurement using a kinds of different instrumentation technologies, calibration strategies and methodologies. Applications can be listed as follows [44]:

- Routine elemental analysis
- Isotope dilution (ID) mass spectrometry
- Isotope ratio and/or composition
- Speciation studies for organometallic compounds
- Heteroatom quantification of proteins
- Characterization and quantification of nanoparticles
- Laser ablation-ICP-MS analysis for solid samples

1.1.2 Certified Reference Materials in Inorganic Analysis

Reference materials (RM) are the key points in the improvements and maintenance of a global measurement system for validation of methods, quality

control purposes, calibration of instruments, assessing laboratory proficiency and value assignment in similar reference materials [59]. Moreover, use of certified reference materials (CRM) provides to establish or confirm the metrological traceability to the SI units which is essential for assuring that measurement results are independent from locations and time. Therefore, use of reference materials and certified reference materials are important to improve the quality of measurements and some of examples for available CRMs for environmental analysis categories (water, soil and air) in terms of elemental content are provided in Table 1.1.

Table 1.1 Some of examples for certified reference materials available for environmental analysis

Producer	CRM code	Matrix	Certified analytes
European Union	ERM- CA713	Waste water	As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se
Turkey	UME CRM 1204	Waste water	As, B, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, V,Zn
European Union	ERM- CA400	Seawater	Hg
European Union	ERM- CA403	Seawater	As, Cd, Co, Cu, Mn, Mo, Ni, Pb
Canada	CASS-6	Seawater	B, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, U, Zn
Canada	NASS-7	Seawater	B, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, U, Zn
NMIA	NMIA MX014	Seawater	As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V
European Union	ERM-CA615	Groundwater	As, Cd, Fe, Hg, Mn, Ni, Pb
Turkey	UME CRM 1201	Spring water	As, Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mn, Na, Ni, P, Pb, Sb, Sn, Sr, V, Zn
USA	NIST SRM 1641e	Water	Hg
USA	NIST SRM 1643f	Water	Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo Na, Ni, Pb, Rb, Se, Sb, Sr, Te, Tl, V, Zn
Canada	AQUA-1	Drinking water	As, Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mn, Mo, Na, Ni, P, Pb, Sb, Sn, Sr, U V, Zn
Canada	SLEW-3	Estuarine water	As, Cd, Cr, Co, Cu, Fe, Mn, Mo, Ni, Pb, U, V, Zn
Canada	SLRS-6	River water	As, Al, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Sr, U, V, Zn
Turkey	UME EnvCRM02	River water	As, Cd, Ni, Pb, Se
European Union	ERM-CC141	Loam soil	As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Zn
European Union	ERM-CC690	Calcareous soil	Ce, Dy, Gd, La, Nd, Sc, Sm. Tb, Tm, Yb, Th, U
USA	NIST SRM 2711a	Soil	Al, As, Ba, Ca, Cd, Co, Cr, Cu, Hg, Fe, K, Mg, Mn, Na, Ni, Pb, P, Sb, Si, Sm, Sr, Ti, U, V, Zn
Turkey	UME EnvCRM03	Soil	As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, V, Zn
European Union	ERM- CC144	Sewage sludge	As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn
USA	NIST SRM 2782	Industrial sludge	As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, Zn
USA	NIST SRM 2781	Domestic sludge	Ag, As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, Zn, N
European Union	ERM- CC580	Estuarine sediment	Total Hg, CH_3Hg^+
USA	NIST SRM 1646a	Estuarine sediment	Al, As, Ca, Cd, Cr, Cu, Fe, K, Li, Mg, Mn, Na, P, Pb, S, Si, Ti, V, Zn
Canada	HISS-1	Marine sediment	As, Al, Be, Ca, Cd, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Se, Sr, Ti, V, Zn
USA	NIST SRM 2702	Marine sediment	Al, As, Ba, Cd, Ce, Cr, Co, Hg, K, La, Mn, Na, Ni, P, Pb, Rb, Sb, Sc, Sr, Th, Ti, Tl V, Zn
European Union	ERM-CZ120	Fine dust (PM10-like)	As, Cd, Pb, Ni
USA	NIST SRM 1649b	Urban dust	Cd, Hg, Pb

1.1.2.1 Production and Certification of Reference Materials

The international standard, ISO 17034:2016, which is aligned with the relevant requirements of ISO/IEC 17025 specifies the general requirements for reference material producers. Technical details including content of certificates and the design of homogeneity, stability and characterization is outlined in ISO Guide 31 and ISO Guide 35:2017. This international standard defines both administrative and technical requirements for reference material producers. In aspects of technical requirements, the outline for production of a certified reference material can be summarized based on the related guidelines as follow and each step has been detailed in the guidelines [59], [60]:

- ***Production planning and control***

  CRM production is planned in regard to material processing, homogeneity test, stability test and characterization and post certification monitoring processes. In the planning stage, production volume, matrix, intended use of the material to be produced, properties of the starting material, and storage conditions for the product is regarded. In addition, available CRMs in the market, metrological traceability, measurement methods, infrastructure and personnel are considered
- ***Material handling and storage***
- ***Feasibility Studies***
- ***Reference material processing***

  Reference material is processed before certification. The stage after providing of raw material is processing reference material to make it ready to be used by customers. The stages of reference material processing and certification period show differences depending on starting material and characteristic property which is going to be certified. The methods to be used in production stage and processing stages which will be followed are decided by considering these differences and evaluation of data obtained from preliminary studies.

- ***Selection of measurement procedures***

  Different measurement procedures might be used in each step of certification considering the purposes of homogeneity, stability and characterization.

- ***Metrological traceability***

  To establish traceability of a certified value to a reference, all measurement results and their uncertainties need to be traceable to this reference. It therefore has to be ensured that the measurement results of all studies refer to the same measurand and their quantity values are comparable.

- ***Homogeneity assessment***

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

- ***Stability assessment***

  Stability test is conducted to determine the inevitable remaining instability in the CRM and to approve the stability of the material. The stability of the units which are exposed to different environmental conditions should be evaluated at defined storage conditions by reference material producers.

- ***Assessment of commutability (if required)***
- ***Characterization***

  There are certain ways suggested in ISO Guide 35:2017 and some of the most preferred ones can be summarized as follow [59]:

  ✓ "*Using a single reference measurement procedure (as defined in ISO/IEC Guide 99) in a single laboratory*"

- ✓ *"Characterization of a non-operationally defined measurand using two or more methods of demonstrable accuracy in one or more competent laboratories*"
- ✓ "*Characterization of an operationally-defined measurand using a network of competent laboratories*"
- ✓ "*Value transfer from a reference material to a closely matched candidate reference material performed using a single measurement procedure performed by one laboratory*"

  "*Characterization based on mass or volume of ingredients used in the preparation of the reference material*"

- ***Data Integrity and evaluation***
- ***Assignment of property value and their uncertainties***
- ***Preparation of certificate***
- ***Post-production monitoring for stability***

1.1.2.2 Isotope Dilution Mass Spectrometry (IDMS): A Potential Primary Method for Characterization a Reference Material

Isotope dilution is simply based on the alteration of the isotope composition of an analyte (element or molecular compound) in unknown sample by addition of known amount of isotopically enriched or labelled form of the same analyte [61]. The isotope ratio in the sample blend, measured by mass spectrometry, provides the concentration of the analyte in sample after simple calculations. IDMS is known to be the most accurate quantification strategy in analytical chemistry [62]–[64].

Isotope dilution principle has been first applied in zoology by using catch-and-release procedures in 1895 by C.G.J Petersen [65]. Basically, a group of individuals is captured alive and marked using inedible marks and then released. After a suitable length of time to allow the marked individuals to mix well with those unmarked, a second capture is performed. From the total number of individuals captured and marked the first time (N_M), the total number of individuals captured the second time (T) and the number of marked individuals

captured again (T) the total population (N_T) in a given area can be estimated using the following equation (2. 1).

$$N_T = N_M \frac{T}{M} \tag{2. 1}$$

Moreover, similar procedure is used to estimate other unknown populations [61]. While capture and release methods which were first recorded in 1895 are much older than the discovery of the isotopes, the first accounts on the use of enriched stable isotopes for ID analysis with mass spectrometry appeared in 1939-1940 for qualitative analysis in protein hydrolysates [66], [67].

The improvements in IDMS for elemental and organic analysis has depended on the availability of isotopically enriched elements and labeled compounds, the technological improvements in mass spectrometers instrumentation and scientific requirements in other disciplines [61]. Application of IDMS in elemental analysis started at the end of 1940s and beginning of 1950s due to the necessary developments in nuclear physics like determination of absolute fission yields, branching ratios, radioactive decay constants and neutron capture cross sections. In early 1970s, the theory for organic IDMS also begun to develop and scientists started to investigate requirements for organic IDMS by comparing established equations for elemental IDMS [68].

- **Potential to be A Primary Analytical Method**

Traceability is defined as "*property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty*"[69]. Moreover, it is demanded that all the analytical methods should be traceable to the units of the International System (SI) either directly or with a provided traceability chain. Primary methods are the ones that directly traceable to the one or more SI units and must possess high metrological quality. To have this, all technical requirements should be well described and fulfilled in the operation of method and a complete measurement uncertainty statement in term of SI units should be provided. 'Isotope dilution mass spectrometry' is accepted by the Consultative Committee for Amount of Substance (CCQM, Comité Consultatif pour la Quantité

de Matière) as a potential primary ratio method that measures the value of a ratio of unknown to a standard of same quantity and whose operation is completely described by a measurement equation [70]. IDMS is mostly preferred in characterization of a reference material as it is the only method that can be applied for trace and ultra-trace determinations providing the traceability to SI units directly with the potential of having smallest measurement uncertainties [71]. IDMS is also started to be used in research laboratories for the validation of routine analytical methods and also decrease total analysis time and increase the analytical quality of measurements [72].

- **Calibration Strategies for Elemental IDMS Applications**

In the field of inorganic analysis, three IDMS calibration methods (single, double, and triple IDMS) can be used [73]. Single IDMS is the first and most popular isotope dilution technique, which is more practical and can be applied provided the mass fraction and isotopic composition of isotopically CRM (iCRM) are known [73], [74]. In this approach, the isotope ratio of the sample blend and the isotope ratio of the mass bias correction solution are measured while rest of the parameters in the equation, except the masses of the sample and the iCRM, are obtained from the iCRM and/or IUPAC certificates. Equation (2. 2) gives the equation for a single IDMS.

In the case of absence of metrologically traceable and certified mass fraction value of iCRM, it needs also to be characterized. Therefore, double (reverse) IDMS (ID^2MS) needs to be applied [73], [74]. If there is a lack of metrological regulation of iCRM for the mass fraction, this case is most commonly used. A primary standard reference material (PSRM) solution made from a primary assay (purity >99.99 percent) element or approved standard reference material is used to describe the mass fraction of iCRM. $\Sigma R_{Y,}$ ΣR_X and ΣR_Z are eliminated in a ID^2MS application unless there is an isotopically difference between the sample and PSRM solution, which is normally the case, and the ID^2MS equation results in equation (2. 3). Due to the cancellation of parameters, the expected calculation variance budget of the ID^2MS is smaller than the single IDMS [64], [71], [74].

$$C_X = C_Y \cdot \frac{m_{y1}}{m_x} \cdot \frac{R_Y - K_{xy} \cdot r_{xy}}{K_{xy} \cdot r_{xy} - R_X} \cdot \frac{\sum (R_i)_X}{\sum (R_i)_Y} \quad (2.\ 2)$$

$$C_X = C_{Z2} \cdot \frac{m_{z2}}{m_x} \cdot \frac{m_{y_1}}{m_{y_2}} \cdot \frac{R_Y - K_{xy1}.r_{xy1}}{K_{xy1} r_{xy1} - R_X} \cdot \frac{R_Y - K_{zy2} r_{zy2}}{K_{zy2} r_{zy2} - R_Y} \quad (2.\ 3)$$

$$C_x = \frac{m_{y_1}}{m_{x_1}} \cdot \left(C_{z_2} \cdot \frac{m_{z_2}}{m_{y_2}} \cdot \frac{(r_{zy_2} - R_z) \cdot (r_{zy_3} - r_{xy_1})}{(r_{xy_1} - R_x) \cdot (r_{zy_3} - r_{zy_2})} + C_{z_3} \cdot \frac{m_{z_3}}{m_{y_3}} \cdot \frac{(r_{zy_3} - R_z) \cdot (r_{xy_1} - r_{zy_2})}{(r_{xy_1} - R_x) \cdot (r_{zy_3} - r_{zy_2})} \right) \quad (2.\ 4)$$

$$K = R_{certified\ value\ or\ IUPAC} / r_{measured} \quad (2.\ 5)$$

Parameter	Unit	Definition
X	-	Sample
Y	-	Isotopically enriched standard, iCRM
Z	-	Primary standard reference material with natural isotopic
xy	-	Blend of X and Y
yz	-	Blend of Y and Z
C_X, C_Y, C_Z	mol/kg	Mass fraction of sample, iCRM and PSRM
m_x	kg	Mass of sample
m_y, m_{y2}, m_{y3},	kg	Mass of isotopically enriched standard
m_y, m_{y2}, m_{y3},	kg	Mass of isotopically enriched standard
m_{z2}, m_{z3}	kg	Mass of PSRM
R_X, R_y, R_Z	-	Isotope ratio in sample, iCRM and PSRM
r_{Xy}, r_{zy2}, R_{zy3}	-	Measured isotope ratio in sample-iCRM (sample blend), iCRM-PSRM (calibration blend)
K_{xy},K_{zy}	-	Mass bias correction factor
ΣR_X, ΣR_Y	-	Sum of all isotope amount ratios of the same denominator

In 2002, Milton et al. developed the third isotope dilution method, which can be used when the certified isotopic composition of an isotopically enriched standard is in question or there is inadequate knowledge [75], [76]. Triple IDMS is the name given to this method of isotope dilution calibration (ID^3MS). Equation (2. 3) is replaced with equation (2. 4) after a third calibration blend of iCRM and PSRM is used to replace R_Y.

- **Technical Aspects of IDMS Applications in Inorganic Analysis**

Inductively coupled plasma mass spectrometry is the most widely used instrument than TIMS and multicollector ICP-MS instruments as it is most likely to be found in laboratories due to more simplicity and cost effectiveness than the others. However, there are some common parameters leading to in accurate results in

IDMS applications and should be considered in sample preparation: Reagent blanks levels, uniformity of isotopes, addition of enriched isotope as possible as early, establishment of isotopic equilibrium after spiking samples. Along with these parameters, there are some critical points that should be taken into account in the measurements of isotope ratio with ICP-MS: spectral interferences, impact of mass bias, detector dead time (detector nonlinearity effects) [61].

As in all other analytical methods for trace analysis, contamination of reagent blank is a potential risk for error in IDMS application and it must be evaluated and corrected carefully. Another important parameter that effect the accuracy of all kinds of measurement in ICP-MS as well as isotope ratio measurements is the possible spectral interferences on isotopes. In IDMS applications, a minimum of two isotopes of element to be determined used and ideally, these isotopes should be free of spectral interferences (isobaric/polyatomic). For polyatomic interferences, double focusing ICP-MS instruments may have some advantages in this respect for some of the elements providing higher resolutions. However, isobaric interferences are still the issue even for double focusing instruments. Therefore, isotopes should be selected considering possible isobaric and polyatomic interferences and also the technological aspects of ICP-MS instruments.

Isotope ratio measurement accuracy is also affected by detector response time (detector dead time) and can result in counting losses if not evaluated appropriately [77]. If isotope pairs have different abundances, calculating the dead time of the instrument may be needed to obtain highly accurate isotope ratio measurements that is independent of analyte concentration [78]. The word "detector dead time" refers to the time taken to recover from an ion impact during which the ion counting system is "blind" to the next incoming ion. In order to obtain high precision on isotope ratio measurements without affected by analyte concentration, it is important to calculate the dead time of the ICP-MS unique to the elements [78]

Another important parameter that influence the accuracy of the measurements is evaluating the mass biases appearing in plasma conditions. Since mass transmittance efficiency varies in inductively coupled mass spectrometry,

measured isotope ratios (r) are often biased [77], and this bias becomes more of a concern for light elements [79]. The different rates of ion transmittance are originated both in the vacuum interface (the nozzle effect) and, in most cases, in the ion lens system (the space charge effect). To adjust for bias in isotope ratio measurements, iCRM or a common standard having natural isotopic composition should be used [80]. Measurements for mass bias correction can be performed by externally (using the same element to be determined) or internally (using different elements close in mass of analyte added to the sample) [81]. For the external correction model, the matrix of the standard used for mass bias correction should be as possible as same with sample blend.

1.1.3 Speciation Analysis

Definition of terms in academia is an important issue to be well understood. Therefore definition of some certain and most widely used terms are given by the International Union for Pure and Applied Chemistry (IUPAC). According the published guideline in 2000, while speciation in chemistry means that "*distribution of an element amongst defined chemical species in a system*", speciation analysis is "*analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample*" [82], [83]. Isotopic distributions (nuclear level), electronic levels and organometallic forms of an element might be chemical species of it [84]. Although the use of total elemental concentration in the assessment of health/environmental risk is a traditional approach, it does not provide useful information anymore for some elements like Cr, As, Hg and Se as particular species of them may have different impact on living organisms in terms of toxicity, bioavailability or metabolic pathways [85]–[89]. For example, although arsenic is known as a toxic element, its toxicity highly depends on its chemical form. The toxicity of arsenic in humans increases in order of arsenobetaine, arsenosugar, dimethylarsinic acid, monomethylarsonic acid, As(V) and As(III) [90]. Many analytical techniques have been developed for the speciation of arsenic species and some of them are summarized in Table 1.2.

It is known that organic forms of mercury (methylmercury or ethylmercury) are more toxic than inorganic forms (Hg^0, Hg^+, Hg^{2+}) [91]. While arsenic and mercury are in the class of toxic elements, the situation is slightly different for chromium and selenium. Chromium can be categorized as essential micronutrient and also toxic element based on its chemical form present in food or environment. While Cr(III) is widely accepted as micronutrient in the human diet and used as a nutritional supplements, Cr(VI) is regulated through the CLP regulation (2008) as it is suspected to be extremely toxic after inhalation and oral exposure on many systems of human being [92]. Moreover, excess and deficiency of selenium have equally adverse health effects and its effects also depends not only to the dose but also its chemical form [93].

Table 1.2 Literature review on species specific analysis for As and Cr

Matrix	Species	Separation Technique	Detection Technique	Reference
Chicken tissue	As(V), As(III), DMA, MMA, AsB, ASA, Rox	Anion Exchange / Hamilton PRP-X100	HPLC-ICP-MS	[94]
Chicken liver, meat and litter	AsB, As(III), DMA, MMA, methyl-3AHPAA, As(V), 3AHPAA, methyl-NAHAA, NAHAA, methyl-Rox, and Rox	Anion Exchange / Hamilton PRP-X110S	HPLC-ICP-MS HPLC-ESI-MS HPLC-ESI-MS/MS	[95]–[98]
Plant	As(III), PAO, PAA, As(V), *o*-APAA, Rox, and ASA	Anion Exchange / IonPac AS7	IC-ICP-MS	[99]
Arugula, dog food, chicken liver, dog urine	MMA, DMA, ASA, Rox, and 4NPAA	Ion Paring / Zorbax SB-AQ	HPLC-HG-AFS	[100]
Urine	As(V) (*iAs, MMA, DMA coeluted*), PDMAO, PMAA, PAA, DPMAO, and DPAA	Reversed Phase/ C4 column	HPLC-ICP-MS	[101]
Porcine and chicken liver	ASA, 4NPAA, and Rox	Reversed Phase/ Luna 5μ C18	HPLC-HG-AFS	[102]
River water	Cr(III), Cr(VI), As(III), As(VI),MMA, Se(IV), Se(VI)	Anion Exchange / Hamilton PRP-X100	LC-ICP-MS	[103]
Animal oil	Cr(III), Cr(VI)	Reversed Phase/ C8 column	HPLC-ICP-MS	[104]
Water	Cr(III), Cr(VI)	Anion Exchange/ IoPac AS7 dionex	IC-ICP-MS	[105]
Seawater, Waste Water, Vinegar	Cr(VI)	Reduction of Cr(VI) at electrode surface	Catalytic adsorptive stripping voltammetry	[106]
Tap water, mineral water, tea, bush branches and leaves	Cr(III), Cr(VI)	Coprecipitation with Ni2+/2-Nitroso-1-naphthol-4-sulfonic acid	FAAS	[107]

ASA: p-Arsanilic acid, AsB : Arsenobetaine, DMA: dimethylarsinic acid, MMA: Monomethylarsonicacid, Rox: 4-Hydroxy-3-nitrophenylarsonic acid (Roxarsone), Methyl-Rox: 4-Hydroxy-3-nitrophenylmethylarsonic acid, methyl-3AHPA: 3-amino-4-hydroxyphenylmethylar sonic acid, 3AHPAA: 3-Amino-4-hydroxyphenylarsonic acid, methyl-NAHAA: N-acetyl-4-hydroxy-phenylmethylarsonic acid, NAHAA: N-acetyl-4-hydroxy-m-arsanilic acid, PAO: Phenylarsine oxide, PAA: Phenylarsonic acid, *o*-APAA: *o*-Aminophenylarsonic acid, 4NPAA: 4-Nitrophenylarsonic acid, PDMAO: Phenyldimethylarsine oxide, PMAA: Phenylmethylarsonic acid Phenyldimethylarsine, DPMAO: Diphenylmethylarsine oxide, DPAA: Diphenylarsonic acid.

1.1.3.1 Selenium

Jöns Jacob Berzelius, known as the "Father of Swedish Chemistry," discovered the element in 1817 [108] and took its place in Mendeljev's table in 1869. He found a stain while handling sulfuric acid, which he initially mistook for tellurium [108]. Selenium (atomic number 34, atomic mass 78.96) is a semi-metallic element that belongs to group XV (chalcogens) and is found in the periodic table between sulfur and tellurium. In certain ways, it resembles those elements. It has allotropic structures and compounds that are analogous to those of sulfur. Selenium has 4 natural oxidation states: Elemental Se (0), Selenide (-2), Selenite (-4) and Selenate (-6). It has six stable isotopes which is listed in Table 1.3 with their atomic masses [109].

Table 1.3 Natural isotopes of selenium

Isotope	Atomic Mass (u)	Natural Abundance
^{74}Se	73.9224767	0.0089
^{76}Se	75.9192143	0.0937
^{77}Se	76.9192143	0.0763
^{78}Se	77.9173097	0.2377
^{80}Se	79.9165221	0.4961
^{82}Se	81.9167003	0.0873

Selenium usually presents in concentrations of 50–90 μg/kg of soil [110], although higher levels have been associated to volcanic, sedimentary, and carbonate rocks. According to its potential oxidation states, selenium can be found in a variety of ways in environmental soils [111]. It's most commonly found as selenides in ores of metals like iron, lead, silver, and copper, where it's found along with sulfides [112]. Few phosphatic rocks, organic-rich black shales, coals, and sulfide mineralization have high selenium concentrations, while the majority of other rock types have very low concentrations [111]. Typical Se content in some of the common rock types are summarized by Fordyce and given in the Table 1.4 [113].

Table 1.4 Typical Se amount in some common rock types

Igneous rocks	Se, mg/kg	**Sedimentary rocks**	Se, mg/kg
Ultramafic	0.05	Limestone	0.03- 0.08
Mafic	0.05	Sandstone	< 0.05
Granitic	0.01-0.05	Shale	0.05-0.06
Volcanic	0.35	Mudstone	0.1-1500
		Phosphates	1-300
		US Coal	0.46-10.5

Many reagents react with selenium chemically, making it possible to incorporate it into organic compounds. Asymmetric or non-symmetric diselenides, on the other hand, are frequently used as starting materials to manufacture of more complex chalcogen-containing derivatives [112]. The amount of selenium present in soil depends on the kind of rock and it also reflects amount of soil in groundwater. Selenium levels in natural water vary from 0.1 to 400 μg/l, with some samples exceeding 6000 μg/l and most dominant species of selenium in water are selenide and selenate [111], [114].

Se is a by-product of the processing of other metals or recovered from sludge accumulated in H_2SO_4 plants and there is no mining in the world special to selenium [115]. Selenium is commonly present in sulfide mineral deposits as a chalcophile element that favors a sulfide host over a silicate host [115].

The more attention was given into geochemistry of selenium as the industrial application of it increased along side with toxicity and health effects of selenium. Selenium is widely being used in electrical components (semi-conductors, cables and contacts) [113]. As it has a photovoltaic (light-to-electricity) and a photoconductive (light-to-conductor) properties, it has been used in photocells, solar cells, and light meters [116]–[118]. The “amorphous selenium detector” in mammographic instruments is a new use of selenium [119].

1.1.3.2 Importance and Health Effects of Selenium on Human

Selenium was thought as a toxic element for 150 year from its discovery,1817 [120]. In 1957, Klaus Schwartz and Calvin Foltz discovered the advantages of selenium for humans and other mammals [121]. The realization of the nutritional importance of selenium increased when any adverse effects were observed on

animal by replacing vitamin E with Se [122] and understanding that selenium is incorporated into glutathione peroxidase (GHPx) [123]. Since then, especially in the last a few decades, there has been a surge interest in selenium as it is an essential micronutrient that has valuable nutritional benefits in trace quantities and it can be harmful at elevated doses to animals and humans [124]. The spectrum of dietary deficiency (40μg day^{-1}) and toxic levels (>400μg day^{-1}) for selenium is one of the narrowest of all the elements (WHO 1996), making it important to thoroughly investigate intakes by humans and other species, emphasizing the importance of recognizing the interactions between environmental consumption and health [111].

Selenium is present as peptide and proteins in the human body. Selenoproteins and Se-containing proteins are the categories of proteins that contain Se. In selenoproteins (SePs), selenium is present as selenocysteine (SeCys) which is the 21st amino acid [125], [126]. There are many functions of these selenoproteins in mammals [126]–[128]. It is also known that selenium has role in fertility, thyroid functions and also has a beneficial effect on mood like depression, anxiety, confusion and hostility [126]. Moreover, it reduces heart disease, strengthens the immune system, has an antagonistic effect on heavy metals has anti-proliferative/anti-inflammatory effects, and [127], [129], [130].

Deficiency of selenium may result in Keshan disease [115], Kashin Beck disease [131], increased viral virulence [132], increase in mortality [133], poorer immune function [128], fertility problems [134], thyroid autoimmune disease [135], Type 2 diabets [136], increase risk of prostate cancer in men [137] and colorectal cancer in women [138].

1.1.3.3 Selenium in Plants

Depletion of microelements in soil is a serious concern that has resulted in the emergence of a slew of human, plant, and animal illnesses linked to micronutrient deficiencies. For the agro-chemical approach to food production, finding innovative solutions to tackle this problem is challenging. Plant micronutrient fertilization is the subject of several research publications and research on

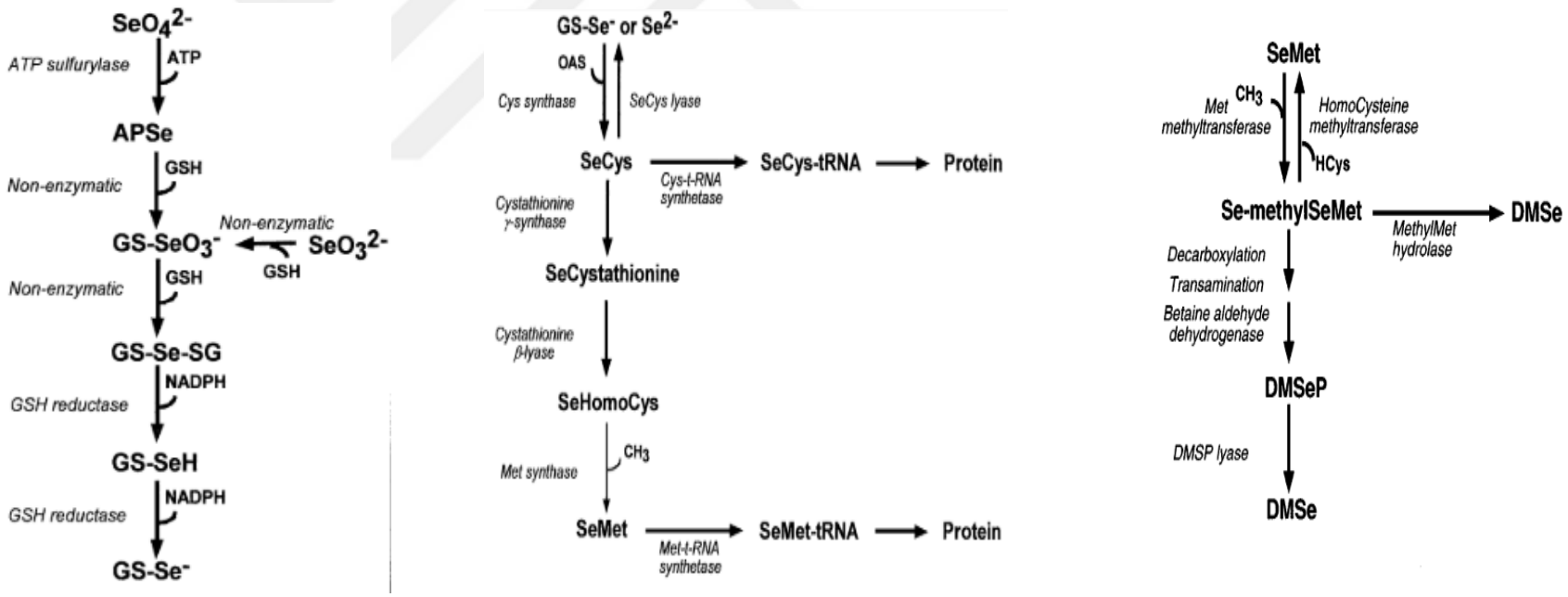
selenium has gained importance for the last few decades as it has roles on human and animal health [139].

Due to the chemical similarities of sulfate (SO_4^{2-}) and selenate (Se(VI)), it has been reported that both species are absorbed by sulfate transporters in the root plasma membrane. While the preferences for uptake of selenate over sulfate varies for each plant, it also affected by some factors such as salinity and the ratio of SO_4^{2-} to Se(VI) concentration in the cultivation medium [124], [140], [141]. The mechanism for selenite absorption, on the other hand, remained unclear and the process was previously considered to be based on passive diffusion rather than being metabolically reliant [142]. However, it was recently discovered that changes on selenite absorption rate depends on the amount of phosphate in the growth media [143]–[145], and that phosphate transporters can take a role in selenite absorption in plants [144].

Since the vegetables are the main dietary source of human and animals, knowledge on selenium compounds present in plants is important. Plants take selenate and/or selenite from the soil. The S pathways in plants assimilate and eliminate selenate (+6) and Se (+4) to selenide (-2) [124], [141], [146]. The activation of selenate by adenosine phosphoselenate (ATP) sulfurylase to adenosine phosphoselenate (APSe), an active form of Se(VI) is the initial rate limiting step and glutathione (GSH) reduce it to selenite [124]. This reduction is followed by further reduction to selenide. The selenide is subsequently converted to Se-Cysteine (SeCys) in a manner that is similar to S-metabolism. SeCys, like its S-analogues, is thought to be converted to Se-Methionine (SeMet) in the same way. In soils containing normal levels of selenium, Se enters the food chain via being incorporated into plant proteins mainly as SeCys or SeMet. Selenomethionine may be further metabolized into Se-adenosyl-Se-Met, Se-MethylSeMethionine (MeSeMet) which can then be broken down into Se-MethylSeCysteine (MeSeCys) and γ-glutamyl-Se-MethylSeCysteine. Moreover, MeSeCys becomes the main organo-selenium species at elevated Se levels in plants while other compounds might be present at much lower levels [147]. Biochemical mechanism of Se(VI) and Se(IV) has been cleared up by Terry et al [124] and

schematic representation of proposed metabolic pathway of them into organo-selenium species are provided in Figure 1.4 and Figure 1.5.

After uptake of Se, the transfer of Se from root to shoot is highly dependent on the species supplied. Selenate, as opposed to selenite or organo-selenium, is considerably easier to transport [148]. The translocation of Se over the compartments in the plant depends on some factors: kinds of plant, its physiological condition, chemical form and concentration of selenium supplied, chemical properties of cultivation medium, especially presence of sulphates and other substances[124].



GSH: glutathione, GS-SeO_3: GSH-conjugated selenite, GS-Se-SG: selenodiglutathione, GS-SeH:selenol, GS-Se^-: gulutathione-conjugated selenide, DMSe: dimethylselenide, DMSeP: dimethylselenoniopropionate

Figure 1.4 Metabolic pathway of inorganic selenium into SeMet and its derivatives in plants [124]

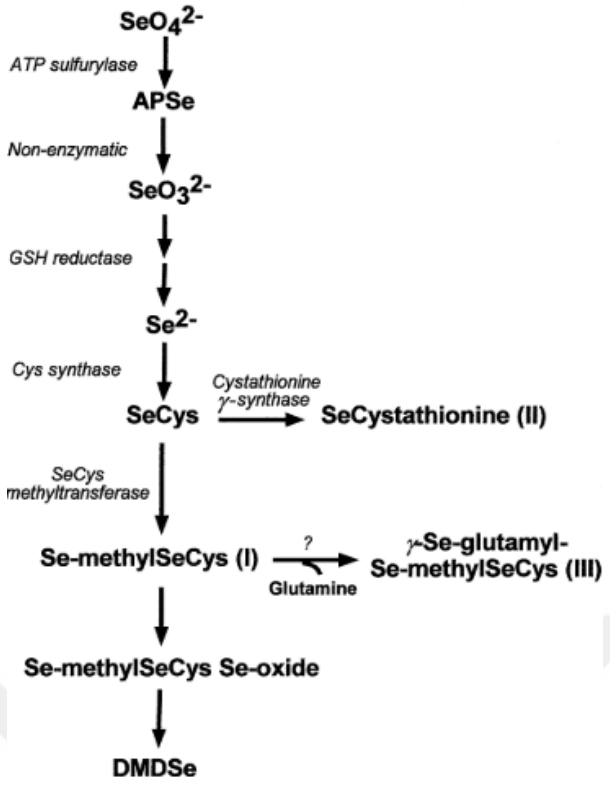


Figure 1.5 Metabolic pathway of inorganic selenium into MeSeCys and its further derivatives in plants[124]

1.1.3.4 Quantification Techniques for Total Selenium

As selenium is becoming increasingly important due to its beneficial effect on human being, determination of selenium is important to food safety and quality control. Therefore, many analytical methods have been developed to achieve precise and sensitive quantification of selenium in different matrixes.

Among spectrometric techniques, HG-AAS [149]–[152] and GF-AAS [153]–[155] are the most widely used techniques due to their low cost and sufficiently low detection limits. Although all selenium species needs to be converted into selenite which forms hydrides of selenium to be determined by HG-AAS, the main advantage of this system is the minimizing possible matrix effects and interferences resulting from sample matrixes.

Atomic florescence spectrometry have been used for determination of total selenium in various matrixes such as vegetables [156], [157], human hair [158], palm oil [159], protein based foods [160]. This technique is also alternative for speciation analysis by coupling with high performance liquid chromatography

(HPLC), gas chromatography (GC) and capillary electrophoresis (CE) [161], and it has many applications for selenium speciation in the literature [39], [162], [163].

Voltammetry as an electroanalytical technique is also used for the determination of selenium in different matrixes. Because of the greater sensitivity and higher signal-to-noise ratio associated with anodic/cathodic stripping voltammetry for trace level measurements, it has been good choice for selenium determination in water [164]. A hanging mercury drop electrode (HMDE) or a thin mercury film electrode as the working electrode is commonly employed in cathodic stripping voltammetry (CSV) [165], [166]. Beside anodic and cathodic stripping voltammetry [164], cyclic voltammetry [167], linear sweep voltammetry, adsorptive differential pulse stripping voltammetry [168], [169], adsorptive stripping voltammetry [170] and square wave voltammetry [166], [171] are also reported to be used for selenium determination. Detection limit at sub ppb and ppt levels have been achieved by hanging mercury drop electrode applying cathodic stripping voltammetry [164].

Although spectrometric techniques such as absorption, florescence and emission [172], [173] and electrochemical techniques have been widely used for selenium determination due to their simplicity, cost and adequate sensitivity for sub-ppb and ppt levels, nowadays, inductively coupled plasma mass spectrometry (ICP-MS) has been the most preferred technique to determine selenium due to its advantages (e.g low detection limits, wide linear dynamic range, capability of multi element analysis, providing information about isotopic relationships [53], [174]–[178]. Development of validated method for Se determination in complex matrixes by ICP-MS is a challenging task due to spectral interferences of ArAr mass on ^{76}Se, ^{78}Se and ^{80}Se and $^{40}Ar^{37}Cl$ masses on ^{77}Se. ^{82}Se also suffers from ^{82}Kr which is present as impurity in Ar gas and $^{81}Br^{1}H$ [179]. Beside the need of elimination these spectral interferences, Se has also relatively high first ionization potential (9.75 eV) resulting in low sensitivity. Determination of Se in biological samples can also be performed by using O_2 which enables formation of SeO^+ or CH_4, H_2 or mixture of H_2 and He which helps to remove argon dimer and argon chloride

species [50]. On the other hand, though polyatomic interferences mentioned above can be eliminated using H_2 gas with a reasonable low detection limits in Se analysis, it generates SeH^+ and BrH^+ ions which interfere with ^{77}Se, ^{78}Se and also ^{80}Se, ^{82}Se, respectively [180], [181]. The effects of interfering hydride ions should be corrected necessarily using mathematical equations as described in literature [182], [183], however, this struggle can also be solved by introducing new type of instrument called as ICP-tandem mass spectrometer (MS/MS) or referred as triple quadrupole ICP-MS (or ICP-QQQ) in 2012 [184].

1.1.3.5 Species Specific Analysis of Selenium

The chemical form of selenium present in samples has a significant impact on its metabolism, transport and bioavailability [185]. Organic selenium species, inorganic selenium species and amino acids/low molecular mass species are the three major categories of selenium species. Any selenium species, such as selenopeptides and selenoproteins, fall outside of these three categories and categorized as forth group [186]. The necessity of determining the specific species of this element in order to understand its metabolism and biological relevance in clinical chemistry, biology, toxicology and nutrition increases the importance of analytical techniques [187]. Various analytical challenges result from the complexity of selenium speciation in environmental and living organisms and C. B'Hymer et. al. summarized the most interested selenium compounds in speciation analysis given in Table 1.5 [188]. Sample pretreatment (like extraction, preconcentration and/or derivatization), separation of species and identification are all part of the analytical procedure for identification and quantification of selenium species. Each step has a great impact on quality of measurements in terms accuracy and precision. Additionally, being present at quite low levels and distributed in many different chemical forms in food and biological samples make selenium speciation analysis difficult. As a conclusion, selenium species must be quantitatively isolated from the matrix without altering their original species form, and they must be accurately characterized in terms of identity and quantity.

Table 1.5 Some of the most interested selenium species in speciation analysis [188]

Chemical name	Formula
Hydrogenselenide	H_2Se (volatile)
Selenous acid (selenite)	SeO_3H_2 (SeO_3^{2-})
Selenic acid (selenate)	SeO_4H_2 (SeO_4^{2-})
Selenocyanate	HSeCN
Trimethylselenonium cation	$(CH_3)_3Se^+$
Dimethylselenide	$(CH_3)_2Se$ (volatile)
Dimethyldiselenide	$(CH_3)Se–Se(CH_3)$ (volatile)
Dimethylseleniumsulfide	$(CH_3)Se–S(CH_3)$ (volatile)
Dimethylseleniumdioxide	$(CH_3)_2SeO_2$ (volatile)
Dimethylselenopropionate	$(CH_3)_2Se^+CH_2CH_2COOH$
Methylselenol	CH_3SeH
Methylseleninic acid	$CH_3Se(O)OH$
Methylselenenic acid	CH_3SeOH
Selenocysteine	$HOOCCH(NH_2)CH_2–Se–H$
Selenomethylcysteine	$HOOCCH(NH_2)CH_2–Se–CH_3$
Selenocystine	$HOOCCH(NH_2)CH_2–Se–Se–CH_2CH(NH_2)COOH$
Selenomethionine	$HOOCCH(NH_2)CH_2CH_2–Se–CH_3$
Selenoethionine	$HOOCCH(NH_2)CH_2CH_2–Se–CH_2CH_3$
γ-Glutamyl-Se-methylselenocysteine	$H_2NCH_2CH_2–CO–NHCH(COOH)CH_2–Se–CH_3$
Selenocystathionine	$HOOCCH(NH_2)CH_2CH_2–Se–CH_2CH(NH_3)COOH$
Selenohomocysteine	$HOOCCH(NH_2)CH_2CH_2–Se–H$
Se-adenoxylselenohomocysteine	$HOOCCH(NH_2)CH_2CH_2–Se–CH_2C_4H_5C_5N_4NH_2$
Selenosugars	Various sugar stuctures
Selenoproteins	Various proteins and enzymes (i.e., GPX, Selenoprotein P, TR)

- **Sample Preparation for Selenium Speciation: Extraction Step**

For the elemental speciation analysis, there are dedicated analytical approaches depending on mobility, stability and bioavailability of the interested metal in matrices and some of the most commonly used extraction techniques are enzymatic hydrolysis, solvent extraction, basic hydrolysis, supercritical fluid extraction, solid phase extraction, derivatization [84]. Extraction of species from a matrix must be performed not only chemically gentle but also efficiently so that integrity of species can be preserved and characterization of species can also be performed successfully. In order to increase Se species recoveries from natural materials, such as plant material and nutritional supplements, different extraction methods have been investigated. To achieve the cleavage of some selenium species which are bound to proteins and peptides is the another challenge in the selenium speciation analysis especially in biological samples. Several extraction methods have been investigated by the many researches and some of them are listed in Table 1.6. As it is seen from the literature review, although the efficiencies of enzymatic hydrolysis vary depending on matrix, type of enzymes used, pH and extraction time, it provides the higher extraction efficiencies as it transform selenium species from protein-bound to soluble forms [189].

Table 1.6 Literature review on selenium speciation analysis in various matrixes

Matrix	Species	Extraction Method	Extraction Solvent	Extraction Efficiency	Separation Technique	Detection Technique	Ref
Brazil Nuts	SeMet	Enzymatic hydrolysis (Proteinase K) ; Mechanical shake for 20 h, at 37℃	Tris-HCl buffer pH 7.5	83%	Ion Paring	HPLC-ICP-MS	[190]
Brazil Nuts	ND	Microwave	[a]Water [b]0.5 M HCl	[a]9 [b]37	Ion Paring	HPLC-ICP-MS	[190]
Yeast	SeMet	Enzymatic hydrolysis ([a]Protease XIV [b]Subtilisin); Probe sonication (ESP)	0.1 M Tris-HCl buffer pH7.5	[a]98% [b]101%	Cation exchange	HPLC-ICP-MS	[191]
Dill	MeSeCys, MeSeMet, TMSe, SeMet, $SeCys_2$ Se(IV), Se(VI)	Acid extraction	0.1 M HCl	~30%	Anion exchange & Ion Pair Reversed Phase & Size Exclusion column	HPLC-ICP-MS	[192]
Garlic, Indian mustard	MeSeCys, SeMet	[a]Acid extraction [b]Buffer extraction [c]Enzymatic hydrolysis (Protease): Mechanical shake for 20 h at 37℃	[a]0.1M HCl [b]25mM ammonium acetate pH 5.6 [c]Water	[a](62-96)% [b](95-107)% [c](103-127)%	Ion Pair Reversed Phase & Size Exclusion column	HPLC-ICP-MS	[193]
Chive	MeSeCys, SeMet, $SeCys_2$, Se(IV), Se(VI)	[a]Acid – alcohol extraction [b] Enzymatic hydrolysis (Proteinase K & Protease XIV); Mechanical shake at 50℃ for 15h+15 h for each enzyme	[a]0.4M perchloric acid-ethanol (8:2) [b]30mM Tris-HCl buffer pH 7.5	[a]~30% [b]~70%	Ion Pair Reversed Phase & Size Exclusion column	HPLC-ICP-MS	[194]
Leek	MeSeCys, SeMet, $SeCys_2$, Se(IV), Se(VI), γ-glut-cyst	Enzymatic hydrolysis (Protease XIV) Mechanical shake for 24 h at 37℃	Water	88%	Anion exchange	HPLC-ICP-MS	[195]
Zea mays	Se(VI), Se(IV)	Enzymatic hydrolysis (Protease XIV) Heating for 24 h at 37℃	30mM Tris-HCl buffer pH 7.0	36%-104%)	Anion exchange	HPLC-ICP-MS	[196]

- **Detection of Selenium Species: Hyphenated Techniques**

After extraction of selenium species completed, next step is determination of the species. Typically, speciation analysis is accomplished using hyphenation techniques, in which a high-efficiency separation technique is used to separate various species of an interested element, and a sensitive detection technique is used to determine the target elemental species at low levels.

Gas chromatography with the combination of different detection systems; usually atomic emission, mass spectrometry and flame photometric detection have been used for volatile Se species such as dimethylselenide, dimethyldiselenide, diethyldiselenide [197], [198]. These systems can also be used for nonvolatile compounds by making them volatile applying derivatization chemistry.

Moreover, as it is seen in Table 1.6, liquid chromatography is the most widely used separation technique for non-volatile and thermally stable Se species in the literature. Depending on analytes, it provides a great deal of flexibility in terms of stationary and mobile phases [189]. The most commonly used stationary phases for separation of low molecular weight selenium compounds are: reversed phase (C8 and C18 stationary phases), cation exchange stationary phase and anion exchange stationary phase. Reversed phase chromatography (RP) is used for separation of neutral and ionic selenium species and mostly ion pairing reagents like heptaflurobutanoic acid, tetrabutylammonium acetate or trifluroacetic acid are used by adding into mobile phases to increase the retention times of hydrophilic selenium species. Ion pair reversed phase liquid chromatography has the benefit of being able to control the retention time of Se species by optimizing the ion pair reagent and its concentration [199]. The disadvantage of ion pair reversed phase liquid chromatography systems is known as having limited resolution for $SeCys_2$ and inorganic selenium species [200]. However, anion exchange chromatography is capable of separation of inorganic selenium species but have difficulty resolving organic selenium compounds [200]. Cation exchange chromatography with use of pyridinium formate as the mobile phase [192], [201], [202] is preferred to use mostly as alternative technique to IP-RP and/or anion exchange chromatography systems. Size exclusion in combination with affinity

chromatography has been widely used for separation of macromolecular selenium species [187]. Most of these separation systems are hyphenated with element specific detection systems involving atomic emission, absorption, florescence or mass spectrometry [203]. Among these detection system, even if it has analytical challenges, ICP-MS is the best and most frequently used one as it has the highest sensitivity.

1.1.4 Traceability and Authentication of Food

1.1.4.1 Importance of Authenticity

Due to many major food adulterations and mislabeling events, food safety and quality have been paid more attention, and determination of geographical origin and food authenticity have become major concerns for food authorities, consumers, farmers and retailers [204], [205]. Therefore, providing origin information on the product label becomes a requirement by the Regulation of the European Parliament and of the Council (EU) No 1169/(2011) of 25 October 2011 [206]. This regulation leads to explosion in research on food authentication to verify the origin label [205], [207]–[211]. The following geographical indications are allowed to be applied to food product under EU regulations [212]:

1. Protected Designation of Origin (PDO) which includes agricultural foods that are produced, processed, and prepared using recognized know how in a specific geographical area.
2. Protected Geographical Indication (PGI) which includes agricultural products and foodstuff that are geographically linked and at least one of the action among production, processing or preparation takes in that region.
3. Traditional Specialties Guaranteed (TSG) which provides traditional character in composition or production methods.

This quality systems allows tracking the origin of product by recalling it if any fraudulent product served as genuine and resulted in any adverse effect in consumers. In recent years, several incidences related to food adulteration have been reported such as addition of oxide forms of lead into paprika, corn syrup into honey or blending two or more different kinds of rices and labeled as Basmati rice

[213]. For financial gain, these adulteration practices using chemical materials or cheap ingredients brings about a serious health risk to consumers, resulting in thousands of hospitalizations in various incidents around the world [214]. In 2007, samples of wheat gluten combined with melamine were detected in numerous U.S. pet food brands, as well as in the human food supply, probably to fraudulently exaggerate results from standard protein content tests. The altered gluten was found to be originated from China, and US officials determined that it came from the Xuzhou Anying Biologic Technology Development Company, headquartered in Xuzhou, China [215]. Moreover, melamine contamination was discovered in large quantities in China's milk supply in 2008. At least six infants have died as a result of infant formula made from melamine-tainted milk, and many more are thought to be affected [215]. Another milk scandal was occurred in India where milk was adulterated with detergent, fat and even urea [215]. In 2005, the contamination of chili powder with the color Sudan 1 which is a carcinogen listed as category 3 by World Health Organization resulted in the recall of hundreds of food products all over the world [216]. In 2011, beside the honey was produced from an artificial sweetener, due to the presence of illegal antibiotics and heavy metals in the honey, millions of pounds of Asian honey were prohibited in Europe [217].

As a result, authentication is critical for both governmental organizations in charge of labeling and industries that must verify incoming batches of raw materials and completed goods for compliance with standards. Food authenticity must also be verified in order to preserve quality and customer satisfaction, as well as perhaps to avoid economic fraud. Therefore, analytical methods which are fast, reliable and component are required to address authentication challenges and assure product quality.

1.1.4.2 Strategies for Authenticity

In the past few years, a drastic progress has been made in analytical techniques, and various analytical approaches have been used to determine food authenticity and detect adulteration. Isotope ratio and elemental analysis, liquid/gas chromatography, spectroscopic methods, sensor techniques and DNA-based

techniques are among the most often utilized techniques for food authenticity and traceability [205].

There are several approaches that have been used for food authentication. One of the most useful approach for authenticity and traceability of primary products entering the food supply chains both in fresh and processed food is DNA markers due to their large number and high stability capacity under production and processing techniques applied during the food-chain [218]. There are two types of markers used in DNA profiling: hybridisation-based markers and Polymerase Chain Reaction (PCR)-based markers, and PCR is regarded to be the faster and more accurate of the two [219]. Another approach is to use fatty acid profiling which is a slower method than some of the others due to the challenges and time taken in sample preparation [220]. However, it has been demonstrated by many researches that it is a promising approach for discriminating between production methods and geographic origin samples [220]–[225]. Beside fatty acids, some other organic molecules can also be good marker for food authentication like polyphenols [226]–[228], triacylglycerol (TAGs) [222], [229] and volatile compounds [230], [231]. Chromatographic analysis such as GC mostly coupled with mass spectrometry or flame ionization detector (FID) and liquid chromatography (LC) coupled with MS or diod array detector (DAD) are the most commonly preferred instruments for performing these analysis in complex food matrices [205].

Examining the variation in the distribution of stable isotopes in the target foodstuff is also an alternative approach that is widely used in the area of food traceability. Isotope ratio mass spectrometry (IRMS) coupled with elemental analyzer (EA) is most commonly used in food traceability studies because isotopic composition of different elements can provide eligible information regarding geography [205], [232]. Stable isotope analysis (SIA) has taken the lead in verifying the authenticity of food origins for producers and regulatory authorities, and has proven especially useful in identifying the origins of adulterated food [233], [234]. Beside isotope ratio of light elements, isotope ratio of some other elements like B, Sr , Nd and Pb have been also investigated for authenticity of food [211], [234]–[236].

Moreover, these elements except boron may provide valuable information related to origin as they are radiogenic [236]. Among them, strontium is commonly studied one in the literature. Strontium has four naturally occurring isotopes and one of them, ^{87}Sr, is a radiogenic isotope which is produced by the β-decay of ^{87}Rb [211]. Therefore, any Sr released into soils, rivers and groundwater has an isotopic signature that reflects its source as $^{87}Sr/^{86}Sr$ ratio varies between different rock/soil types. On the other hand, as the fractionation of heavier elements is not as much as light elements during translocation, radiogenic elements can be good marker in authentication studies. Boron has two naturally stable isotopes and isotopic composition of this element may vary depending on growth environments, soils, water and fertilizers [234]. As boron is a relatively lighter element, more attention should be paid to use as a geographical marker. In 2013, boron has been used together with Sr isotopes as excellent indicator for the origin of coffee beans by Liu H. et al. [237]. On the other hand, it has been showed that isotopic fractionation of boron present in even the different compartment of a bell pepper that resulted in wide B isotope variability [238]. Isotopic composition of any food sample can be determined with thermal ionization mass spectrometry (TIMS) and multi collector inductively couple plasma mass spectrometry (MC-ICP-MS) with more precise measurements and ICP-MS instruments with less precise measurements [211], [239].

Multi element profiling (trace element analysis) is the another approach that is widely used for origin determination [211], [213], [234], [237], [240]–[244]. Animal tissues have a multi element composition that is similar to that of the plants they eat. The vegetation reflects the bio-available and mobilized nutrients present in the underlying soils from which they were farmed in terms of composition [245]. There are various studies conducted for food authenticity using multi element profiling approach since mineral elements in food are more stable and less effected during processing and storage period than other compound [206]. ICP-MS and ICP-AES instruments are the most preferred analytical techniques as they are capable of determining multi element in a single run with the powerful detection limits [232]. In recent studies, multi element profiling is combined with isotope ratio measurements to improve the accuracy of

authentication of food [229], [246]. Moreover, the mineral and trace element profile of plants is determined by the soil type and ambient circumstances under which they are grown. Therefore, in some studies, determination of trace element and mineral content together with isotope ratios in plants and provenance soils have been proposed to assure the geographical origin of food samples [206], [209], [247], [248].

1.2 Objective of the Thesis

The main principle of this thesis was to develop and demonstrate the application of analytical techniques by inductively coupled plasma mass spectrometry. The first objective of the thesis was to produce and certificate a reference material related to an environmental matrix to make the measurement capabilities of national and international laboratories improve. For this purpose, seawater was selected as the target matrix since certified seawater reference material is only available from other NMIs. A primary measurement methods with the highest metrological quality was developed for the determination of ultra-trace elements like As, Cd, Cu, Cr, Hg, Fe, Ni, Pb and Zn in seawater and used for certification of a seawater reference material. The production and certification of this reference material have been performed in accordance with ISO 17034 and ISO Guide 35 by TÜBİTAK UME.

The second objective of this thesis was to conduct a detail research on metabolization of different selenium species by plants. For this purpose, leek (Allium Porrum) was cultivated in hydroponic medium fortified with inorganic selenium species. The uptake rate, translocation, biotransformation, bioavailability, and bioaccessibility of selenite and selenate by the leek were investigated. For the measurements of total selenium and selenium speciation analysis, sensitive analytical methods were developed by ICP-MS/MS and HPLC-ICP-MS/MS, respectively.

In the last chapter of the thesis, it was aimed to perform a preliminary research by applying multi element profiling using inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) to walnut samples and to their provenance soils for their

authentication. This study was aimed to find out crucial markers that can be used in an extensive research in the future.

1.3 Hypothesis

In the first chapter of the thesis, a candidate certified reference seawater material which was also used as the material for key (CCQM-K155) and a parallel-run pilot (CCQM-P196) comparison study organized by Inorganic Analysis Working Group was aimed to be produced in accordance with EN ISO 17034 and ISO Guide 35. The main motivation of this part of the study was to eliminate the foreign dependency of the certified seawater reference material needs of the national laboratories of Turkey. In this project, as the value assignment of the candidate material was planned to be conducted by TÜBİTAK UME, there were two options according to the guides: either use of more than one analytical methods by one laboratory or use of primary measurement technique by one laboratory. In this study, it was aimed to develop and validate an SI traceable primary reference method to be used for certification measurements of the candidate material. Since the operation of isotope dilution mass spectrometry which is a potential primary technique is well defined and understood, a full uncertainty statement can be written in SI units and a traceability chain to be clearly identified in terms of the mole and the kilogram in the shortest way. Therefore, isotope dilution mass spectrometry, IDMS, was aimed to be used as a potentially primary method for Cd, Cr, Cu, Fe, Ni, Pb and Zn. Moreover, in order to achieve the highest accuracy and precision and be able to apply IDMS as a primary method, developing a matrix separation procedure was one the main challenging point in the method development. In addition to those parameters, a method was developed applying matrix matched calibration technique to be used for the characterization of As in seawater by ICP-MS/MS in which the high potential of molecular interference of $^{40}Ar^{35}Cl$ on ^{75}As is a challenge. Homogeneity, short term stability, long term stability and characterization measurements of all targeted analytes were aimed to be performed using these validated SI traceable analytical techniques.

Selenium is an essential element with significant nutritional advantages in trace amounts but is toxic at high levels, [124] and Turkey is one of the countries whose

the daily intake amount has been observed to be less than recommended [249]. Hence, in the second chapter, it was aimed to cultivate selenium enriched food which has been consumed widely in Turkey and provide detailed investigation on the metabolization of selenium in the plant. It is well known that due to their nutritional properties and biological activities, which are dependent on chemicals such as organosulphur compounds and methylated selenium species containing aminoacids, *Allium* species are the most preferred vegetables [250], [251]. In this chapter, the motivation was to cultivate selenium enriched *Allium Porrum* (leeks) which can be achieved by free of differences in soil compositions. Hydroponic leek culture with high and low levels of Se(IV) and Se(VI) fortification, as well as their metabolization by the plant in terms of uptake, translocation, biotransformation, bioavailability and bioaccessibility were aimed to be studied.

In recent years, protected geographical indication, protected designation of origin and traditional specialties guaranteed have been gaining importance in all over world to be able to verify the origin of foods and their labels provided [205], [207]–[211]. In the last chapter of this thesis, it was aimed to conduct a research to find out the any geographical indicator by performing multi element profiling study in walnut samples and also their soils of origin as walnut is one of the most consumed food in Turkey. The relationship between walnuts and their soils of origin was evaluated according to the one-to-one correlation coefficient of the elements and the correlation coefficient of different elements pairs.

2
MATERIALS AND METHODS

2.1 Instrumentation

2.1.1 Instrumentation for Certification of Trace Elements in Seawater Certified Reference Material

An inductively coupled mass spectrometry with triple quadrupole (ICP-MS/MS, 8800 ICP-QQQ, Agilent Technologies, Japan) was used to conduct all of the certification measurements on UME CRM 1206. Two quadrupole units are located between the octupole reaction cell and can be used to reduce polyatomic interference by combining a collision gas (H_2, He, O_2, NH_3) and a reactive gas (O_2 or NH_3) that selectively react with either the interfering or target ion. A Peltier device was used to cool a double pass quartz spray chamber to 2.0 °C, which was fitted with a MicroMist nebulizer. To avoid salt accumulation in the nebulizer and injector, a dual argon humidifier (Elegra, Glass Expansion) was used. To reduce oxide formation, the high matrix introduction system (HMI) was also turned on. The MassHunter software's automated tuning tool was used to optimize tuning parameters for each analyte. A simulated matrix was used for each analyte to choose the reaction or collision cell gas, and the operating conditions were also optimized depending on the isotope pair(s). Oxygen cell gas was used as a collision gas for Cd in a single quad mode, as well as a reaction gas for Cr and As mass-shifting. For Cu and Fe, collision cell gases of helium and NH_3 were used, respectively. In single quad mode, hydrogen cell gas was used as a collision gas for Ni and Zn.

Annually calibrated and SI traceable via TÜBITAK UME, an analytical balance with a resolution of 0.01 mg (Sartorius MSA225S-100-DA) was used in the entire sample preparation and regular performance monitoring of the balance was carried out using calibrated E2 class weights. Colloidal particles were separated from supernatant solutions using a centrifuge (Beckman Coulter, Allegra X-15R).

2.1.2 Instrumentation for Analysis of Leek Samples

Total selenium and speciation analysis was conducted by Agilent ICP-MS/MS described in 2.1. In total Se analysis, mass shift with O_2 was applied to eliminate/reduce spectral interferences resulting from matrix and optimization of parameters was performed using ^{76}Se, ^{78}Se and ^{80}Se isotopes. Instrumental settings for total and speciation analysis for Se are given Table 2.10.

For total selenium determination, samples were digested using temperature and pressure controlled Cem Mars Microwave system.

Chromatographic separations were performed using Agilent 1100 HPLC system. HPLC and ICP-MS/MS is coupled by using a PEEK tubing. A C18 column (Phenomenex Synergi Hydro-RP, 250 x 4.60mm, 4μ) column and strong anion exchange column (Hamilton PRP-X-100, 250 x 4.6mm, 10μm) were utilized in the speciation analysis.

2.1.3 Instrumentation for Authentication Study

A double focusing magnetic sector field inductively couple plasma mass spectrometry, SF-ICP-MS (Element 2, Finnigan MAT, Bremen, Germany) was used for total elemental determination in soil and walnut samples. The sample introduction system consisted of a PFA nebulizer mounted onto cyclonic PFA spray chamber for determination of total elemental mass fractions in digested soil samples and a glass cyclonic spray chamber equipped with seaspray nebulizer for the determination of total elemental mass fractions in digested walnut samples.

Total elemental mass fraction determination was also performed by Spectro Arcos brand inductively couple plasma optical emission spectrometer, ICP-OES, with axial plasma. The results for As, B, Cd, P, Sb and Sr obtained by ICP-OES which provide better precision with respect to ICP-MS was used in the data set. A 50 mL PTFE cyclonic TFE spray chamber combined with teflon mira mist nebulizer was used for sample introduction in this instrument.

Walnut samples were digested by temperature-controlled MARS Xpress (CEM Corporation, Matthews, USA) and soil samples were digested by using temperature and pressure controlled CEM MARS microwave digestion system.

All sample preparations were performed gravimetrically via SI traceable balance (Mettler Toledo AX205). Daily performance of balance was checked by using E2 class (Häfner Gewichte GmbH, Germany) reference weights.

2.2 Chemicals and Materials

2.2.1 Chemicals and Materials for the Certification of Trace Elements in Seawater Certified Reference Material

The Milestone subPUR sub-boiling distillation method was used to obtain ultrapure acid from Emsure grade nitric acid (Merck, 65%). The PURELAB Flex device from Elga Veolia was used to produce ultrapure de-ionized water (18.2 MΩ·cm resistivity). The co-precipitation reagent was selected as triethylamine solution (99.7% purity, Across Organics). The list of the certified reference materials, isotopically enriched certified reference materials used in this study is provided in Table 2.1.

Trace Science's ^{60}Ni enriched isotopic material (>95% purity) was used in the IDMS application for Ni. The mass bias correction in the measurements of isotopic composition of Pb in sample and standard was performed via NIST SRM 981 while NIST SRM 982 was used for the same purpose in the measurements of sample and calibration blends. In the double IDMS and triple IDMS applications, SI traceable certified reference materials (NIST SRM 3100 series) were used as primary standard reference materials (PSRM). NIST SRM 3103a was used to create calibration plots for As measurements.

Sample preparation for recovery studies of coprecipitation procedures was done gravimetrically in ISO 7 while the sample preparation for ID-ICP-MS measurements was performed gravimetrically in ISO 6. The trueness of the developed method was investigated using two different origin matrix CRM provided in Table 2.1.

Table 2.1 List of the standards used in the certification of trace elements in seawater certified reference material

Standards	Manufacturer	Standard No
Arsenic Standard Solution	NIST	SRM 3103a
Cadmium Standard Solution	NIST	SRM 3108
Chromium Standard Solution	NIST	SRM 3114
Copper Standard Solution	NIST	SRM 3112a
Iron Standard Solution	NIST	SRM 3126a
Nickel Standard Solution	NIST	SRM 3136
Lead Standard Solution	NIST	SRM 3128
Zinc Standard Solution	NIST	SRM 3168a
Cadmium Isotopic Reference Material	IRMM	IRMM 621
Chromium Isotopic Reference Material	IRMM	IRMM 624
Copper Isotopic Reference Material	IRMM	IRMM 632
Iron Isotopic Reference Material	IRMM	IRMM-620
Nickel Isotopic Reference Material	Trace Science	-
Lead-206 Spike Assay and Isotopic	NIST	SRM 991
Zinc Isotopic Reference Material	IRMM	IRMM 654
Common Lead Isotopic Reference Material	NIST	SRM 981
Equal-Atom Lead Isotopic Reference Material	NIST	SRM 982
Trace Elements in Seawater	NMIA	NMIA MX014
Nearshore Seawater Certified Reference Material for Trace Metals and other Constituents	NRC	CASS-6

New 15 mL polypropylene centrifuge tubes were exposed to cleaning procedure before to use in sample preparation. Steps in the cleaning procedure are; (1) Fill with 5.0% (v/v) HNO_3 (analytical grade), (3) fill with ultrapure water, (4) fill with 2.0% sub-boiled HNO_3, (5) fill with ultrapure water, and (6) dry in ISO 4 ultra-clean laminar flow cabinet.

2.2.2 Chemical and Materials for Analysis of Leek Samples

The whole list of chemicals with the specifications and their manufacturers used in the analysis of leek samples is provided in Table 2.2. Deionized water produced by Elga Veolia PURELAB Flex system used throughout the study. Sub-boiled HNO_3 described in 2.2.1 and concentrated H_2O_2 was used in mineralization of samples.

NIST SRM 3149 Selenium was used as calibration solution by diluting with 1.0% HNO_3 (v/v) in the analysis of total selenium. The certified reference material,

NIST SRM 1573a tomato leaves, was used for validation of the mineralization program and optimizing the operating conditions of the ICP-MS/MS for total Se determination.

For the speciation analysis, 0.1 g/kg Se for inorganic selenium species and 0.01 g/kg Se for the other organo-selenium species were prepared by weighing the certain amounts of Na_2SeO_3, Na_2SeO_4, Selenomethionine (SeMet), seleno-DL-cystine ($SeCys_2$) and Se-(methyl)selenocysteine (MeSeCys) into HDPE bottles and dissolving them in deionized water. Stock solutions have been stored at +4 °C and working standard solutions were prepared daily by serial dilution. All sample and standard preparation steps were performed gravimetrically throughout the study.

Enzymatic digestion of leek samples were performed by using the solution which Proteinase K, Protease XIV were added to Tris-HCl buffer solution (pH 7.5) containing 1.0 mM $CaCl_2$. Pancreatin, pepsin and α-amylase were used for the simulation of gastrointestinal digestion system of human together with NaCl. The pH adjustment in the simulation of gastrointestinal digestion was performed by using HCl and $NaHCO_3$.

1.0% (v/v) heptafluorobutyric acid (HFBA) was used as a mobile phase for reverse phase – ion pairing chromatography (RP-IP-HPLC) and citrate buffer (prepared from diammonium hydrogen citrate anhydrous) at pH 5.5 was used for strong anion exchange chromatography (SAX). Methanol was added to the mobile phases at the concentration of 3.0% (v/v).

Table 2.2 The list of chemicals used in analysis of leek samples

Chemicals	Manufacturer	Additional information
HNO_3	Merck	Emprove, 67%
HCl	Merck	37% ultrapure
H_2O_2	Merck	Emprove , 35%
HFBA	Alfa Aesar	99%
Methanol	JT Baker	Ultra gradient HPLC grade
Na_2SeO_3	Alfa Easer	Min 99%
Na_2SeO_4	Alfa Easer	Anyhdrous 99.8% +
Selenomethionine	Sigma	-
Seleno-DL-cystine	Sigma	-
Se-(methyl)selenocysteine	Sigma	95%
Proteinase K	Sigma	from Tritirachium album
Protease XIV	Sigma	Bacterial, from Streptomyces griseus
Tris-HCl	ITW Reagents	min 99%
$CaCl_2$	Alfa Aeser	dried powder, 97%,
Pancreatin	Sigma Life Science	from porcine pancreas (USA, 4 x USP)
Pepsin	Sigma Life Science	from porcine gastric mucosa (USA, 250 units/mg solid)
α-amylase	Sigma Life Science	from Bacillus subtilis (USA, ≥1.500 units/mg solid)
NaCl	Merck	-
$NaHCO_3$	Sigma Life Science	-
Diammonium hydrogen citrate anhydrous	Sisco Research Laboratories	Min 98%

2.2.3 Chemical and Materials for Provenance Study

Samples were digested by using Ultrapure NORMATOM 68% (w/w) nitric acid (VWR, Belgium) and Suprapure 65% (w/w) nitric acid (Merck, Germany) for walnut and soil samples, respectively. During the optimization of digestion procedures for soil samples, 37% (w/w) HCl (Merck, Germany), 48% (w/w) HF (Merck, Germany), 30% (w/w) H_2O_2 (Merck, Germany), 70 % (w/w) $HClO_4$, 4.0% (w/w) H_3BO_3 were also used. De-ionized water (18.2 MΩ·cm resistivity) produced by Elga Veolia PURELAB Flex system was used throughout the study. NIST SRM 3100 series of mono-elemental solutions (National Institute of standards & Technology, USA) were used for the quantification of analytes. UMECRM 1202 “Elements in Hazelnut”, NIST SRM 2387 “Peanut Butter” and NIST SRM 2711a “Montana II Soil” were used for the validation of methods.

Only new and cleaned polypropylene falcon tubes (15 or 50 mL centrifuge tubes, VWR International) were systematically pre-cleaned by keeping them with full of

analytical grade 15% HNO_3 (v/v) and deionized water overnight for both steps. Microwave vessels were also cleaned by digesting 10 mL of analytical grade 65% HNO_3 (w/w) and they were kept overnight by filling with de-ionized water.

2.3 Experimental

2.3.1 Certification of Trace Elements in Seawater Certified Reference Material

UME CRM 1206 is a certified reference material for trace level elements in seawater. UME CRM 1206 was also used as the sample of international key comparison CCQM K155 Elements in Seawater which has been organized in 2019-*cont*. This international comparison was a collaboration of TÜBİTAK UME and Government Laboratory of Hong Kong, China (GLHK).

2.3.1.1 Material Processing and Process Control

The sampling of the raw material for seawater-based reference material was performed by TÜBITAK Marmara Research Vessel of Environment and Cleaner Production Institute on 09 August, 2018 and 15 August, 2018 in the Marmara Sea with the experts of marine scientist of MAM and technical experts of UME. The samples were pumped using immersible pump into 10 L precleaned high density polyethylene drums. Raw materials for candidate certified reference materials were collected from two different location of the Marmara Sea (40° 46.200’ N ; 029° 12.956’ E and 40 31,423 N ; 027 11, 333 E). Approximately 100 L seawater from each location was sampled into the pre-cleaned drums and all the drums were acidified to obtain pH 1.6 with sub boiled HNO_3 and stored in 4.0 °C until the further processing steps take place.

In order to determine natural levels of these two different batch, subsampling was performed from each drums (20 sampling) into pre-cleaned falcon tubes in the amount of 40 mL. Analyses 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 given in Table 2.3 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.

Table 2.3 Background and target levels in seawater used in processing

Analyte	Background level in raw material[1]	Target level in processed material
As	1.7 ± 0.1	2.5
Cd	0.06 ± 0.01	0.5
Cr	0.2 ± 0.1	2.5
Cu	1.7 ± 0.1	2.5
Fe	-[2]	5.0
Hg	0.05 ± 0.02	0.2
Ni	1.3 ± 0.1	5.0
Pb	0.06 ± 0.06	1.0
Se	N.D	2.0
Zn	3.7 ± 1.1	10

[1]mean value and its standard deviation belongs to 10 drums sampled at 15 August 2018
[2]could not be measured accurately by proposed multielement ICP-MS/MS method

Whole processing of reference materials including spiking, homogenization and filling were 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 the filling of the drums with distilled water and run the homogenization system for several times. Drums were left for 5 days with full of distilled 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% (v/v) 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 was rinsed with several liters of seawater.

Cleanness of 250 mL low density polyethylene (LDPE) for 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, Bremen, Germany). The results indicated that concentrations 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 bottles and re-measured by HR-ICP-MS to make sure that the background levels were minimized. After this step, developed cleaning procedure was applied to the whole batch.

Cleaning procedures included 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 membrane (Pall Corp, Supor® Membrane, AcroPack™ 1000, PN 12992) which was 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 and dispatched for gamma irradiation at 25 kGy. All the bottles were placed into aluminized PET sachets after gamma irradiation and placed at reference temperature room.

2.3.1.2 Triethylamine Assisted $Mg(OH)_2$ Co-precipitation Method Development

Co-precipitation techniques have become popular because they are cost-effective and practical for determining elements in seawater [252]–[259]. Since one of the major concerns in the application of ultra-trace analysis is the availability of high-purity reagent, ammonium hydroxide (NH_4OH) has become the most popular reagent in co-precipitation techniques. However, one of the major disadvantages is that some elements such as Cu, Cd, Co, Ni, and Zn form soluble ammonia complexes, limiting the use of ammonia as a co-precipitation agent. As a result, greater effort is needed to achieve high recovery for these elements. The

details of the method development is also provided in the papers published in the scope of this thesis [260], [261].

I. Preparation of treated matrix matched seawater

The same origin of raw material mentioned in 2.3.1.1 was used to produce treated matrix matched seawater (TMMS) which was used in method development studies. TEA supported co-precipitations were used to extract trace elements from 1.0 L of seawater. A 40-mL aliquot of seawater was transferred into a 50-mL pre-cleaned centrifuge tube and combined with 0.40 mL TEA for the treatment process. Supernatant solutions were transferred to 50 mL centrifuge tubes after centrifuging at 10000 rpm for 30 minutes. In order to remove excess TEA, these solutions were heated to 110 °C for 60 minutes in an ISO 4 laminar fume hood using digestion blocks (DigiPrep, SCP Science). All of the evaporated samples were collected in a 1.0 L of PFA container

The mass fraction of Mg, Ca, Na and K as major electrolytes in non-treated and treated seawater were determined using triple quadrupole ICP-MS/MS. While the difference in mass fractions of Ca, K, and Na was insignificant, the amount of Mg in the treated seawater sample was approximately 50% as compared to the initial seawater matrix. The amount of Mg is a crucial parameter since matrix separation is dependent on $Mg(OH)_2$ co-precipitation. To obtain TMMS for use in method development studies, 1.48 g $MgSO_4$ was added to treated seawater to adjust Mg concentration to approximately 700 mg/L. TMMS were gravimetrically spiked with the target element at the level of UME CRM 1206 using NIST SRM 3100 series standards in order to optimize co-precipitation conditions with TEA. Table 2.4 show the approximate spike concentrations.

Table 2.4 Theoretical spiked levels of investigated analytes in TMMS

Analyte	Mass Fraction, ng/g
As	3.0
Cd	0.5
Co	6.0
Cr	6.0
Cu	2.0
Fe	15.0
Mn	6.0
Mo	6.0
Ni	5.0
Pb	1.0
Se	2.0
V	6.0
Zn	7.0

II. Optimization of removal efficiency of salinity and recovery rate of analytes by TEA/$Mg(OH)_2$ co-precipitation

Efficiency of matrix removal was a critical point for precise and accurate isotope ratio measurements (IRM). Even though complete recovery is not needed for IDMS applications as long as isotopic equilibrium is formed in the blends prior to any sample manipulation, high recovery values help to reduce uncertainties in more accurate ratio measurements. As a result, TMMS was used to conduct optimization studies for the TEA and co-precipitation steps. Experiments were carried out with 5.0 g TMMS without any preconcentration. ICP-MS/MS was used to evaluate the samples using an internal standard (^{115}In) via an external calibration plot.

Co-precipitation in Single Step:

TEA was applied to 5.0 g of TMMS in various quantities ranging from 50 to 150 μL. The samples were diluted to 5.0 g with ultrapure water after pellets were dissolved in 0.60 mL 6.9% (w/w) HNO_3. Although quantitative recovery for the target analytes was achieved in the range of 91% to 104% even with the addition of 50 μL TEA, total salinity which consist of mainly Mg was not low enough by using single phase co-precipitation, as shown in Figure 2.1 and Figure 2.3. As a

result, an additional co-precipitation phase was investigated in order to reduce the overall salinity of the final solutions.

Co-precipitation in Two Step:

The main purpose of the second step as co-precipitation step was to vary the Mg amount in the first step while keeping the TEA quantity constant in the second step, because the efficiency of co-precipitation is dependent on the amount of Mg in the solution. As a result, the effects of adding 50, 60, and 80 μL of TEA to 5.0 g of TMMS during the first step of co-precipitation were investigated. Pellets from the initial co-precipitation were dissolved in 500 μL of 6.9% (w/w) HNO_3, then diluted to 2.0 mL with de-ionized water. The samples were then centrifuged after adding 40 μL of TEA at the second co-precipitation step. After dissolving the pellets in 500 μL of 6.9% (w/w) HNO_3, the solutions were diluted to 5.0 mL with de-ionized water. As it is shown in Figure 2.2, the salinity in the solutions was lowered to 0.03 g/L, compared to 8.9 g/L (original seawater) by employing the second co-precipitation stage.

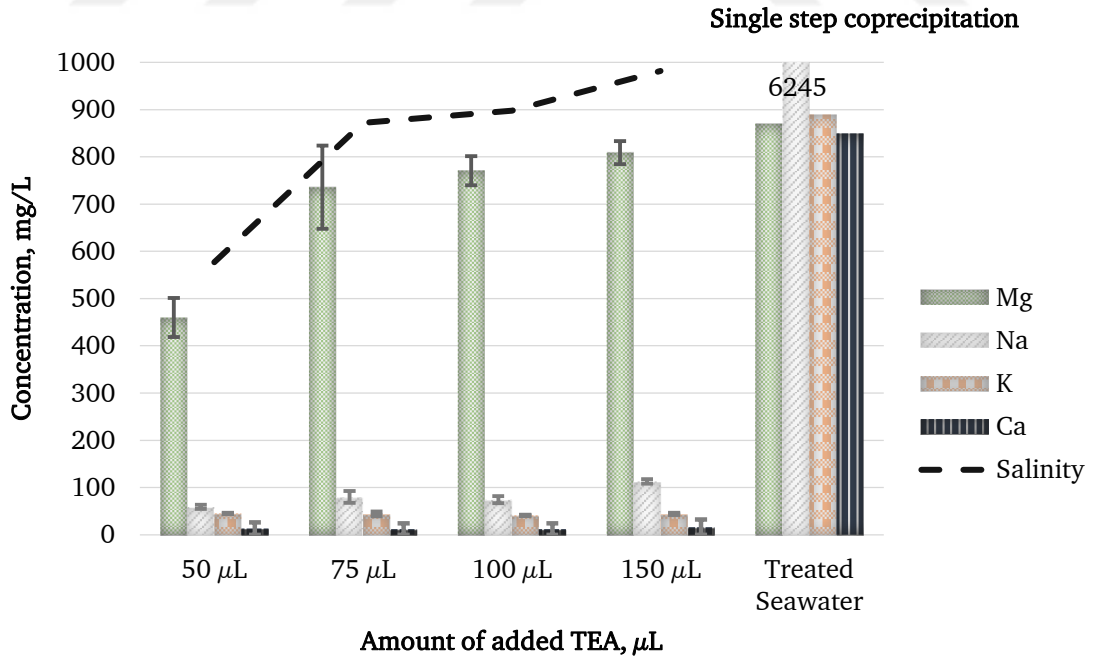


Figure 2.1 Total salinity of the supenatant solutions after applying single step co-precipitation

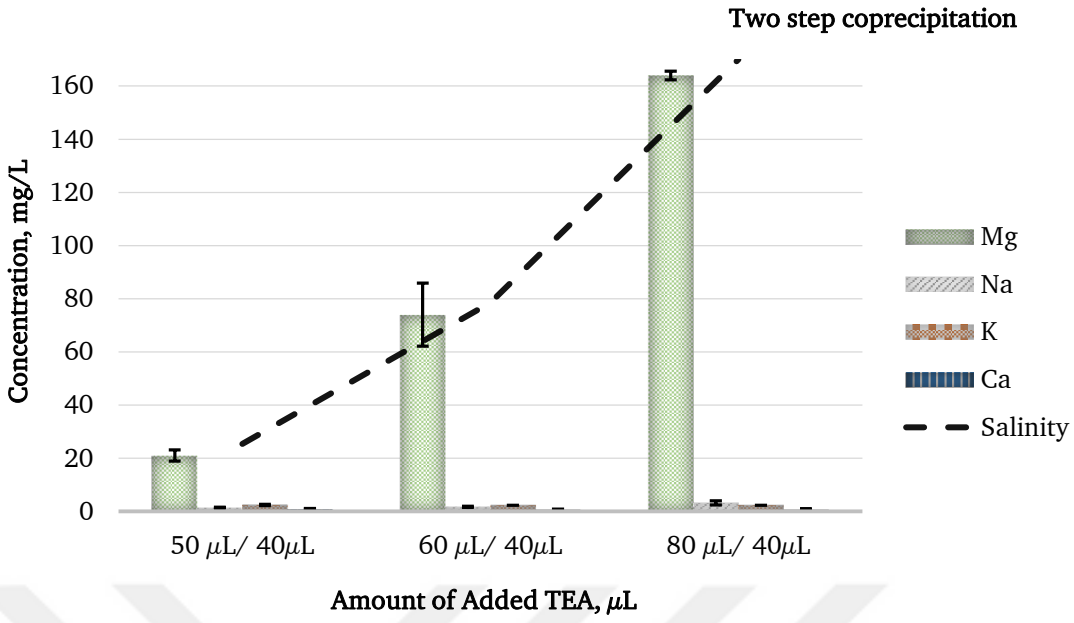


Figure 2.2 Total salinity of the supernatant solutions after applying two step co-precipitation

As demonstrated in Figure 2.1 and Figure 2.2, the recovery obtained by a single step co-precipitation approach is more reproducible and comparatively higher than the ones obtained by applying co-precipitation procedure twice. However, recovery values for the latter application are acceptable to obtain high sensitivity for ratio measurements by triple quad ICP-MS. TEA/$Mg(OH)_2$ strategy was used in two steps (50 and 40 μL) to assess target analytes in seawater using ID-ICP-MS.

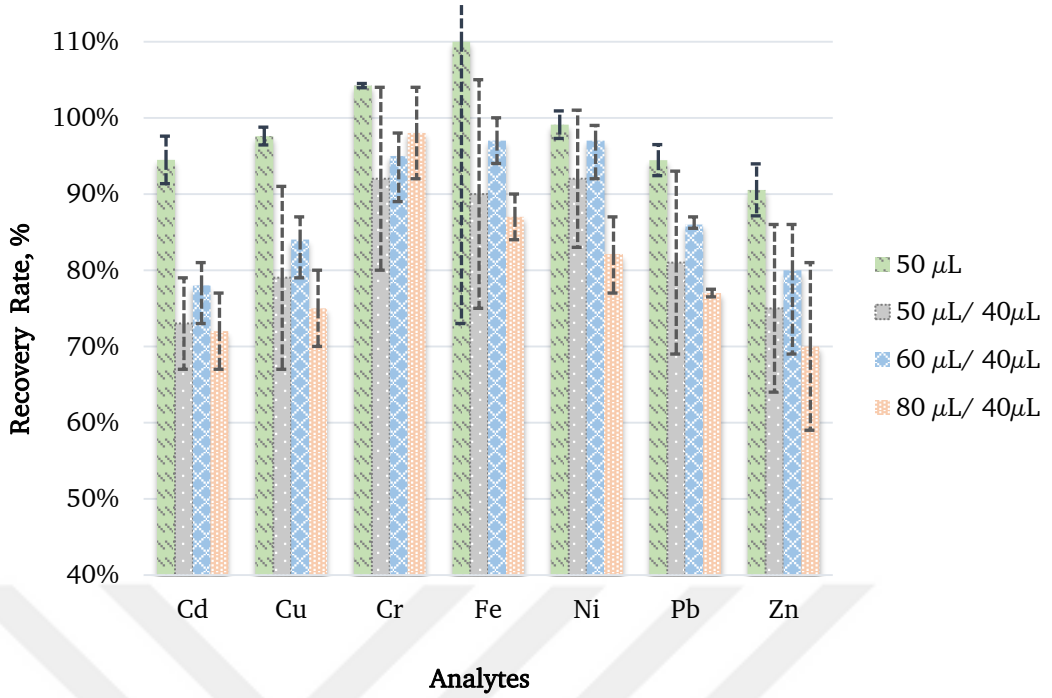


Figure 2.3 TEA assisted $Mg(OH)_2$ co-precipitation recovery studies

Hence, two steps (50/40 μL) TEA/$Mg(OH)_2$ strategy was combined with ID-ICP-MS method for determination of Cd, Cu, Cr, Fe, Ni, Pb and Zn.

2.3.1.3 IDMS Measurements as a Primary Reference Technique for Cd, Cr, Cu, Fe, Ni, Pb and Zn

Since the operation of isotope dilution mass spectrometry is well known and understood, it is widely used to characterize the certified value to reference materials. In this way, total uncertainty statement is expressed in terms of SI units. Establishment of traceability chain can be set in terms of two SI units, the mole and kilogram, in a shortest way and that was used to get high quality analytical results for the characterization of reference materials [64].The aim of the method used in the characterization of UME CRM 1206 was to achieve traceable and highly reliable quantification with the lowest measurement uncertainty.

Important parameters such as isotopic equilibrium, isotope uniformity must be met in order to use IDMS as a primary method. The most critical points in this study was detailed in following sections:

- **Detector Dead Time**

It is critical to establish the detector response (dead) time of the instrument specific to the elements in order to give high accuracy isotope ratio measurements irrespective of analyte mass fraction. In this study, spiking of samples was performed to get the ratio of 1.00 ± 0.05 for $^{52}Cr/^{50}Cr$, $^{63}Cu/^{65}Cu$, $^{60}Ni/^{62}Ni$ and $^{206}Pb/^{208}Pb$ in order to reduce the dead time impact on signals. However, it was was established by using the Method 2 [78] for Cd, Fe and Zn as the ratio of $^{113}Cd/^{111}Cd$, $^{56}Fe/^{57}Fe$ and $^{66}Zn/^{68}Zn$ were adjusted to 0.20 ± 0.05, 4.0 ± 0.2 and 0.50 ± 0.05, respectively.

- **Mass Bias Correction**

Possible mass biases [64], [77], [79], particularly happen in use of collision/reaction cell gases, are an another important parameter affecting the accuracy in IRM [262]. The effects of mass biases in measured isotopes were evaluated in all three calibration strategies for iron measurements.

In this study, the solutions for mass bias correction was prepared from the sample itself using TEA/$Mg(OH)_2$ coprecipitation process to ensure that the matrix of sample blends and the mass bias solution were as similar as possible. The concentration of element in unknown sample was matched in the mass bias solution, in addition to the matrix. Mass bias correction was applied in a bracketing approach [76]. Since R_y and R_x were obtained from the certificates of iCRM and IUPAC, respectively, the mass bias correction is an important parameter, particularly in single IDMS applications. However, it was discovered that if an exact match in the isotope ratio of sample and calibration blends is obtained in the use of ID^2MS and ID^3MS, this correction can be ignored. The RSD% of the mass bias solution calculated over the four-hour series is 0.7% (n=8), indicating that the isotope ratio measurements by ICP-MS/MS is stable.

- **Effective reduction conditions of polyatomic interferences for highly accurate isotope ratio measurements**

In the application of IDMS, an element must have at least two available isotopes that are preferably fully free of any isobaric or polyatomic interference. The

thorough investigation for eliminating or reducing possible polyatomic interferences is the most important parameter for accurate IRM. As stated in section 2.3.1.2, the major analytes Mg, Na, K, and Ca in the residual matrix were 21 ± 2, 1.4 ± 0.2, 2.4 ± 0.2 and 0.60 ± 0.07 mg/L, respectively. Aside from these elements, there could be other interfering elements that are not taken into account when optimizing co-precipitation conditions. Table 2.5 lists the target analytes in UME CRM 1206 as well as the most likely polyatomic interferences.

Some interferences, such as MoO and ZrO on Cd isotopes, are difficult to eliminate even using the high resolution mode of a sector field ICP-MS instrument. As a result, if applying mathematical corrections is not preferred due to the target measurement uncertainty budget, using a reaction/collision cell may be required. ICP-MS/MS, on the other hand, has a significant advantage in terms of effectively eliminating polyatomic interferences because it has not only four cell gases (O_2, NH_3, He and H_2), but also an additional quadrupole ion selection guide that allows the instrument to operate in MS/MS mode and also in terms of stability during the measurement sequence when dealing with considerably high matrix samples. The instrument's stability is assured by using a high matrix introduction mode and an argon humidifier unit to support the nebulizer and sampler cones. Before optimizing the cell gas flow rate, the Masshunter program of the ICP-MS/MS was used to tune the settings for each analyte independently. The removal effectiveness of polyatomic interferences was assessed using both the background equivalence concentration (BEC) and the IRM. Therefore, target analytes in simulated matrix solutions and 1.0% HNO_3 matrix under optimized conditions were measured, separately. The normalized K values (IUPAC value/measured ratio) were used to determine the ratio measurement results, and the results are summarized in Table 2.6 - Table 2.8.

Table 2.5 The most probable polyatomic interferences on target analytes

Isotopes	Potential Polyatomic Interferences					
^{110}Cd	$^{70}Zn^{40}Ar$	$^{94}Mo^{16}O$	$^{94}Zr^{16}O$			
^{111}Cd	$^{75}As^{36}Ar$	$^{95}Mo^{16}O$	$^{93}Nb^{18}O$			
^{112}Cd	$^{96}Mo^{16}O$	$^{96}Ru^{16}O$	$^{96}Zr^{16}O$			
^{113}Cd	$^{97}Mo^{16}O$					
^{114}Cd	$^{74}Se^{40}Ar$	$^{98}Ru^{16}O$	$^{98}Mo^{16}O$			
^{63}Cu	$^{47}Ti^{16}O$	$^{27}Al^{36}Ar$	$^{23}Na^{40}Ar$	$^{45}Sc^{18}O$	$^{15}N^{16}O^{16}O^{16}O$	$^{14}N^{17}O^{16}O^{16}O$
^{65}Cu	$^{49}Ti^{16}O$	$^{25}Mg^{40}Ar$	$^{23}Na^{23}Na^{18}O^{1}H$			
^{50}Cr	$^{34}S^{16}O$	$^{32}S^{18}O$	$^{14}N^{36}Ar$	$^{10}B^{40}Ar$		
^{52}Cr	$^{12}C^{40}Ar$	$^{36}Ar^{16}O$				
^{53}Cr	$^{37}Cl^{16}O$	$^{13}C^{40}Ar$	$^{36}Ar^{16}O^{1}H$		$^{35}Cl^{18}O$	
^{56}Fe	$^{40}Ar^{16}O$	$^{40}Ca^{16}O$				
^{57}Fe	$^{41}K^{16}O$	$^{39}K^{16}O$	$^{40}Ar^{16}O^{1}H$			
^{60}Ni	$^{44}Ca^{16}O$	$^{24}Mg^{36}Ar$	$^{20}Ne^{40}Ar$			
^{62}Ni	$^{46}Ti^{16}O$	$^{22}Ne^{40}Ar$	$^{14}N^{16}O^{16}O^{16}O$			
^{66}Zn	$^{26}Mg^{40}Ar$	$^{36}Ar^{14}N^{16}O$	$^{50}V^{16}O$	$^{50}Ti^{16}O$	$^{48}Ti^{18}O$	
^{66}Zn	$^{54}Fe^{12}C$	$^{53}Cr^{13}C$	$^{54}Cr^{12}C$	$^{50}Cr^{16}O$		
^{68}Zn	$^{52}Cr^{16}O$	$^{56}Fe^{12}C$	$^{32}S^{36}Ar$	$^{28}Si^{40}Ar$	$^{55}Mn^{13}C$	

Table 2.6 Investigation of cell gases performances for Cd and Cu based on BEC and Isotope ratio measurements

	Mode	Optimum gas flow rate	Isotopes	BEC, μg/L	K_{Matrix}	K_{Matrix} / K_{HNO3}
Cd ($Cd/^{111}Cd$)	No gas	-	^{110}Cd	0.77	2.430 ± 0.053	2.445
			^{111}Cd	0.37	1	
			^{112}Cd	0.34	0.945 ± 0.026	0.932
			^{113}Cd	0.24	0.854 ± 0.032	0.842
			^{114}Cd	0.25	0.864 ± 0.027	0.843
	H_2-SQ	8.5	^{110}Cd	0.03	1.145 ± 0.053	1.165
			^{111}Cd	0.05	1	
			^{112}Cd	0.010	0.891 ± 0.045	0.876
			^{113}Cd	0.012	0.940 ± 0.029	0.887
			^{114}Cd	0.007	0.917 ± 0.056	0.836
	O_2-SQ	75	^{110}Cd	5.4	15.22 ± 1.19	15.406
			^{111}Cd	0.01	1	
			^{112}Cd	1.2	2.313 ± 0.236	2.224
			^{113}Cd	0.008	1.024 ± 0.067	0.954
			^{114}Cd	0.007	1.054 ± 0.092	0.980
	He-SQ	10	^{110}Cd	0.2	2.291 ± 0.179	2.330
			^{111}Cd	0.14	1	
			^{112}Cd	0.14	0.720 ± 0.014	0.726
			^{113}Cd	0.15	0.655 ± 0.020	0.639
			^{114}Cd	0.16	0.679 ± 0.060	0.621
Cu	No gas	-	^{63}Cu	0.08	0.921 ± 0.003	0.977
			^{65}Cu	0.12		
	He-SQ	7	^{63}Cu	0.02	0.908 ± 0.006	0.998
			^{65}Cu	0.03		
	NH_3 /He-Mass Shift	25/1	^{63}Cu - $^{97}Cu(NH_3)_2$	0.02	0.918 ± 0.005	1.002
			^{65}Cu - $^{99}Cu(NH_3)_2$	0.02		

Table 2.7 Investigation of cell gases performances for chromium based on BEC and isotope ratio measurements

	Mode	Optimum gas flow rate	Isotopes	BEC, $\mu g/L$	K_{Matrix}	K_{Matrix} / K_{HNO3}
Cr ($^{52}Cr/^{50}Cr$)	No gas	-	^{52}Cr	0.15	0.360 ± 0.006	0.457
			^{50}Cr	0.14		
	H_2-SQ	8.5	^{52}Cr	0.200	0.952 ± 0.009	0.905
			^{50}Cr	0.081		
	He-SQ	10	^{52}Cr	0.054	0.869± 0.026	0.946
			^{50}Cr	0.390		
	H_2-MSMS	8.5	^{52}Cr	0.062	0.879 ± 0.014	1.035
			^{50}Cr	0.052		
	O_2-MSMS	50	^{52}Cr	0.012	1.150 ±0.018	1.041
			^{50}Cr	0.026		
	He-MSMS	8	^{52}Cr	0.011	0.926 ± 0.009	1.005
			^{50}Cr	0.021		
	NH_3/He-MSMS	15/3	^{52}Cr	0.020	0.889 ± 0.005	1.020
			^{50}Cr	0.021		
	O_2-Mass Shift	50	^{52}Cr-	0.008	0.974± 0.019	1.007
			^{50}Cr-	0.040		
	NH_3/He-Mass Shift	50/1	^{52}Cr - $^{86}Cr(NH_3$	0.006	0.839 ± 0.030	1.013
			^{50}Cr- $^{84}Cr(NH_3$	0.008		

Table 2.8 Investigation of cell gases performances for iron based on BEC and isotope ratio measurements

	Mode	Optimum gas flow rate	Isotopes	BEC, μg/L	K_{Matrix}	K_{Matrix} / K_{HNO3}
Fe ($^{56}Fe/^{57}Fe$)	H_2-SQ	7	^{56}Fe	0.024	1.417 ± 0.021	1.244
			^{57}Fe	3.4		
	O2-SQ	60	^{56}Fe	0.045	1.351 ± 0.016	1.218
			^{57}Fe	2.6		
	He-SQ	11	^{56}Fe	0.025	1.313 ± 0.014	1.156
			^{57}Fe	2.3		
	NH3/He-SQ	35/6	^{56}Fe	0.030	1.085 ± 0.029	1.000
			^{57}Fe	0.035		
	H2-MS/MS	9	^{56}Fe	0.021	1.150 ±0.018	1.059
			^{57}Fe	0.80		
	O2-MS/MS	80	^{56}Fe	0.03	1.115 ± 0.019	1.060
			^{57}Fe	0.80		
	He-MS/MS	11	^{56}Fe	0.03	1.160 ±0.016	1.053
			^{57}Fe	0.66		
	NH3/He-MS/MS	50/7	^{56}Fe	0.028	1.038 ± 0.052	1.030
			^{57}Fe	0.026		

- **Optimization of ICP-MS/MS for Cd isotope ratio measurements**

To eliminate the potential polyatomic interferences mentioned in Table 2.5, different cell gases were investigated. Since ^{111}Cd is the enriched isotope to be used in IDMS, ^{110}Cd, ^{112}Cd, ^{113}Cd, and ^{114}Cd were investigated to find the best isotope pair. Using single quad (SQ) mode, interference reduction efficiency was investigated using H_2, Q_2, and He cell gases.

Background equivalence concentrations were determined using 100 μg/L Zr, 250 μg/L Mo, 20 μg/L Se, 20 μg/L Zn, 20 μg/L As and 1.0 μg/L Cd in 1.0% (v/v) nitric acid as standard solution and 20 μg/L Zn, 250 μg/L Mo, 20 μg/L Se, 100 μg/L Zr, 20 μg/L As in 1.0% (v/v) HNO_3 as blank solution. After deciding the optimal cell gas flow rates for each tune mode, isotope ratio measurements of the same solutions with 1.0 g/L Cd in 1.0% (v/v) HNO_3 were carried out in the same order to ensure the trueness of the isotopic measurements. For the solution without matrix, the K values were found to be in the range of 1.094-0.924.

Measured isotope ratios (Cd/^{111}Cd) revealed that use of H_2 cell gas in single quad (H_2-SQ) mode effectively reduced interferences on ^{110}Cd, ^{111}Cd, ^{112}Cd, ^{113}Cd and ^{114}Cd. Furthermore, as shown in Figure 2.4, while formation rate of ZrO increased in the use of O_2 cell gas in single quad (O_2-SQ) mode, interference reduction efficiency on ^{111}Cd, ^{113}Cd and ^{114}Cd was found to be more effective to the former. For ^{111}Cd, ^{113}Cd and ^{114}Cd, the BEC was reduced to 10 ng/L, 8 ng/L, and 7 ng/L, respectively, from 370 ng/L, 240 ng/L, and 250 ng/L. Therefore, ^{113}Cd/^{111}Cd and ^{114}Cd/^{111}Cd were chosen and analyzed in ICP-MS/MS using the O_2-SQ mode.

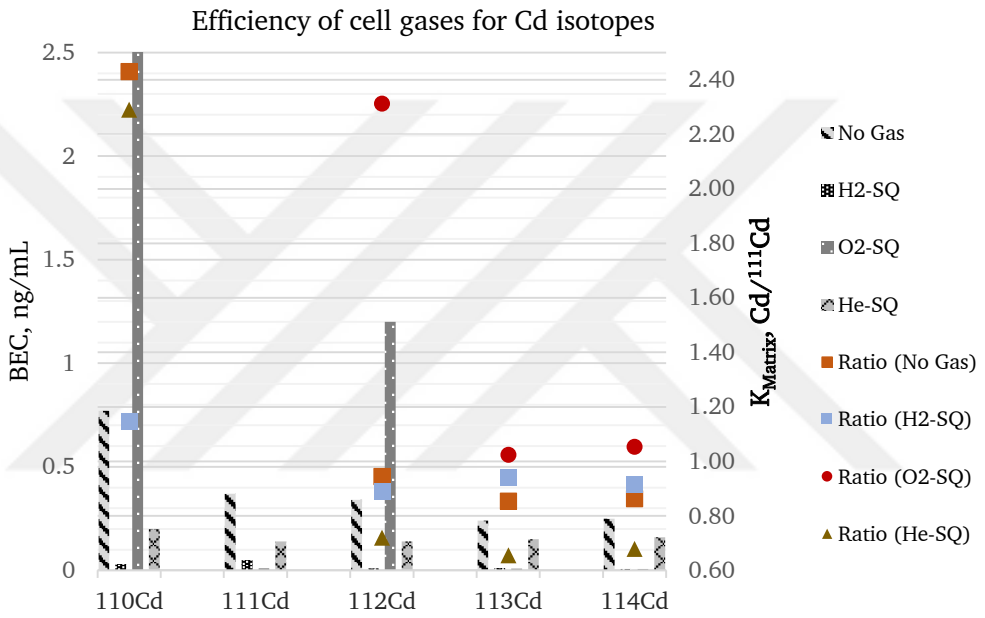


Figure 2.4 Performance of cell gases on Cd isotopes in SQ mode of ICP-MS/MS

- **Optimization of ICP-MS/MS for Cr isotope ratio measurements**

A simulated matrix of 100 ng/mL S and 1.0 mg/L B in 1.0% nitric acid (v/v) was used, and 3.0 ng/mL Cr and its blank were measured in this matrix solution. For Cr isotopes, interferences resulting from plasma should be taken into account in addition to possible polyatomic interferences caused by matrix. As a consequence, mass shift with reaction gases (O_2 and NH_3/He) was studied in addition to the instrument's SQ mode and MS/MS modes. The instrument was run in SQ mode to investigate the gases H_2, He, and O_2. The use of O_2 cell gas was eliminated because it resulted in a very high BEC values on ^{50}Cr. In the MS/MS mode, it was discovered that interferences from matrix and plasma can be effectively reduced

below 60 ng/L as BEC for ^{50}Cr and ^{52}Cr. Although mass shift using NH_3/He (Cr – $Cr(NH_3)_2$) was found to be the most effective way of reducing interference, mass shift using O_2 (Cr – CrO) was chosen due to the more accurate data obtained on IRM, as seen in Figure 2.5 and Table 2.7.

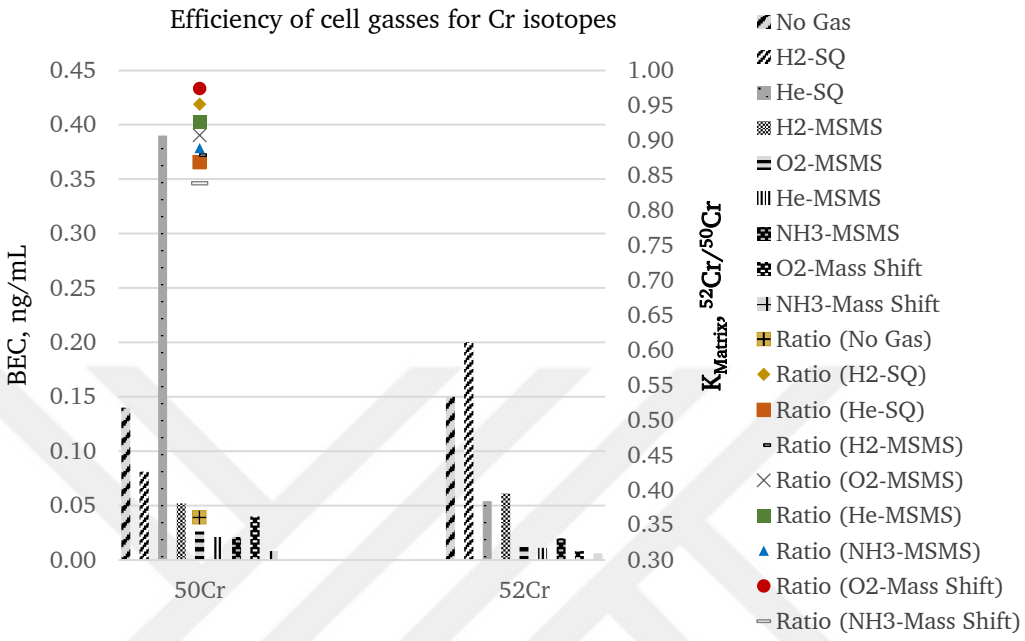


Figure 2.5 Cell gases performance on Cr isotopes

- **Optimization of ICP-MS/MS for Cu isotope ratio measurements**

The prevalent interferences in seawater matrix for Cu isotope measurements are predicted to be $^{23}Na^{40}Ar$ and $^{25}Mg^{40}Ar$ on ^{63}Cu and ^{65}Cu, respectively. Moreover, a simulated matrix including 100 ng/mL Al, 100 ng/mL Ti, and 100 ng/mL Sc, as well as 20 mg/L Mg, 2.0 mg/L Na and 1.0 ng/mL Cu was applied in the investigation of cell gases performances on the reducing polyatomic interferences. The most successful methods for eliminating possible interferences were He in single quad (He-SQ) mode and mass shift using NH_3 cell gas (Cu-$Cu(NH_3)_2$). Despite the fact that both approaches were equal in terms of BEC and ratio measurement precision, He-SQ mode was chosen for the IDMS measurements.

- **Optimization of ICP-MS/MS for Fe isotope ratio measurements**

The most abundant polyatomic interferences on ^{56}Fe and ^{57}Fe are $^{40}Ar^{16}O$ and $^{40}Ar^{16}O^{1}H$, respectively. Furthermore, highly precise isotope analysis becomes more difficult at sub-ppb levels of Fe, as the concentration of Ca that interferes as

$^{40}Ca^{16}O$ and K that interferes as $^{41}K^{16}O$ and $^{39}K^{18}O$ in co-precipitated seawater matrix is just a sub-ppm [263]. Background equivalent concentration (BEC) and isotope ratios were calculated using four different cell gasses in both SQ and MS/MS modes of triple quadrupole ICP-MS to investigate the removal efficiency of these interferences.

A simulated matrix solution containing 2.0 mg/L Na, 1.0 mg/L Ca, 3.0 mg/L K and 15.0 μg/L Fe in 1.0% (v/v) HNO_3 and a background solution including 3.0 mg/L K, 1.0 mg/L Ca and 2.0 mg/L Na in 1.0% (v/v) HNO_3 was used during the optimization measurements.

Any cell gases in SQ mode, with the exception of NH_3/He, could not achieve as precise ratio measurements as MS/MS mode. Since polyatomic interferences on ^{56}Fe are easier to be removed by almost any cell gas, also in SQ mode, the aim of this optimization was to reduce interferences on the ^{57}Fe isotope (Figure 2.6).

The most effective cell gas was found to be NH_3/He in either the SQ or MS/MS mode, according to both BEC values and IRM. For IDMS applications, single quad mode was chosen as it offered higher signal intensities and a lower relative standard deviation for IRM. Even though the polyatomic interferences on ^{56}Fe and ^{57}Fe were less than 30 ng/L, a systematic background corrections on measured intensities were performed.

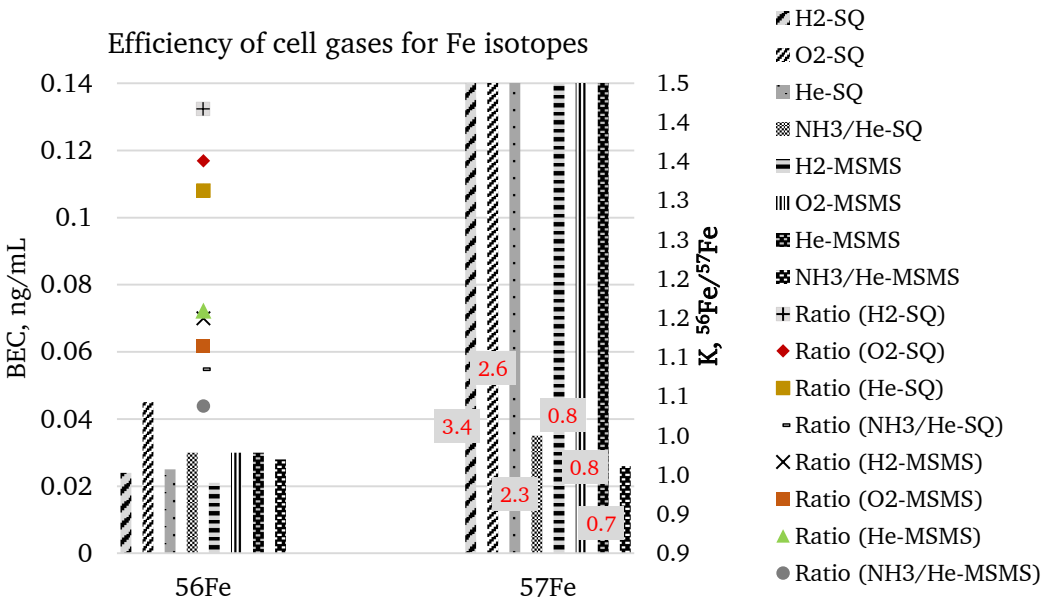


Figure 2.6 Cell gases performance on Fe isotopes

- **Optimization of ICP-MS/MS for Ni and Zn isotope ratio measurements**

A solution containing 5.0 ng/mL Ni in 20 mg/L Mg and 1.0 mg/L Ca in a 1.0% (v/v) HNO_3 was tested for the investigation of potential polyatomic interferences on ^{60}Ni and ^{62}Ni using four cell gases in SQ mode. Although it was concluded that a cell gas was not required for very precise Ni isotope ratio measurements, He cell gas was used in SQ mode as it improves sensitivity by approximately 30% with respect to no gas mode. Isotope ratio measurement of Zn ($^{66}Zn/^{68}Zn$) was also performed using cell gas in H_2-SQ mode due to the similar observation.

2.3.1.4 Detection Power of TEA/$Mg(OH)_2$-IDMS

The analytical blank must be evaluated carefully in order to accurately assess the analytes at ultra-trace levels. IDMS was used to evaluate reagent blanks for each element. In order to taken all the parameters into account for the assignment of blank level of the procedure, a 90 µL TEA was added to tubes and spiked as done for the sample blends. The total volume of nitric acid used in solving pellets was added to tubes and diluted with deionized water to 5.0 g after evaporation of solution near to dryness at 110 °C in an ISO Class 4 Class II B2. The reagent level was determined by six separate blank blends and the detection limit was calculated as three times of standard deviation obtained from the blank solutions ($3xSD_{blank}$).

2.3.1.5 Development of a Method for Determination of Arsenic in Seawater

ID-ICP-MS as a potentially primary technique can only be applied to elements which have at least two isotopes. In this project, the background level of raw material given in Table 2.3 was determined as (1.7 ± 0.1) μg/kg by matrix matched calibration technique and then seawater was spiked by NIST SRM 3103a Arsenic (As) standard solution which contains approximately 1.6 mol/L nitric acid to get 2.5 μg As/kg and homogenized as described in 2.3.1.1. As the mass fraction of arsenic was at low ppb, its determination was only possible with ICP-MS with the lowest measurement uncertainty.

- **Optimization of ICP-MS/MS for As**

In order to optimize parameters in ICP-MS/MS to get the highest sensitivity with minimized interference of $^{40}Ar^{3}5Cl$ on ^{75}As isotopes, background equivalent concentration (BEC) were measured using 10 ppm Ca, 10 ppm K and 0.35% NaCl in 1.0% (v/v) HNO_3 as simulated blank matrix and 10 mg/L Ca, 10 mg/L K, 0.35% NaCl and 5.0 μg/L As in 1.0 % (v/v) HNO_3 as simulated matrix. Hydrogen (H_2-SQ) and He (He-SQ) gas were used as collision cell gas in SQ mode and O_2 gas was used as reaction cell gas to shift mass of As isotope from 75 to 91 ($^{75}As^{16}O$). All the tune parameters were firstly optimized by using 5.0 μg/L As in 1.0 % HNO_3 and optimum cell gases was investigated under these optimized conditions by using described solutions. Background equivalence concentrations downs from 2.8 μg/L obtained in no gas mode to 0.03 μg/L, 0.04 μg/L and 0.01 μg/L for H_2, He and O_2 modes, respectively. Oxygen mass shift mode was chosen for the analysis as sensitivity was approximately 2.1 and 2.7 times better than others, respectively. The optimized tune parameters of ICP-MS/MS are given in Table 2.9.

Table 2.9 Optimum ICP-MS/MS parameters for As measurements in seawater

Nebulizer	Micromist glass nebulizer
Spray chamber	Scott type-double pass
RF applied power (W)	1550
Sampling depth (mm)	8.2
Nebulizer pump (rps)	0.2
Scan Type	MS/MS
Nebulizer gas flow rate (L min^{-1})	0.70
Dilution gas flow rate (L min^{-1})	0.60
Reaction Gas	O_2
Cell gas flow rate (%)	40
Extract 1 (V)	-65
Extract 2 (V)	-200
Omega bias (V)	-115
Omega lens (V)	4.4
Octopole bias (V)	-5
KED (V)	-7
Wait time offset (ms)	0
Sweeps/replicate	50
Integration time/mass (s)	1.9998
Number of replicates	10
Total analysis time/sample (min)	0.4

- **Four-point matrix matched external calibration technique**

For highly accurate and precise measurements, four-point matrix matched external calibration technique was used for the determination of total As in sea water. Matrix induced high Cl^- interference is eliminated by shifting the mass of ^{75}As to the mass of $^{75}As^{16}O$ with the O_2 reaction gas as described above.

Seawater samples were gravimetrically diluted 10 times by 1.0% (v/v) HNO_3. The equal amount of sample (1/10) was also added into calibration standards. The preparation of calibration standards for matrix matched external calibration method is summarized in Table 2.10 Calibration standards should be prepared for each different sea water matrix.

Table 2.10 . Preparation of calibration solution for matrix matched external calibration

	STD 0	STD 1	STD 2	STD 3
Added matrix amount, g	0.5	0.5	0.5	0.5
Target As concentration to be added, ng/g	0	$(C_{sample}/10)$-$C_{sample}/10)/5)$	$(C_{sample}/10)$	$(C_{sample}/10)+(C_{sample}/10)/5)$
Total amount of solution, g	5.0	5.0	5.0	5.0

2.3.1.6 Estimation of Measurement Uncertainty

Every uncertainty components (bottom up approach) were taken into account according to ISO/GUM and Eurachem guides to calculate combined uncertainty [264], [265]. Evaluation of measurement uncertainty was performed by using a specialized program, Gum Workbench® software, which applies the principles provided in DIN/ISO/BIPM Guidelines [266].

2.3.1.7 Establishment of Metrological Traceability

To determine the measurement accuracy, a procedure must be validated, which also includes estimation of measurement uncertainty and demonstration of the traceability chain for results. While traceability does not guarantee measurement accuracy, a well- demonstration of traceability chain is obligatory for the reference material certification, according to ISO 17034 and ISO Guide 35. Since the operation of isotope dilution mass spectrometry is well defined and understood,

it can be used to assign the certified value of reference materials. The use of a calibrated and SI traceable balance and certified reference materials provided by NMIs was used to create traceability of all target analyte (As, Cd, Cr, Cu, Fe, Ni, Pb, and Zn) amount content in this study.

The measurement results of target Cd, Cr, Cu, Fe, and Zn by a single IDMS are traceable to SI unit mole via RMM-621, IRMM-632, IRMM-624, and IRMM-654 enriched certified reference material (iCRM) manufactured by the European Commission Joint Research Center and used in the preparation of sample blends, as well as to kg via a SI traceable calibrated balance and metrological control of the sample blends. As the traceability to kg for double and triple IDMS measurements was obtained in a similar manner, traceability to mole has been developed for double IDMS with the iCRM and NIST SRM 3100 series. Traceability to the mole in the triple IDMS application, on the other hand, is established to SI units using only the NIST SRM 3100 series.

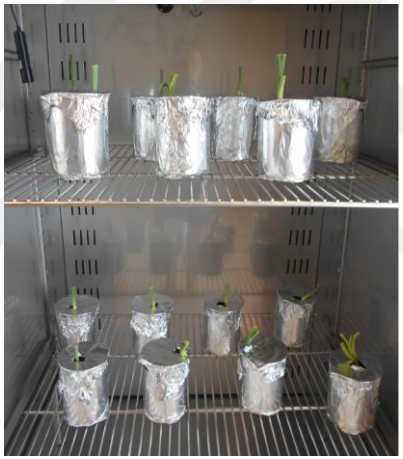
Traceability of As measurements which were performed by matrix matched standard addition using ICP-MS/MS was also established via NIST SRM 3103a and gravimetric sample and standard preparation.

2.3.2 Experimental for Analysis of Leek Samples

2.3.2.1 Hydroponically Cultivation of Leek Samples

The leek samples were obtained from organic bazaar and hydroponically cultured in climatic chamber as seen in Figure 2.7 and Figure 2.8. Only autoclaved labwares were used for cultivation. Leek samples were cut into 12 cm lengths from the root and rinsed with tap water before being rinsed with deionized water. At least five plants for each level described in Table 3. 16 together with three control plants were placed in a climatic chamber at 25 °C for 14 days with 14 hours of light and 10 hours of darkness. In order to compare the differences in metabolization of Se(IV) and Se(VI), a group of plants were grown in fortified 20 μM Se (Level-1) and 40 μM Se (Level-2) nutritional solutions. Additional groups of leek samples were independently cultured in Level 3 (280 μM Se(IV), Level 4 (450 μM Se(IV)), Level 5 (200 μM Se(VI)) and Level 6 (325 μM Se(VI)) nutritional solutions for

further research of metabolization at increased inorganic selenium supplementation. In able to evaluate the selenium uptake rate, 400 μL nutritional solution from Level-1 and Level-2 was sampled periodically after nutritional solutions was set to initial weight by tap water. By the end of cultivation period, the plants were harvested and carefully rinsed with tap water and then deionized water before split into roots, stems and leaves. All the parts were cut into smaller pieces to increase the surface area of the samples in able to perform lyophilization more efficiently. After lyophilization, all the parts of leek were grinded to obtain homogenized powder and store at -20 ˚C.



3rd day of implamentation

14th day of implamentation

Figure 2.7 Cultivation of leek samples in climatic chamber

3rd day after implementation

5rd day after implantation

8th day after implantation

12th day after implantation

14th day after implantation

Figure 2.8 An example for growing period of a leek supplemented by Se(IV)

2.3.2.2 Analysis of Total Selenium

Total selenium quantities was determined in the parts of dried leeks, nutritional solutions, and enzymatic and gastrointestinal digested leek samples. Except nutritional solutions, other two kind of samples were digested in CEM Mars Microwave system by using the digestion program given in Table 2.11.

Table 2.11 Microwave digestion program for leek samples

Level	Temperature (°C)	Ramping time (min)	Hold time (min)
1	Room temperature - 135	5	-
2	135 - 180	5	20

The measurements of total selenium in all kinds of samples were performed by matrix matched external calibration method and all the measurements were performed gravimetrically.

NIST SRM 1573a *Tomato Leaves* were used to check method's trueness. A total of 500 mg of CRM was put into digestion bombs, and 4.0 mL sub-boiled HNO_3, 0.50 mL 30% (w/w) H_2O_2 and 2.0 mL deionized H_2O were added into samples. Deionized water was used to dilute the digested samples to 10 g. Use of oxygen mass shift mode was found to be necessary in able to eliminate all interferences resulting from matrix components. The calibration graphs obtained during the analysis of total Se in NIST SRM 1573a are given in Figure 2.9. The recovery of the NIST SRM 1573a was found as 102.3 % ± 0.8 % (n=4) by using O_2 mass shift mode - ICP-MS/MS. Therefore, all the analysis of digested samples was performed in O_2 mass shift mode of the instrument under optimized conditions given Table 2.12. The ICP-MS/MS limits of detection ($3sd+C_{blank}$) and quantification ($10sd+C_{blank,}$) for Se was found to be 2.2 ng/g and 3.5 ng/g , respectively.

Table 2.12 ICP-MS/MS parameters for total and speciation analysis of selenium

	Total Se Analysis	Se Speciation Analysis
Nebulizer	Micromist glass nebulizer	
Spray chamber	Scott type-double pass	
RF power (W)	1550	
Nebulizer pump (rps)	0.2	0.3
Reaction Gas	O_2	H_2
Scan Type	MS/MS	MS/MS
Masses monitored	80-96, 78-94, 76-92	80-80
Nebulizer gas flow rate (L min^{-1})	1.20	1.10
Extract 1 (V)	-8	3.5
Extract 2 (V)	-200	-200
Omega bias (V)	-100	-110
Omega lens (V)	6.6	6.6
Q1 enterance	-2.0	-13.0
Q1 exit	-3.0	1.0
Q1 bias	-2.0	0.0
Q1 prefilter bias	-32.0	-14.0
Q1 postfilter bias	-40.0	-20.0
Cell gas flow rate (%)	40	3.0
OctP bias (V)	-3.5	1.0
OctP RF (V)	150	170
KED (V)	-5.0	-8.0
Sweeps/replicate	100	-
Integration time/mass (s)	0.5	-
Number of replicates	10	-
Total analysis time/sample (min)	1.0	-

The analyses of nutritional solutions were performed by applying matrix matched external calibration technique and representative calibration graphs are given in Figure 2.10.

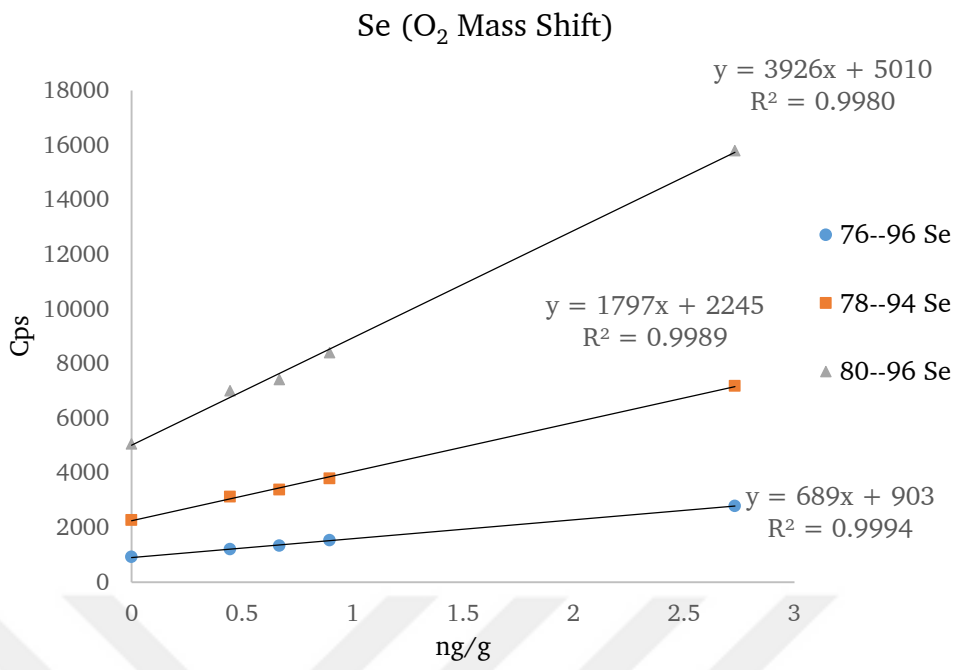


Figure 2.9 Calibration graphs of ^{76}Se, ^{78}Se and ^{80}Se obtained for the analysis of NIST SRM 1573a

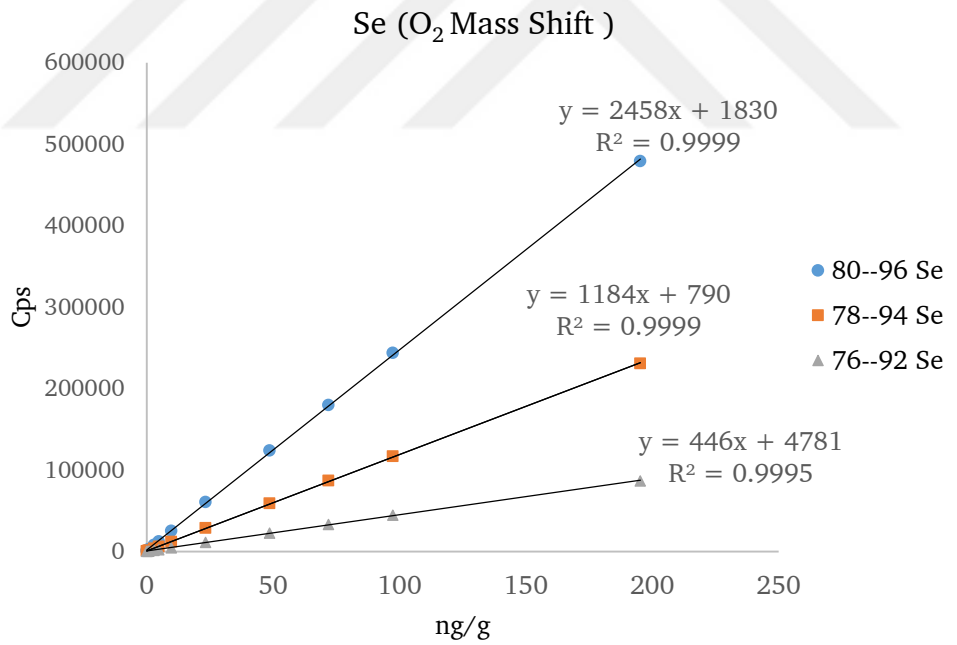


Figure 2.10 Representative calibration graphs of ^{76}Se, ^{78}Se and $^{8}0Se$ obtained for total Se in the analysis of nutritional solutions

10 mg of powder leaves and stems were put into digested bombs together with 2.0 mL sub-boiled HNO_3, 1.0 mL 30% H_2O_2 and 1.0 mL H_2O. Deionized water was used to dilute the digested materials to 10 g.

For enzymatically extracted solutions of leek samples, 2.0 mL of solution was weighted into bombs together with and 2.0 mL sub-boiled HNO_3, 1.0 mL 30% H_2O_2 and deionized water was used to dilute the digested samples to 10 g. Before being introduced to the ICP-MS/MS, the digested samples were further diluted by a factor of two.

Approximately, 0.25 mL of gastrointestinal digested samples were also mineralized using of 2.0 mL sub-boiled HNO_3, 1.0 mL 30% H_2O_2 and 2.0 mL H_2O into vessels. The same dilution procedures were used for digested samples as they were for enzymatically extracted samples.

Representative calibration graphs for total Se analysis during analysis of enzymatically extracted and gastrointestinal digested samples are given in Figure 2.11.

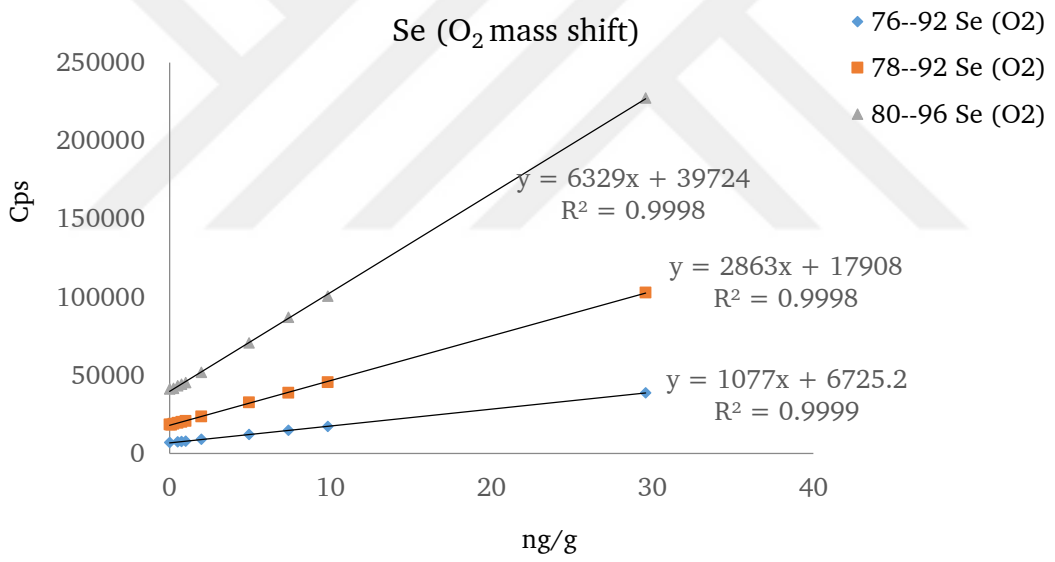


Figure 2.11 Representative calibration graphs of ^{76}Se, ^{78}Se and ^{80}Se obtained for total Se in the analysis of enzymatically extracted samples

2.3.2.3 Enzymatic Extraction Procedure

In order to release selenoamino acids, enzymatic hydrolysis of proteins was applied. The extraction procedure was adapted from the study conducted by E. Kapolna et al. [194] and slightly modified. A batch of extraction solution containing 5.0 mg/each sample of Proteinase K and Protease XIV were prepared using 30 mM Tris-HCl (pH 7.5) containing 1.0 mM $CaCl_2$. Approximately 10 mg of samples were weighed into centrifuge tubes and 5.0 mL of extraction solution

were added into the tubes. Samples were shaken for 18 hours at 50 °C. The final solutions were then centrifuged and filtered using 0.45 μm PVDF filter.

2.3.2.4 Gastrointestinal Digestion Procedure

Human gastrointestinal digesting system was simulated *in vitro* to figure out the fraction extracted in the intestine and accessible for subsequent biological processing which is called as bioaccessibility. Approximately 0.05 g of dried stems and leaves together with 500 μL of gastric juice (1.0% w/v pepsin in 150 mM NaCl, adjusted pH to 2.0 using 37% HCl) were placed into micro centrifuge tubes and incubated for 4.0 h at 37 °C [267]. After the gastric digestion, solutions were adjusted to pH 6.8 by using $NaHCO_3$ and incubated for another 4.0 h at 37 °C by adding of 500 μL intestinal juice (0.04% (w/v) amylase in 150 mM NaCl, 3.0% w/v pancreatin,). After intestinal digestion, samples were centrifuged at 14000g for 30 min at 4.0 °C and supernatant was gently pipette transferred to another micro centrifuge tube. Until analysis, all the samples were stored at -20 °C.

2.3.2.5 Speciation Analysis

To investigate the selenium species in enzymatically and gastrointestinal digested samples, two different chromatographic separations were applied by HPLC-ICP-MS under the optimized conditions. The speciation analyses were performed by matrix matched external calibration method and all the measurements were performed gravimetrically.

A. Strong Anion Exchange (SAX) HPLC-ICP-MS/MS

Strong anion exchange column was used for the determination of selenite and selenate in extracted/digested solutions (Table 2.13).

Table 2.13 Optimum parameters for SAX-HPLC

Column	Hamilton PRP-X100 (250 x 4.1 mm, 10 μm)	
Mobile Phase (MP)	(A)	0.50 mM citrate buffer including 3.0% (v/v) MeOH (pH 5.5)
	(B)	10.0 mM citrate buffer including 3.0% (v/v) MeOH (pH 5.5)
Gradient Program	0-4 min – 100% MP-A (1.0 mL/min) 4-5 min – 100% MP-A – 100% MP-B (1.2 mL/min) 5-11 min – 100% MP-B (1.2 ml /min) 11-12 min – 100% MP-B - 100% MP-A (1.0 mL/min) 12-13 min – 100% MP-A (1.0 mL/min)	
Injection Volume	20 μL	
Calibration	Matrix matched external calibration	

A typical linear calibration graph obtained for Se(IV) and Se(VI) in the analysis of extracted samples are shown in Figure 2.12 and Figure 2.13. The calibration plots were linear in the range of 0.50-80 ng/g Se with the regression coefficients (R^2) of 0.999 or better for both analytes. The retention time of selenocystine ($Se(Cys)_2$) was closed to dead volume and as seen in Figure 2.14 and Figure 2. 15, the resolution of first two peek in which the latter one belongs to MeSeCys was not good enough to be used in quantification measurements. Therefore, all three organo-selenium species were quantified by using ion-pair reverse phase chromatography described in below.

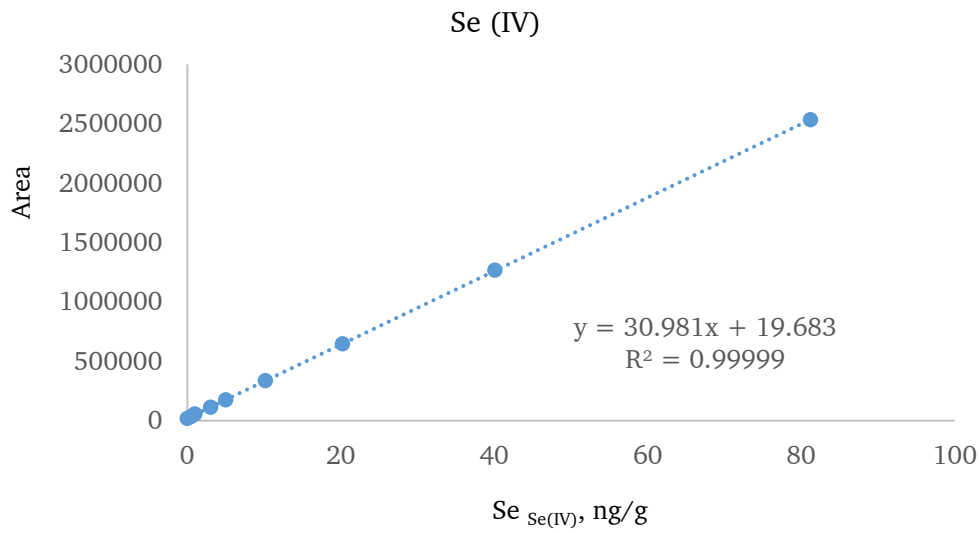


Figure 2.12 Linear calibration plot of Se(IV) obtained by SAX-HPLC-ICP-MS/MS

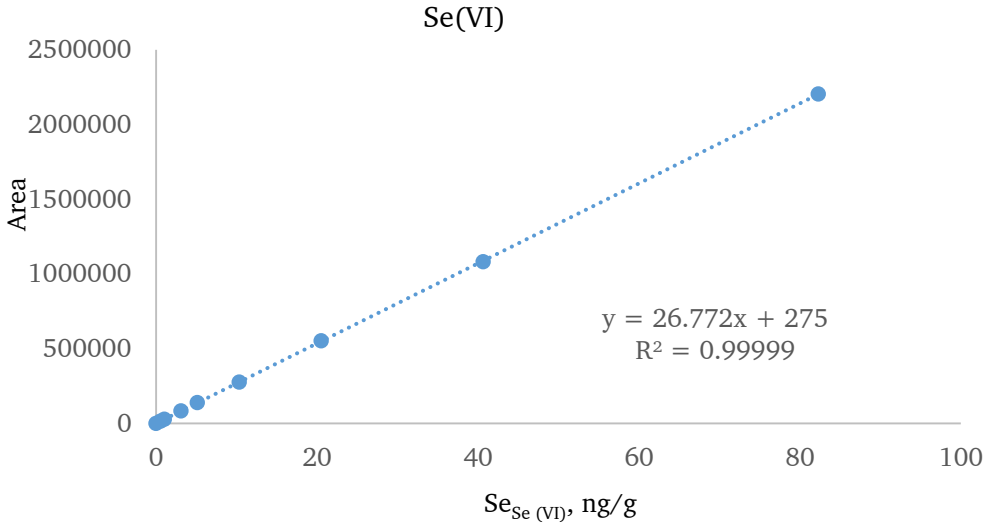


Figure 2.13 Linear calibration plot of Se(VI) obtained by SAX-HPLC-ICP-MS/MS

300 ng/kg standard in 3.0% (v/v) methanol was used to derive the system's detection power in terms of LOD (3sd+C_{blank}) and LOQ (10sd+C_{blank}) for Se(IV) and Se(VI) and the results are given in Table 2.14.

Table 2.14 Detection and quantification limits of SAX-ICP-MS/MS system

	LOD, ng/g *(n=6)*	LOQ, ng/g *(n=6)*
Se(IV)	0.48	0.68
Se(VI)	0.43	0.58

Typical chromatograms are given in Figure 2.14 and Figure 2.15 obtained for enzymatically digested sample and gastrointestinal digested sample, respectively.

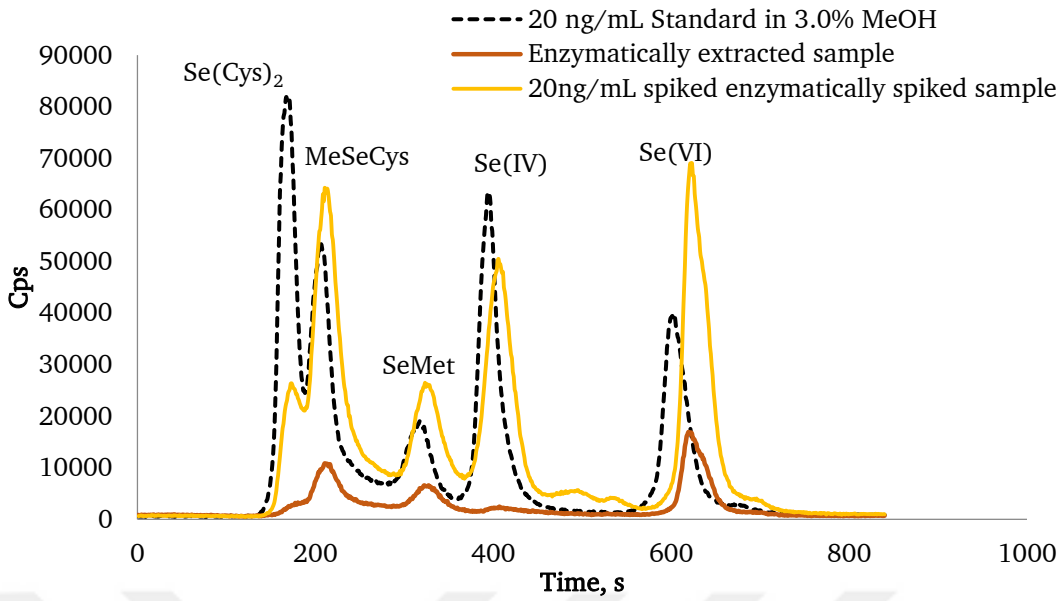


Figure 2.14 Chromatogram obtained by SAX-HPLC-ICP-MS/MS for 20 ng/mL mix standard of selenium species, enzymatically extracted sample and spiked enzymatically extracted sample

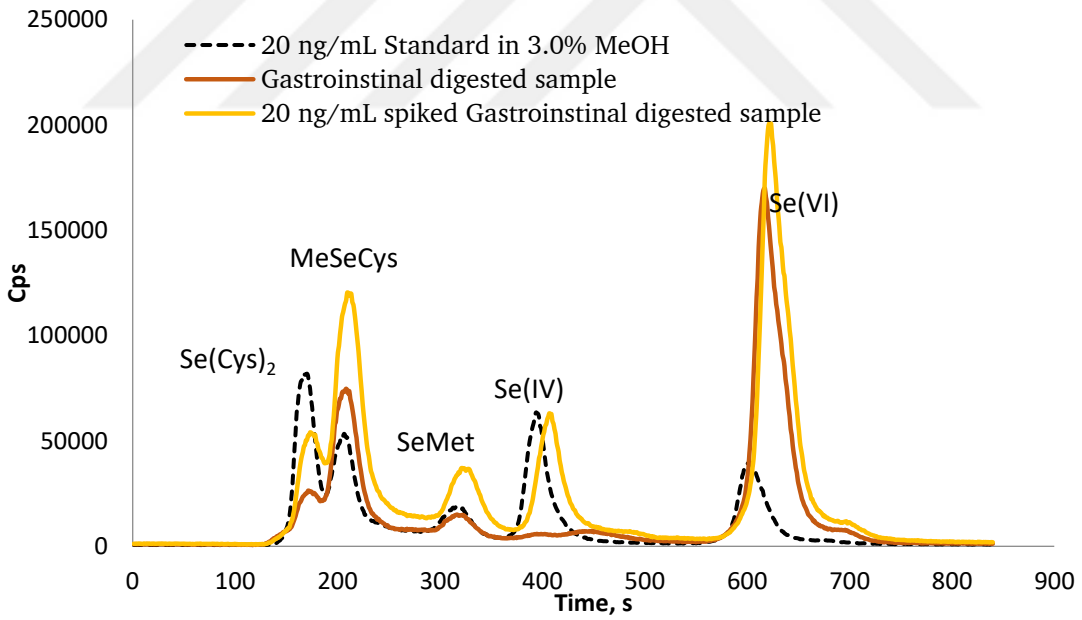


Figure 2.15 Chromatogram obtained by SAX-HPLC-ICP-MS/MS for 20 ng/mL mix standard of selenium species, gastrointestinal digested sample and spiked gastrointestinal digested sample

B. Ion Pair Reverse Phase (IP-RP) HPCL-ICP-MS/MS

Selenocystine, SeMet and MeSeCys were determined by RP-IP-HPLC system using PhenomenexSynergi Hydro-RP C18 (250 x 4.60mm, 4μ) column. Isoctoratic elution was applied in the pumping of mobile phase to the system and 0.1% (v/v) HFBA in 3.0% methanol (pH 6.0) used as the ion pair reagent. The injection volume and calibration strategy kept same as SAX-HPLC-ICP-MS/MS system.

As Se(IV) and Se(VI) were eluted very close to dead volume, only $Se(Cys)_2$, SeMet and MeSeCys were quantified by using this chromatographic separation system.

A typical linear calibration graphs obtained for SeMet, MeSeCys and $Se(Cys)_2$ in the analysis of extracted samples are shown in Figure 2.16, Figure 2.17 and Figure 2.18 respectively. The calibration plot was found to be linear in the range of 0.50-200 ng/g Se with regression coefficient (R^2) of 0.999 for SeMet and MeSeCys and 0.99 for $Se(Cys)_2$.

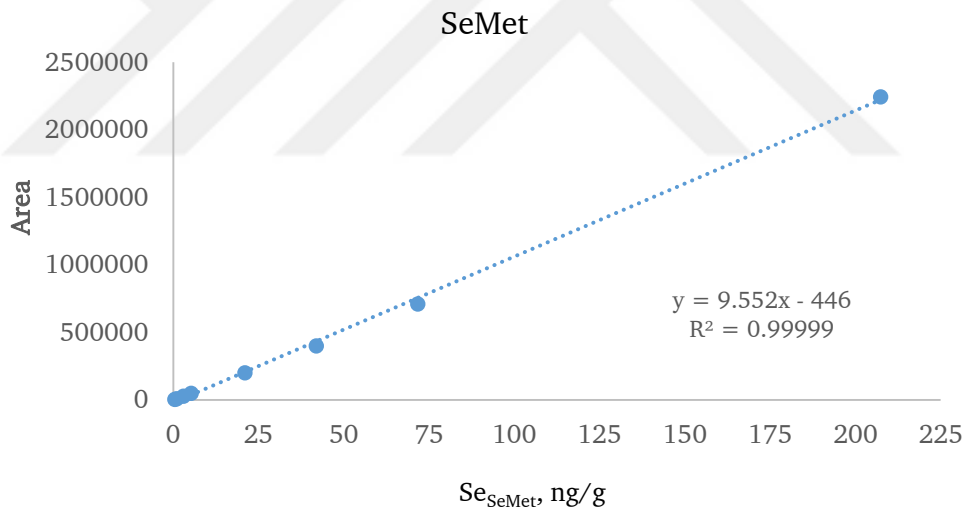


Figure 2.16 Linear calibration plot of SeMet obtained by IP-HPLC-ICP-MS/MS

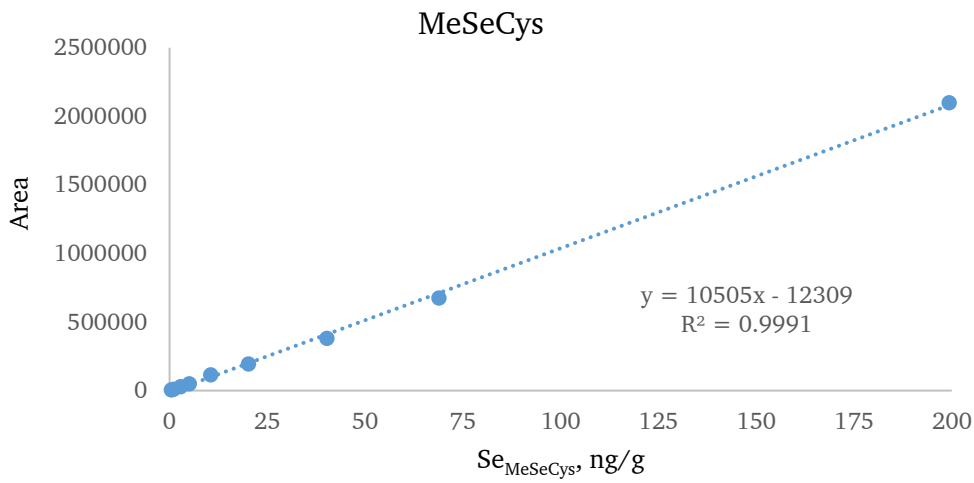


Figure 2.17 Linear calibration plot of MeSeCys obtained by IP-HPLC-ICP-MS/MS

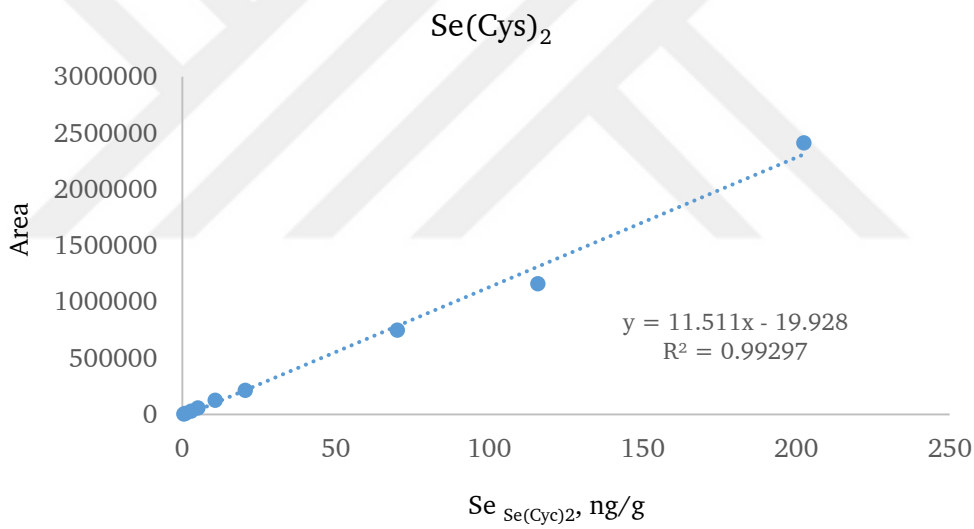


Figure 2.18 Linear calibration plot of $SeCys_2$ obtained by IP-HPLC-ICP-MS/MS

100 ng/kg standard in 3.0% (v/v) methanol was used to compute the system's limit of detection and quantification values (Table 2.15) for MeSeCys, SeMet and Se(Cys). The calculations were performed using the same approach stated above.

Table 2.15 Detection and quantification limits of IP-HPLC-ICP-MS/MS system

	LOD, ng/g *(n=6)*	LOQ, ng/g *(n=6)*
MeSeCys	0.39	0.74
SeMet	0.22	0.30
$SeCys_2$	0.15	0.27

Typical chromatograms are given in Figure 2.19 and Figure 2.20 obtained for standard in 3% MeOH, enzymatically extracted and gastrointestinal digested sample.

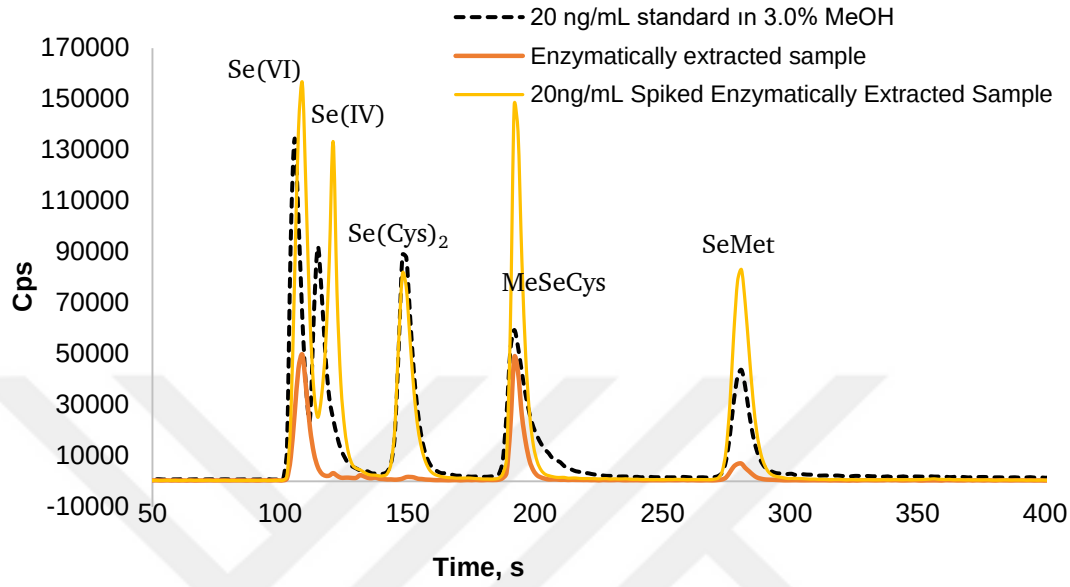


Figure 2.19 Chromatogram obtained by IP-HPLC-ICP-MS/MS for 20 ng/mL mix standard of selenium species, enzymatically extracted sample and spiked enzymatically extracted sample

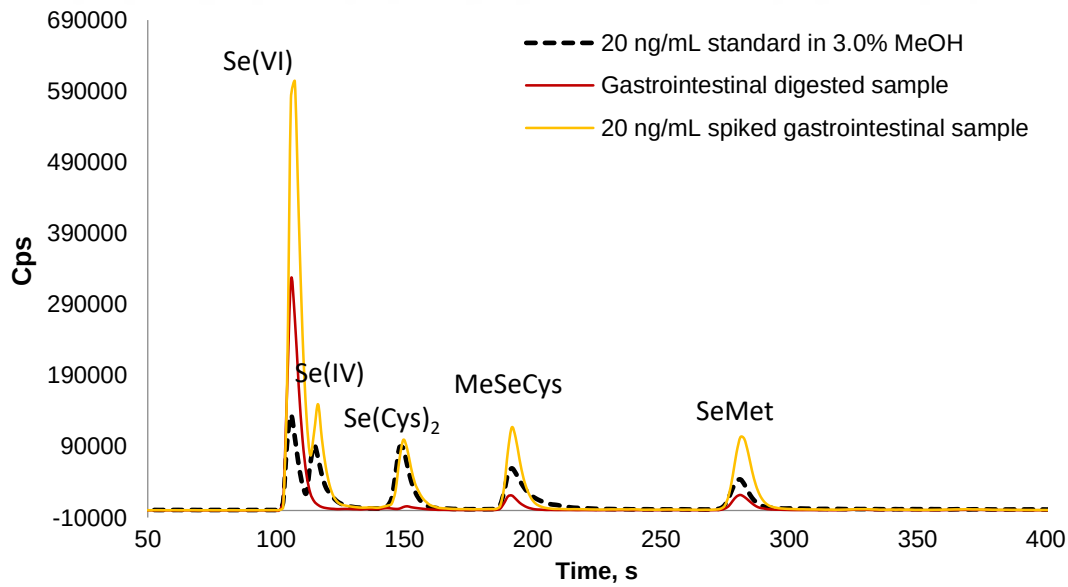


Figure 2.20 Chromatogram obtained by IP-HPLC-ICP-MS/MS for 20 ng/mL mix standard of selenium species, gastrointestinal digested sample and spiked gastrointestinal digested sample.

2.3.2.6 Statistical Evaluation

The standard deviations and mean values given in the tables/figures were computed using leek samples that were cultivated individually. Except bioavailability study, while five replicates for fortified samples were used in data set, 3 replicates for Level 1 and Level 2 and 5 replicates for the rest of the studied levels in control samples were used. For the bioavailability study, the standard deviations and mean values were calculated on three independent cultivated leek sample.

As the data set has less than 29 observations, Mann Withney U Test, a non-parametric alternative of independent samples t-test, was utilized in statistical analysis to demonstrate a significant difference between two groups. Unless otherwise specified, all assessments were carried out with a 95 % confidence interval (CI).

2.3.3 Experimental for Provenance Study of Walnuts

2.3.3.1 Sampling and Processing of Walnut and Soil Samples

A total of 17 walnut samples of 2017 products and 13 soil samples were collected. The origins of samples are given in Table 2.16 Soil samples were collected from at least three different points around the tree in which walnut samples were collected so that representative characterization of soil can be obtained in measurements. Soil samples were sampled 5-10 cm below surface area and sealed in plastic bags. In order to homogenize soil samples, all sub-samples were mixed. Homogenized samples were sieved by Retsch AS200 Vibratory Sieve Shaker using sequentially sieves with sizes of 2.0 mm, 1.0 mm and 500 μm (Figure 2.21). The fractions with particle size <500 μm were processed further. These samples were dried at 70 ℃ at oven to increase the efficiency of milling. Sieved and well dried soil samples were milled by using Planetary Mill PULVERISETTE 5 and sealed in polypropylene bags.

Table 2.16 Origin of walnut samples

Sample Code	Origin of Samples	Walnut	Soil
1	Turkey/Erzincan	+	+
2	Turkey/İzmit	+	
3	Turkey/Malatya	+	+
4	Turkey/Erzincan	+	+
5	Turkey/ İstanbul	+	+
6	Turkey/Bursa	+	+
7	Lübnan	+	+
8	Turkey/Fethiye	+	+
9	USA/Pecan	+	
10	USA /California	+	
11	Turkey/Kaman-Kırşehir	+	+
12	Turkey/Kütahya	+	+
13	Turkey/Balıkesir	+	+
14	Turkey/Elazığ	+	+
15	Turkey/Yalova	+	+
16	Turkey/Elmalı -Antalya	+	+
17	Ukraine	+	

>2.0mm

>1.0 mm

>0.5mm

< 0.5 mm

Figure 2.21 Representative photos of sieved soil

Walnut samples were collected with their outside shells. They were crashed and walnut kernels were gathered. Some walnuts were sampled with their green skins. These walnuts were left for drying at room temperature or they were kept at 35 °C in drying oven to speed up the process. After that green skin was peeled and walnut kernels were gathered by crashing outside shells. If walnut kernels were found as damp, they were kept at 35 °C in drying oven to make the processing of material easier. At least 20 g of walnut kernel were grinded by using blender (IKA M 20) which is made of stainless steel. Blade and reservoir of blender were cleaned by washing with hexane and then with deionized water to minimize the risk of cross contamination. Blended samples were sealed into PP bags as seen in Figure 2.22.

Figure 2.22 Representative photos of grinded walnut kernel samples.

2.3.3.2 Microwave Digestion Procedure for Walnut Samples

Digestion procedure for walnut samples was optimized by using UME CRM 1202 *Elements in hazelnut*. Digestion procedure was tested with CEM MARS 5 (12 vessels) and also CEM MARS Xpress (40 vessels) systems. Although the former system is pressure and temperature controlled, the latter one is only temperature controlled. Digestion performance of two systems was investigated to complete the mineralization step in a shorter time as there is excess number of samples. For both digestion techniques 1.0 g sample was used. In digestion of UME CRM 1202 samples by CEM MARS 5 (Digestion Program-1), 10.0 mL ultrapure HNO_3 (60% w/w) was used whereas 10.0 mL suprapure HNO_3 (65% w/w) was used for CEM MARS Xpress (Digestion Program-2). Samples were pre-digested overnight and microwave digestion procedure was applied. After digestion was completed, samples were diluted by deionized water to 50 mL PP tubes gravimetrically.

For the Digestion-1, mineralization consists of two steps as described in Table 2.17 and Table 2.18. On the other hand, only first step (Table 2.17) was applied in Digestion-2. Digestion efficiency of two techniques was tested for As, B, Ba, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Sr, P, Pb, Sb, Sr and Zn and results have been discussed in section 3.3.2.

Table 2.17 First Step of Microwave Digestion Program for walnut samples

Level	Temperature (°C)	Ramping time (min)	Hold time (min)
1	Room temperature - 150	10	10
2	150 - 190	10	20

Table 2.18 Second Step of Microwave Digestion Program for walnut samples

Level	Temperature (°C)	Ramping time (min)	Hold time (min)
1	Room temperature - 150	10	10
2	150 - 200	10	15

2.3.3.3 Microwave Digestion Procedure for Soil Samples

Ideally, a complete digestion has to be performed for the determination of both elemental mass fraction and isotopic composition of soil samples. Thirteen soil samples collected from thirteen different places of Turkey mean that all these soil samples have completely different compositions from each other. Therefore, optimum digestion procedure has to be established.

Four different digestion procedures were planned to be compared by using NIST SRM 2711a "Montana II Soil" and two different soil samples which were collected from Istanbul (No 5) and Bursa (No 6). Acid compositions and applied microwave digestion temperature programs of these procedures are given in below. As it is seen in Table 2.19, four different acid compositions with three different temperature and pressure-controlled microwave digestion program which are given in Table 2.20 - Table 2.22 were tested for the digestion of soil. In order to evaluate the performance of these four digestion procedures, three independent subsamples from NIST SRM 2711a "Montana II Soil", No 5 and No 6 were digested with three procedural blanks belongs to each procedure.

Table 2.19 Summary of tested digestion procedures for mineralization of soil

	Procedure 1	Procedure 2	Procedure 3	Procedure 4
Sample amount, *g*	0.45	0.45	0.45	0.45
65% HNO_3 *(w/w), mL*	7.0	8.0	6.0	4.0
37% HCl *(w/w), mL*	2.0	-	-	1.0
48% HF *(w/w), mL*	1.0	-	1.0	2.0
30% H_2O_2 *(w/w), mL*	-	2.0	-	2.0
70 % $HClO_4$ *(w/w), mL*	-	-	1.0	-
4 % H_3BO_3 (w/w), *mL*	-	-	-	12,5
Microwave Digestion Program	A	A	B	C

Table 2.20 Microwave digestion program A

	Level	Temperature (°C)	Ramping time (min)	Hold time (min)
Step 1	**1**	Room temperature - 170	6	4
	2	170 - 200	10	40

Table 2.21 Microwave digestion program B

	Level	Temperature (°C)	Ramping time (min)	Hold time (min)
Step 1	**1**	Room temperature - 110	10	10
	2	110 - 150	15	10
	3	150 - 170	10	15

Table 2.22 Microwave digestion program C

	Level	Temperature (°C)	Ramping time (min)	Hold time (min)
Step 1	**1**	Room temperature - 140	20	5
	2	140 - 200	15	30
Step 2	**1**	Room temperature - 140	20	5
	2	140 - 180	10	15

Program C was applied to Procedure 4. After the completion of first step, 4.0% (w/w) boric acid was added into to digestion vessels and second step of microwave digestion program was run.

2.3.3.4 Statistical Evaluation

Correlation analysis is used to interpret the relationship between two independent variables. Pearson's r or Spearman's Rho tests can be used to perform correlation analysis. Pearson's r is a parametric test and its non-parametric counterpart is the

Spearman's Rho test. The estimated correlation coefficient shows the direction and magnitude of the relationship. If the estimated coefficient is significantly positive (negative), one can conclude that two variables move in the same (opposite) direction.

3
RESULTS AND DISCUSSION

3.1 Production of Trace Elements in Seawater Certified Reference Material

3.1.1 Development of Reference Method for the Elements Interested

3.1.1.1 Evaluation of Detection Power of TEA/$Mg(OH)_2$ – IDMS

Each IDMS approach's procedural blanks were calculated separately, as defined in 2.3.1.4. As shown in Table 3.1, the proposed method's reagent blank level was found to be better than the method produced using TEA as a reagent in $Mg(OH)_2$ precipitation [254]. Cd, Cu, Cr, Fe, Ni, Pb, and Zn blank values correspond to 1.4%, 5.4%, 5.6%, 0.6%, 1.5%, 8.7%, and 2.6% of matrix certified reference material (CASS-6) which have lower certified mass fractions than NMIA MX014 and UME CRM 1206, respectively. With the exception of Cd and Pb, the analytical procedure is valid for CASS-6 without the need for preconcentration of analytes. Despite the fact that the blank levels for Cd and Pb were low enough, due to instrumental sensitivity limitations, these elements had to be preconcentrated.

While detection limits of TEA/ $Mg(OH)_2$ technique were found to be compatible with NH_3/$Mg(OH)_2$ techniques except for Cu [268] and Cr [257], detection limits of the technique are very satisfactory for UME CRM 1206 certification. TEA/$Mg(OH)_2$ technique provided higher recovery efficiencies for Cd and Cu, which were above 70% when compared to NH_3/$Mg(OH)_2$ technique [268], while comparable recovery efficiency for Pb. With (92±7) % and (75±11) % recovery efficiencies, the former may also provide high recovery efficiencies for Ni and Zn. Z. Arslan et al. [254] have also shown the higher recovery efficiencies of the TEA assisted co-precipitation technique. These high recovery efficiencies can aid in avoiding sample preconcentration, allowing for analysis with smaller sample intakes.

Without applying background correction, the procedural blank level and detection limit for iron were found to be 48 ng/kg (0.86 nM) and 41 ng/kg (0.73 nM), respectively. However, the applying background correction for iron was found to be necessary. Therefore, all the signals was corrected based on the background signals which measured systematically during sequences. The background corrected signals were also used in the calculations of procedural blank. The characteristic procedural blank concentration and detection limit were measured using a direct IDMS and were reported as 9 ng/kg (0.16 nM) and 11 ng/kg (0.20 nM), respectively. The procedural blank level of the suggested method is roughly 20 times improved with respect to the recently published results [254] in which TEA was utilized for co-precipitation (Table 3.1). J. Wu et al. reported that $NH_3/Mg(OH)_2$ co-precipitation techniques can achieve lower detection limits as the procedural blank levels of $TEA/Mg(OH)_2$ co-precipitation are largely consistent with that proposed technique in the literature [252], [269].

The reported blank values obtained by techniques using commercially available SeaFAST systems which equipped with Nobias-chealate PA-1 resin are provided in Table 3.1. The main disadvantages of the three different resins given in Table 3.1 are not being selective to chromium and determination can only be performed by coprecipitation approaches.

Table 3.1 Comparison of most commonly used analytical methods in terms of blank levels

Matrix Removal Technique	Method	Sample intake, g	Preconcentration Factor	Instrument	Cd	Cu	Cr	Fe	References
					ng/L	*ng/L*	*ng/L*	*ng/L*	
TEA/$Mg(OH)_2$ Coprecipitation	ID^3MS	5.0	1	ICP-MS/MS	0.3	28	5	*9*	This Study
TEA $Mg(OH)_2$ Coprecipitation	External Calibration	10	10	Q-ICP-MS	2.0	70	25	*204*	[254]
NH_3/$Mg(OH)_2$ Co-precipitation	Additional Calibration	50	10	Q-ICP-MS	N.A	N.A	1	*5.6*	[257]
NH_3/$Mg(OH)_2$ Co-precipitation	Single IDMS	1.3	13	Q-ICP-MS	0.7	1.7	N.A	*N.A*	[268]
NH_3/$Mg(OH)_2$ Co-precipitation	Single IDMS	1.4	14	HR-ICP-MS	N.A	N.A	N.A	*6.8*	[269]
NH_3/$Mg(OH)_2$ Co-precipitation	Single IDMS	14	140	HR-ICP-MS	N.A	N.A	N.A	*2.5*	[269]
SPE (Nobias-Chealate PA-1)	External Calibration	20-40	8-16	ICP-MS/MS	0.04	1.9	N.A	*7.8*	[270]
SPE (Nobias-Chealate PA-1)	External Calibration	10	25	ICP-SF-MS	0.04	2.2	N.A	*N.A*	[271]
SPE (Nobias-Chealate PA-1)	Standard Addition Calibration	40	10	ICP-SF-MS	0.9	2.1	N.A	*1.7*	[272]
SPE (Nobias-Chealate PA-1)	External Calibration	120	8	Q-ICP-MS	N.D	N.D	N.A	*1.9*	[273]
SPE (Nobias-Chealate PA-1)	Additional Calibration	9	200	HR-ICP-MS	N.A	0.8	N.A	*3.6*	[274]
SPE (Nobias-Chealate PA-1)	External Calibration	120	8	HR-ICP-MS	<0.22	1.1	N.A	*1.8*	[275]

N.D: not detected, N.A: not applicable

Table 3.1 Comparison of most commonly used analytical methods in terms of blank levels-continuous

Matrix Removal Technique	Method	Sample intake, g	Preconcentration Factor	Instrument	Ni	Pb	Zn	References
					ng/L	*ng/L*	*ng/L*	
TEA/$Mg(OH)_2$ Coprecipitation	ID^3MS	5.0	1	ICP-MS/MS	6.1	0.9	32	This Study
TEA $Mg(OH)_2$ Coprecipitation	External Calibration	10	10	Q-ICP-MS	65	62.0	125	[254]
NH_3/$Mg(OH)_2$ Co-precipitation	Additional Calibration	50	10	Q-ICP-MS	N.A	0.9	37	[257]
NH_3/$Mg(OH)_2$ Co-precipitation	Single IDMS	1.3	13	Q-ICP-MS	N.A	0.1	N.A	[268]
NH_3/$Mg(OH)_2$ Co-precipitation	Single IDMS	1.4	14	HR-ICP-MS	N.A	N.A	N.A	[269]
NH_3/$Mg(OH)_2$ Co-precipitation	Single IDMS	14	140	HR-ICP-MS	N.A	N.A	N.A	[269]
SPE (Nobias-Chealate PA-1)	External Calibration	20-40	8-16	ICP-MS/MS	3.1	0.1	1.6	[270]
SPE (Nobias-Chealate PA-1)	External Calibration	10	25	ICP-SF-MS	2	0.3	7.0	[271]
SPE (Nobias-Chealate PA-1)	Standard Addition Calibration	40	10	ICP-SF-MS	11.7	0.2	1.7	[272]
SPE (Nobias-Chealate PA-1)	External Calibration	120	8	Q-ICP-MS	N.D	0.32	6.6	[273]
SPE (Nobias-Chealate PA-1)	Additional Calibration	9	200	HR-ICP-MS	1.53	N.A	7.5	[274]
SPE (Nobias-Chealate PA-1)	External Calibration	120	8	HR-ICP-MS	1.00	0.06	7.9	[275]

N.D: not detected, N.A: not applicable

3.1.1.2 Evaluation of Analytical Performances of Calibration Strategies

The method used to characterize a candidate certified reference material should be traceable, highly precise, and have the lowest measurement uncertainty possible. For inorganic analysis, isotope dilution mass spectrometry (IDMS) is a potential primary technique that offers low uncertainty and a well-established traceability chain to SI units. As a result, IDMS was chosen to be used in the characterization of UME CRM 1206 for the analytes Cd, Cu, Cr, Fe, Ni, Pb, and Zn, taking into account the restrictions specified in ISO 17034 for the characterization of a candidate certified reference material. The development and validation of ID-ICP-MS methods for the target analytes have been published by Ari et al. [260].

For inorganic research, there are three different IDMS calibration strategies: single IDMS, double IDMS, and triple IDMS. In the literature, all these calibration strategies have been identified and discussed [73]–[75]. On the basis of Fe measurements, the analytical performances of three calibration strategies were evaluated in a seawater matrix and the results of this study has been published by Ari et al.[261]

3.1.1.3 Trueness of Calibration Strategies

Two kind of matrix CRMs, CASS 6 Nearshore Seawater Certified Reference Material for Trace Metals and Other Constituents and NMIA MX014 coastal seawater, were studied to determine the accuracy of three calibration strategies. Due to the lower level of Fe in the seawater matrix compared to the candidate certified reference content, CASS-6 was chosen to determine the measurement trueness of the process. Since CASS 6 has a pH of 1.6, any adjustment of pH was necessary, and co-precipitation was completed by adding the proportional amount of TEA (20 μL for 2.0 g of CASS 6) in relation to the sample amount used in the first step. In the second step, the addition of TEA was applied drop by drop until solutions produced noticeable turbidity, as the concentration of Mg changes for each seawater matrix. NMIA MX014 has a higher mass fraction than the candidate CRM. Since the pH of 1.0 g MX014 was approximately 0.7, it was modified to pH 1.6 by adding ultrapure water just before applying the co-precipitation procedure,

and the same co-precipitation technique was used as defined for the previous CRM preparation.

CRM measurements were assessed not only on the basis of percentage recovery, but also on the basis of ERM Application Note 1 [276], which demonstrated that the certified and measured values of these matrix CRMs are not substantially different within their expanded uncertainties. Table 3.2 compares three different calibration methods based on Fe measurement; Table 3.3, Table 3.4 and Table 3.5 compare the analytical performance of the ID^3MS framework for all target elements.

Table 3.2 Trueness of three IDMS calibration strategies based on Fe measurements

	Certified Value, ng/g (k=2)	***IDMS***		***ID²MS***		***ID³MS***	
		Measured Value, ng/g (k=2)	Recovery, %	Measured Value, ng/g (k=2)	Recovery, %	Measured Value, ng/g (k=2)	Recovery, %
CASS -6 (n=5)	1.53 ± 0.12	1.521 ± 0.032	99.4 ± 1.9	1.525 ± 0.027	99.6 ± 1.8	1.536 ± 0.038	100.4 ± 2.4
ERM Application Note 1		No significant difference		No significant difference		No significant difference	
NMIA MX014 (n=4)	21.7 ± 0.32	21.32 ± 0.26	99.0 ± 0.2	21.60 ± 0.14	99.5 ± 0.1	21.70 ± 0.14	100.0 ± 0.2
ERM Application Note 1		No significant difference		No significant difference		No significant difference	
UME CRM 1206 (*n=12*)		12.70 ± 0.16		12.728 ± 0.084		12.732 ± 0.062	

Table 3.3 Sensitivity and precision of TEA/$Mg(OH)_2$-ID^{3}MS for target analytes

	Sensitivity		Precision	
Analyte	*LOD, ng/kg*	*Reagent Blank, ng/kg*	*u(w)[1] %*	*u(b)[2] %*
Cd	0.4	0.3	0.35%	0.89%
Cu	34	28	0.41%	0.69%
Cr	7	5.5	0.49%	0.90%
Fe	9	11	0.20 %	0.13%[3]
Ni	1.1	6.1	0.09%	0.43%
Pb	0.4	0.9	0.13%	0.68%
Zn	15	32	0.20%	0.34%

[1]Combined uncertainty on repeatability
[2]Combined uncertainty on intermediate precision
[3]Relative standard deviation on average values of three independent day measurement results

Table 3.4 Trueness of TEA/$Mg(OH)_2$-ID^{3}MS for target analytes – NMX014

	Trueness			
Analyte	**MX014 Certified value, ng/g**	**MX014 Measured value, ng/g** (*n=3, k=2*)	**ERM Application Note 1[1]**	**Recovery of MX014, %**
Cd	1.318 ± 0.034	1.282 ± 0.023	Passed	97.3 ± 1.2
Cu	2.90 ± 0.25	2.989 ± 0.057	Passed	103.5 ±1.5
Cr	2.613 ± 0.075	2.606 ± 0.063	Passed	99.7 ± 0.9
Fe	21.70 ± 0.32	21.70 ± 0.14	Passed	100.0 ± 0.2
Ni	3.66 ± 0.10	3.584 ± 0.075	Passed	97.9 ± 1.0
Pb	2.467 ± 0.065	2.437 ± 0.016	Passed	98.3 ± 0.3
Zn	-	-	-	-

[1] Passed: no significant difference was observed between the certified value and measured value

Table 3.5 Trueness performance of TEA/$Mg(OH)_2$-ID^3MS for target analytes-CASS 6

Trueness				
Analyte	**CASS 6 Certified value, ng/g**	**CASS 6 Measured Value, ng/g** (*n=4, k=2*)	**ERM Application Note 1**[1]	**Recovery of CASS 6, %**
Cd	0.0213 ± 0.0018	-	-	-
Cu	0.520 ± 0.032	0.529 ± 0.020	Passed	101.8 ± 3.7
Cr	0.098 ± 0.016	0.097 ± 0.006	Passed	98.5 ± 2.5
Fe	1.53 ± 0.12	1.536 ± 0.038	Passed	100.4 ± 2.4
Ni	0.410 ± 0.040	0.4214 ± 0.0042	Passed	102.8 ± 0.7
Pb	0.0104 ± 0.0040	-	-	-
Zn	1.24 ± 0.18	1.213 ± 0.032	Passed	97.8 ± 2.1

[1] Passed: no significant difference was observed between the certified value and measured value

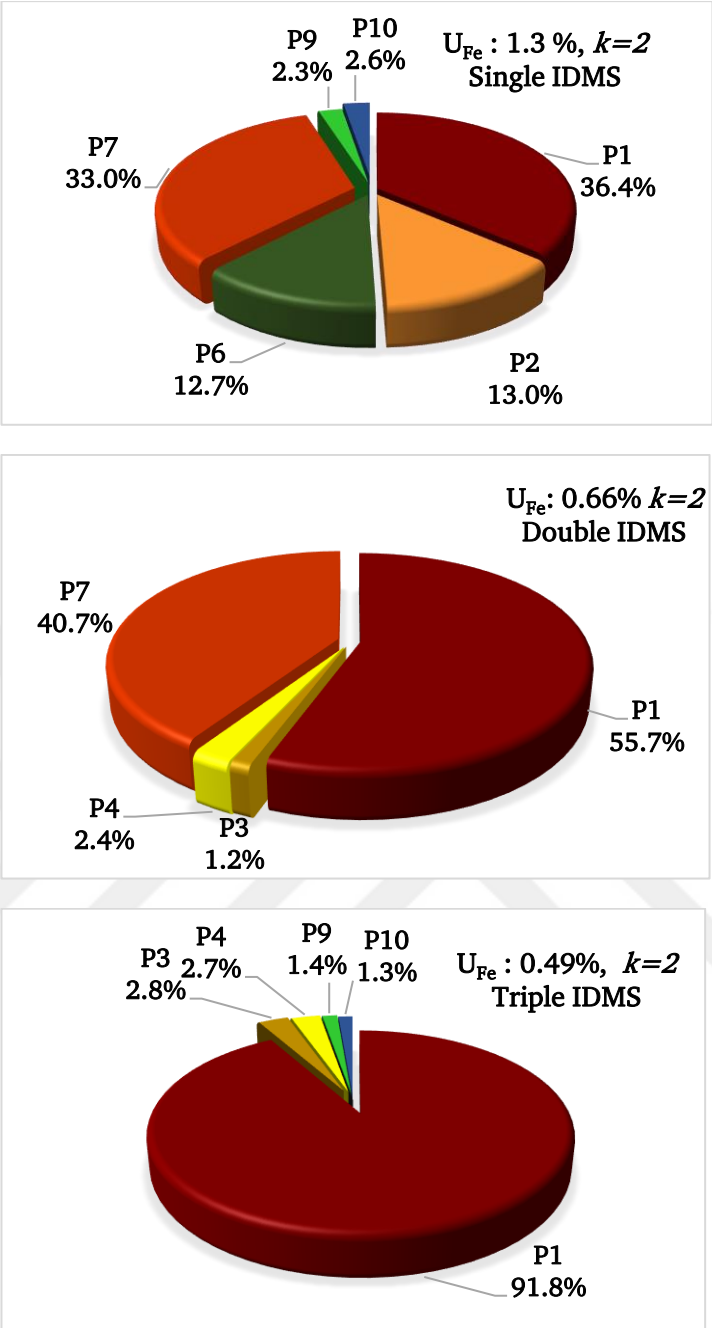
3.1.1.4 Precision of Calibration Strategies

Precision is essential for the characterization of a candidate CRM given expanded uncertainty on certified reference material, in addition to the method's trueness. Therefore, the UME CRM 1206 findings were used to assess the precision of all calibration strategies. Ten replicates per day were used in the data set (2 days for single IDMS, 3 days for ID^2MS and ID^3MS). One-way ANOVA was used for the assessment. For single, ID^2MS, and ID^3MS, the relative standard uncertainty of repeatability (within day precision) was 0.16%, 0.19% and 0.20%, respectively. Moreover, while all three methods had similar within-day precision, the relative standard uncertainty of between day precision (intermediate precision) or single IDMS and ID^2MS was measured as 0.37% and 0.21%, respectively. This value could not be determined for ID^3MS as intermediate precision was less than repeatability ($MS_{between} < MS_{within}$). However, the relative standard deviation on three-day average values was found to be 0.13% for ID^3MS. The calculation of measurement uncertainty took into account the intermediate precision of calibration approaches.

3.1.1.5 Evaluation of Uncertainty Budgets of Single IDMS, ID^2MS and ID^3MS

Since there was no substantial difference between the measurement results for two CRMs and certified values, all three UME CRM 1206 data sets were compared in terms of average value closeness and uncertainty. Three units of candidate CRM and four independent sub-samples per unit were used for characterization measurements.

Single IDMS was found to have an average value of (12.70±0.16) ng/g. The 1.3% relative increased uncertainty (k=2) is within the normal range of 1–2% for single IDMS applications [253]. The main parameters in the total uncertainty budget were determined to be sample preparation (weighing) (36.4%), precision on IRM of mass bias correction solution (12.7%), IUPAC values (13.0%), and between day precision (33.0%). This study also confirmed a significant reduction in measurement uncertainty of ID^2MS as the other author claimed in previous studies [73], [74] . As seen in the Figure 3.1, the double IDMS uncertainty budget is 0.66% (k=2), which is roughly half of the single IDMS uncertainty budget. Sample preparation (weighing) (55.7 %) and between day precision (40.7%) were reported to be the main contributors of the double IDMS measurement uncertainty budget. Since a second series of calibration blend is applied to the measurements in triple IDMS, the measurement uncertainty is likely to be higher than that of double IDMS [73]. However, applying triple IDMS with 0.49% expanded measurement uncertainty improved the precision.



	Uncertainty Contribution Parameters
P1	Sample preparation (Weighing)
P2	Uncertainty on IUPAC (col 9) isotopic abundance of Fe
P3	Uncertainty on $^{56}Fe/^{57}Fe$ ratio of sample blends
P4	Uncertainty on $^{56}Fe/^{57}Fe$ ratio of calibration blends
P5	Uncertainty on $^{56}Fe/^{57}Fe$ ratio of K for calibration blends
P6	Uncertainty on $^{56}Fe/^{57}Fe$ ratio of K for sample blends
P7	Uncertainty on intermediate precision
P8	Uncertainty on $^{56}Fe/^{57}Fe$ ratio of procedural blank blends
P9	Uncertainty on background correction
P10	Other

Figure 3.1 Comparison of uncertainty budget of IDMS, ID^2MS, ID^3MS

As it can be seen in Figure 3.1, the gravimetric sample preparation step contributed the highest (91.8 %) to the total uncertainty budget. Therefore, applying metrological gravimetric principles to IDMS applications using substitution weighing against E2 class mass standards will lower the measurement uncertainty. However, this is only feasible for single IDMS since the metrological weighing process takes a long time for ID^2MS and ID^3MS.

While the mean values of the three methods are consistent within their respective uncertainties (Figure 3.2), triple IDMS has a better intermediate precision and subsequent uncertainty budget than single IDMS and ID^2MS. As a result, the characterization value of UME CRM 1206 is assigned with the value of ID^3MS (12.732 ±0.062 ng/g) measurements.

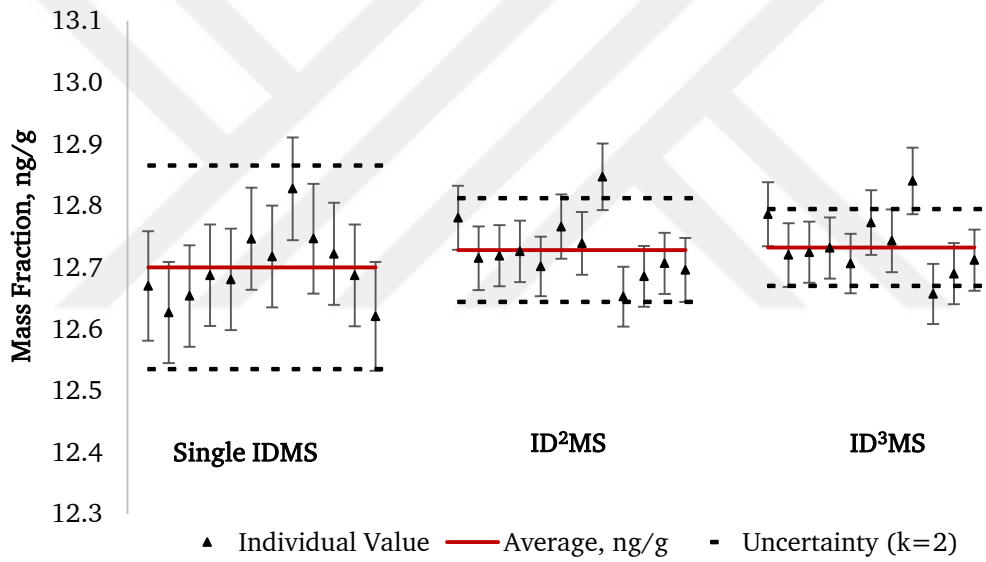


Figure 3.2 The results Fe in UME CRM 1206 obtained by different calibration approaches

Following a thorough analysis of iron measurements with different calibration techniques, the entire certification of Cd, Cr, Cu, Ni, Pb, and Zn was completed using TEA assisted $Mg(OH)_2$ coprecipitation combined with ID^3MS (TEA/$Mg(OH)_2$-ID^3MS).

3.1.2 Validation of Matrix Matched External Calibration - ICP-MS/MS Method for of Total As in Sea Water

Validation of the method was performed by evaluating LOD, LOQ, selectivity, linearity, working range, repeatability, intermediate precision and also trueness. Limit of detection and quantification (Table 3.6) were calculated using six blank solutions which exposed to all sample preparation steps in the method and were calculated as 3xSD and 10xSD where SD is the standard deviation of the measurement results obtained for blank solutions, respectively.

Table 3.6 Sensitivity and precision of matrix matched external calibration –ICP-MS/MS for total As determination in seawater

Analyte	Sensitivity		Precision	
	LOD, ng/g	*LOQ, ng/g*	*u(w)[1] %*	*u(b)[2] %*
As	0.02	0.07	0.31%	0.10%

[1]Combined uncertainty on repeatability
[2]Combined uncertainty on intermediate precision

Linearity and working range were evaluated based on the target samples which are studied during the validation of method. UME CRM 1206, NMIA MX014 and CASS 6 were studied and linearity which is described as correlation coefficient for these three different seawater matrices was always found be >0.999. Linear calibration plots for each matrix are given in Figure 3.3 - Figure 3.5. Based on these calibration plots, the tested working range for the method is 1 - 4 ng/g.

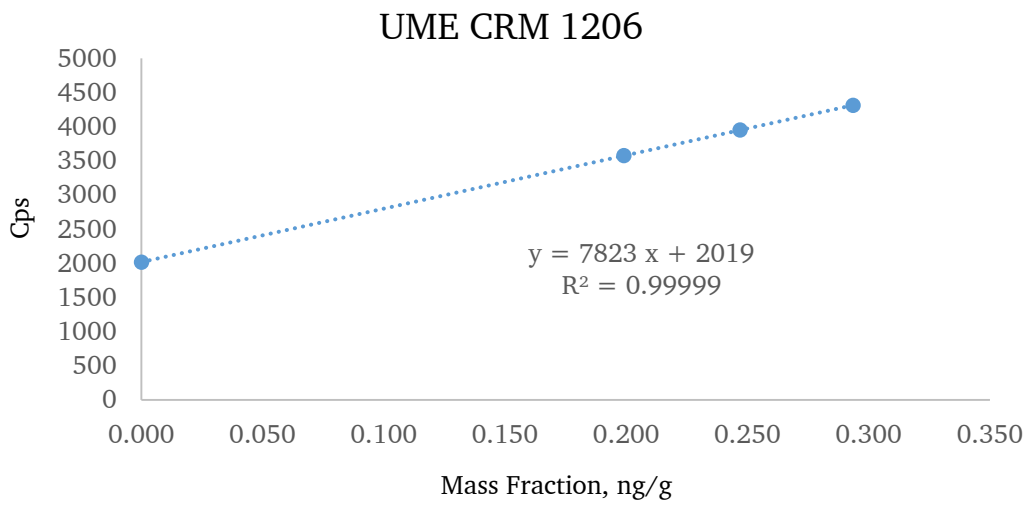


Figure 3.3 Calibration plot obtained for UME CRM 1206

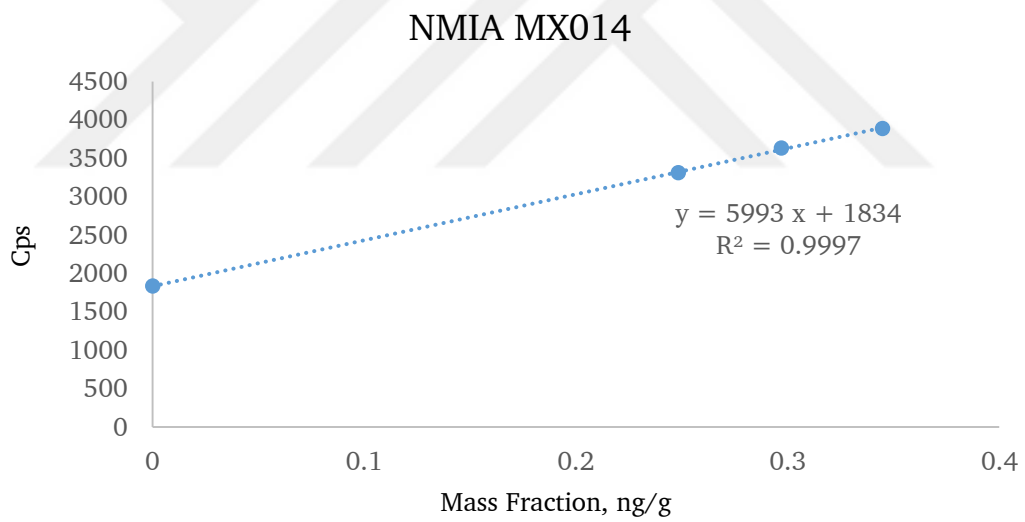


Figure 3.4 Calibration plot obtained for NMIA MX014

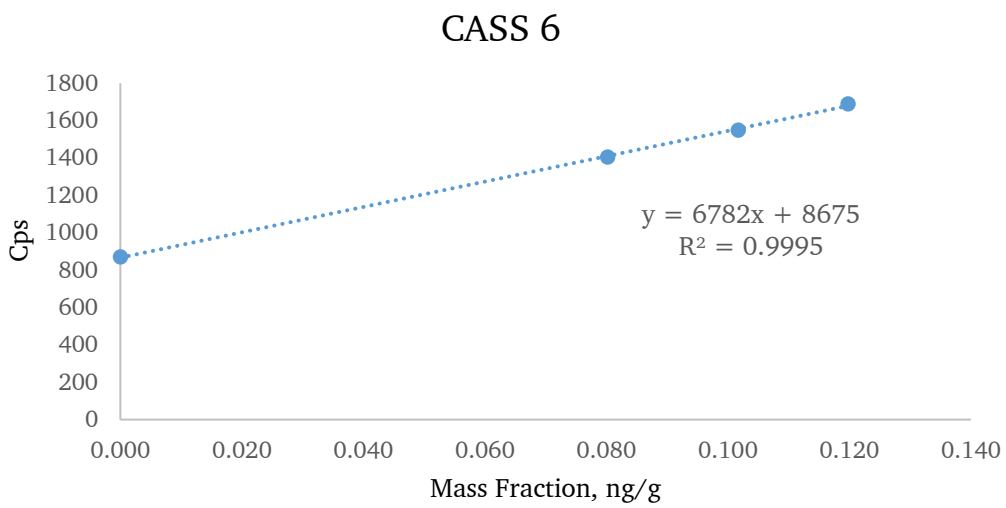


Figure 3.5 Calibration plot obtained for CASS 6

Selectivity of the method was investigated using different collision/reaction cell gases as described in section 2.3.1.5. Background equivalence concentration for the matrix consist of 10 mg/L Ca, 10 mg/L K and 0.35% NaCl in 1.0% (v/v) HNO_3 was found to be 0.01 μg/L by using oxygen mass shift mode of ICP-MS/MS which is the demonstration for selectivity of ^{75}As in 1/10-fold diluted seawater matrix.

Precision of the method was evaluated with the data set consisted of six replicates per each independent measurement. Three independent measurements with new (independent) calibration and sample preparation were performed to validate the precision of method. Intermediate precision and repeatability of the method were calculated using one-way ANOVA as given in Table 3.7.

Trueness of the method was studied with two certified reference materials and as seen in Table 3.8, the results were found to be in well agreement with the certified one and their uncertainties. Estimation of measurement uncertainty for characterization study was obligatory according to ISO 17034 and it was calculated as it is described in section 2.3.1.6. Intermediate precision of the method (u_b; 0.10%) was also taken into consideration. The expanded measurement uncertainty ($k=2$) was calculated as 1.3% for UME CRM 1206.

Table 3.7 Evaluation of method precision for determination of total As in seawater

Measurement Results

Rep 1	*Rep 2*	*Rep 3*	*Rep 4*	*Rep 5*	*Rep 6*
2.47016	2.52367	2.50212	2.51323	2.49684	2.54850
2.52459	2.53545	2.52190	2.51407	2.53503	2.50492
2.52589	2.54525	2.50942	2.54386	2.52135	2.51025

Sum of Squares of each results: 114.25
Square of sum / (P*N): 114.24

Summary

Groups	*Count*	*Sum*	*Average*	*Variance*
Row 1	6	15.055	2.509	0.00
Row 2	6	15.136	2.523	0.00
Row 3	6	15.156	2.526	0.00

N = 3 nb (number of between-day-replicates)
P = 6 nw (number of within-day replicates)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000963	2	0.00048143	1.32747042	0.29456927	3.68232034
Within Groups	0.005440	15	0.00036267			
Total	0.006403	17				

Overall average	2.519249
s(w)	0.019044
s(w) (relative)	0.76%
s(b)	0.004449
s(b) (relative)	0.18%
s(i)(relative) %RSDi	0.78%
u_w (absolute)	0.007775
u_w (relative)	0.31%
u_b (absolute)	0.002569
u_b (relative)	0.10%

Table 3.8 Trueness of matrix matched external calibration –ICP-MS/MS for total As determination in seawater

Trueness								
	MX014				CASS 6			
Analyte	Certified Value, *ng/g*	Measured Value, ng/g (*n=3, k=2*)	ERM Application Note 1[1]	Recovery of MX014, %	Certified Value, ng/g	Measured Value, ng/g (*n=3, k=2*)	ERM Application Note 1[1]	Recovery of CASS 6, %
As	2.96 ± 0.26	3.02 ± 0.07	Passed	102.1 ± 0.5	1.02 ± 0.1	1.127 ± 0.041	Passed	110 ± 1.0

[1] Passed: no significant difference was observed between the certified value and measured value

3.1.3 Between and Within Unit Homogeneity

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, random stratified sampling scheme (RSS) covering whole batch was used in the selection of 10 bottles for the between unit homogeneity study (Master Unit No: 20, 78, 117, 149, 168, 221, 267, 298, 339, 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 samplings were taken from each unit. TEA/$Mg(OH)_2$-ID^3MS method was applied for the between unit homogeneity measurement using ICP-MS/MS. Therefore, the measurements were performed under the high precision conditions and all sub-samples were introduced to the ICP-MS such a randomized order.

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

i. Regression analyses in order to evaluate the potential trends in each analytical run at 95% and 99% confidence levels. 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 (3.1) where trends significant at least a 95 % confidence level:

$$C_{Corrected} = C_{Measured} - b \cdot i \tag{3.1}$$

where;

b : slope of the linear regression,

i : position of the result in the analytical sequence.

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

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

iv. 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.

Estimation of uncertainty contribution of material homogeneity is evaluated using Analysis of Variance (ANOVA).

The following equation (3.2) is used for repeatability of method (s_{wb}) and equation (3.3) is used for the calculation of standard deviation between units (s_{bb}).

$$s_{wb} = \sqrt{MS_{within}} \tag{3.2}$$

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

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

$$s_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \tag{3.3}$$

$MS_{between}$: 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 which contributes to uncertainty covering method repeatability using equation (3.4).

$$u^*_{bb} = \frac{s_{wb}}{\sqrt{n}} \sqrt[4]{\frac{2}{\nu_{MSwithin}}} \tag{3.4}$$

$\nu_{MSwithin}$: 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 (3.2), (3.3) and (3.4) 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. As there was no certain technical explanation for unit outliers belong to Fe and Cr (Table 3.9), between unit homogeneity was modeled

as a rectangular distribution (equation (3.5)) and following equation was applied for rectangular standard uncertainty of homogeneity.

$$u_{rect} = \frac{|Outlier\ value - Average\ value|}{\sqrt{3}} \tag{3.5}$$

As the results of technically evaluated data set are given in Table 3.9, the results of the between-unit variation are given in the Table 3.10 and the biggest value between s_{bb}, u^*_{bb} and u_{rect} was assigned as u_{bb}, uncertainty of homogeneity.

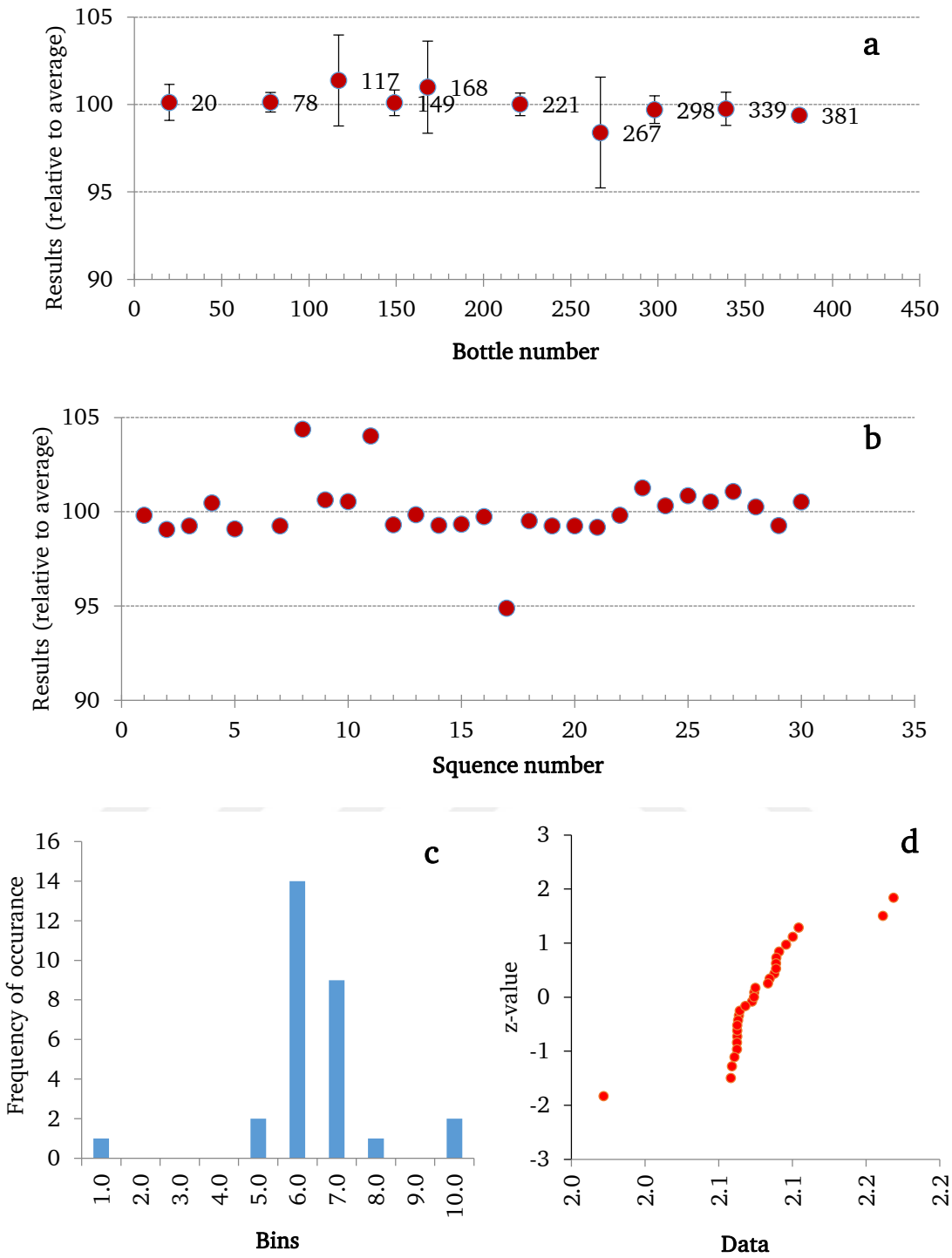
Table 3.9 Summary of technically evaluated data set of homogeneity study

Analyte	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 3.10 Results of homogeneity study

Analyte	$s_{wb,rel}$,%	$s_{bb,rel}$, %	$u^*_{bb,rel}$, %	$u_{rec,rel}$, %	$u_{bb,rel}$,%
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 graphical representation of homogeneity data set belongs to all target analytes are given in Figure 3.6- Figure 3.14.



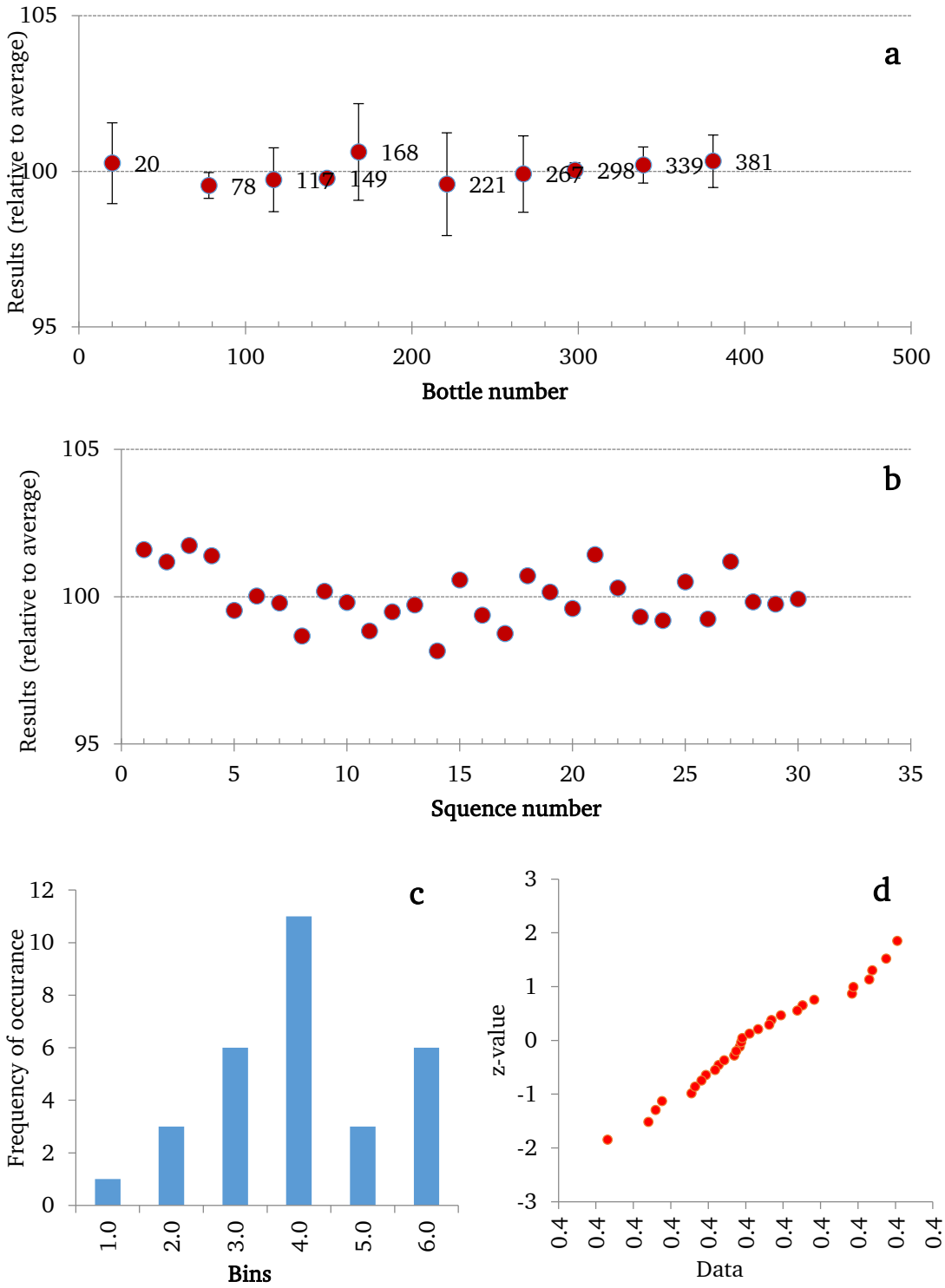
a. Graph of filling sequence order

b. Graph of analytical sequence order

c. Histogram

d. Normal Probability Plot

Figure 3.6 Homogeneity graph of As



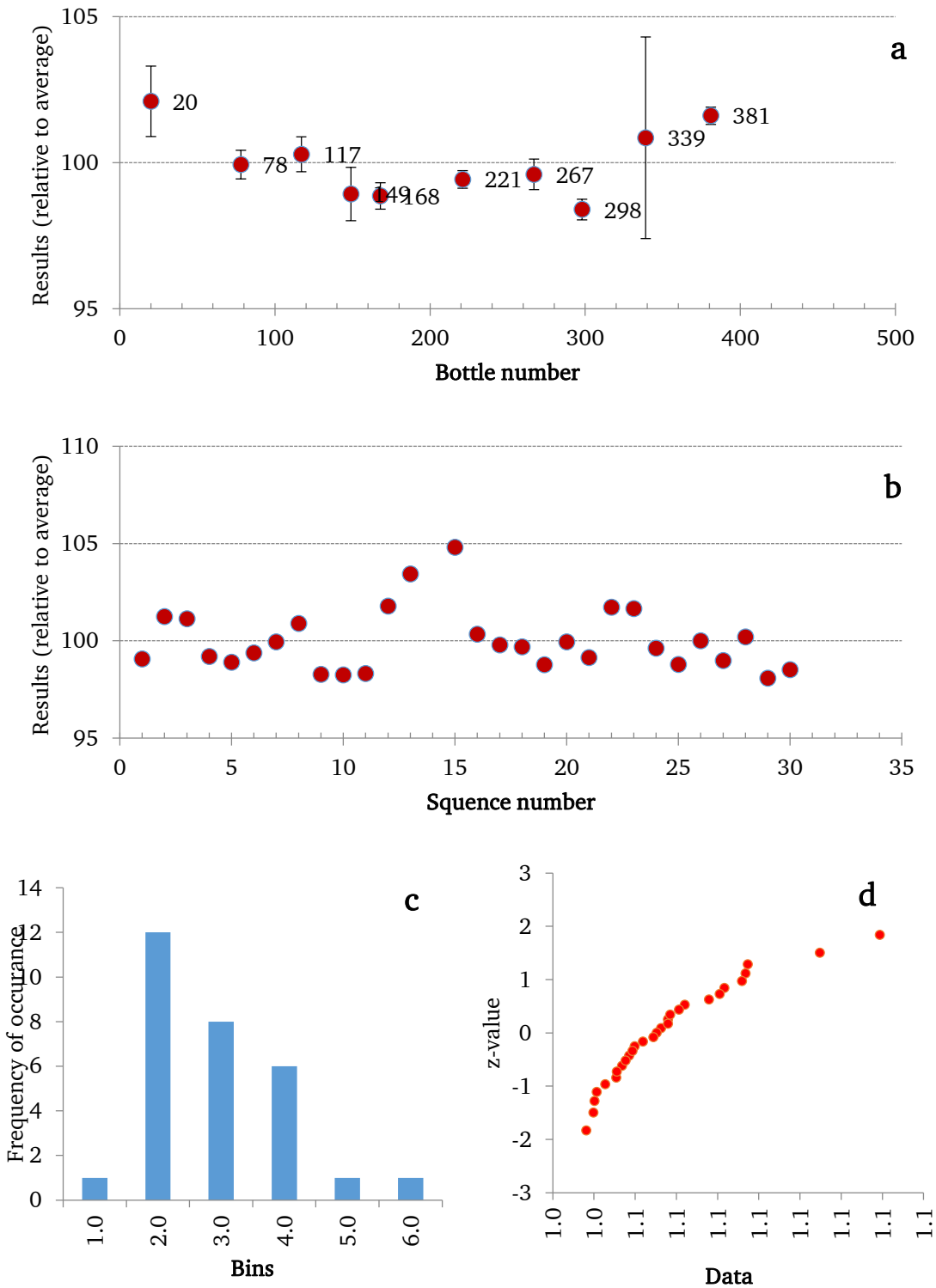
a. Graph of filling sequence order

b. Graph of analytical sequence order

c. Histogram

d. Normal Probability Plot

Figure 3.7 Homogeneity graph of Cd



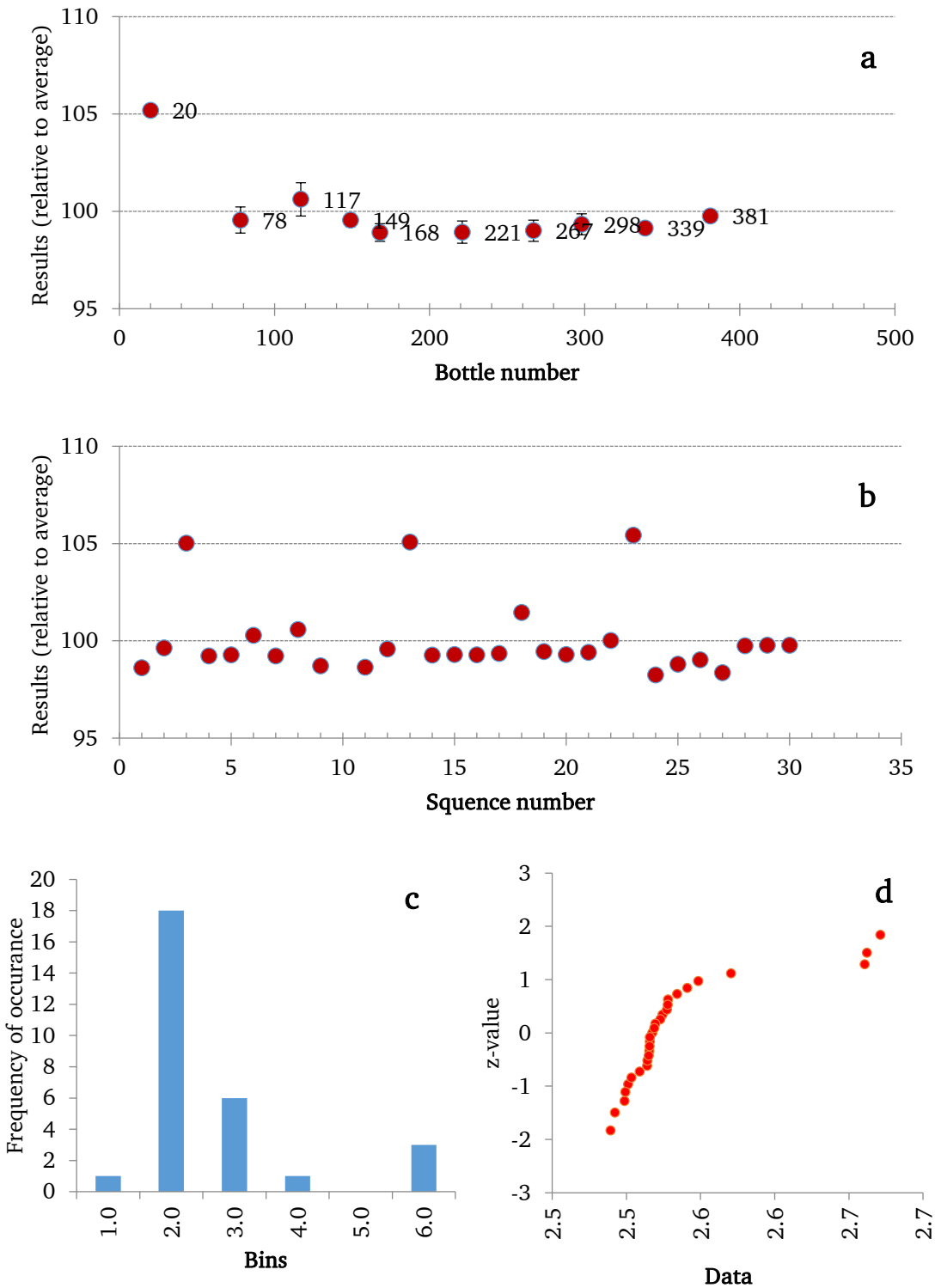
a. Graph of filling sequence order

b. Graph of analytical sequence order

c. Histogram

d. Normal Probability Plot

Figure 3.8 Homogeneity graph of Cu



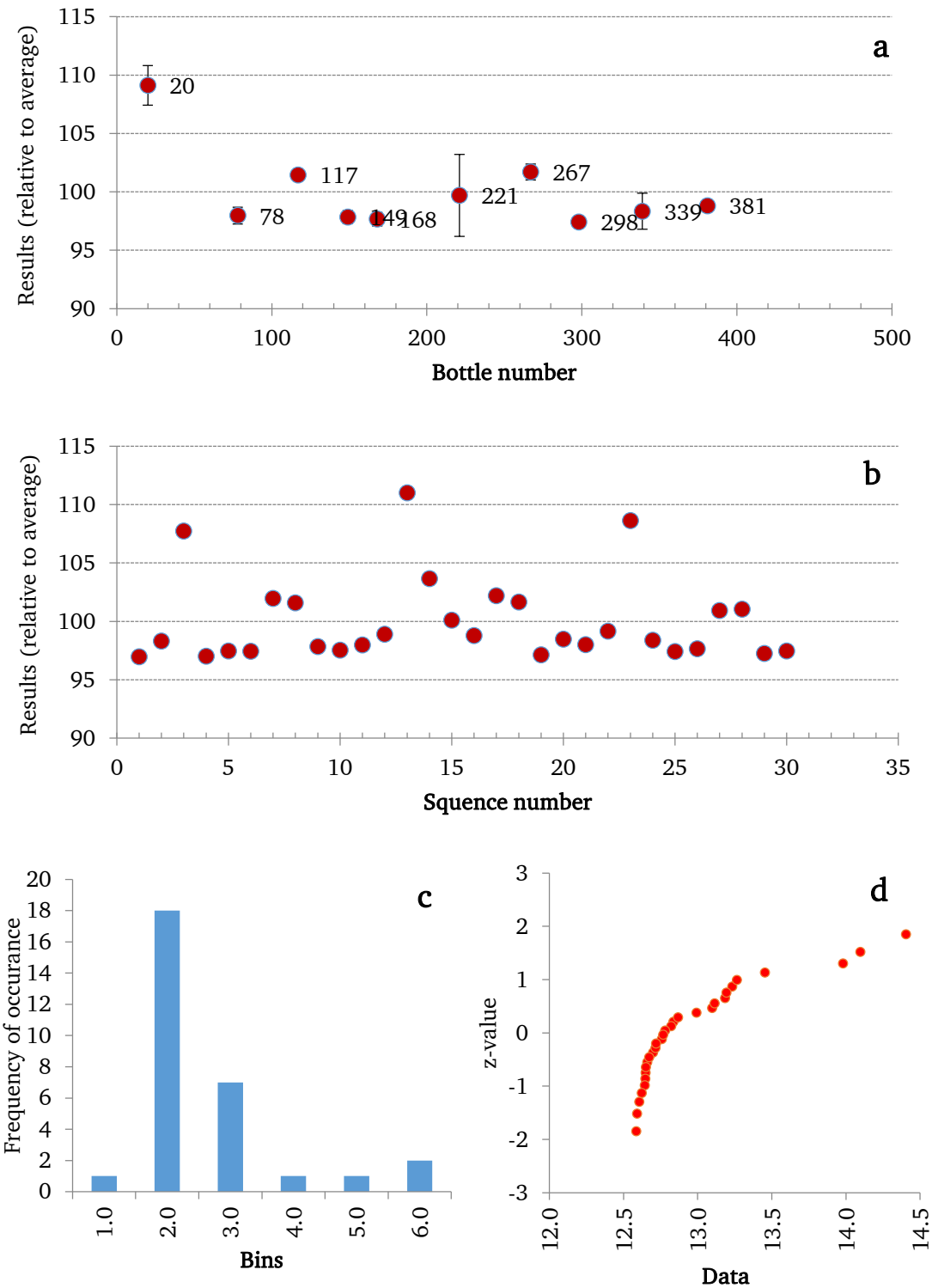
a. Graph of filling sequence order

b. Graph of analytical sequence order

c. Histogram

d. Normal Probability Plot

Figure 3.9 Homogeneity graph of Cr



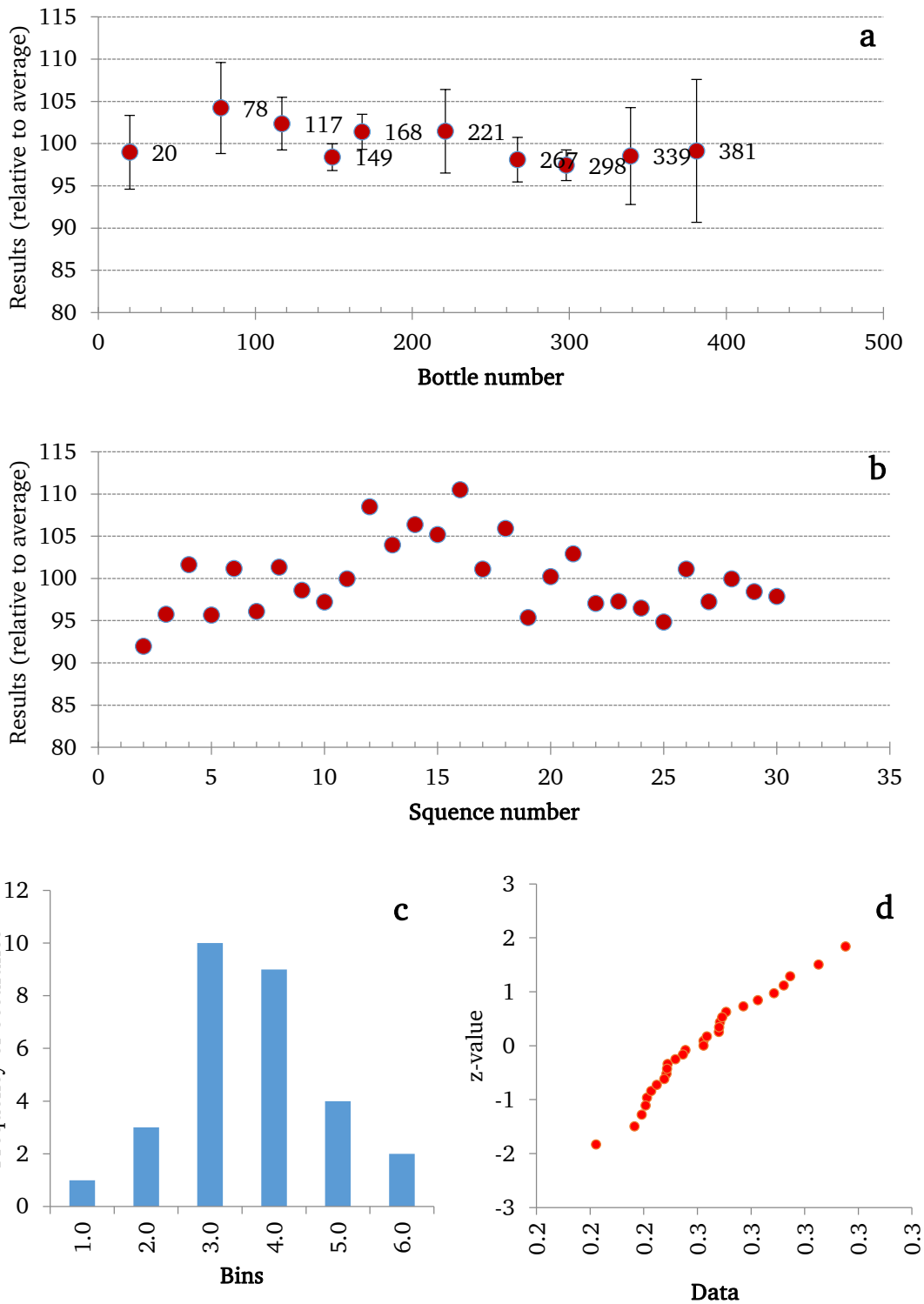
a. Graph of filling sequence order

b. Graph of analytical sequence order

c. Histogram

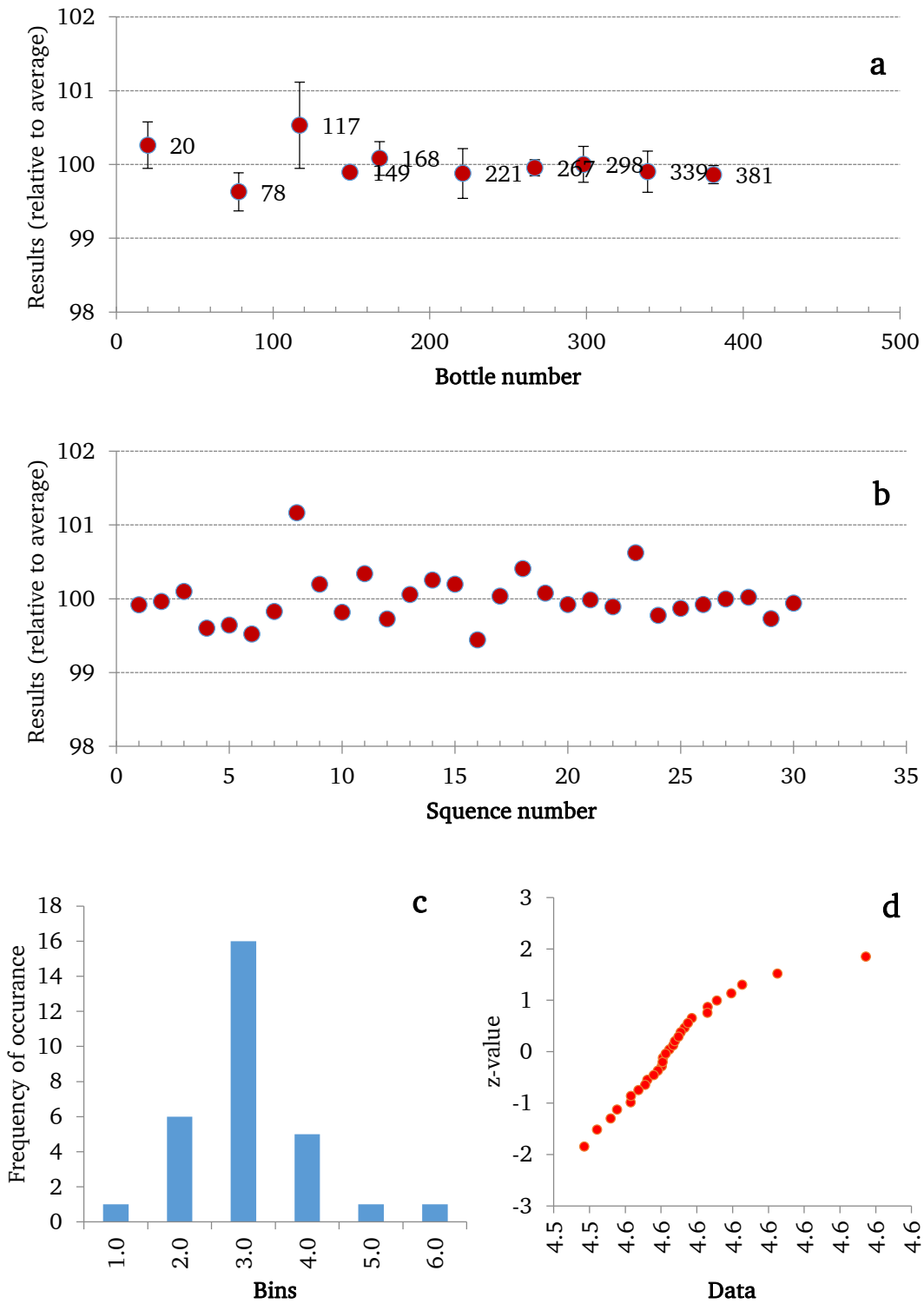
d. Normal Probability Plot

Figure 3.10 Homogeneity graph of Fe



a. Graph of filling sequence order
b. Graph of analytical sequence order
c. Histogram
d. Normal Probability Plot

Figure 3.11 Homogeneity graph of Hg



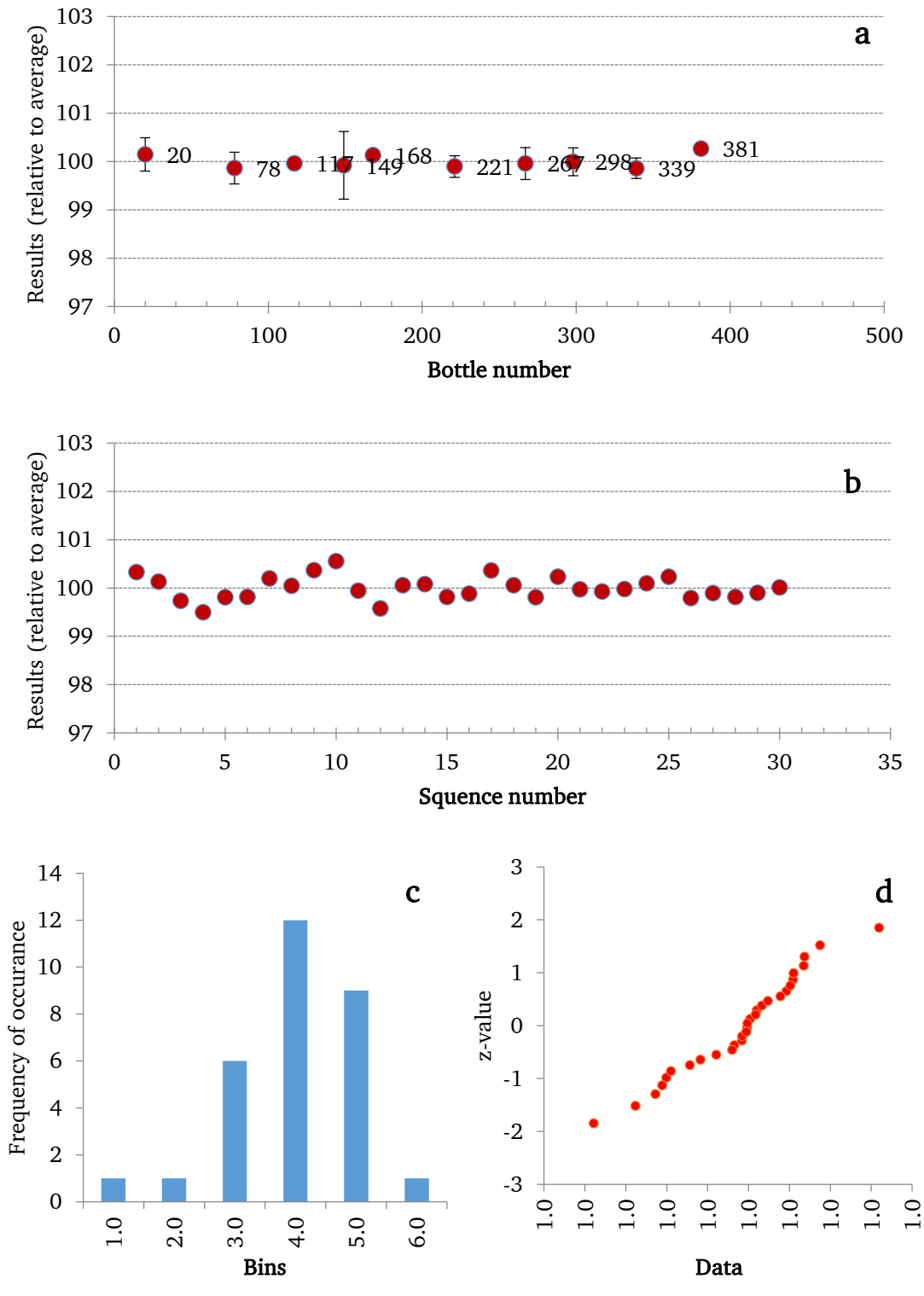
a. Graph of filling sequence order

b. Graph of analytical sequence order

c. Histogram

d. Normal Probability Plot

Figure 3.12 Homogeneity graph of Ni



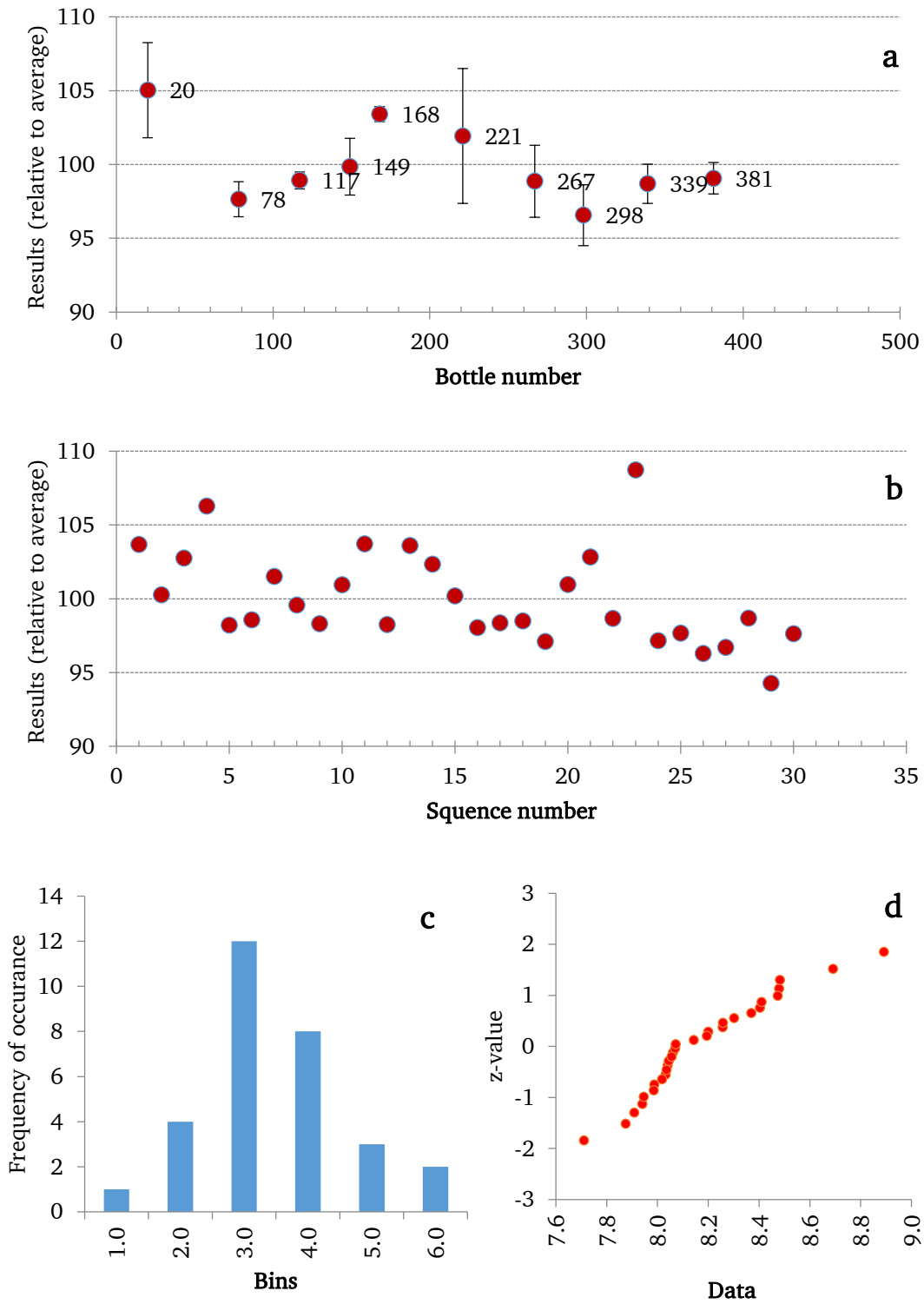
a. Graph of filling sequence order

b. Graph of analytical sequence order

c. Histogram

d. Normal Probability Plot

Figure 3.13 Homogeneity graph of Pb



a. Graph of filling sequence order

b. Graph of analytical sequence order

c. Histogram

d. Normal Probability Plot

Figure 3.14 Homogeneity graph of Zn

Establishment of within unit homogeneity was necessary to declare the amount of analyte in each individual aliquots of sample from a single unit containing same amount of analyte. 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 can be used in an 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.

As the material is a solution, any heterogeneity within unis is expected. Nevertheless, as the homogeneity study was performed by using 5 mL for Cr, Cd, Ni, Cu, Pb, Zn, Fe and 1.0 mL for As, it is recommended that minimum those sample amounts should be used in any analysis .

3.1.4 Assessment of Short Term and Long Term Stability

The measurement design of the stability studies followed an isochronous scheme as described below [277] and the uncertainty contribution of stability of material was calculated as described by Linsinger et al. [278]. The bottles used for stability analysis were selected using RSS. Stability measurements were performed by TEA/$Mg(OH)_2$-ID^3MS method using ICP-MS/MS for Cr, Cd, Ni, Cu, Pb, Zn, Fe, cold vapor-double IDMS by HR-ICP-MS for Hg and matrix match external calibration by ICP-MS/MS for As determination. In order to obtain proper uncertainty related to stability of the material, three replicates for each unit were analyzed and all the analysis for each parameter were completed in a randomized unit/replicate to distinguish any analytical trend form a material degradation that may occur within storage period.

I. Short Term Stability

For the short term stability measurements, STS, according to the designed test temperatures and time periods, 14 units were selected by RSS from the whole batch produced. Short term stability tests were performed for 1, 2 and 4 weeks at pre-defined test temperatures,+18 °C and +60 °C. Two units for each time period were used for STS. 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 there was significant instability even for a one week at 60 °C.

The evaluation of stability measurements were carried out for each temperature and Grubbs' test at confidence levels of 95% and 99% were applied, separately. One outlier for 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 short term stability dataset, the mass fractions versus time were plotted and the regression lines were calculated in order to check the significant trends to indicate the 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 short term stability measurements are summarized in Table 3.11 and graphical representations of data are also provided in Figure 3.15 - Figure 3.34. 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 to be stable in exposure to 60 °C for four weeks. Therefore, additional test temperatures were investigated for Zn. It was found that statistically degradation of Zn was found at 40 °C after two weeks as seen in Figure 3.33. Therefore, uncertainty related to short term stability of material was calculated for two weeks at 40 °C and 60 °C for Zn and other analytes, respectively. Uncertainty contribution resulting from STS was calculated by applying following equation (3.6) [59].

$$u_{sts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \times t \qquad (3.6)$$

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 3.11 Summary of results for short term stability test

Analyte	$u_{sts,rel}$[1] (%)				Significance of the trend on a %95 confidence level				Number of individual outlying results at %95 confidence level			
	T1	*T2*	*T3*	*T4*	*T1*	*T2*	*T3*	*T4*	*T1*	*T2*	*T3*	*T4*
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	-	-

[1]Standard uncertainty has been calculated for two week
T1=18 °C, T2=30 °C, T3=40 °C, T4=60°C

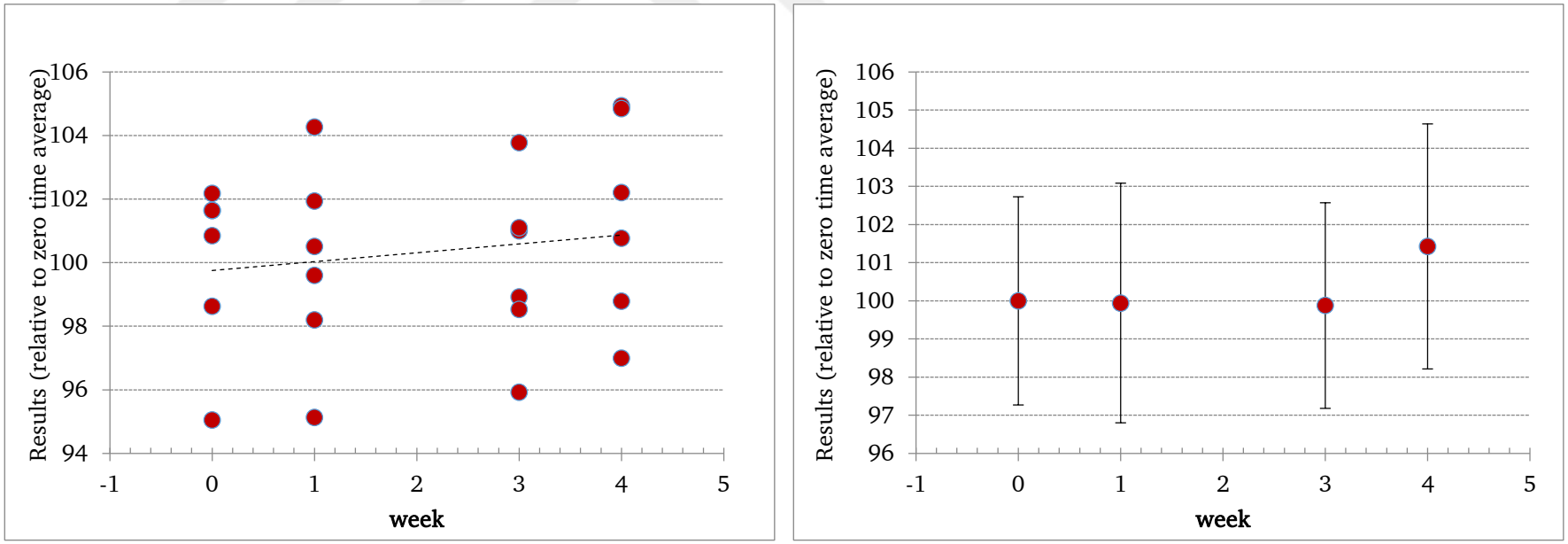


Figure 3.15 Short term stability at 18 °C for As

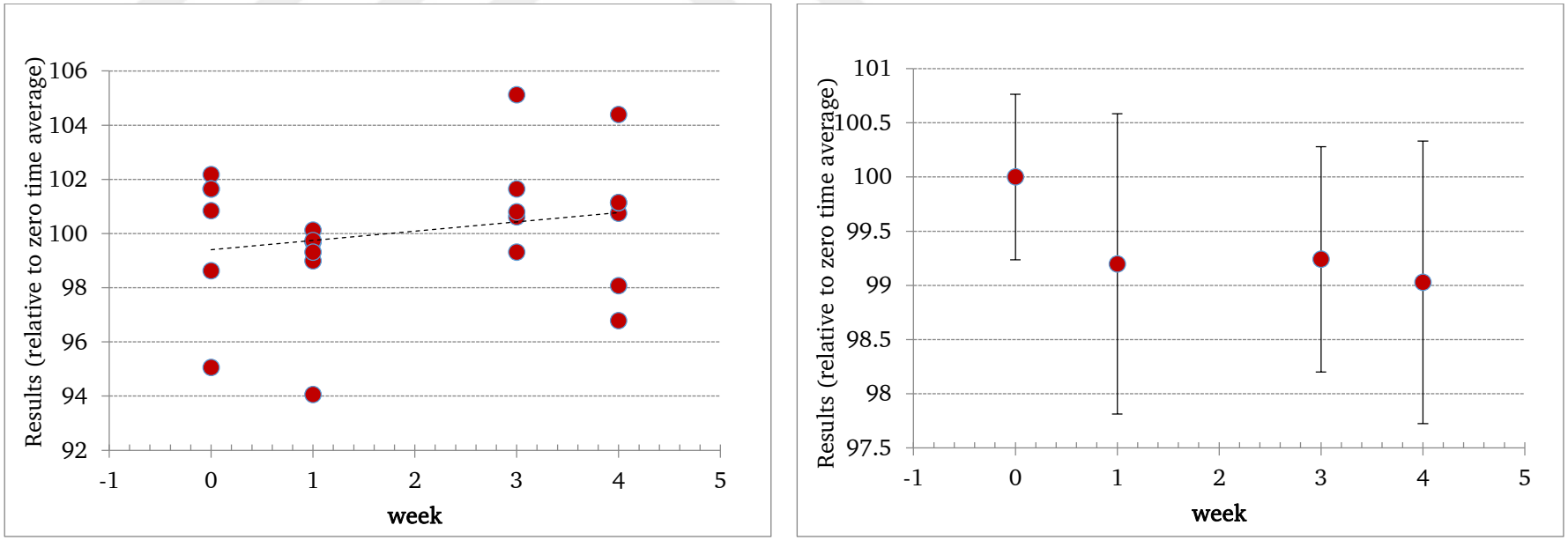


Figure 3.16 Short term stability at 60 °C for As

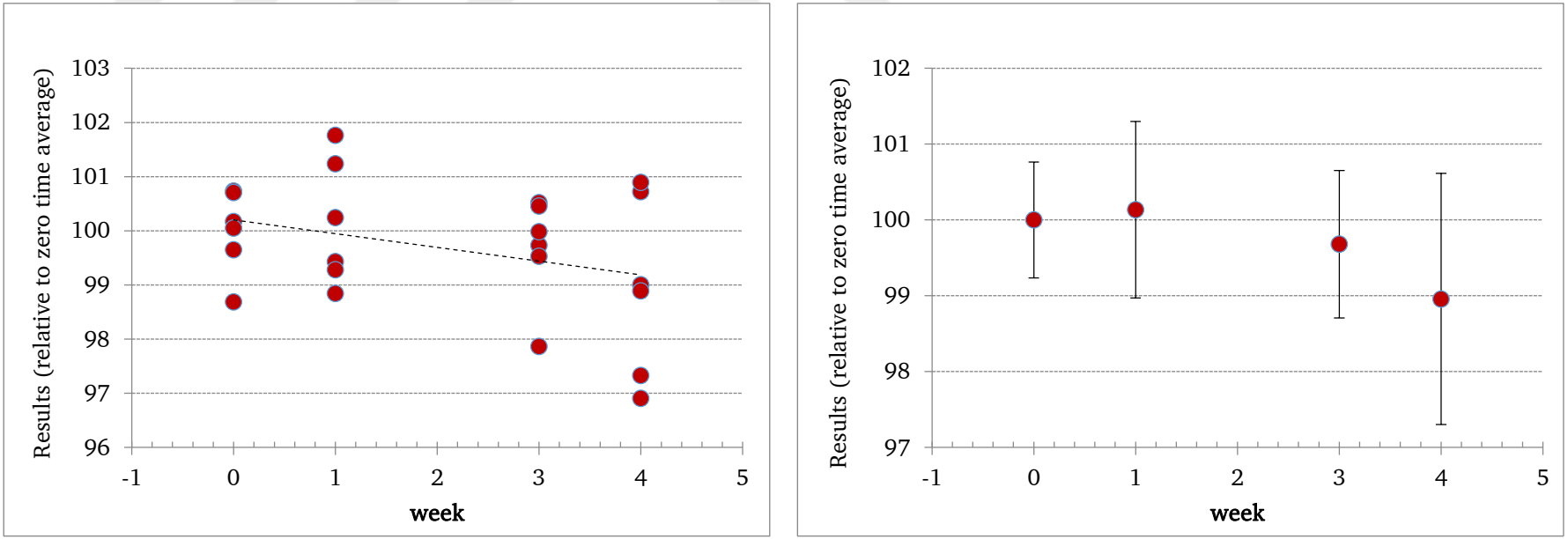


Figure 3.17 Short term stability at 18 °C for Cd

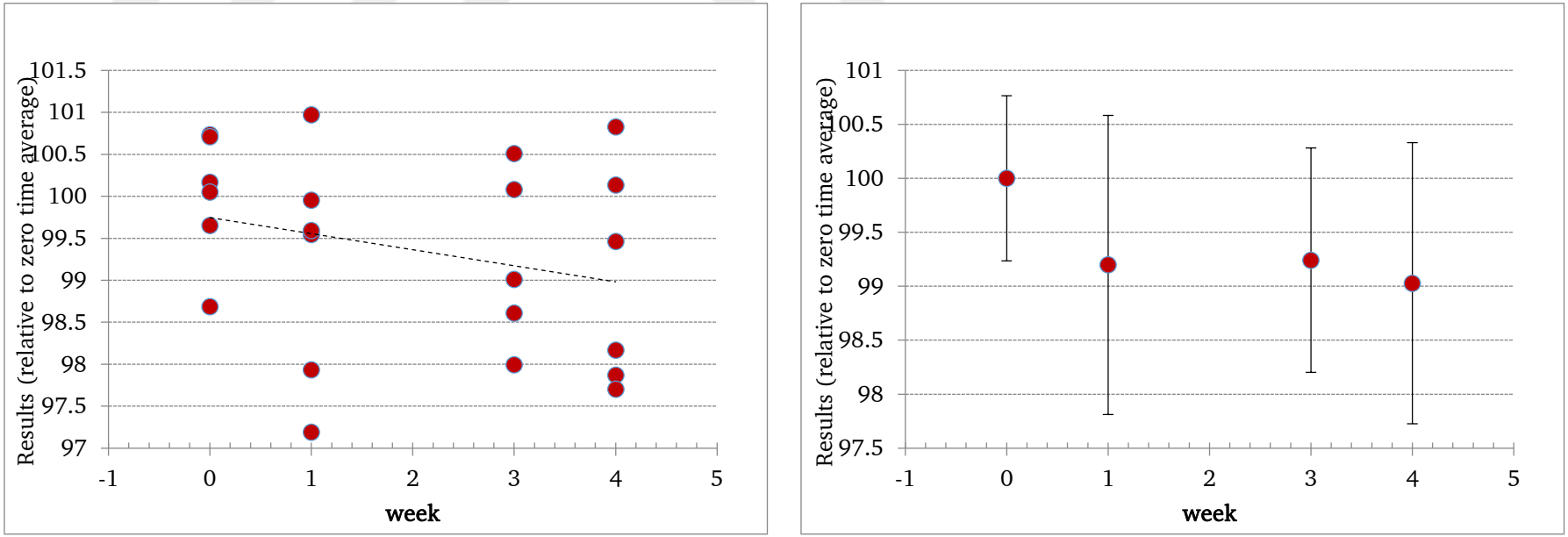


Figure 3.18 Short term stability at 60 °C for Cd

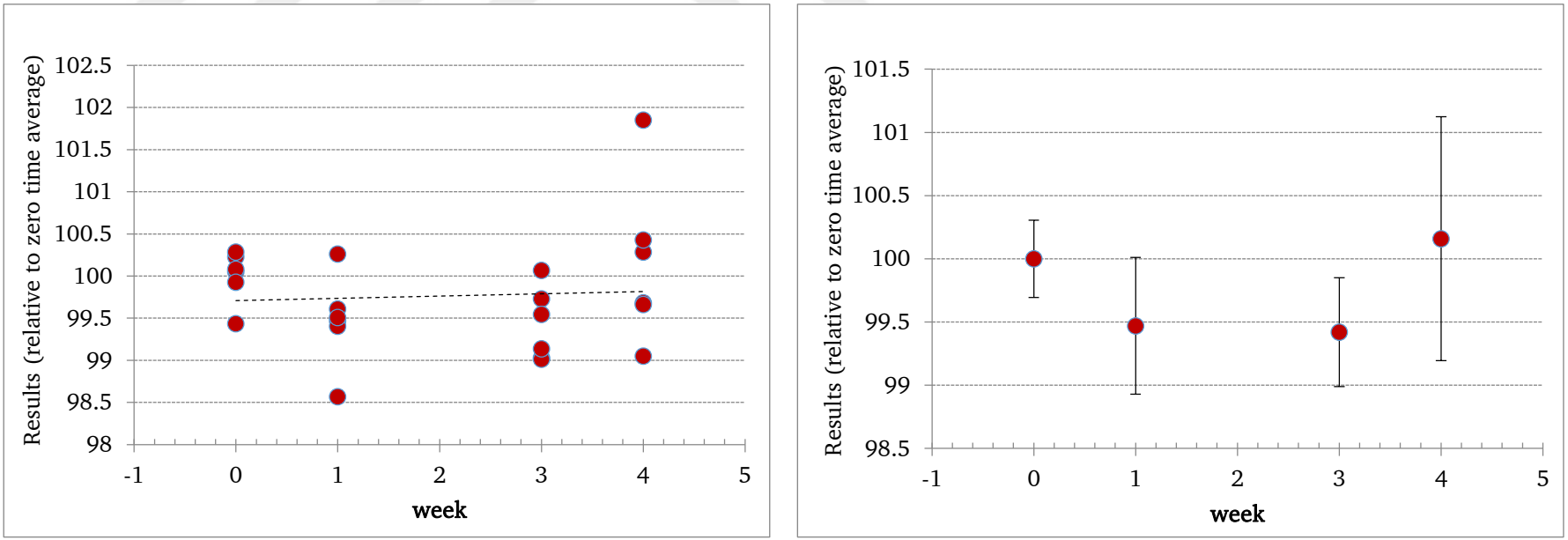


Figure 3.19 Short term stability at 18 °C for Cr

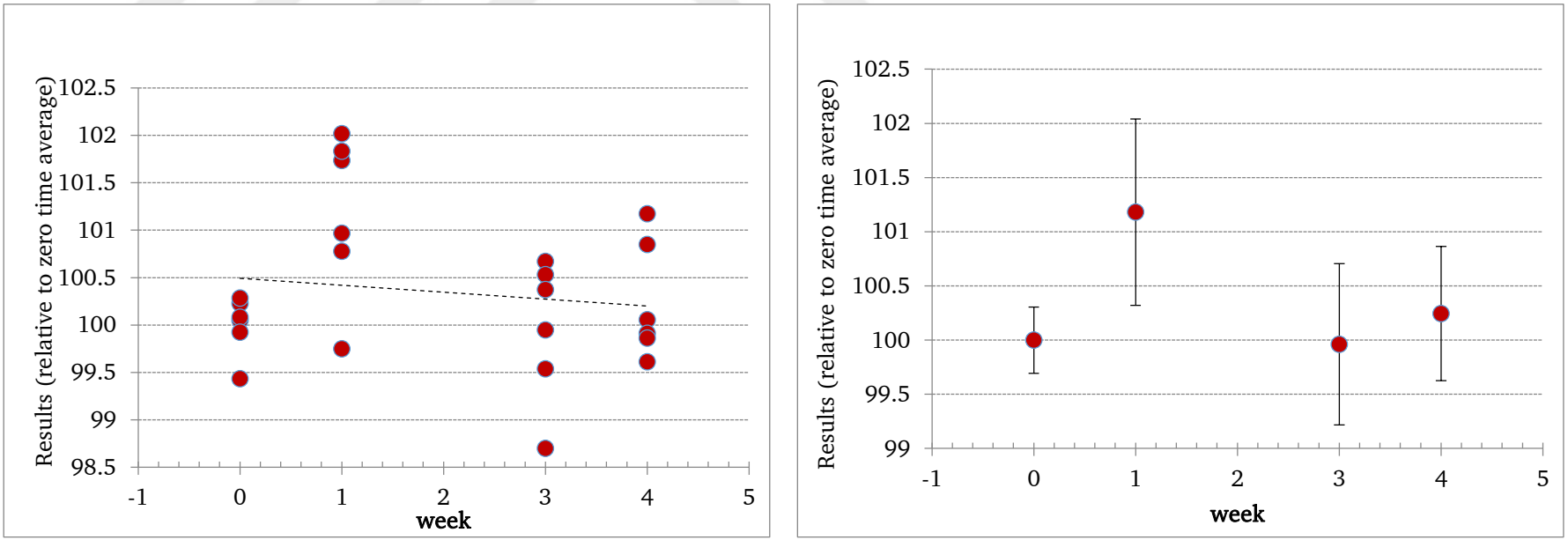


Figure 3.20 Short term stability at 60 °C for Cr

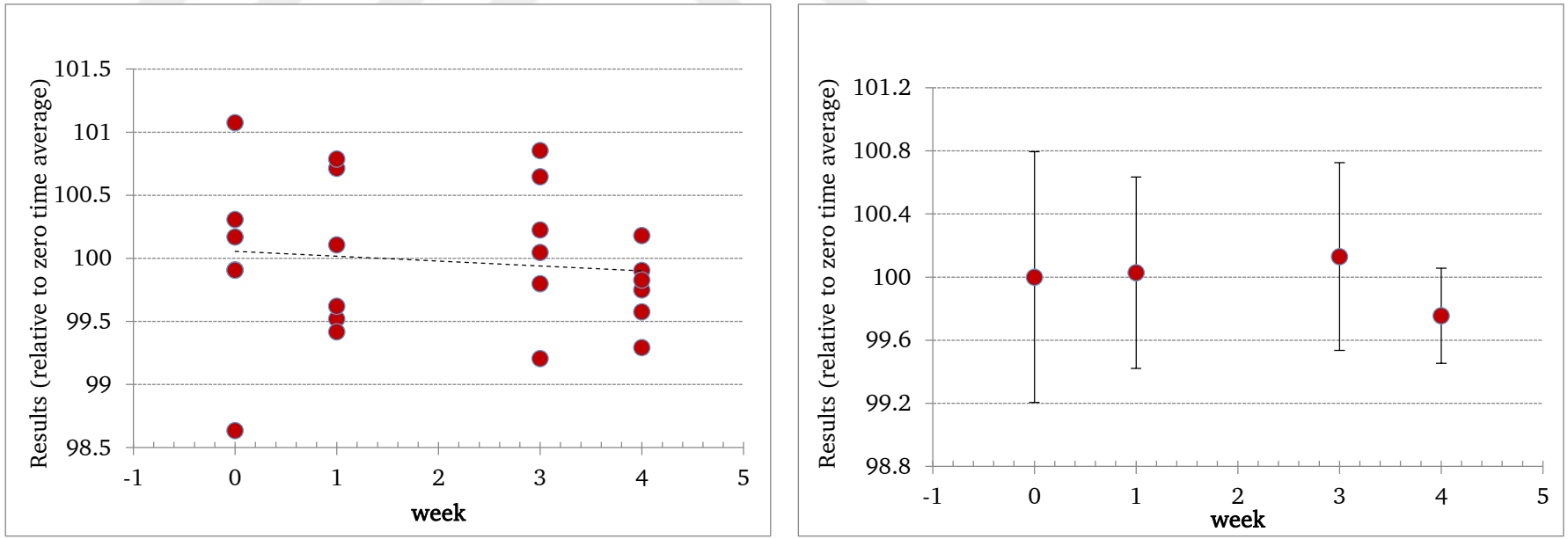


Figure 3.21 Short term stability at 18 °C for Cu

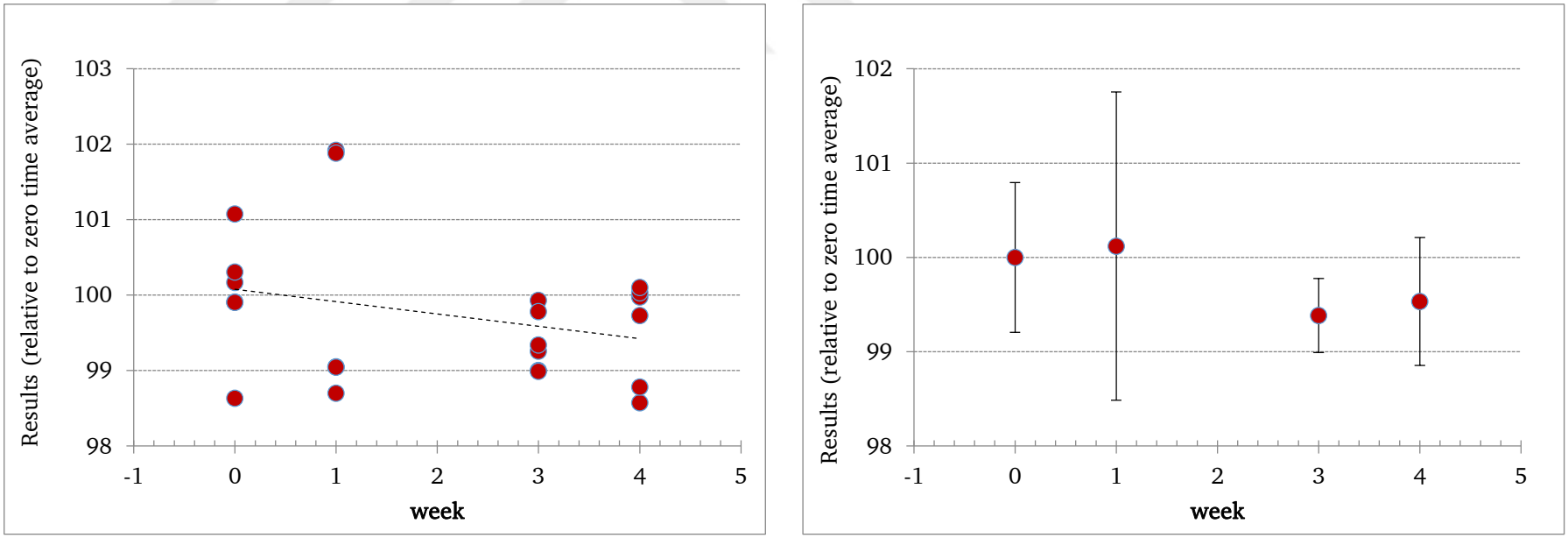


Figure 3.22 Short term stability at 60 °C for Cu

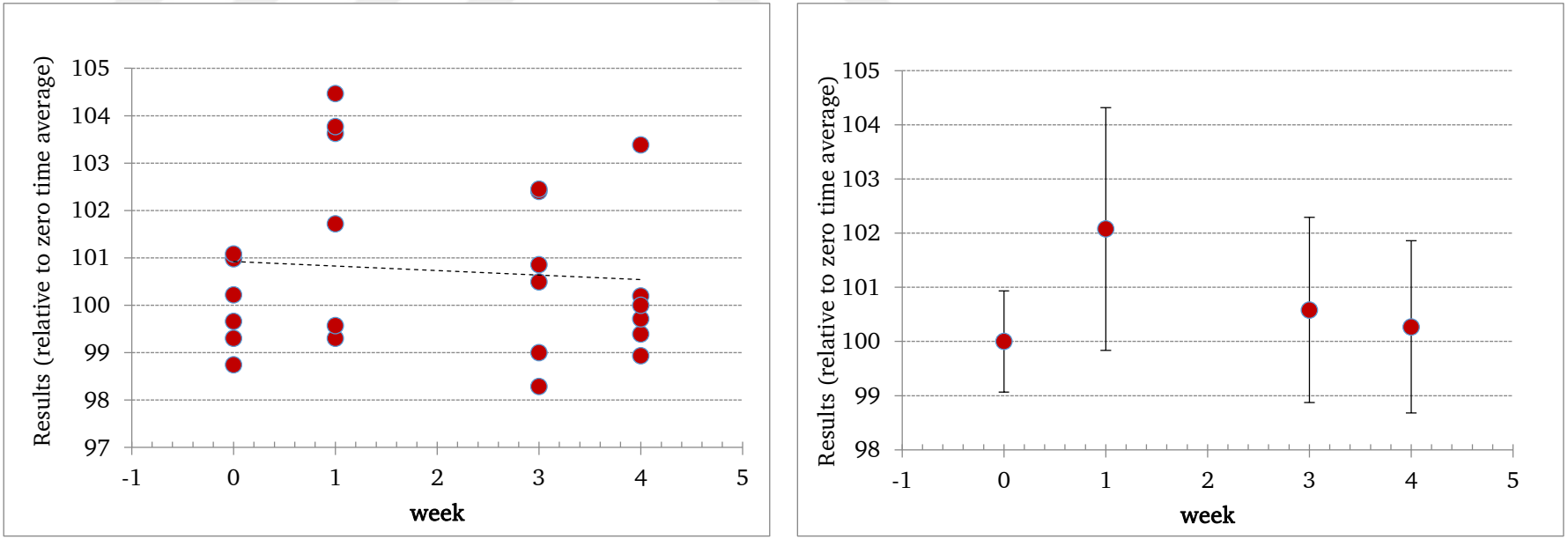


Figure 3.23 Short term stability at 18 °C for Fe

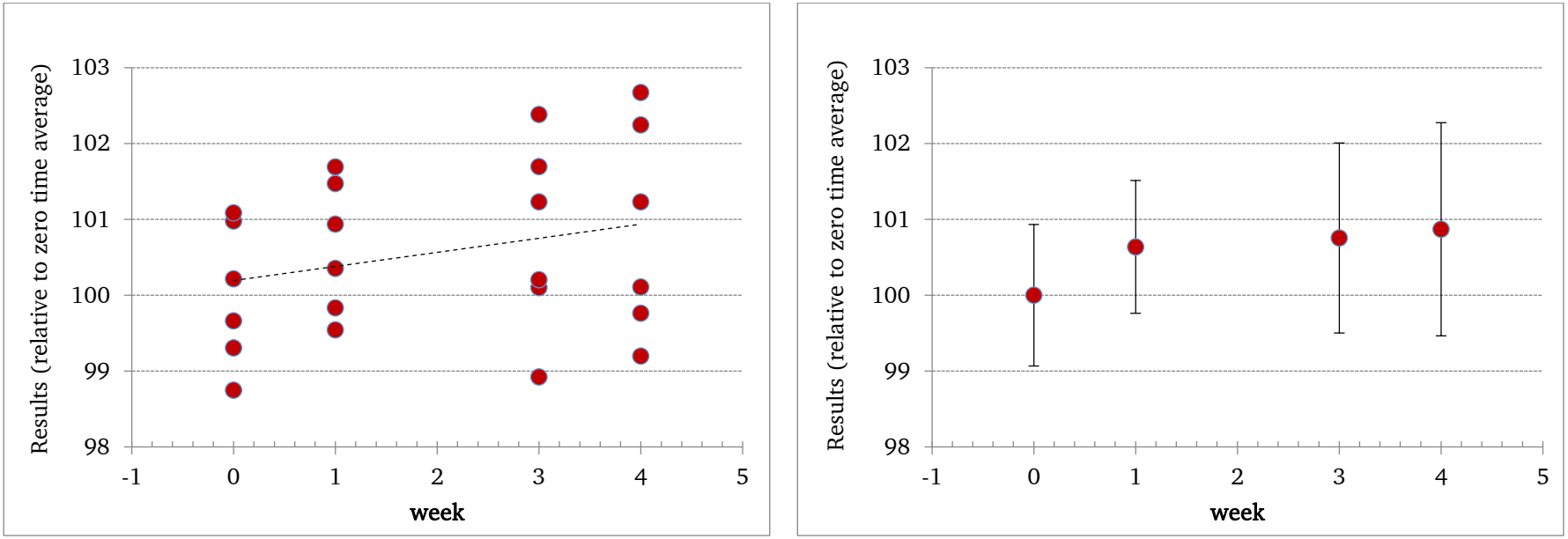


Figure 3.24 Short term stability at 60 °C for Fe

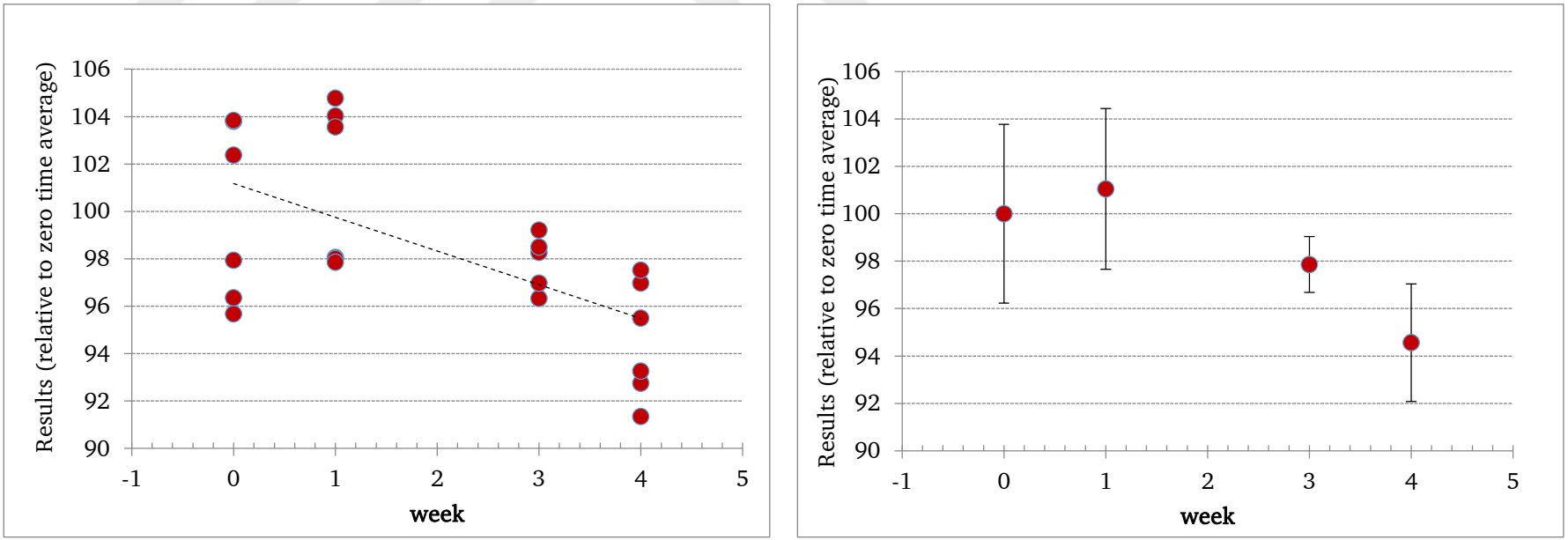


Figure 3.25 Short term stability at 18 °C for Hg

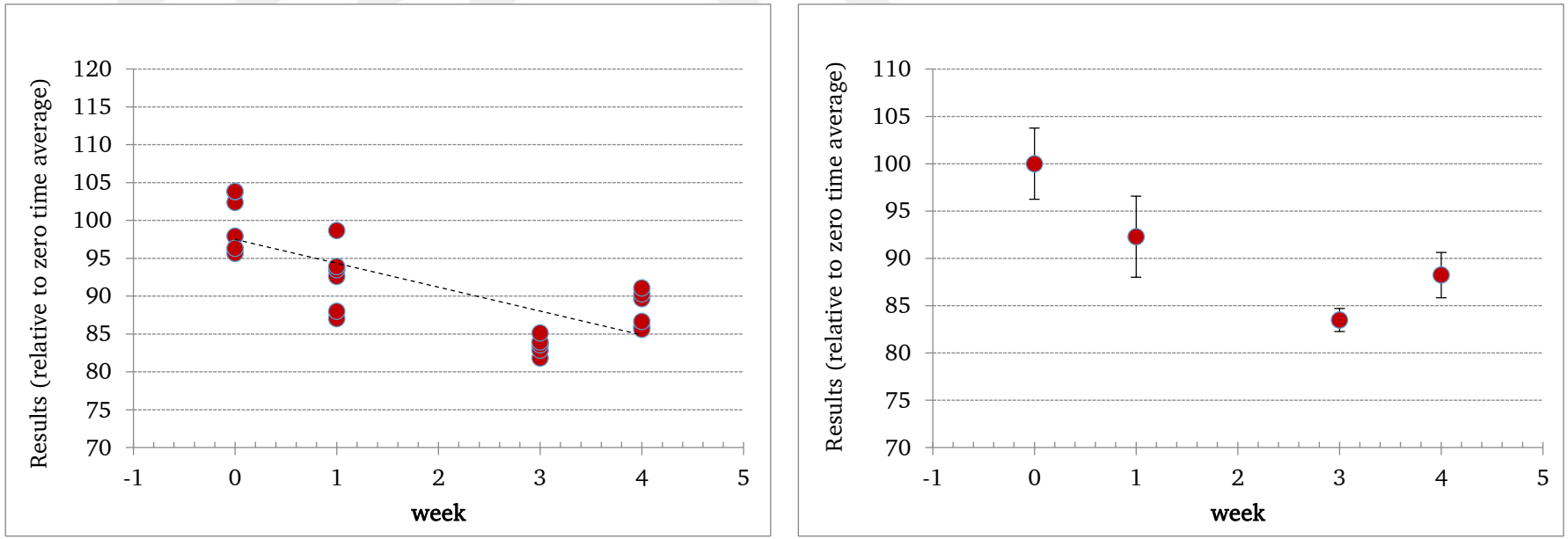


Figure 3.26 Short term stability at 60 °C for Hg

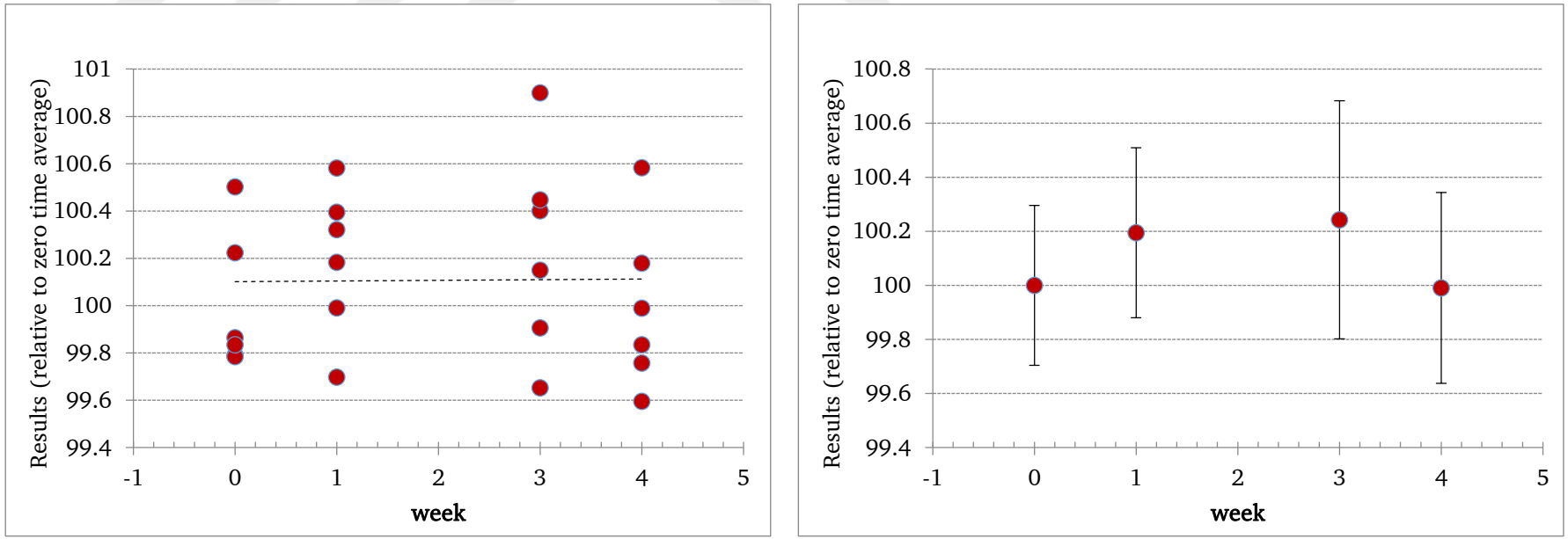


Figure 3.27 Short term stability at 18 °C for Ni

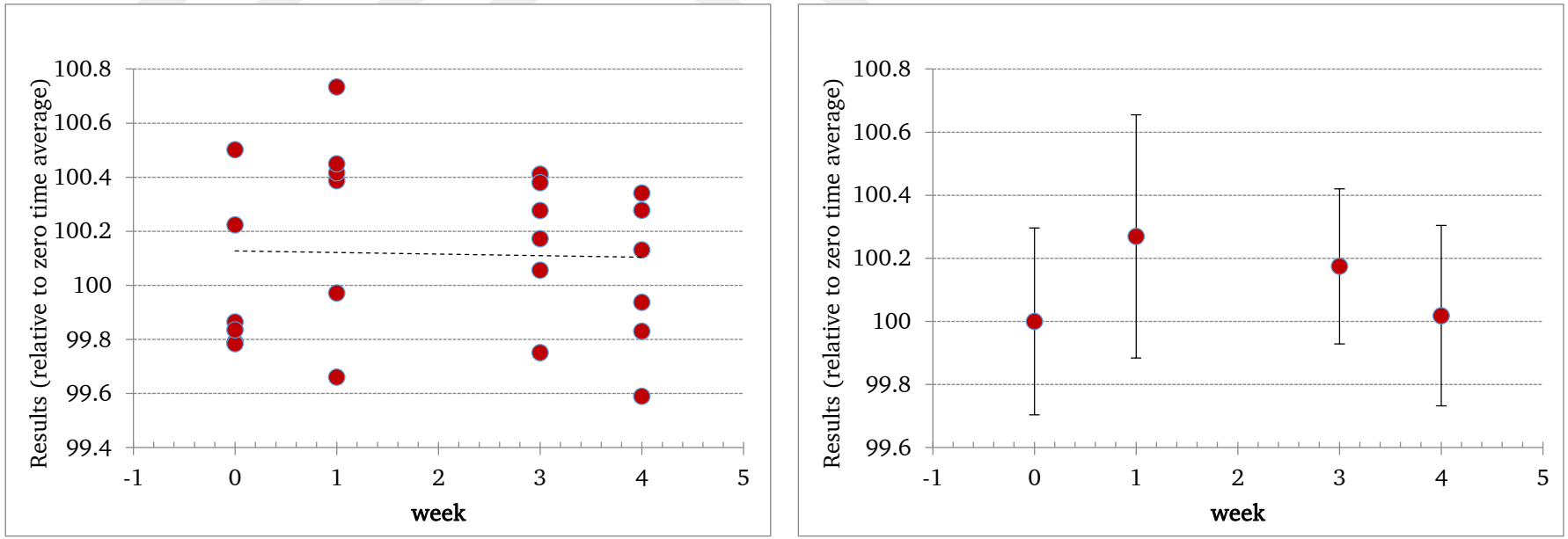


Figure 3.28 Short term stability at 60 °C for Ni

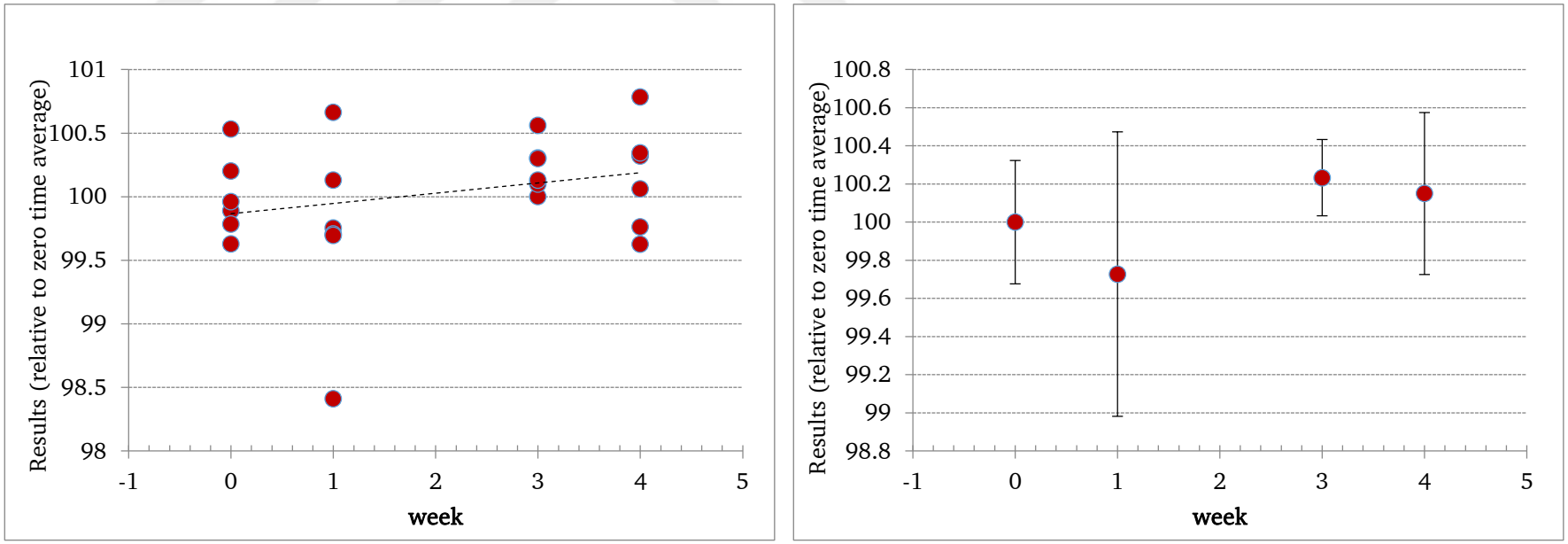


Figure 3.29 Short term stability at 18 °C for Pb

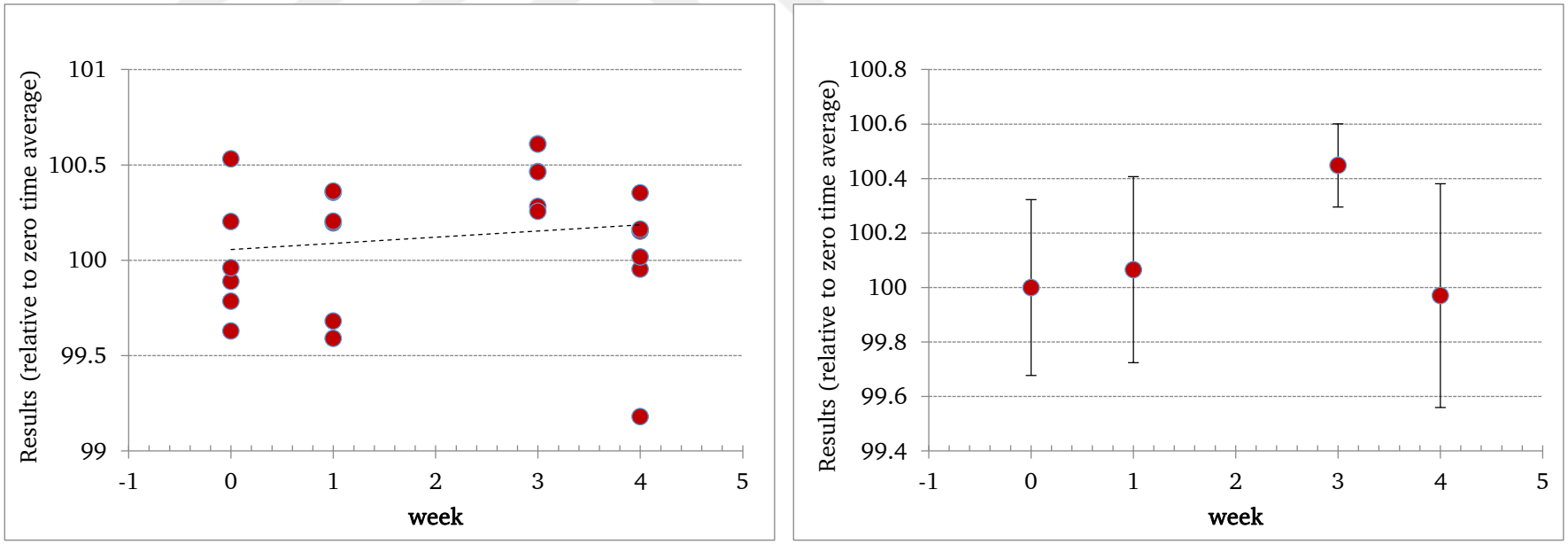


Figure 3.30 Short term stability at 60 °C for Pb

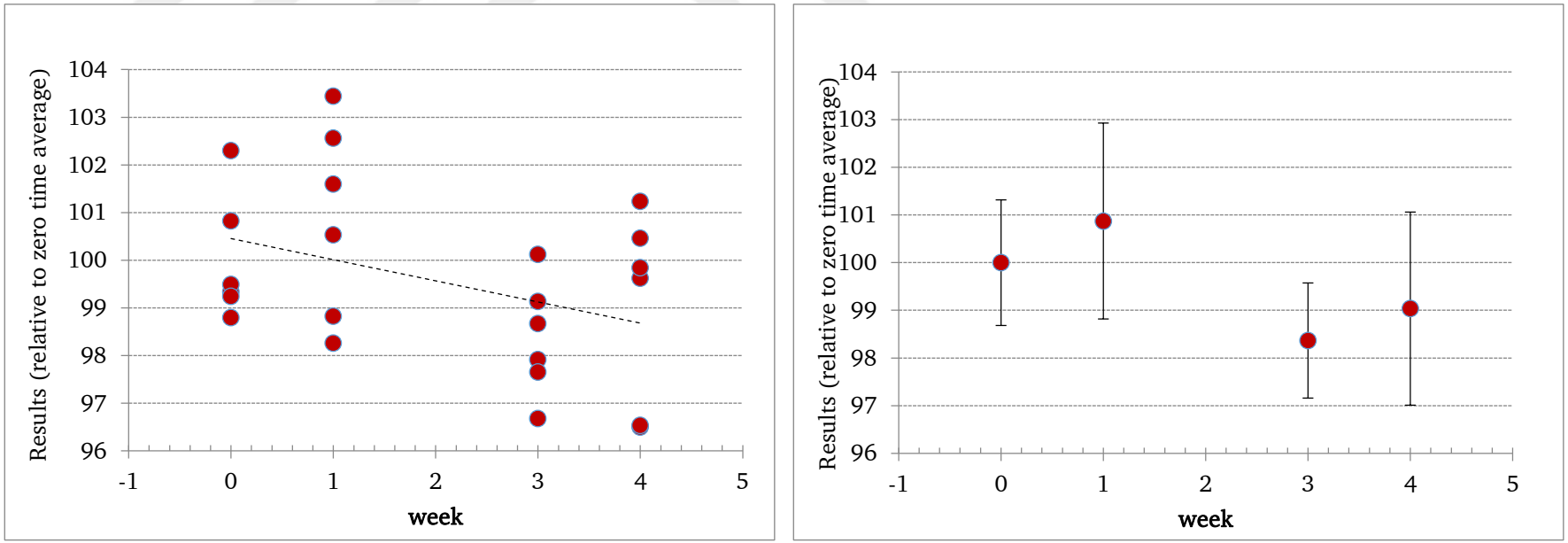


Figure 3.31 Short term stability at 18 °C for Zn

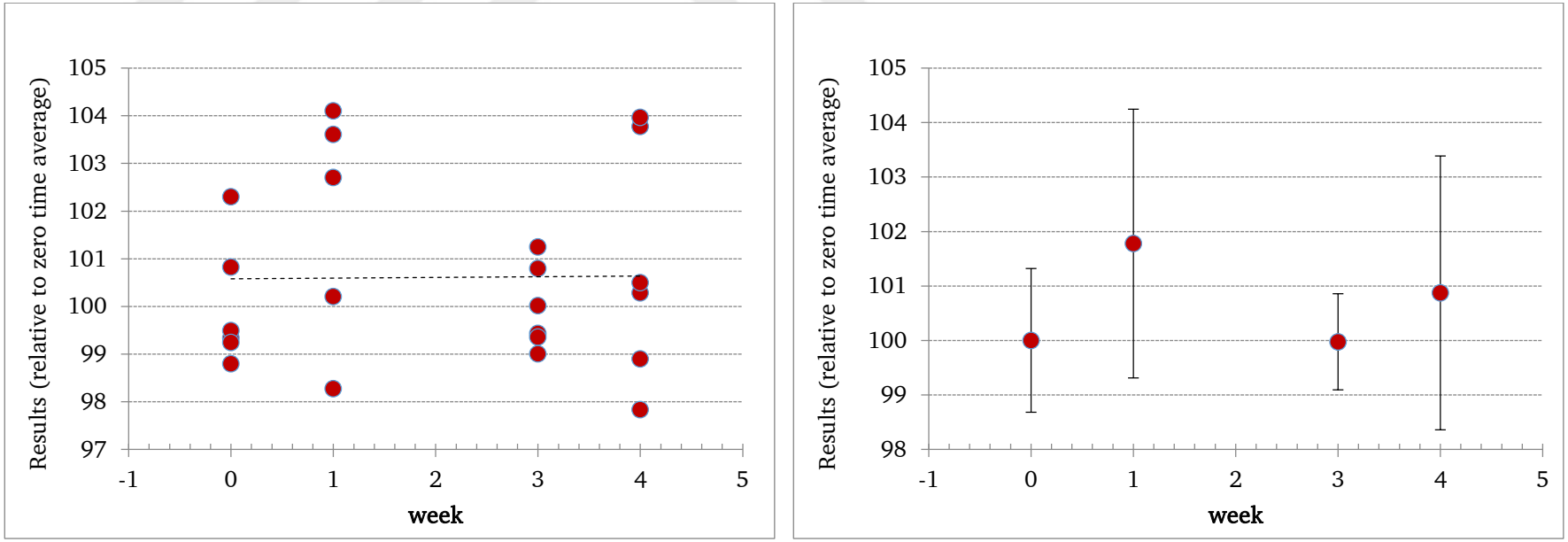


Figure 3.32 Short term stability at 30 °C for Zn

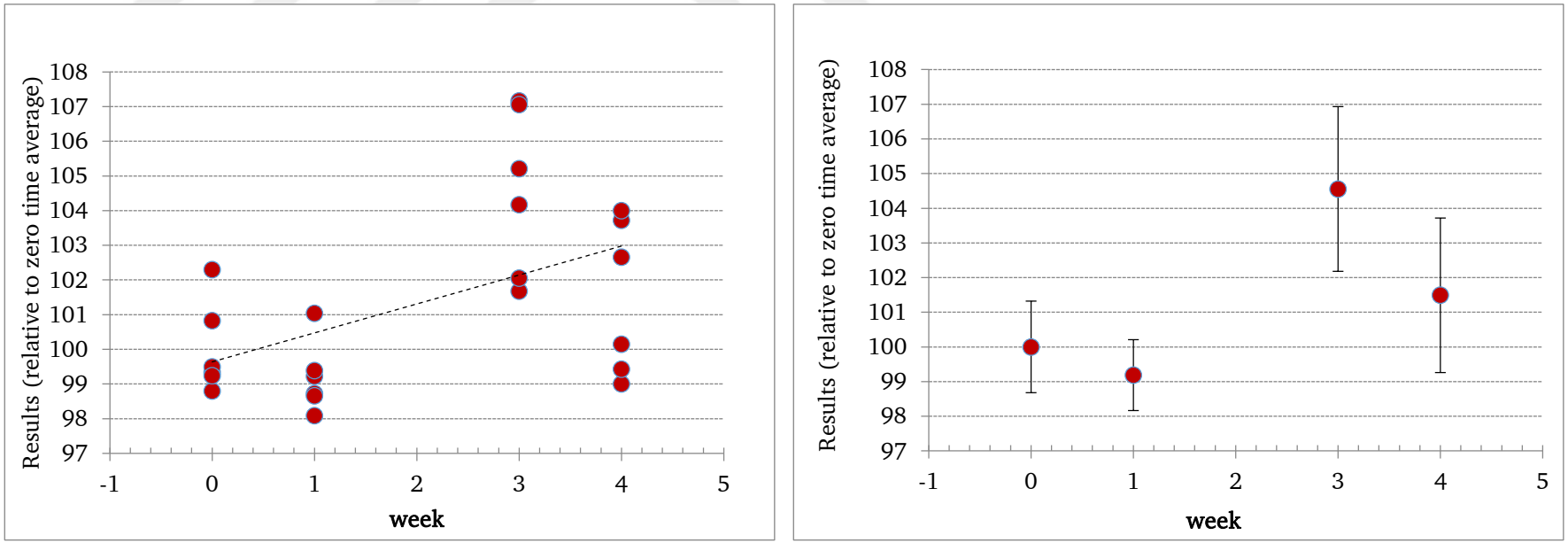


Figure 3.33 Short term stability at 40 °C for Zn

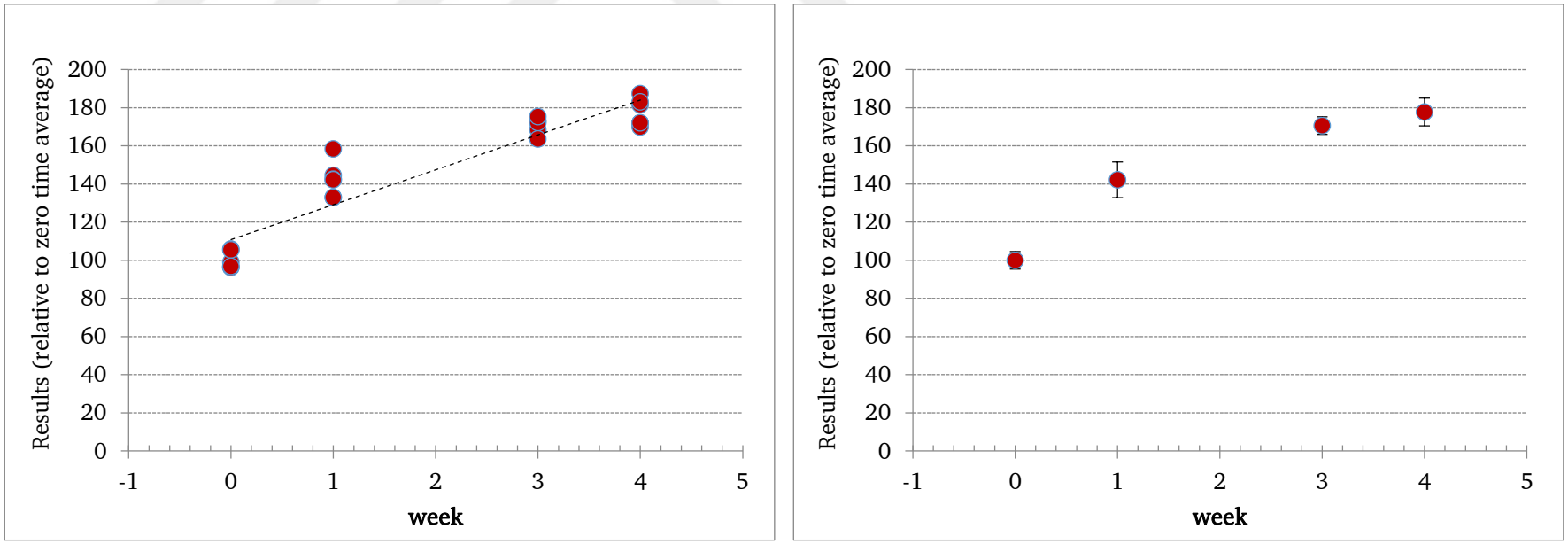


Figure 3.34 Short term stability at 60 °C for Zn

II. Long Term Stability

The uncertainty resulting from shelf life of the candidate CRM at 18 °C has been calculated by performing the long term stability measurements (LTS). 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 long term stability analysis. The reference temperature has been set to 4.0 °C and each unit were transferred to reference temperature at the end of the time period at 18 °C.

All the data obtained for LTS was screened for outlier using single Grubbs test at 95% and 99% confidence levels. Two outlying individual results for Cr, one outlying individual results for Cu, Fe and Zn were found. 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 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 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 belongs to unit no 142 was actually significantly higher than the rest of the bottle averages. As the uncertainty resulting from homogeneity for chromium was calculated in a way of covering bottle outliers, this data set belong to unit no 142 was removed completely in able 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). However, 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 (3.7), [59]. The shelf life of UME CRM 1206 certified reference material was defined as 12 months after sales date at 18 °C and the uncertainty contribution of LTS was calculated based on this period (Table 3.12). Graphical representation of the data related to each analyte is given Figure 3.35-Figure 3.42. Additionally, post-certification monitoring is going to be done in certain periods.

$$u_{lts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \times t \tag{3.7}$$

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 ay

Table 3.12 Summary of results for long term stability test

Analyte	$u_{lts,rel}$[1] (%)	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	-

[1]Standard uncertainty has been calculated for one year

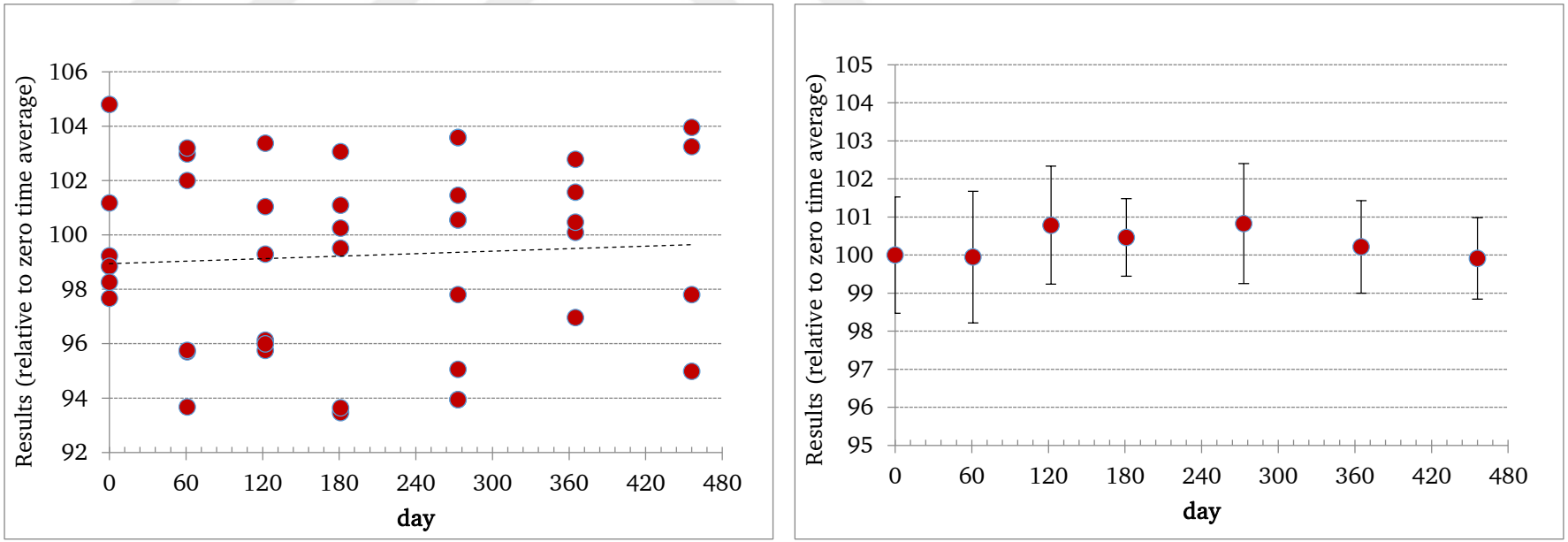


Figure 3.35 Long term stability for As

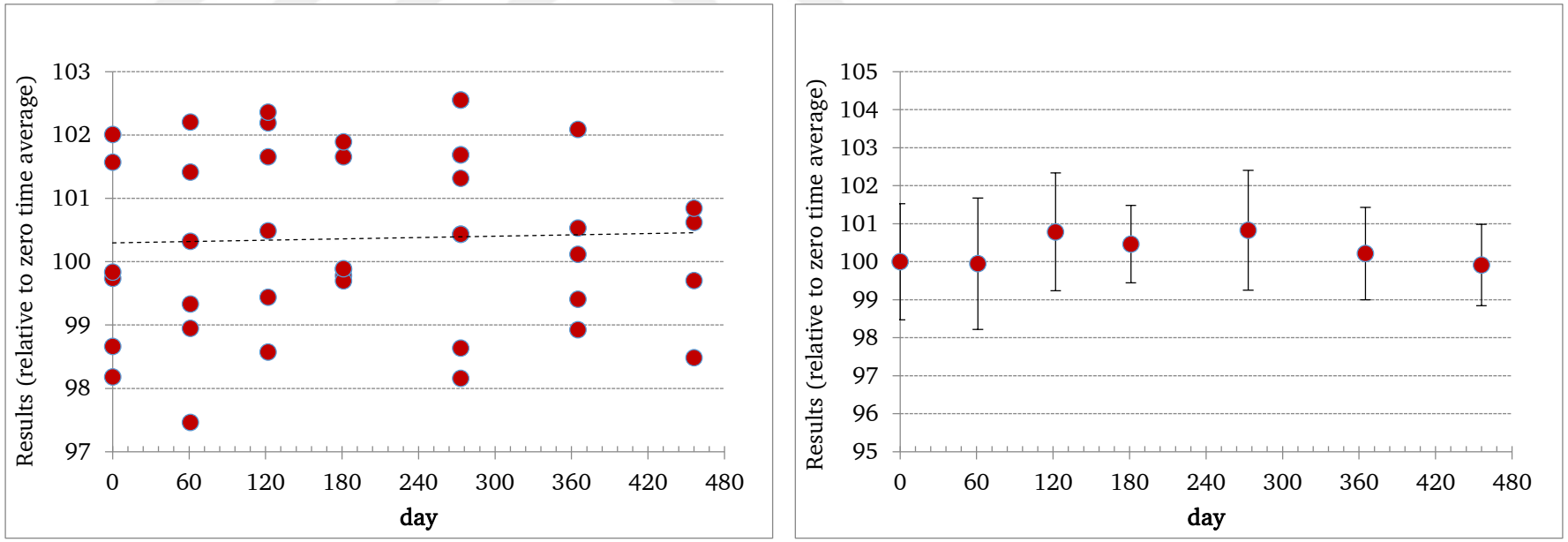


Figure 3.36 Long term stability for Cd

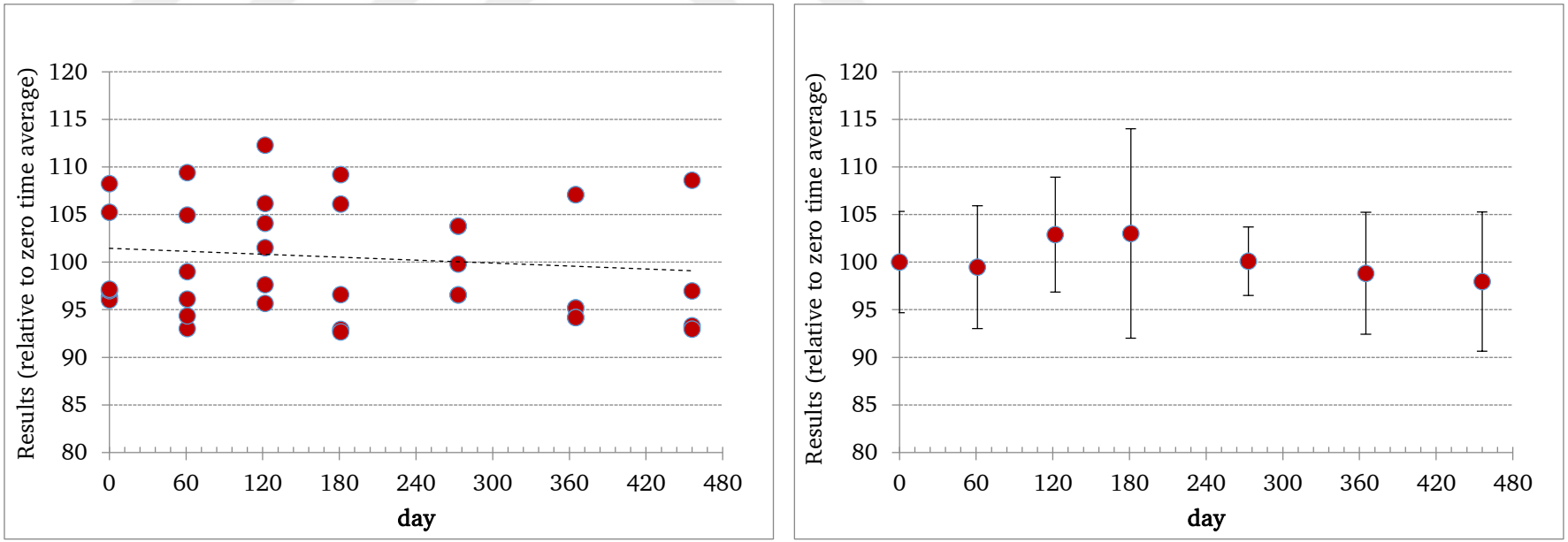


Figure 3.37 Long term stability for Cr

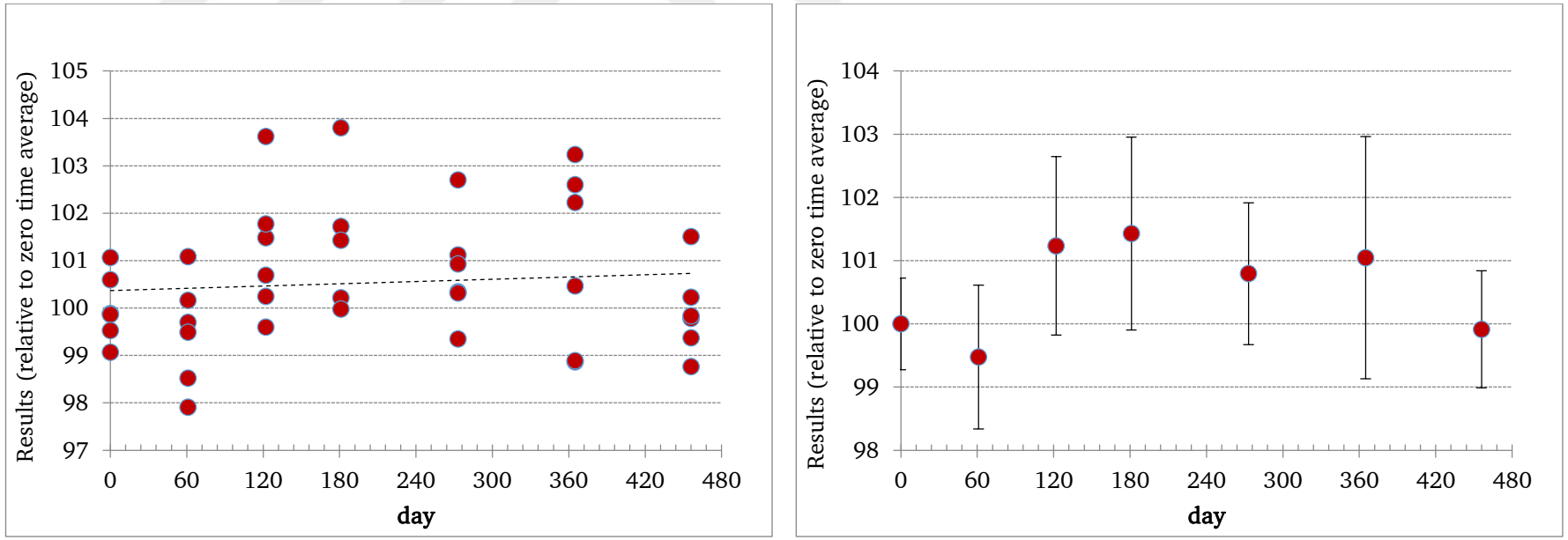


Figure 3.38 Long term stability for Cu

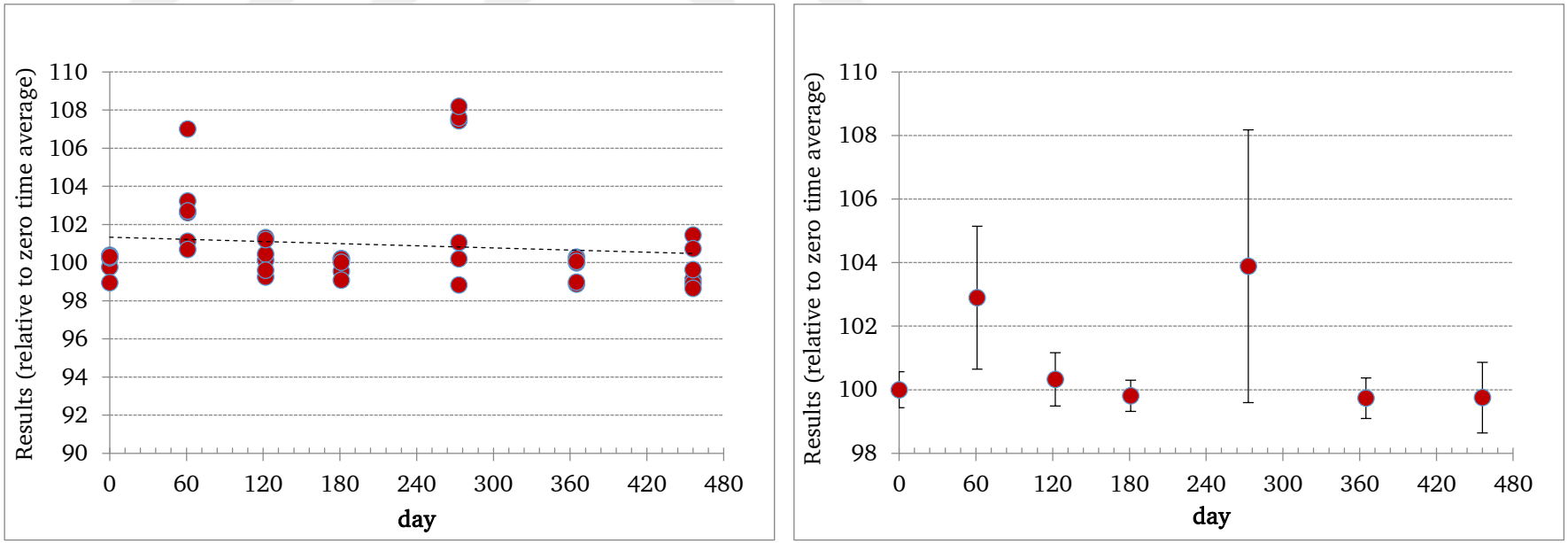


Figure 3.39 Long term stability for Fe

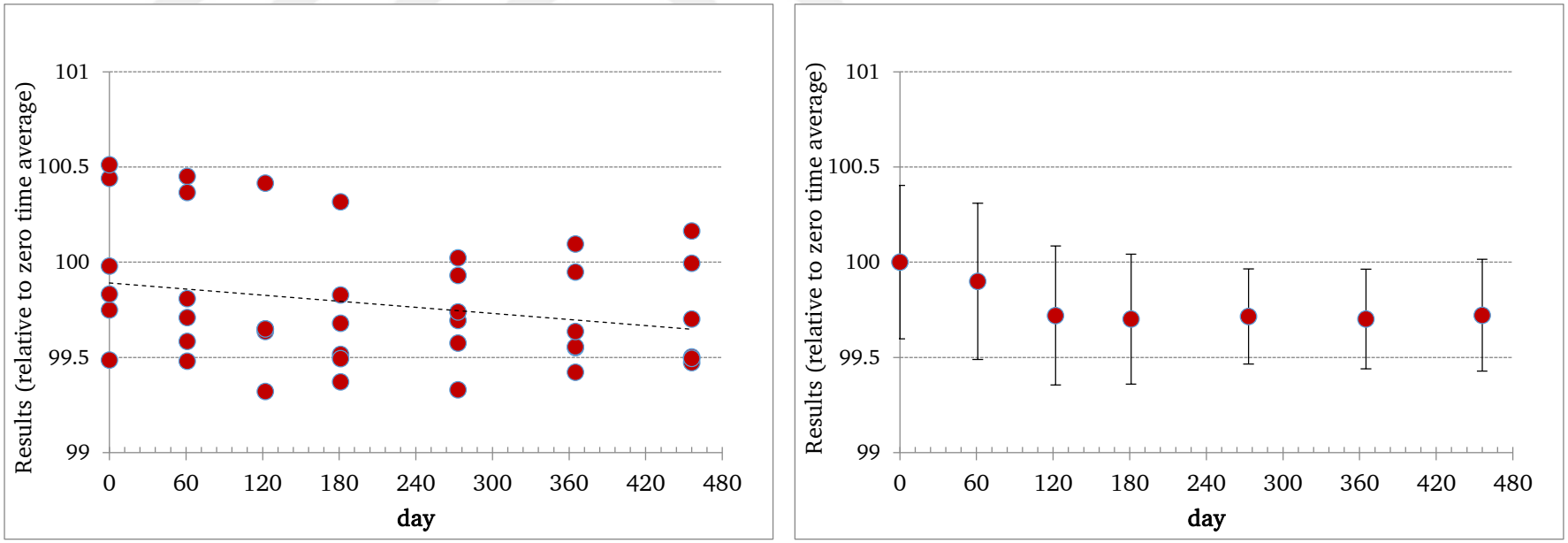


Figure 3.40 Long term stability for Ni

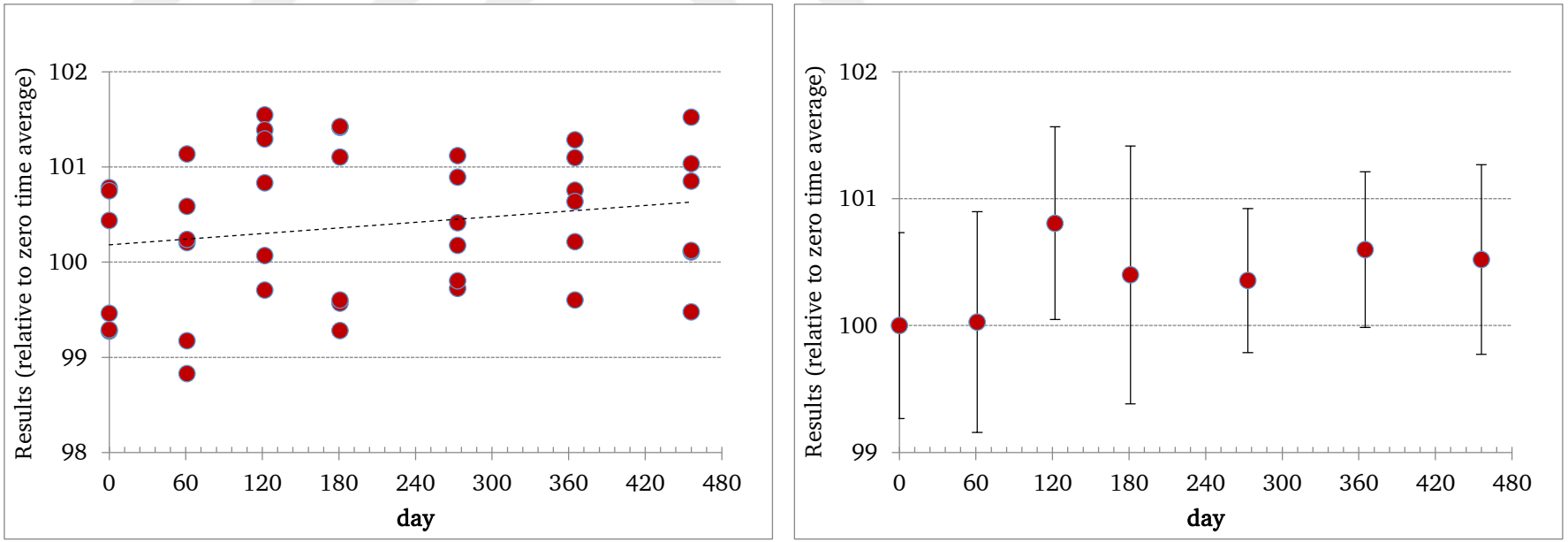


Figure 3.41 Long term stability for Pb

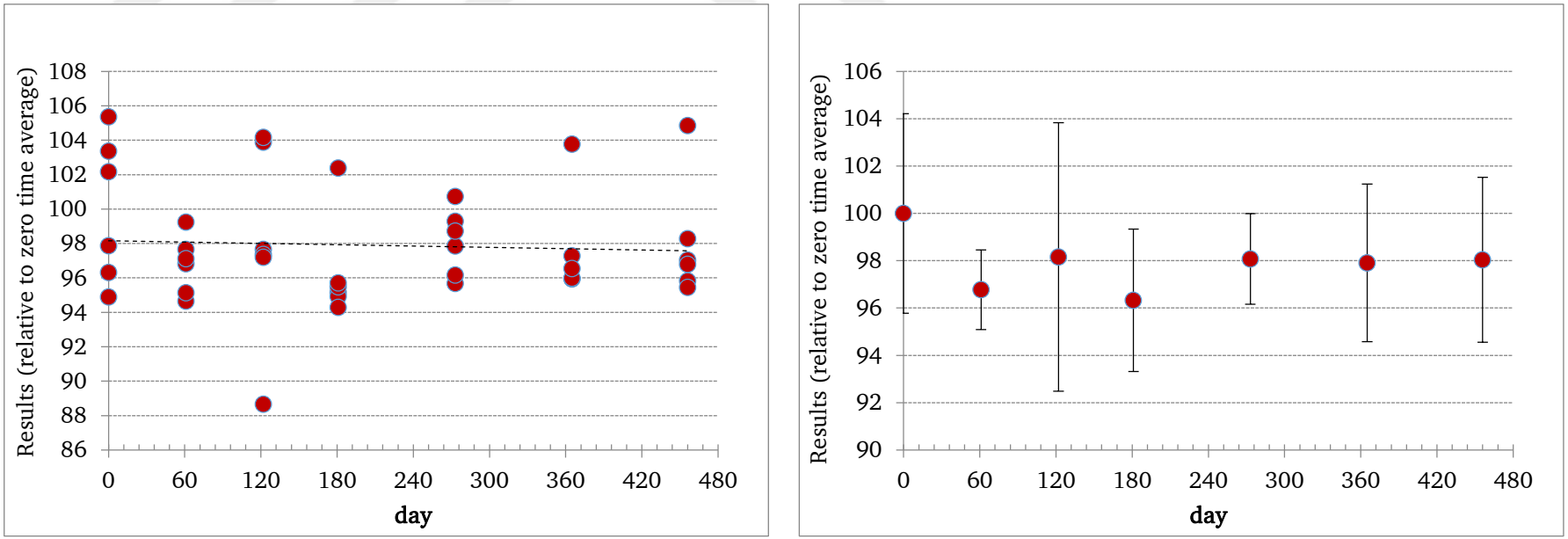


Figure 3.42 Long term stability for Zn

3.1.5 Characterization

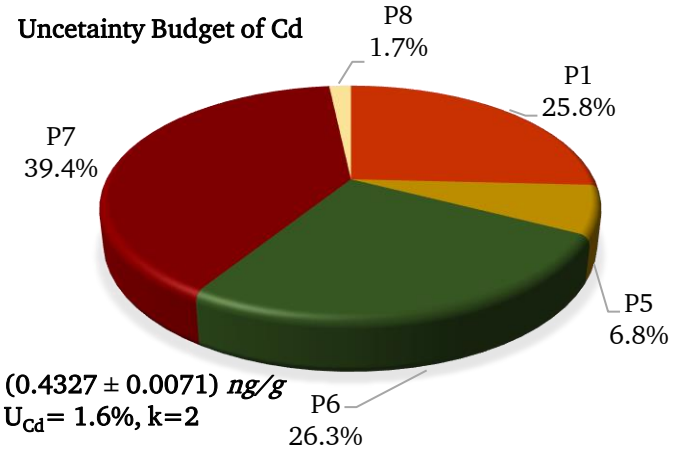
In this study, a reference method by a single laboratory was applied in the characterization of candidate reference material with an introduction of primary reference method. The combination of triple isotope dilution mass spectrometry (TEA/$Mg(OH)_2$-ID^3MS) and triethylamine assisted $Mg(OH)_2$ co-precipitation strategy was used for this purpose. The measurement results were calculated by using both MS Excel files and also Gum Workbench® software [266] and these calculations provided double checking of the results independently. As the summary of characterization results is given in Table 3.13, measurement uncertainty budgets of Cd, Cu, Cr, Ni, Pb and Zn are summarized in Figure 3.43, Figure 3.44 and Figure 3.1 for Fe.

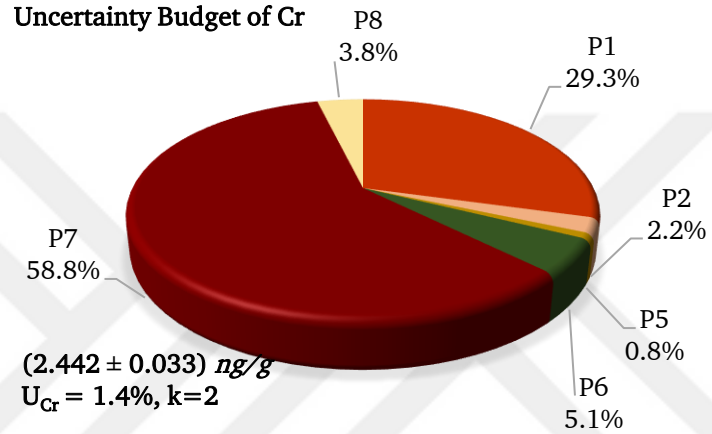
Table 3.13 Summary of UME CRM 1206 characterization measurements

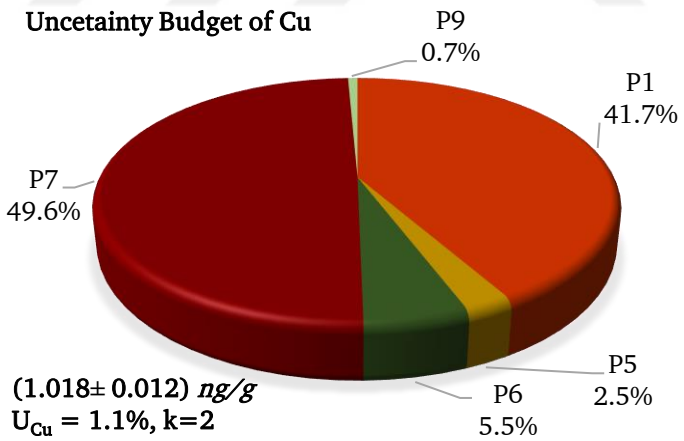
	Value, ng/g (n=12)	U_{char} *(k=2)*
Cd	0.4327	0.0071
Cu	1.018	0.012
Cr	2.442	0.033
Fe	12.732	0.062
Ni	4.568	0.037
Pb	1.068	0.016
Zn	8.521	0.075

The relative expanded uncertainties for Cd, Cr, Cu, Fe, Ni, Pb and Zn were calculated as 1.6%, 1.4%, 1.1%, 0.49%, 0.81%, 1.5% and 0.88%, respectively. With the exception of Fe and Ni, the between day precision was found to be the main contributor to the uncertainty budget, and for Fe and Ni it was sample preparation (weighing). The traditional weighing procedure was used in this study and the overall uncertainty budget may be lowered by using the metrological weighing procedure. At these concentrations, the contribution of IRM on sample and calibration blends was observed to be less than the uncertainty originating from the sample preparation for Cd measurements. The second most significant source of uncertainty was determined to be overall precisions on ratio

measurements. The UME CRM 1206's elemental composition was presented as 1.018 ± 0.012 ng/g for Cu, 0.4327 ± 0.0071 ng/g for Cd, 12.732± 0.0062 ng/g for Fe, 2.442 ± 0.033 ng/g for Cr, 1.068 ± 0.016ng/g for Pb, 4.568 ± 0.037ng/g for Ni and 8.521 ± 0.075 ng/g for Zn.

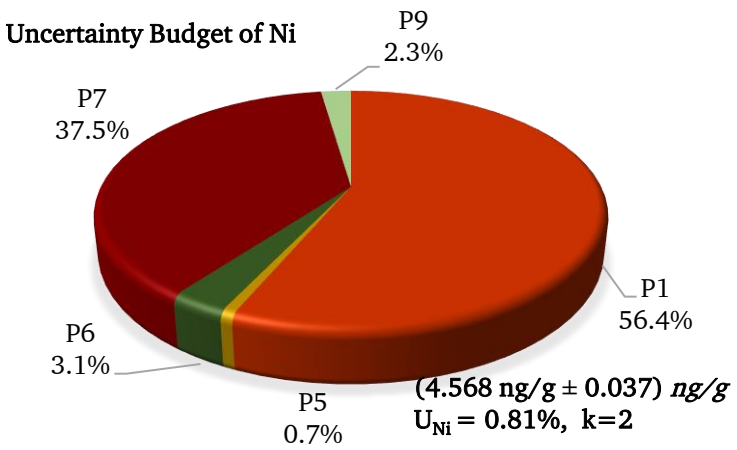


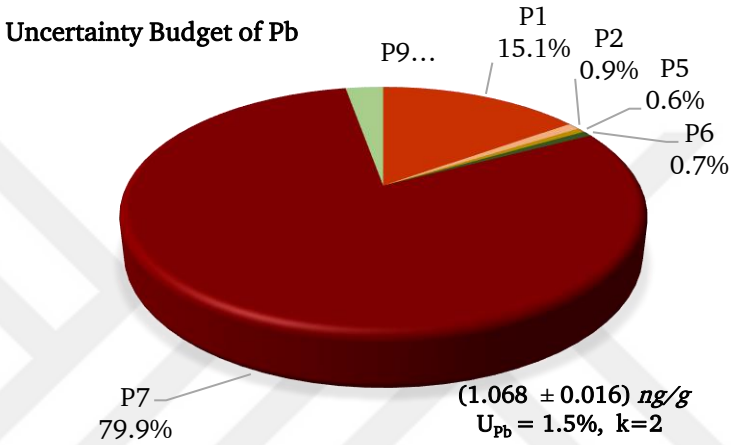


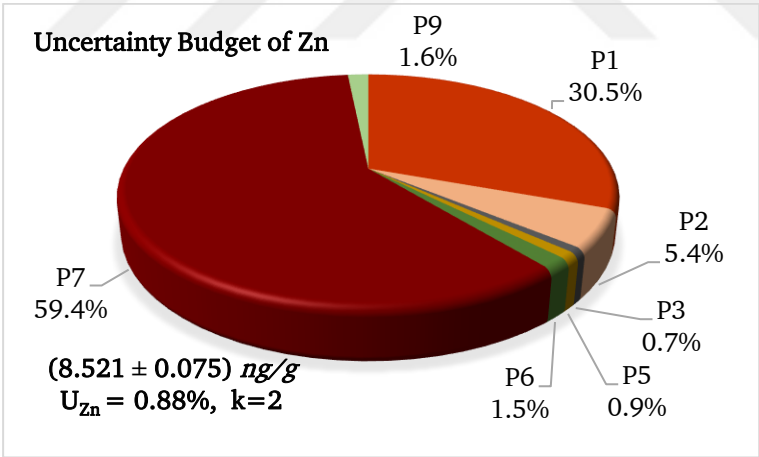


Code	Parameters contributes to uncertainty budget
P1	Sample Preparation (Weighing)
P2	Primary standard reference material
P3	Uncertainty on IUPAC (col 9) isotopic abundance of analyte
P4	Isotopic abundance measurements
P5	Measurements of sample blends ratio
P6	Measurements of calibration blends ratio
P7	Intermediate precision
P8	Reagent blank
P9	Sum of other parameters' contribution

Figure 3.43 Summary of measurements uncertainty budgets for Cd, Cr and Cu







Code	Parameters contributes to uncertainty budget
P1	Sample Preparation (Weighing)
P2	Primary standard reference material
P3	Uncertainty on IUPAC (col 9) isotopic abundance of analyte
P4	Isotopic abundance measurements
P5	Measurements of sample blends ratio
P6	Measurements of calibration blends ratio
P7	Intermediate precision
P8	Reagent blank
P9	Sum of other parameters' contribution

Figure 3.44 Summary of measurements uncertainty budgets for Ni, Pb and Zn

3.1.6 Property Value and Uncertainty Assignment

The uncertainty component of the certified value is composed of the uncertainty contributions from the characterization study (u_{char}), the homogeneity study (u_{bb}), the short-term stability study (u_{sts}) and the long-term stability study (u_{lts}). The uncertainty of the CRM were determined by combining the components affecting value of the assigned uncertainty are calculated using the following equation [59]:

$$U_{CRM} = k\sqrt{u_{char}^2 + u_{bb}^2 + u_{sts}^2 + u_{lts}^2} \tag{3.8}$$

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 3.14 and the contribution of each parameter to U_{CRM} is given in Table 3.15.

Table 3.14 Certified value and uncertainty components

Analyte	Certified Value, µg/kg	$u_{char,rel}$ (%)	$u_{bb,rel}$ (%)	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)	U_{CRM}, µg/kg, *(k=2)*	$U_{CRM,rel}$ % *(k=2)*
As[1]	2.52	0.65	0.56	0.74	1.38	0.10	4.0
Cd	0.433	0.82	0.34	0.29	0.56	0.010	2.32
Cr	2.44	0.68	2.87	0.21	2.64	0.20	7.9
Cu	1.018	0.57	0.78	0.24	0.51	0.022	2.21
Fe	12.73	0.24	5.27	0.28	0.90	1.37	10.7
Ni	4.568	0.41	0.18	0.08	0.12	0.043	0.94
Pb	1.068	0.74	0.08	0.09	0.28	0.017	1.61
Zn	8.52	0.44	1.62	1.13	1.34	0.42	4.9

[1]This value is provided as informative value in the certificate

Table 3.15 Percent contribution of each uncertainty parameter to U_{CRM}

Analyte	u_{char}	u_{bb}	u_{lts}	u_{sts}
As	13.3%	9.8%	59.7%	17.2%
Cd	56.6%	9.7%	26.4%	7.1%
Cr	2.9%	52.4%	44.3%	0.3%
Cu	25.9%	48.5%	20.7%	4.6%
Fe	0.2%	97.0%	2.8%	0.3%
Ni	76.1%	14.7%	6.5%	2.9%
Pb	85.5%	1.0%	12.2%	1.3%
Zn	3.3%	44.6%	30.5%	21.7%

3.2 Metabolization of Inorganic Selenium by Leek

3.2.1 Cultivation of Leek

Cultivation of leek samples was performed as described in section 2.3.2.1. The fortification of Se(IV) and Se(VI) was performed at the at planting stage (0. Day) with concentration given in Table 3.16. Leek samples were divided into root, stem and leaf just after harvested. All the parts of each leek samples were weighed before and after lyophilization and total moisture content (n=46) of root, stems and leaves were found to be 70.5 ± 9.0%, 82.4 ± 8.4% and 88.4 ± 2.4%, respectively. The results of this research has been published by Ari et al.[279].

The effect of inorganic selenium fortification on growth were examined via average dry masses of leek samples. The dried masses of root, stem and leaf of each sample and overall dried masses of leeks fortified by low and high level of selenium are given in Table 3.17 - Table 3.19. Total dried mass of a leek was calculated as sum of the masses of each part (Table 3.20). The growth effects of inorganic selenium culture on leek samples are depicted graphically in Figure 3.45.

Table 3.16 Fortification type and amount of leek samples

Code of Level	Type of fortification	Amount of fortification, μM
1	Se(IV), Se(VI)	20
2	Se(IV), Se(VI)	40
3	Se(IV)	280
4	Se(IV)	450
5	Se(VI)	200
6	Se(VI)	325

Table 3.17 Dried mass of root, stem and leaf of a leek samples belongs to control group

		Level 1 & Level 2			Level 4 & Level 6		
		Root, g	Stem, g	Leaf, g	Root, g	Stem, g	Leaf, g
Control Group	1	0.40	0.35	0.49	0.26	0.25	0.23
	2	0.40	0.24	0.26	0.48	0.32	0.32
	3	0.41	0.51	0.32	0.26	0.27	0.11
	4	-	-	-	0.45	0.50	0.37
	5	-	-	-	0.32	0.35	0.45
Average, g		0.40	0.37	0.36	0.35	0.34	0.30
sd, g		0.01	0.14	0.12	0.10	0.10	0.13

Level 1: 20 μM Se, Level 2: 40 μM Se, Level 4: 450 μM Se(IV), Level 6: 325 μM Se(VI)

Table 3.18 Dried mass of root, stem and leaf of a leek samples cultivated by selenite

		Level - 1			Level - 2			Level-4		
		Root, g	Stem, g	Leaf g	Root, g	Stem, g	Leaf, g	Root, g	Stem, g	Leaf, g
Se(IV) fortified	1	0.71	0.68	0.78	0.72	0.54	0.41	0.42	0.30	0.29
	2	0.35	0.29	0.39	0.35	0.41	0.28	0.32	0.30	0.27
	3	0.58	0.61	0.59	0.59	0.73	0.63	0.40	0.39	0.26
	4	0.49	0.30	0.39	0.69	0.90	0.62	0.39	0.47	0.11
	5	0.38	0.39	0.45	0.33	0.31	0.33	0.30	0.27	0.23
Average, g		0.50	0.46	0.52	0.54	0.58	0.46	0.37	0.34	0.23
Sd		0.15	0.18	0.17	0.19	0.24	0.16	0.05	0.08	0.07

Level 1: 20 μM Se, Level 2: 40 μM Se, Level 4: 450 μM Se(IV)

Table 3.19 Dried mass of root, stem and leaf of a leek samples cultivated by selenate

		Level - 1			Level - 2			Level-6		
		Root, g	Stem, g	Leaf, g	Root, g	Stem, g	Leaf, g	Root, g	Stem, g	Leaf, g
Se(VI) fortified	1	0.28	0.37	0.23	0.47	0.55	0.62	0.73	0.70	0.23
	2	0.24	0.27	0.23	0.70	0.47	0.56	0.68	0.61	0.37
	3	0.46	0.48	0.28	0.76	0.62	0.27	0.51	0.48	0.28
	4	0.31	0.38	0.29	0.29	0.35	0.25	0.48	0.36	0.15
	5	0.40	0.43	0.45	0.63	0.75	0.87	0.46	0.52	0.30
Average, g		0.34	0.39	0.30	0.57	0.55	0.51	0.57	0.53	0.27
Sd		0.09	0.08	0.09	0.19	0.15	0.26	0.12	0.13	0.08

Level 1: 20 μM Se, Level 2: 40 μM Se, Level 6: 325 μM Se(VI)

Table 3.20 Dried mass of whole leek sample fortified by selenium species

		Level-1	Level-2	Level-4 & Level-6
Control Group	Average, g	1.13 ± 0.20	1.13 ± 0.20	0.99 ± 0.29
Se(IV) fortified	Average, g	1.48 ± 0.48	1.57 ± 0.55	0.94 ± 0.10
Se(VI) fortified	Average, g	1.02 ± 0.23	1.63 ± 0.49	1.37 ± 0.28

Level 1: 20 μM Se, Level 2: 40 μM Se, Level 6: 325 μM Se(VI)

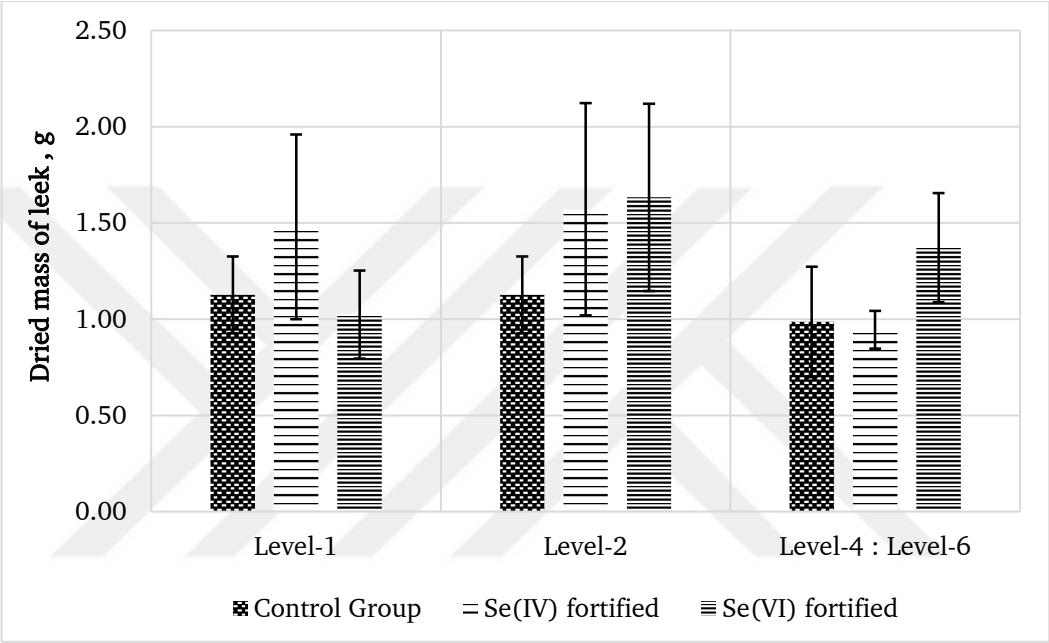


Figure 3.45 Growth effect of selenium species on leek

To investigate the positive and negative effects of Se(IV) and Se(VI) supplementation at low (Level-1, Level-2)and high levels (Level-4, Level-6) on leek samples, statistical evaluations for masses of each part of leek samples including root and entire plant in control groups were performed, as seen in the Table 3.21-Table 3.29. This study found that fortifying leek samples with Se(IV) and Se(VI) at low levels had no significant effect on samples at 95% CI on none of the parts of plant and on whole plant. Moreover, though there was no significant difference for selenite fortification at Level-4 on root, stem, leaf and whole plant, fortification by selenate at Level-6 had a positive significant growth effect on root (p value = 0.021 < 0.05) and stem (p value = 0.028 < 0.05). However, this fortification did not provide significant difference on leaves and finally on whole plants.

Table 3.21 Descriptive statistics on masses for Se(IV) and Se(VI) at level-1 fortification

Group		N	Minimum	Maximum	Mean	Std. Deviation
1	Root, g	3	0.40	0.41	0.4033	0.00577
	Stem, g	3	0.24	0.51	0.3667	0.13577
	Leaf, g	3	0.26	0.49	0.3567	0.11930
	Whole, g	3	0.90	1.24	1.1267	0.19630
2	Root, g	5	0.35	0.71	0.5020	0.14789
	Stem, g	5	0.29	0.68	0.4540	0.18036
	Leaf, g	5	0.39	0.78	0.5200	0.16673
	Whole, g	5	1.03	2.17	1.4760	0.48118
3	Root, g	5	0.24	0.46	0.3380	0.09011
	Stem, g	5	0.27	0.48	0.3860	0.07829
	Leaf, g	5	0.23	0.45	0.2960	0.09044
	Whole, g	5	0.74	1.28	1.0200	0.22760

Group 1: Control, Group 2: Selenite, Group 3: Selenate
Significant figures are kept as reported in statistical evaluation

Table 3.22 Results of test statistics on masses for selenite at level-1 fortification

	Root	Stem	Leaf	Whole
Mann-Whitney U	6.000	5.000	3.000	6.000
Wilcoxon W	12.000	11.000	9.000	12.000
Z	-0.450	-0.745	-1.350	-0.450
Asymp. Sig. (2-tailed)	0.653	0.456	0.177	0.653

Table 3.23 Results of test statistics on masses for selenite at level-2 fortification

	Root	Stem	Leaf	Whole
Mann-Whitney U	4.000	6.000	4.000	5.000
Wilcoxon W	19.000	12.000	19.000	20.000
Z	-1.069	-0.447	-1.050	-0.750
Asymp. Sig. (2-tailed)	0.285	0.655	0.294	0.453

Table 3.24 Descriptive statistics on masses for selenite and selenate at level-2 fortification

Group		N	Minimum	Maximum	Mean	Std. Deviation
1	Root, g	3	0.40	0.41	0.4033	0.0058
	Stem, g	3	0.24	0.51	0.3667	0.1358
	Leaf, g	3	0.26	0.49	0.3567	0.1193
	Whole, g	3	0.90	1.24	1.1267	0.1963
2	Root, g	5	0.33	0.72	0.5360	0.1854
	Stem, g	5	0.31	0.90	0.5780	0.2389
	Leaf, g	5	0.28	0.63	0.4540	0.1629
	Whole, g	5	0.97	2.21	1.5680	0.5488
3	Root, g	5	0.29	0.76	0.5700	0.1904
	Stem, g	5	0.35	0.75	0.5480	0.1511
	Leaf, g	5	0.25	0.87	0.5140	0.2595
	Whole, g	5	0.89	2.25	1.6320	0.4854

Group 1: Control, Group 2: Selenite, Group 3: Selenate
Significant figures are kept as reported in statistical evaluation

Table 3.25 Results of test statistics on masses for selenite at level-2 fortification

	Root	Stem	Leaf	Whole
Mann-Whitney U	6.000	3.000	4.000	4.000
Wilcoxon W	12.000	9.000	10.000	10.000
Z	-0.450	-1.342	-1.043	-1.050
Asymp. Sig. (2-tailed)	0.653	0.180	0.297	0.294

Table 3.26 Results of test statistics on masses for selenate at level-2 fortification

	Root	Stem	Leaf	Whole
Mann-Whitney U	3.000	2.500	5.000	3.000
Wilcoxon W	9.000	8.500	11.000	9.000
Z	-1.350	-1.500	-0.745	-1.350
Asymp. Sig. (2-tailed)	0.177	0.134	0.456	0.177

Table 3.27 Descriptive statistics on masses for selenite and selenate at level-4 and level- 6 fortification

Group		N	Minimum	Maximum	Mean	Std. Deviation
1	Root, g	5	0.26	0.48	0.3540	0.1048
	Stem, g	5	0.25	0.50	0.3380	0.0988
	Leaf, g	5	0.11	0.45	0.2960	0.1311
	Whole, g	5	0.64	1.32	0.9880	0.2862
2	Root, g	5	0.30	0.42	0.3660	0.0527
	Stem, g	5	0.27	0.47	0.3460	0.0826
	Leaf, g	5	0.11	0.29	0.2320	0.0716
	Whole, g	5	0.80	1.05	0.9440	0.0999
3	Root, g	5	0.46	0.73	0.5720	0.1240
	Stem, g	5	0.36	0.70	0.5340	0.1292
	Leaf, g	5	0.15	0.37	0.2660	0.0820
	Whole, g	5	0.99	1.66	1.3720	0.2875

Group 1: Control, Group 2: Level -4 Selenite, Group 3: Level 6-Selenate
Significant figures are kept as reported in statistical evaluation

Table 3.28 Results of test statistics on masses for selenite at level-4 fortification

	Root	Stem	Leaf	Whole
Mann-Whitney U	11.500	11.500	8.000	10.000
Wilcoxon W	26.500	26.500	23.000	25.000
Z	-0.210	-0.210	-0.946	-0.524
Asymp. Sig. (2-tailed)	0.834	0.834	0.344	0.600

Table 3.29 Results of test statistics on masses for selenate at level-6 fortification

	Root	Stem	Leaf	Whole
Mann-Whitney U	1.500	2.000	10.000	5.000
Wilcoxon W	16.500	17.000	25.000	20.000
Z	-2.312	-2.193	-.525	-1.576
Asymp. Sig. (2-tailed)	0.021	0.028	0.599	0.115

3.2.2 Investigation of Selenium Uptake

Because selenate and sulfate have chemical similarities, it has generally been assumed that , sulfate transporters can help plants uptake selenate, and that the selectivity of those transporters changes with ratio of the sulphate to Se(VI) amount in the culture medium [140], [141]. On the other hand, Se(IV) uptake mechanism was not clear up to recent. Previously, it was believed that mechanism of selenite uptake was based on passive diffusion rather than being metabolically

dependent [142]. Recent studies showed that Se(IV) uptake rate changes depending on the concentration of phosphate in growth medium [143]–[145] and phosphate transporters may play a role in Se(IV) uptake by plants [144].

The benefit of hydroponic growth is being a practical way of understanding for selenium uptake by plants. Total selenium amount in cultivation medium (nutritional solution) at cultivated day (0. day) and harvested day (14. day) was measured to figure out total Se uptake. The uptaken amount in relation to exposed selenite amount was determined to be in the range of 60% for Level-1 and 40% for Level-2 and it increased to (95±7)% with Level-3 exposure. In Level-4 exposure, however, it decreased to (39±7)%. The relative uptake of Se(VI) from fortified nutritional solutions, on the other hand, could not exceed 30% with the exception of Level-5 exposure ((92±2)%)

The nutritional solutions sampled systematically from the cultivation medium were also undertaken to assess Se(IV) and Se(VI) uptake rate by leeks throughout the cultivation period. Since leek samples from the same origin were used in cultivation by Se(IV) and Se(VI) at Level-1 and Level-2, and the ratios were same for each species, evaluating the uptake rate of selenium species for these levels is more appropriate. ICP-MS/MS was used to analyze all of these nutritional solutions in order to examine leek selenium uptake behavior. While selenite uptake began on the 2nd day with 15% and finished with the range of 35%-75% at 14th day, noteworthy Se(VI) uptake did not start until 6th day as seen in Figure 3.46 and Figure 3.47. It was noticed that as the exposure concentration in the nutritional solution increased from 20 μM to 40 μM Se(VI), the average intake rate increased, but could not exceed 30%.

In this study, it can be concluded that selenite can be absorbed almost two times more efficient than selenate (˜30%) by leek samples at both two different fortification level and absorption of selenite much easier than selenate by leek samples.

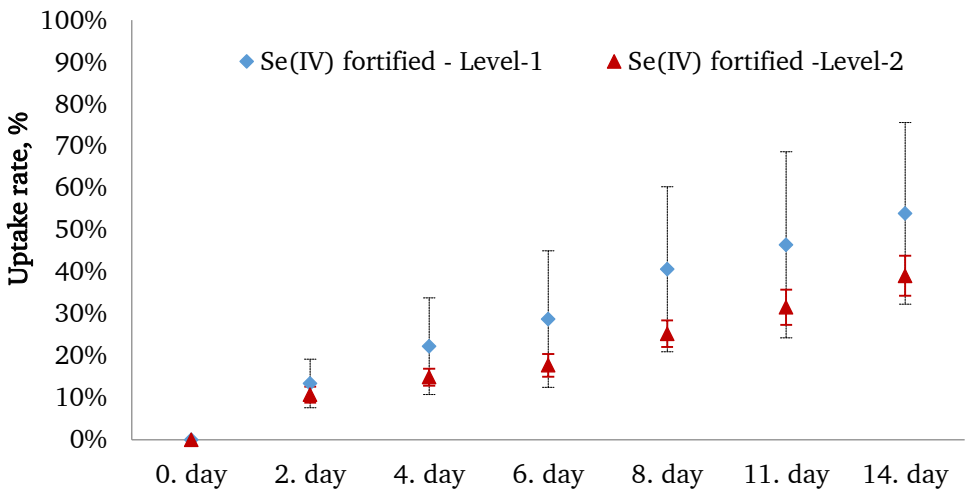


Figure 3.46 Selenite uptake rate by leeks sample

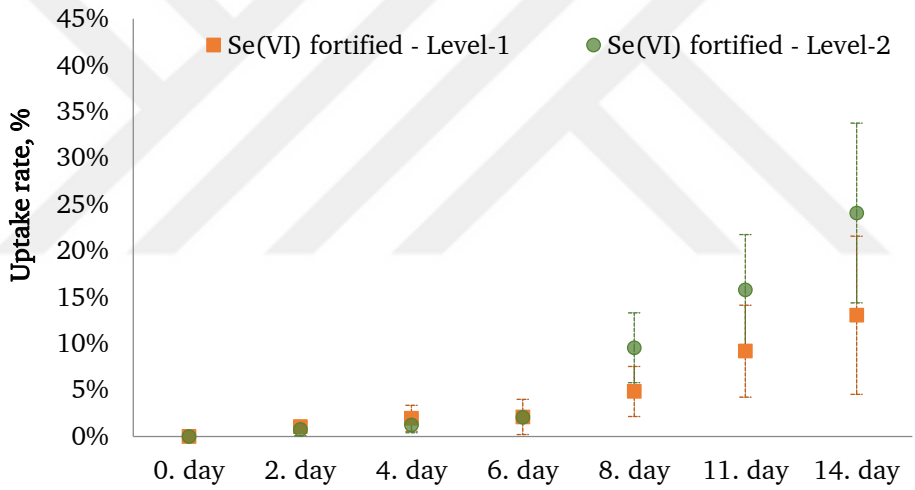


Figure 3.47 Selenate uptake rate by leeks sample

The differences in uptake rate of Se(IV) and Se(VI) between different exposure levels might be resulted from the presence of varied sulfate and phosphate transporters in leek samples and differences in ratio of selenium to transporter as well.

Theoretically, accumulated selenium concentration was also calculated by division of uptake amount which was measured as described above into complete dry mass of leek (root+stem+leaves). This study was performed for each level of fortified leek samples and average value of 5 leek samples was presented in Figure 3.48.

With the exception of the highest doses applied in Se(VI) fortification (325μM, Level-6), Se content in leek rose as exposure doses increased, as it can be seen in Figure 3.48. Plants fed with selenite, on the other hand, show a more pronounced rise in this tendency.

Different capabilities of the ATP sulfurylase enzyme catalyzing the synthesis of APS and APSe in certain cultivation batches might explain why the accumulation rate in Level-6 is lower than other levels [124], [146]. On the other hand, this study revealed that leek may be classified as Se hyper accumulator plant since they could accumulate above 1.0 Se g/kg in dried mass [280] when fortified with Se(IV) at high levels (Figure 3.48).

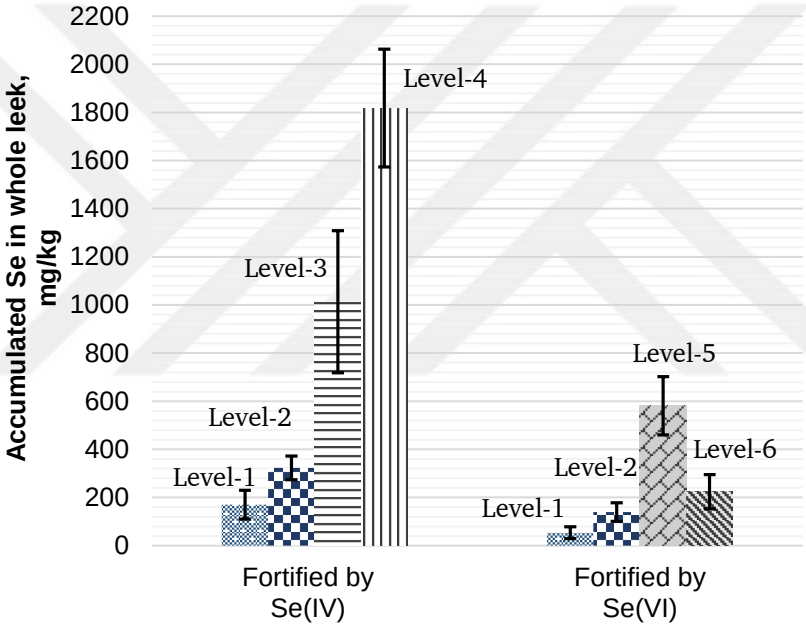


Figure 3.48 Selenium accumulation capacity of leek samples

3.2.3 Selenium Bioaccessibility in Leek

Selenium bioaccessibility can be evaluated by investigating total amount of selenium and species in samples. For this purpose, total selenium amount in leaves and stems was measured as described in section 2.3.2.2 and the speciation analyses were performed as described in 2.3.2.5 as well.

3.2.3.1 Total Selenium Accumulation in Edible Parts of Leek

Although the uptake rate experiments showed that leeks can accumulate high amount of Se, additional research about translocation mechanism of Se in leek is

needed to determine whether Se is present in edible parts. As a result, the edible parts of leek samples (leaf and stem) were mineralized and total selenium amount was evaluated using ICP-MS/MS.

Summary of measurement results obtained for five leek samples is given in Table 3.30. The concentration of Se in control plants for stem and leaf were determined as (11±2) mg/kg and (10±3) mg/kg, respectively. The levels of total Se amount in control group is already high considering the other plants investigated in literature [192], [194], [281] Selenium enrichment was achieved by a factor of 3 to 10 even with fortification at Level-1 and Level-2 with Se(IV) and Se(VI) whereas more significant enrichment was obtained by fortification at higher levels for both species. As it can be seen in Table 3.31 and Table 3.32, with respect to control group, there is a significant difference ($p=0.025 < 0.05$) between control group and Level-1 Se(IV) fortified leek samples while fortification with Se(VI) at Level-1 did not resulted in any significant difference ($p=0.297 > 0.05$). Furthermore, in comparison to the control group, fortification with higher level of selenium, independent of the species, resulted in statistically significant accumulation in leek samples.

Table 3.30 Total Se amount in edible parts of leek samples cultivated by inorganic selenium species

LEAF	Fortified by Se(IV) *Se_{total}, mg/kg, (n=5)*
[1]Control group	11 ± 2
20 μM (Level-1)	38 ± 16
40 μM (Level-2)	50 ± 15
280 μM (Level-3)	169 ± 50
450 μM (Level-4)	120 ± 31

LEAF	Fortified by Se(VI) *Se_{total}, mg/kg,(n=5)*
[1]Control group	11 ± 2
20 μM (Level-1)	28 ± 21
40 μM (Level-2)	101 ± 86
200 μM (Level-5)	257 ± 173
325 μM (Level-6)	242 ± 146

STEM	Fortified by Se(IV) *Se_{total}, mg/kg,(n=5)*
[1]Control group	10 ± 3
20 μM (Level-1)	35 ± 16
40 μM (Level-2)	48 ± 14
280 μM (Level-3)	222 ± 54
450 μM (Level-4)	151 ± 72

STEM	Fortified by Se(VI) *Se_{total}, mg/kg, (n=5)*
[1]Control group	10 ± 3
20 μM (Level-1)	17 ± 10
40 μM (Level-2)	49 ± 24
200 μM (Level-5)	156 ± 85
325 μM (Level-6)	81 ± 50

[1]n=13

Furthermore, comparison between the accumulated amount of selenium in the cases of Se(VI) and Se(IV) fortification was also statistically compared for each level. Mann-Whitney-U test revealed that there was a significant difference at 90% confidence level ($p=0.076 < 0.1$) between Se(IV) fortified and Se(VI) fortified leek samples at Level-1 and fortification by Se(IV) resulted in more Se accumulation in leek. Moreover, this difference was found to be insignificant in the elevated levels as seen in Table 3.33-Table 3.35. On the other hand, though selenate uptake rate which did not mostly exceed 30% is not as high as selenite by leek, total Se results revealed that selenium enrichment was not significantly differ in leek samples which is a sign for higher mobility of selenate. On the contrary, in the cases of fertilization of soil with selenite and selenate, accumulation of selenium in leek [195] and other Allium species [281] were described as being distinct, with more selenate deposition in the plants. The reason of less bioavailability of selenite than selenate for plants cultivated on soil is stronger absorption of former by iron oxides and/or hydroxides and better solubility of latter in water [195].

Table 3.31 Descriptive statistics on total Se (mg/kg) in edible parts for Se(IV) and Se(VI) at level-1 and level-2 fortification

Group	Levels	N	Minimum	Maximum	Mean	Std. Deviation
1	Level-1 Level-2	3	4.30	39.30	21.0000	17.5548
2	Level-1	5	48.40	125.20	72.6800	30.2436
	Level-2	5	77.89	145.87	98.1920	27.3810
3	Level-1	5	17.90	105.00	44.6400	34.8240
	Level-2	5	20.06	256.18	150.7140	107.6157

Group 1: Control, Group 2: Selenite, Group 3: Selenate
Significant figures are kept as reported in statistical evaluation

Table 3.32 Results of test statistics total Se in edible parts for selenite and selenate at level-1 fortification

	Group 1 & 2	Group 1 & 3	Group 2 & 3
Mann-Whitney U	0.000	4.000	4.000
Wilcoxon W	6.000	10.000	19.000
Z	-2.236	-1.043	-1.776
Asymp. Sig. (2-tailed)	0.025	0.297	0.076

Group 1: Control, Group 2: Selenite, Group 3: Selenate

Table 3.33 Results of test statistics total Se in edible parts for selenite and selenate at level-2 fortification

	Group 1 &2	Group 1 &3	Group 2 &3
Mann-Whitney U	0.000	1.000	10.000
Wilcoxon W	6.000	7.000	25.000
Z	-2.236	-1.938	-0.522
Asymp. Sig. (2-tailed)	0.025	0.053	0.602

Group 1: Control, Group 2: Selenite, Group 3: Selenate

Table 3.34 Descriptive statistics on total Se (mg/kg) in edible parts for selenite and selenate at higher level fortification

Group	Levels	N	Minimum	Maximum	Mean	Std. Deviation
1	Level 3&5	5	15.30	27.70	21.8200	5.1722
	Level 4&6	5	11.58	30.74	19.2402	7.8434
2	Level 3&5	5	254.80	490.90	390.4800	94.7123
	Level 4&6	5	190.43	440.58	270.9500	100.1218
3	Level 3&5	5	90.50	743.60	412.5600	254.7538
	Level 4&6	5	157.15	639.22	323.8840	186.0772

Group 1: Control, Group 2: Selenite, Group 3: Selenate
Significant figures are kept as reported in statistical evaluation

Table 3.35 Results of test statistics on total Se in edible parts for selenite and selenate at high level fortification

	Group 1&2		Group 1&3		Group 2&3	
	Level 3&5	Level 4&6	Level 3&5	Level 4&6	Level 3&5	Level 4&6
Mann-Whitney U	0.000	0.000	0.000	0.000	11.000	10.000
Wilcoxon W	15.000	15.000	15.000	15.000	26.000	25.000
Z	-2.611	-2.611	-2.611	-2.611	-0.313	-0.522
Asymp. Sig. (2-tailed)	0.009	0.009	0.009	0.009	0.754	0.602

Group 1: Control, Group 2: Selenite, Group 3: Selenate

Translocation of selenium species was evaluated by comparing translocation factors (TF) which was calculated using the fallowing the Equation 3.9

$$TF = \frac{C_{stem}}{C_{Leaf}} \tag{3. 9}$$

where C_{stem} and C_{leaf} are total amount of Se in stem and leaf, respectively.

The calculated translocation factors belong to different exposure concentration of selenite and selenate is tabulated in Table 3.36 and graphical representation is shown in Figure 3.49.

Table 3.36 Translocation factor of Se species in leek at different exposure concentration

	Fortified by Selenite ([1]n=5)	**Fortified by Selenate** ([1]n=5)
Control Group	0.966 ± [2]0.565	
Level-1	0.930 ± 0.202	0.812 ± 473
Level-2	0.975 ± 0.197	1.217 ± 1.460
Level-3	1.363 ± 0.343	
Level-5		0.675 ± 0.151
Level-4	1.232 ± 0.328	
Level-6		0.485 ± 0.447

[1]represents the number of leek samples investigated
[2]n=13

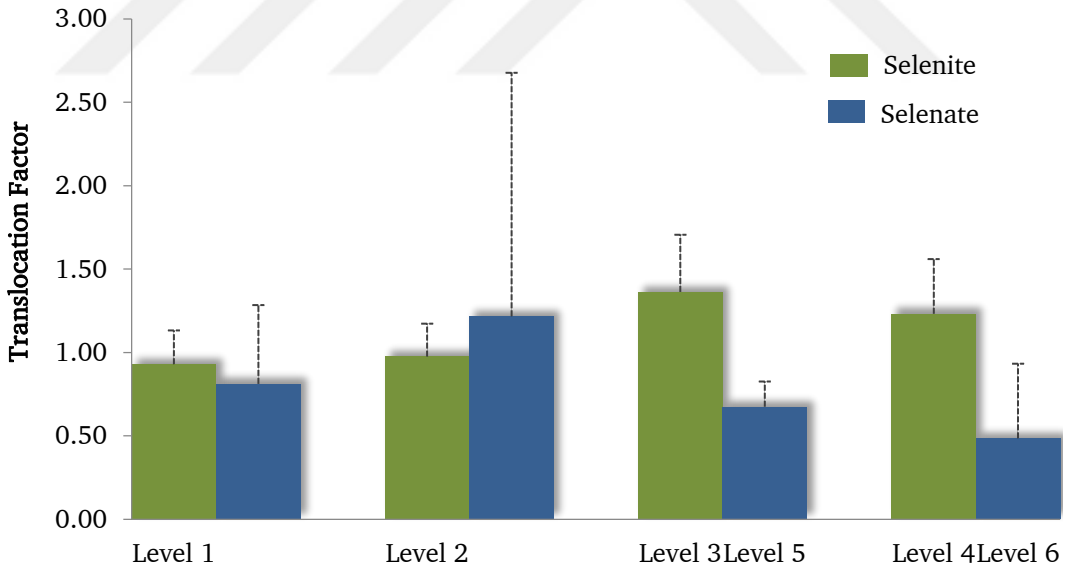


Figure 3.49 Graphical representation of translocation of inorganic selenium in leek

Statistical evaluations given in Table 3.37 and Table 3.38 revealed that localization of selenium in stem and leaf did not differ from each other at low levels supplementation with Se(IV) or Se(VI). However, as the molarity of Se(VI)

and Se(IV) increase in the cultivation medium, total amount of selenium in leaf and stems became to be different. This difference showed that selenate localized more in leaves than stems at elevated level supplementation. The reason of this phenomena can be explained as the rate of conversion of Se(IV) into low mobility organo-selenium species in plant tissue is faster than Se(VI) in roots. Similar conclusions were also reported for , green onion, lettuce, indian mustard, rice, sugar beet and broccoli [148], [282], [283].

Table 3.37 Descriptive statistics on translocation of selenium in edible parts

	Group	N	Minimum	Maximum	Mean	Std. Deviation
Level-1	1	5	0.61	1.13	0.9297	0.2025
	2	5	0.41	1.52	0.8116	0.4726
Level-2	1	5	0.67	1.18	0.9752	0.1973
	2	4	0.32	1.08	0.5775	0.3392
Level-3 Level-5	1	5	0.99	1.92	1.3627	0.3428
	2	5	0.49	0.87	0.6746	0.1515
Level-4 Level-6	1	5	0.86	1.66	1.2318	0.3280
	2	5	0.12	1.26	0.4852	0.4473

Group 1: Selenite, Group 2: Selenate
Significant figures are kept as reported in statistical evaluation

Table 3.38 Results of test statistics on translocation of selenium in edible parts

	Level-1	Level-2	Level-3&5	Group 4&6
Mann-Whitney U	9.000	4.000	0.000	3.000
Wilcoxon W	24.000	14.000	15.000	18.000
Z	-0.731	-1.470	-2.611	-1.984
Asymp. Sig. (2-tailed)	0.465	0.142	0.009	0.047

The difference between the theoretical Se amount calculated using measurement results of Se amount in nutritional solutions as shown in Figure 3.48 and total Se measured in stems and leaves of cultivated leek samples by Se(IV) fortification further revealed that selenium transportation to edible sections could not occured efficiently, and proposing that the most of uptaken selenium was likely remained in roots.

3.2.3.2 Results of Enzymatic Extraction

Total Se in extracted solutions was used to determine the extraction efficiencies of each leek sample's leaves and stems. Extracted solutions digestion were carried out as described in the section 2.3.2.2, and ICP-MS/MS was utilized to quantify total Se in the solution using matrix matched external calibration technique under optimized tune parameters given in Table 2.10. The results are provided in Table 3.39- Table 3.42.

Table 3.39 Extraction efficiencies determined for Level-1 fortification by Se(IV) and Se(VI)

Level of Fortification	Sample Code	[1]Extraction Efficiency	Sample Code	[1]Extraction Efficiency
Level- 1 Control Group	Leaf-1	72%	Stem-1	81%
	Leaf-2	67%	Stem-2	38%
	Leaf-3	54%	Stem-3	57%
Level-1 Fortified by Se(IV)	Leaf-1	51%	Stem-1	32%
	Leaf-2	49%	Stem-2	42%
	Leaf-3	54%	Stem-3	73%
	Leaf-4	73%	Stem-4	76%
	Leaf-5	65%	Stem-5	79%
Level-1 Fortified by Se(VI)	Leaf- 1	53%	Stem- 1	63%
	Leaf- 2	83%	Stem- 2	77%
	Leaf- 3	66%	Stem- 3	72%
	Leaf- 4	92%	Stem- 4	81%
	Leaf- 5	80%	Stem- 5	77%

[1]RSD < 5.8% (n=3; from a single enzymatic extract belongs to Level-1)

Table 3.40 Extraction efficiencies determined for Level-2 fortification by Se(IV) and Se(VI)

Level of Fortification	Sample Code	[1]Extraction Efficiency	Sample Code	[1]Extraction Efficiency
Level- 2 Control Group	Leaf-1	72%	Stem-1	81%
	Leaf-2	67%	Stem-2	38%
	Leaf-3	54%	Stem-3	57%
Level-2 Fortified by Se(IV)	Leaf-1	67%	Stem-1	73%
	Leaf-2	62%	Stem-2	70%
	Leaf-3	63%	Stem-3	51%
	Leaf-4	48%	Stem-4	62%
	Leaf-5	61%	Stem-5	64%
Level-2 Fortified by Se(IV)	Leaf- 1	79%	Stem- 1	87%
	Leaf- 2	67%	Stem- 2	80%
	Leaf- 3	67%	Stem- 3	66%
	Leaf- 4	82%	Stem- 4	78%
	Leaf- 5	76%	Stem- 5	83%

[1]RSD < 5.8% (n=3; from a single enzymatic extract belongs to Level-1)

Table 3.41 Extraction efficiencies determined for Level-3 and Level-5 fortification

Level of Fortification	Sample Code	[1]Extraction Efficiency	Sample Code	[1]Extraction Efficiency
Control Group of Level 3 & Level 5	Leaf-1	74%	Stem-1	56%
	Leaf-2	80%	Stem-2	58%
	Leaf-3	77%	Stem-3	55%
	Leaf-4	78%	Stem-4	72%
	Leaf-5	92%	Stem-5	23%
Level-3 Fortified by Se(IV)	Leaf-1	86%	Stem-1	66%
	Leaf-2	82%	Stem-2	74%
	Leaf-3	81%	Stem-3	80%
	Leaf-4	87%	Stem-4	71%
	Leaf-5	76%	Stem-5	70%
Level-5 Fortified by Se(IV)	Leaf-1	96%	Stem-1	85%
	Leaf-2	102%	Stem-2	91%
	Leaf-3	98%	Stem-3	95%
	Leaf-4	105%	Stem-4	89%
	Leaf-5	87%	Stem-5	91%

[1]RSD < 5.8% (n=3; from a single enzymatic extract belongs to Level-1)

Table 3.42 Extraction efficiencies determined for Level-4 and Level-6 fortification

Level of Fortification	Sample Code	[1]Extraction Efficiency	Sample Code	[1]Extraction Efficiency
Control Group of Level 4 & Level 6	Leaf-1	20%	Stem-1	87%
	Leaf-2	33%	Stem-2	62%
	Leaf-3	26%	Stem-3	46%
	Leaf-4	63%	Stem-4	26%
	Leaf-5	23%	Stem-5	-
Level-4 Fortified by Se(IV)	Leaf-1	62%	Stem-1	14%
	Leaf-2	78%	Stem-2	68%
	Leaf-3	75%	Stem-3	51%
	Leaf-4	77%	Stem-4	53%
	Leaf-5	63%	Stem-5	64%
Level-6 Fortified by Se(IV)	Leaf-1	74%	Stem-1	62%
	Leaf-2	84%	Stem-2	78%
	Leaf-3	31%	Stem-3	82%
	Leaf-4	75%	Stem-4	97%
	Leaf-5	104%	Stem-5	56%

[1]RSD <5.8% (n=3; from a single enzymatic extract belongs to Level-1)

3.2.3.3 Biotransformation of Selenite and Selenate in Leek

Considering the beneficial or toxic effects of selenium, chemical form of selenium in edible parts of plant is critical for more efficient selenium fortification in crops [284], [285]. The total selenium content in extracted solution, as well as the findings of speciation analysis in the same solutions were used to evaluate biotransformation of inorganic selenium species in this study. While Se(VI) remains mostly as it is in leaves and stems, the biotransformation rate of Se(IV) at each level was found to be more than 90% (Table 3.43).

Table 3.43 Biotransformation of inorganic species uptaken by leek

Biotransformation Rate, %				
		Control Group ([1]n=5)	Fortified by Se(IV) ([1]n=5)	Fortified by Se(VI) ([1]n=5)
Leaf	Level 1	78 ± [2]23	95 ± 2	52 ± 15
	Level 2		96 ± 1	59 ± 31
	Level 3	53 ± 20	92 ± 7	
	Level 4	99 ± 3	95 ± 2	
	Level 5	53 ± 20		31 ± 27
	Level 6	99 ± 3		30 ± 13
Stem	Level 1	49 ± [2]43	94 ± 4	45 ± 12
	Level 2		96 ± 3	45 ± 31
	Level 3	51 ± 31	91 ± 4	
	Level 4	61 ± 28	84 ± 8	
	Level 5	51 ± 31		60 ± 12
	Level 6	61 ± 28		39 ± 26

[1]Represents the number of leek samples investigated
[2]n=3

The experimental data also proved that metabolization of selenite into organo-selenium species is more effective and Se(VI) remains mostly as it is in leek samples cultivated by $Se(VI)^-$ fortification as it was stated in literature [194], [286], [287].

3.2.3.4 Characterization of Selenium Species in Leek

Enzymatically extracted leaves and stems were also exposed to speciation analyses to investigate the which kind of selenium species were come into existence by cultivation of leek samples enriched in selenite or selenate fortified medium at different concentrations. As the determination of selenite and selenate were quantified by SAX-HPLC-ICP-MS/MS, $Se(Cys)_2$, MeSeCys and SeMet were determined by IP-HPLC-ICP-MS/MS system. The findings of analyzing the leaves and stems of leek samples fortified by selenite and selenate at various doses are presented in Table 4.44 and Table 4.45, respectively. The amount of $Se(Cys)_2$ in the extracted solutions was below LOQ, hence it was not given in those tables.

The recovery rate of sum of the all quantified Se species to total Se amount in these solutions were also evaluated using equation 3.10.

$$R_{EE} = \frac{C_{SSE}}{C_{TE}} \quad (3.10)$$

where,

$R_{EE:}$ recovery rate in enzymatically extract, C_{TE}: total concentration of Se in extract, C_{SSE}:, sum of four Se species in extract quantified by speciation analysis.

The recovery rates in leaves of control group, samples cultivated by fortification of Se(IV) and Se(VI) were found as 133±61% (n=13), 80±15% (n=19) and 95±8% (n=19), respectively. Furthermore, the recovery rates in stems for those samples were recorded as 116±25% (n=13), 94±27% (n=19) and 100±13% (n=19), respectively. As provided in Table 4.43, practically all kinds of selenium species were effectively quantified in the samples cultivated by Se(VI) fortification, however there might be some undefined organo-selenium species in the samples cultivated by Se(IV) in which the rate of biotransformation is above 90%.

Overall data demonstrated that leek samples cultivated with low levels selenium fortification had accumulated less amount of inorganic selenium in edible parts, with MeSeCys and SeMet being the most prominent organo-selenium species in Se fortified leek samples, as shown in Table 3.44 and Table 3.45. Though, the amount of organo-selenium species increase as Se fortification amount increase in leek samples, approximately 10-15 times higher inorganic selenium species were also accumulated in whole edible parts in especially Se(VI) fortified leeks (Table 3.46). The reason of this might be the limiting metabolization steps that take place from root to leaves. Therefore, hydroponic cultivation of leeks with low level Se fortification is more preferable. Additionally, it was already known and also observed in this study that as selenate must be reduced to selenite in metabolization pathway, metabolization of selenate is more difficult. Therefore, it tend to be accumulated as selenate equal or more than organo-selenium species. In conclusion, cultivation of leek samples with low level of selenite fortification is recommended in order to get more healthy and nutritional food.

Table 3.44 Summarized measurement results for selenium species determined in the extracts of leaves

LEAF ([1]n=5)	[3]Se (IV), mg/kg	[4]Se (VI), mg/kg	[5]$Se_{MeSeCys}$, mg/kg	[6]Se_{SeMet}, mg/kg
[2]Control group	2.18 ± 1.74	N.D.	3.37 ± 1.78	2.18 ± 1.36
20 μM Level-1: Se (IV)	0.94 ± 0.28	0.14 ± 0.01	6.51 ± 3.66	5.05± 1.74
20 μM Level-1: Se (VI)	0.65 ± 0.34	11.2 ±13.5	3.33 ± 2.31	4.48 ± 4.50
40 μM Level-2 : Se(IV)	1.09 ± 0.39	0.24 ±0.09	9.96 ± 5.14	10.8 ± 3.88
40 μM Level-2 : Se(VI)	1.02 ± 0.51	35.0 ± 43.2	5.64 ± 3.61	10.2± 7.0
280 μM Se (IV) (Level-3)	8.39 ± 5.23	3.02 ± 0.59	83.5 ± 42.4	33.4 ± 14.5
450 μM Se (IV) (Level-4)	3.68 ± 1.75	0.97 ± 0.16	54.4 ±26. 6	16.1 ± 5.2
200 μM Se (VI) (Level-5)	6.23 ± 2.51	204 ± 170	29.7 ±13.5	25.2 ± 12.5
325 μM Se (VI) (Level-6)	1.84 ± 0.98	132 ± 131	30.2 ± 10.9	36.6 ± 27.8

[1]represents the number of leek samples investigated
[2]n=13
[3]RSD < 5.4% (n=3; derived from a single Level-1 enzymatic extract)
[4]RSD <4.9% (n=3; derived from a single Level-1 enzymatic extract)
[5]RSD <6.4% (n=3; derived from a single Level-1 enzymatic extract)
[6]RSD <2.9% (n=3; derived from a single Level-1 enzymatic extract)
N.D.: Not Detected

Table 3.45 Summarized measurement results for selenium species determined in the extracts of stems

STEM ([1]n=5)	[3]Se (IV), mg/kg	[4]Se (VI), mg/kg	[5]$Se_{MeSeCys}$, mg/kg	[6]Se_{SeMet}, mg/kg
[2]Control group	2.05 ± 1.73	N.D.	4.15 ± 4.40	0.61 ± 0.39
20 μM Level-1: Se(IV)	0.77 ± 0.15	0.28 ± 0.34	18.7 ± 9.0	1.29 ±1.03
20 μM Level-1: Se(VI)	0.72 ± 0.51	5.94 ± 2.66	5.18 ± 4.30	0.91 ± 0.56
40 μM Level-2 : Se(IV)	0.96 ± 0.60	0.16 ± 0.15	34.6 ± 15.6	3.97 ± 2.32
40 μM Level-2 : Se(VI)	0.38 ± 0.39	23.5 ±14.7	18.1± 14.0	3.45 ± 2.65
280 μM Se(IV) (Level-3)	13.4 ±4.1	0.28 ± 0.63	111 ± 52	17.8± 6.6
200 μM Se(VI) (Level-5)	5.82 ± 1.44	55.9 ± 38.2	54.7±19.4	13.8 ± 8.1
450 μM Se(IV) (Level-4)	16.5 ± 19.5	N.D.	43.0 ± 30.0	6.36 ± 4.62
325 μM Se(VI) (Level-6)	1.77 ± 1.32	49.3±68.0	6.88 ± 5.75	2.54 ± 0.87

[1]represents the number of leek samples investigated
[2]n=13
[3]RSD < 5.4% (n=3; derived from a single Level-1 enzymatic extract)
[4]RSD <4.9% (n=3; derived from a single Level-1 enzymatic extract)
[5]RSD <6.4% (n=3; derived from a single Level-1 enzymatic extract)
[6]RSD <2.9% (n=3; derived from a single Level-1 enzymatic extract)
N.D.: Not Detected

Table 3.46 Total amount of selenium species in edible parts of leek

Whole edible part ([1]n=5)	Se(IV), mg/kg	Se(VI), mg/kg	$Se_{MeSeCys}$, mg/kg	Se_{SeMet}, mg/kg
[2]Control group	3.4 ± 3.2	N.D.	7.5 ± 5.5	2.8 ± 1.4
20 μM Level-1: Se(IV)	1.7 ± 0.2	0.5 ± 0.3	25.3 ± 11.4	6.3 ± 2.4
20 μM Level-1: Se(VI)	1.4 ± 0.8	17.2 ± 16.0	8.5 ± 6.0	5.4 ± 5.0
40 μM Level-2 : Se(IV)	2.0 ± 0.6	0.3 ± 0.3	44.6 ± 19.9	14.7 ± 5.8
40 μM Level-2 : Se(VI)	1.4 ± 0.6	58.4 ± 50.4	23.8 ± 17.1	13.7 ± 9.5
280 μM Se(IV) (Level-3)	21.8 ± 8.3	1.0 ± 2.2	194 ± 17	51.2 ± 17.2
200 μM Se(VI) (Level-5)	12.1 ± 3.4	260 ± 205	84.4 ± 24.1	39.0 ± 20.2
450 μM Se(IV) (Level-4)	20.2 ± 20.0	0.6 ± 0.5	97.4 ± 54.9	22.5 ± 7.0
325 μM Se(VI) (Level-6)	3.2 ± 3.4	181 ± 133	37.1 ± 7.5	39.1 ± 28.0

[1]represents the number of leek samples investigated
[2]n=13
N.D.: Not Detected

3.2.4 Selenium Bioavailability form Leek

In this study, selenium bioavailability refers to the fraction of soluble selenium released after gastrointestinal (GI) digestion and is available for subsequent processes of absorption through human intestinal mucosa. This soluble fraction was estimated by simulating gastrointestinal digestion system of human beings as it described in section 2.3.2.4 and three representative leek samples from each fortification level were investigated. After simulated gastrointestinal digestion, the total Se quantity in leaves and stems was evaluated as described in 2.3.2.2 and bioavailability rate was calculated using the following equation:

$$Bioavailability\ Rate = \frac{C_{TGI}}{C_T} \text{ x } 100\% \qquad (3.11)$$

where,
C_{TGI} : total amount of Se in gastrointestinal digest, C_T : total amount of Se in dried sample

The results are summarized in Table 3.47.

Table 3.47 Bioavailability of selenium from leaves and stems of Se enriched leeks

LEAF	**Bioavailability Rate, %** ([1]n=3)	**STEM**	**Bioavailability Rate, %** ([2]n=3)
20 μM **Level-1: Se(IV)**	76 ± 18	20 μM **Level-1: Se(IV)**	79 ± 29
20 μM **Level-1: Se(VI)**	95 ± 5	20 μM **Level-1: Se(VI)**	89 ± 6
40 μM **Level-2 : Se(IV)**	68 ± 11	40 μM **Level-2 : Se(IV)**	71 ± 16
40 μM **Level-2 : Se(VI)**	90 ± 7	40 μM **Level-2 : Se(VI)**	90 ± 2
280 μM (Level-3: Se(IV)	85 ± 1	280 μM (Level-3: Se(IV)	90 ± 8
200 μM (Level-5 Se(VI)	92 ± 3	200 μM (Level-5 Se(VI)	91 ± 5
450 μM (Level-4 : Se(IV)	81 ± 7	450 μM (Level-4 : Se(IV)	59 ± 16
325 μM (Level-6 : Se(VI)	69 ± 18	325 μM (Level-6 : Se(VI)	85 ± 2

[1]$RSD_{total\ Se}$ <1.4 % (n=3; derived from a single Level-4 gastrointestinal digest)
[2]$RSD_{total\ Se}$< 0.7 % (n=3; derived from a single Level-6 gastrointestinal digest)

As it can be seen in the Table 3.47, bioavailability rate of selenium in leaves and stems fortified by selenate is obviously higher than fortified by selenite leeks. As a result, while selenite's biotransformation into organo-selenium compounds in leek were clearly higher than selenate's, the bioavailability of selenite derived selenium species in different parts of leek is slightly lower than selenate derived selenium species. Furthermore, because fortification with Se(VI) resulted in remarkable accumulation of selenium in selenate form, as stated in section 3.2.3.4, it can be concluded that selenate gets more easily available in human intestinal mucosa after gastrointestinal digestion in this matrix. This theory was also supported by speciation analyses for gastrointestinal digested samples as seen in Table 3.48 and Table 3.49.

Equation 3.12 was used to evaluate the recovery rates of all Se species measured to total Se amount in the gastrointestinal digest sample.

$$R_{GIE} = \frac{C_{SSGI}}{C_{TGI}} \quad (3.12)$$

where,

R_{GIE} : Recovery rate in gastrointestinal digest, C_{TGI} : Total concentration of Se in gastrointestinal digest, C_{SSGI} : Sum of four Se species in gastrointestinal digest.

The recovery rates in leaves of Se(IV) fortified samples and Se(VI) fortified samples were found to be 76±8% (n=12) and 93±15% (n=12), respectively and similar recovery rates in stems were found to be 76±12% (n=12) for Se(IV) fortified and 93±6% (n=12) for Se(VI). Therefore, it can be concluded that there are some certain amount of organo-selenium species that could not be quantified by chromatographic analysis in Se(IV) supplemented samples which have higher biotransformation rate capacity as shown in Table 3.43.

While leaves fortified with lowest level of Se(VI) exhibited an similar distribution of Se(VI) and MeSeCys, selenate was found to be the most prevalent species in GI digested leave fortified with Se(VI) at higher levels as shown in Table 3.48. Selenate and MeSeCys, on the other hand, were found to be in equal and as the most dominant species at stems in the leeks treated with Se(VI) as shown in Table 3.49. Furthermore, in leaves and stems of leek samples supplemented by Se(IV), the most dominant species was found to be MeSeCys.

Table 3.48 Summarized measurement results for selenium species determined in the gastrointestinal digested leaf of leeks

LEAF [1] n=3	[2]Se(IV), mg/kg	[3]Se(VI), mg/kg	[4]$Se_{MeSeCys}$, mg/kg	[5]Se_{SeMet}, mg/kg
20 μM **Level-1: Se(IV)**	1.7 ± 0.4	N.D.	14.7 ± 7.3	2.1 ± 0.6
20 μM **Level-1: Se(VI)**	1.1 ± 0.3	5.6 ± 3.8	6.9 ± 3.5	1.0 ± 0.5
40 μM **Level-2 : Se(IV)**	1.3 ± 0.3	N.D.	18.2 ± 4.4	5.1 ± 1.7
40 μM **Level-2 : Se(VI)**	0.9 ± 0.8	83 ± 27	36.5 ± 8.4	9.7 ± 1.3
280 μM Se(IV) (Level-3)	9.7 ± 4.6	3.1 ± 3.3	82 ± 53	12.3 ± 5.7
200 μM Se(VI) (Level-5)	3.5 ± 0.3	165 ± 78	28 ± 11	9.3 ± 4.9
450 μM Se(IV) (Level-4)	6.3 ± 1.0	0.6 ± 0.1	65± 23	5.9 ± 3.0
325 μM Se(VI) (Level-6)	7.9 ± 1.0	166 ± 178	41.1 ± 5.4	21 ± 17

[1]represents the number of leek samples investigated
[2]RSD < 3.7% (n=3; derived from a single Level-6 gastrointestinal digest
[3]RSD < 1.1% (n=3; derived from a single Level-6 gastrointestinal digest)
[4]RSD < 4.3% (n=3; derived from a single Level-6 gastrointestinal digest)
[5]RSD < 3.5% (n=3; derived from a single Level-6 gastrointestinal digest)
N.D.: Not Detected

Table 3.49 Summarized measurement results for selenium species determined in the gastrointestinal digested stem of leeks

STEM[1] n=3	Se(IV), mg/kg	Se(VI), mg/kg	$Se_{MeSeCys}$, mg/kg	Se_{SeMet}, mg/kg
20 μM **Level-1: Se(IV)**	1.7 ± 0.7	0.2 ± 0.3	21.6 ± 7.0	0.5 ± 0.3
20 μM **Level-1: Se(VI)**	1.0 ± 0.2	3.3 ± 0.8	3.9 ± 1.2	0.4 ± 0.1
40 μM **Level-2 : Se(IV)**	2.2 ± 0.8	N.D.	19.7 ± 6.9	2.2 ± 0.7
40 μM **Level-2 : Se(VI)**	2.2 ± 0.3	23.4 ± 8.1	29 ± 11	2.6 ± 0.7
280 μM Se(IV) (Level-3)	14.5 ± 3.4	1.5 ± 0.6	120 ± 49	9.1 ± 2.4
200 μM Se(VI) (Level-5)	8.7 ± 4.1	63 ± 29	54.5 ± 7.0	8.1 ± 3.5
450 μM Se(IV) (Level-4)	15.1 ± 6.2	N.D.	44 ± 31	5.6 ± 3.4
325 μM Se(VI) (Level-6)	9.3 ± 0.5	27 ± 11	13.5 ± 0.8	1.5 ± 0.2

[1]represents the number of leek samples investigated
[2]RSD < 3.7% (n=3; derived from a single Level-6 gastrointestinal digest)
[3]RSD < 1.1% (n=3; derived from a single Level-6 gastrointestinal digest)
[4]RSD < 4.3% (n=3; derived from a single Level-6 gastrointestinal digest))
[5]RSD < 3.5% (n=3; derived from a single Level-6 gastrointestinal digest)
N.D.: Not Detected

3.3 Multi-element Composition in Walnuts and Provenance Soils for Geographical Traceability

3.3.1 Method Validation for Determination of Total Elemental Mass Fraction in Walnut

An analytical method using SF-ICP-MS was developed for determination of total elemental mass fraction in walnut samples. Method development and validation was studied using UME CRM1202 *Elements in Hazelnut* and NIST 2387 *Peanut Butter* and UMECRM 1202 *Elements in Peanut*.

The samples were digested using the pressure and temperature-controlled microwave digestion system (Digestion Program-1) as described in section 2.3.3.2. In order to minimize matrix effects, matrix matched external calibration technique was applied. Additionally, internal standard (^{115}In) was also used to be able to minimize effects of evaporation during measurement period and instant performance changes in plasma conditions and/or sample introduction systems. Sample preparation and determination of As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Sr, P, Pb, Sb, Sr and Zn were subdivided in three groups concerning best working ranges of each elements in ICP-MS and also procedural blank levels. These groups are given in Table 3.50. For trace, minor and major elements, samples were diluted 1.4, 10 and 200 folds before introducing to ICP-MS. All sample preparation was performed gravimetrically.

Limit of detection, limit of quantification, repeatability (within day precision), intermediate precision (between day precision), accuracy, linearity, working range and selectivity have been investigated in the method validation.

Table 3.50 Analytes and working range of method in peanut

Groups	Analyte	Isotope	Resolution Mode	Working Range of the Method, ng/g
TRACE	As	^{75}As	HR	2 - 17
	B	^{10}B, ^{11}B	MR	8000 - 60000
	Cd	^{112}Cd, ^{114}Cd	LR	4 - 28
	Cr	^{52}Cr, ^{53}Cr	MR	100 - 500
	Na	^{23}Na	LR	500 - 3400
	Pb	^{206}Pb, ^{207}Pb, ^{208}Pb	LR	2-14
	Sb	^{121}Sb, ^{123}Sb	LR, MR	0.4 - 3
	Analyte			**Working Range of the Method, μg/g**
MINOR	Ba	^{135}Ba, ^{137}Ba	MR	3.0 - 37
	Co	^{59}Co	MR	0.1 - 1.7
	Cu	^{63}Cu, ^{65}Cu	MR	9 - 109
	Ni	^{60}Ni, ^{62}Ni	MR	0.9 - 10.5
	Sr	^{86}Sr, ^{88}Sr	MR	3.5 - 41.5
	Zn	^{66}Zn, ^{67}Zn, ^{68}Zn	MR	11 - 137
	Analyte			**Working Range of the Method, μg/g**
MAJOR MAJOR	Ca	^{42}Ca,44Ca	MR, HR	763 - 3047
	Fe	^{56}Fe, ^{57}Fe	MR	20 - 78
	Mg	^{24}Mg, ^{25}Mg, ^{26}Mg	MR	778 - 3111
	Mn	^{55}Mn	MR	48-193
	K	^{39}K	HR	2585 - 10328
	P	^{31}P	MR	3100 - 12300

Linearity and working range were evaluated based on UME CRM 1202 and NIST CRM 2711a which are studied during the validation of method. Linearity which is described as correlation coefficient within the working range given in Table 3.50 was found to be >0.999.

As it is seen in Table 3.51, sensitivity of the proposed method was demonstrated using LOD ($3s+C_{blank}$), LOQ ($10s+C_{blank}$) and precision is reported as repeatability (within day precision) and intermediate precision (between day precision). Precision of the method for most of the analytes was found to be satisfactory considering the purpose of the study. However, the combined uncertainty on repeatability of Cr, Pb and Sb and the combined uncertainty on intermediate precision of As were found to be relatively higher than others as the natural levels of them quite low in sample. Low precisions belong to these analytes resulted in relatively high measurement uncertainty budgets.

Trueness of the method was studied using mentioned certified reference materials and in the absence of suitable CRM/SRM, gravimetrical spiking into sample was

performed to show the recoveries of some elements. As it seen in Table 3.52 - Table 3.54, trueness of the method was found higher than 95.0±5.0% for the all analytes.

Measurement of uncertainty has been calculated using top down approach according to EURACHEM/CITAC Quantifying Uncertainty in Analytical Measurement [264] and also Evaluation of measurement data – Guide to the expression of uncertainty JCGM 100:2008 [265]. The measurement uncertainty results for total elemental mass fraction determination in walnut samples are given in Table 3.55.

Table 3.51 Sensitivity and precision of the method for multi elemental analysis in walnuts by HR-ICP-MS

		Sensitivity		Precision	
		LOD, *(n=15)*	LOQ, *(n=15)*	*u(w)*[3] *%*	*u(b)*[4] *%*
Trace Group, ng/g	As	0.8	1.8	5.63	8.42
	B	225	526	0.82	0.77
	Cd	0.3	0.7	1.55	2.61
	Cr	10	25	16.5	$MS_{between}<MS_{within}$
	Na	838	1549	3.54	$MS_{between}<MS_{within}$
	[1]Pb	0.6	1.1	15.7	$MS_{between}<MS_{within}$
	Sb	0.4	1.2	14.1	$MS_{between}<MS_{within}$
Minor Group, ng/g	Ba	36	73	0.78	0.46
	[2]Co	2.4	4.5	0.71	1.12
	Cu	19	39	0.66	0.74
	Ni	62	100	2.02	$MS_{between}<MS_{within}$
	Sr	19	35	1.04	3.17
	Zn	889	1938	0.53	0.91
Major Group, mg/kg	Ca	76	157	1.02	2.19
	Fe	2.3	4.7	0.30	0.99
	K	18	32	1.58	$MS_{between}<MS_{within}$
	Mg	66	153	0.30	0.99
	Mn	0.06	0.1	0.67	0.70
	P	12	28	0.46	$MS_{between}<MS_{within}$

[1]n=13
[2]n=12
[3]Combined uncertainty on repeatability
[4]Combined uncertainty on intermediate precision

Table 3.52 Trueness of the method for multi elemental analysis in walnuts by HR-ICP-MS using NIST SRM 2387 Peanut Butter

	Certified value, mg/kg U (*k=2*)	Measurement Result, mg/kg *(n=6)*	Recovery %
Na	4890 ± 140	4625 ± 270	94.6 ± 5.5
Ca	411 ± 18	406 ± 12	98.8 ± 2.8
Fe	16.4 ± 0.8	15.8 ± 0.9	96.4 ± 5.7
K	6070 ± 200	5954 ± 57	98.1± 0.9
P	3378 ± 92	3364 ± 15	100 ± 0.4
Mg	1680 ± 70	1722 ± 12	103 ± 0.7
Mn	16.0 ± 0.6	16.1 ± 0.3	101 ± 1.6
Cu	4.93 ± 0.15	4.81 ± 0.06	97.6 ± 1.3
Zn	26.3 ± 1.1	25.5 ± 0.6	97.0 ± 2.1

Table 3.53 Trueness of the method for multi elemental analysis in walnuts by HR-ICP-MS using UME CRM 1202 Elements in Hazelnut

	Certified value, U (*k=2*)	Measurement Result *(n=9)*	Recovery %
B, *mg/kg*	16.8 ± 2.2	15.7 ± 0.3	93.5 ± 1.8
[1]**Ba**, *mg/kg*	5.8 ± 0.3	5.8 ± 0.2	100.2 ± 3.1
Ca, *mg/kg*	1550 ± 110	1424 ± 24	97.2 ± 2.0
Cd, *µg/kg*	6.4 ± 0.9	6.2 ± 0.3	96.5 ± 4.7
Co, *µg/kg*	278 ± 28	271 ± 7	97.5 ± 2.6
Cu, *mg/kg*	16.4 ± 1.0	16.0 ± 0.3	97.6 ± 1.8
Fe, *mg/kg*	36.1 ± 2.9	36.3 ± 1.0	100.5 ± 2.6
[1]**K**, *mg/kg*	5890 ± 550	5713 ± 177	97.0 ± 3.0
Mg, *mg/kg*	1540 ± 150	1469 ± 66	95.4 ± 4.3
Mn, *mg/kg*	95.3 ± 6.3	91.2 ± 2.7	95.7 ± 2.8
Ni, *mg/kg*	1.60 ± 0.17	1.53 ± 0.08	95.8 ± 4.9
[1]**P**, *mg/kg*	3240 ± 890	3051 ± 91	94.2 ± 2.8
Sr, *mg/kg*	6.68 ± 0.46	6.34 ± 0.18	94.9 ± 2.7
Zn, *mg/kg*	20.4 ± 1.8	19.8 ± 0.7	97.0 ± 3.3

[1]Informative Value

Table 3.54 Trueness of the method for multi elemental analysis in walnuts by HR-ICP-MS obtained by gravimetrically spiking before digestion

	Natural level determined in UME CRM 1202, ng/g	Spiked level, ng/g	Recovery, % *(n=6)*
As	6.5	25	93.3± 3.6
Cr	50	55	95.3 ± 9.2
Pb	5.1	25	95.2± 1.8
Sb	0.4	25	98.4± 3.2

Table 3.55 Estimated measurement uncertainty values for determination of total elemental content in walnut

	Mass Fraction	u *(k=1)*	U *(k=2)*	% U *(k=2)*
As, *ng/g*	6.7	0.7	1.4	20
B, *mg/kg*	16.1	1.3	2.6	16
Ba, *mg/kg*	5.74	1.30	2.60	4.6
Ca, *mg/kg*	1521	49	98	6.4
Cd, *ng/g*	6.0	0.66	1.32	21
Co, *ng/g*	281	11	23	8.1
Cr, *ng/g*	50	9	17	34
Cu, *mg/kg*	16.4	0.5	1.0	6.3
Fe, *mg/kg*	35.6	1.4	2.7	7.5
K, *mg/kg*	5942	169	339	5.7
Mg, *mg/kg*	1527	48	95	6.2
Mn, *mg/kg*	94.3	3.2	6.4	6.8
Na, *ng/g*	902	47	93	10
Ni, *ng/g*	1790	75	150	8.4
P, *mg/kg*	3242	85	170	5.2
Pb, *ng/g*	4.7	0.8	1.5	31
Sb, *ng/g*	0.44	0.05	0.1	28
Sr, *mg/kg*	6.83	0.29	0.58	8.6
Zn, *mg/kg*	21.0	1.0	2.0	9.7

3.3.2 Evaluating the Performances of Two Digestion Program for Walnut Mineralization

The validated method described in the section 3.3.1 was also tested with CEM MARS Xpress system (Digetion-2). This digestion system is only temperature controlled and 40 samples can be digested in a single run while the other one (Digetion-1) is temperature and pressure controlled with 12 vessels. This study was performed to fasten sample preparation step. Three replicates of UMECRM 1202 samples were digested with two techniques as described in the section 2.3.3.2. Three replicates of procedural blanks were also exposed to the same procedures. Six samples and six procedural blanks were analyzed at the same sequence with the developed method. The performance of Digestion-2 was evaluated by applying ERM Application Note [276] for the certified parameters present in UME CRM 1202 and student t-test was used for the rest.

It was proofed that there were no significant differences in the digestion performance between Digestion-1 and Digestion-2 techniques for any of analyte investigated as seen in Table 3.56. Therefore, Digestion-2 was used for mineralization of walnut samples.

Table 3.56 Evaluation of digestion techniques for walnut mineralization

	Results obtained by Digestion-1			Results obtained by Digestion-2					UMECRM 1202	ERM Application Note 1			Student t-test			
Analyte	Mass fraction	s	RSD	Mass fraction	s	RSD	U % *(k=2)*	U *(k=2)*	Certified Value U *(k=2)*	Δ	U_{Δ}	Result	t value	df	$t_{critical}$	Result
As, ng/g	8.1	1.3	16%	7.4	0.3	4.2%	20%	1.5	-				0.94	4.00	2.78	Passed
B, mg/kg	[1]17.8	1.7	9.5%	18.1	1.0	5.3%	16%	2.9	16.8 ± 2.2	1.3	3.6	Passed	0.26	3.00	3.18	Passed
Ba, mg/kg	8345	43	0.5%	8248	364	4.4%	4.6%	379	-				0.46	4.00	2.78	Passed
Ca, mg/kg	1477	21	1.4%	1505	3	0.2%	6.4%	96	1550 ± 110	45	146	Passed	2.37	4.00	2.78	Passed
Cd, ng/g	[1]6.8	0.1	1.7%	6.5	0.1	1.1%	21%	1.4	6.4 ± 0.9	0.1	1.6	Passed	2.76	3.00	3.18	Passed
Co, ng/g	289	7	2.5%	289	15	5.2%	8.1%	23	278 ± 28	11	36	Passed	0.06	4.00	2.78	Passed
Cr, ng/g	46	30	65%	48	7	15.6%	25%	12	-				0.13	4.00	2.78	Passed
Cu, mg/kg	16.4	0.2	0.9%	16.2	0.2	0.9%	6.3%	1.0	16.4 ± 1	0.2	1.4	Passed	1.71	4.00	2.78	Passed
Fe, mg/kg	32.3	0.8	2.3%	32.7	0.5	1.6%	7.5%	2.5	36.1 ± 2.9	3.4	3.8	Passed	0.66	4.00	2.78	Passed
K, mg/kg	5165	104	2.0%	5260	90	1.7%	5.7%	300	-				1.19	4.00	2.78	Passed
Mg, mg/kg	1411	17	1.2%	1424	15	1.1%	6.2%	88	1540 ±150	116	174	Passed	0.99	4.00	2.78	Passed
Mn, mg/kg	88.3	1.9	2.2%	88.5	2.5	2.8%	6.8%	6.0	95.3 ± 6.3	6.8	8.7	Passed	0.09	4.00	2.78	Passed
Na, ng/g	695	43	6.1 %	849	26	3.0%	10%	85	-				5.34	4.00	2.78	Passed
Ni, mg/kg	1.58	0.04	2.8%	1.57	0.04	2.8%	8.4%	0.13	1.60 ± 0.17	0.03	0.22	Passed	0.29	4.00	2.78	Passed
P, mg/kg	2915	40	1.4%	2973	14	0.5%	5.2%	155	-				2.37	4.00	2.78	Passed
Pb, ng/g	[1]5.3	0.5	9.2%	5.0	1.3	26.6%	32%		-				0.31	3.00	3.18	Passed
Sr, mg/kg	6.37	0.13	2.1%	6.27	0.19	3.0%	8.6%	0.5	6.68 ± 0.46	0.41	0.71	Passed	0.77	3.00	3.18	Passed
Zn, mg/kg	20.4	0.2	1.0%	19.7	0.5	2.8%	9.7%	1.9	20.4 ± 1.8	0.7	2.6	Passed	2.09	4.00	2.78	Passed

[1]n=2

3.3.3 Total Elemental Mass Fractions of Walnut Sample

Five independent subsamples from each walnut sample were digested. Digestion of whole samples (17 samples with 5 subsamples; 85 digestions in total) was completed in five runs with three procedural blank samples for each. Measurement of the digested samples was performed as described in section 3.3.1. Moisture content of walnut samples were determined by keeping them at 75 °C for 12 hours. Summary of dry-mass basis mass fractions of the samples are given in Table 3.57 -Table 3.59 as trace, minor and major elements, respectively. Mass fractions of Cr, Na, Pb and Sb in most of the walnut samples were found to be below limit of quantification.

Table 3.57 Total elemental mass fractions of trace groups in walnut samples

Analyte	As			B			Cd			Cr		
LOD	0.8	ng/g		255	ng/g		0.3	ng/g		10	ng/g	
LOQ	1.8			526			0.7			25		
Sample Code (n=5)	Mass Fraction µg /kg	%RSD	s.u	Mass Fraction mg /kg	RSD%	s.u	Mass Fraction µg /kg	%RSD	s.u	Mass Fraction µg /kg	%RSD	s.u
1	2.4	22%	0.2	20.8	2.9%	0.3	1.00	6.1%	0.03	<LOQ		
[1]2	<LOQ	-	-	16.0	1.2%	0.1	<LOQ	-	-	<LOQ		
3	<LOQ	-	-	9.21	3.1%	0.13	1.44	3.5%	0.02	<LOQ		
4	<LOQ	-	-	19.7	3.6%	0.3	1.06	4.3%	0.02	[1]28	43%	5.9
5	2.0	18%	0.2	15.2	1.7%	0.1	8.33	2.8%	0.11	<LOQ		
6	14	4.3%	0.3	12.2	2.5%	0.1	1.03	6.5%	0.03	<LOQ		
7	<LOQ	-	-	16.3	3.0%	0.2	0.69	6.2%	0.02	<LOQ		
8	<LOQ	-	-	14.7	1.9%	0.1	<LOQ	-	-	<LOQ		
9	2.5	15%	0.2	6.91	1.1%	0.03	65	1.4%	0.4	<LOQ		
10	3.0	11%	0.2	10.7	2.7%	0.1	1.57	1.5%	0.01	<LOQ		
11	16	4.4%	0.3	19.3	1.0%	0.1	0.79	4.3%	0.02	<LOQ		
12	[2]98	4.7%	2	14.4	2.3%	0.1	0.69	3.1%	0.01	<LOQ		
13	7.7	7.0%	0.2	11.2	2.4%	0.1	<LOQ	-	-	<LOQ		
14	3.1	34%	0.5	9.94	1.3%	0.06	0.80	2.6%	0.01	<LOQ		
15	7.1	10%	0.3	11.7	1.6%	0.1	<LOQ	-	-	<LOQ		
16	4.6	20%	0.4	12.4	4.2%	0.2	<LOQ	-	-	<LOQ		
17	4.1	23%	0.4	13.3	2.7%	0.2	<LOQ	-	-	<LOQ		

[1]n=4

Table 3.57 Total elemental mass fractions of trace groups in walnut samples-*continuous*

Analyte	Na			Pb			Sb		
LOD	838	ng/g		0.6	ng/g		0.4	ng/g	
LOQ	1549			1.1			1.2		
Sample Code (n=5)	Mass Fraction μg /kg	%RSD	s.u	Mass Fraction μg /kg	%RSD	s.u	Mass Fraction μg /kg	RSD%	s.u
1	<LOQ	-	-	2.1	29%	0.3	<LOQ	-	-
2	<LOQ	-	-	<LOQ	-	-	<LOQ	-	-
3	<LOQ	-	-	<LOQ	-	-	<LOQ	-	-
4	<LOQ	-	-	1.1	10%	0.05	<LOQ	-	-
5	<LOQ	-	-	<LOQ	-	-	<LOQ	-	-
6	<LOQ	-	-	<LOQ	-	-	<LOQ	-	-
7	[1]3092	1.7%	27	4.7	12%	0.2	<LOQ	-	-
8	<LOQ	-	-	<LOQ	-	-	<LOQ	-	-
9	2036	5.3%	48	2.3	10%	0.1	<LOQ	-	-
10	<LOQ	-	-	1.1	19%	0.1	<LOQ	-	-
11	<LOQ	-	-	1.4	15%	0.1	<LOQ	-	-
12	<LOQ	-	-	<LOQ	-	-	<LOQ	-	-
13	<LOQ	-	-	<LOQ	-	-	<LOQ	-	-
14	<LOQ	-	-	<LOQ	-	-	<LOQ	-	-
15	2252	3.2%	33	<LOQ	-	-	<LOQ	-	-
16	<LOQ	-	-	<LOQ	-	-	<LOQ	-	-
17	<LOQ	-	-	3.9	60%	1.1	<LOQ	-	-

[1]n=4

Table 3.58 Total elemental mass fractions of minor groups in walnut samples

Analyte	Ba			Co			Cu		
LOD	36	ng/g		2.4	ng/g		19	ng/g	
LOQ	73			4.5			39		
Sample Code (n=5)	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction ng/g	RSD%	s.u	Mass Fraction mg/kg	%RSD	s.u
1	2.35	5.8%	0.06	58	5.2%	1.4	9.38	2.2%	0.09
2	1.03	3.8%	0.02	48	2.6%	0.6	15.7	1.0%	0.07
3	0.51	4.0%	0.01	53	2.2%	0.5	13.8	0.8%	0.05
4	4.26	0.7%	0.01	134	1.0%	1	11.6	0.7%	0.03
5	5.50	0.7%	0.02	1659	1.3%	10	17.5	1.2%	0.09
6	5.98	1.6%	0.04	265	2.4%	3	13.1	0.9%	0.05
7	1.99	10%	0.09	111	1.7%	0.8	13.4	1.8%	0.11
8	[1]1.46	1.7%	0.01	17	3.8%	0.3	9.66	0.3%	0.02
9	0.87	1.8%	0.01	129	1.7%	1.0	7.20	1.2%	0.04
10	4.61	0.8%	0.02	87	1.3%	0.5	13.7	0.7%	0.04
11	[1]3.45	1.6%	0.03	106	3.5%	1.7	11.5	1.1%	0.06
12	4.16	1.2%	0.02	39	2.9%	0.5	12.1	1.3%	0.07
13	3.88	1.0%	0.02	111	1.4%	0.7	16.9	0.6%	0.04
14	11.6	0.8%	0.04	114	1.7%	0.9	16.6	0.7%	0.05
15	1.45	1.3%	0.01	39	1.4%	0.2	12.9	1.1%	0.06
16	4.83	0.7%	0.01	29	3.6%	0.5	10.7	0.4%	0.02
17	2.25	2.1%	0.02	51	1.0%	0.2	10.9	2.3%	0.11

[1]n=4

Table 3.58 Total elemental mass fractions of minor groups in walnut samples- *continuous*

Analyte	Ni			Sr			Zn		
LOD	62	ng/g		19	ng/g		889	ng/g	
LOQ	100			35			1938		
Sample Code (n=5)	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction mg/kg	%RSD	s.u
1	4.73	2.1%	0.05	2.63	1.4%	0.02	18.9	1.5%	0.1
2	4.22	1.8%	0.03	2.46	2.1%	0.02	43.2	1.0%	0.2
3	1.62	1.2%	0.01	4.92	0.7%	0.02	36.4	1.2%	0.2
4	2.13	3.0%	0.03	4.40	1.2%	0.02	37.9	0.8%	0.1
5	4.74	1.6%	0.03	4.20	0.6%	0.01	35.3	0.7%	0.1
6	2.43	2.5%	0.03	1.21	1.3%	0.01	34.4	1.2%	0.2
7	1.76	1.5%	0.01	1.28	2.7%	0.02	40.1	1.5%	0.3
8	4.15	0.9%	0.02	1.82	0.5%	0.004	18.0	0.7%	0.1
9	6.26	1.8%	0.05	7.24	1.1%	0.03	36.9	1.3%	0.2
10	5.06	0.5%	0.01	7.78	1.1%	0.04	30.1	0.9%	0.1
11	2.60	1.3%	0.02	3.46	1.5%	0.02	38.9	1.1%	0.2
12	2.62	1.9%	0.02	3.24	1.0%	0.01	29.2	0.6%	0.1
13	1.07	2.0%	0.01	6.02	0.8%	0.02	42.1	1.3%	0.2
14	2.21	1.1%	0.01	18.3	1.5%	0.12	33.8	0.5%	0.1
15	1.55	1.5%	0.01	4.27	1.8%	0.03	28.5	1.4%	0.4
16	2.40	2.2%	0.02	6.62	0.4%	0.01	17.6	0.7%	0.1
17	2.39	2.8%	0.03	3.30	3.7%	0.05	25.9	0.9%	0.1

Table 3.59 Total elemental mass fractions of major groups in walnut samples

Analyte	Ca			Fe			K		
LOD	76	*mg/kg*		2.3	*mg/kg*		18	*mg/kg*	
LOQ	157			4.7			32		
Sample Code (n=5)	Mass Fraction mg /kg	%RSD	s.u	Mass Fraction mg /kg	%RSD	s.u	Mass Fraction mg /kg	%RSD	
1	1083	3.8%	18	21.9	2.3%	0.2	3844	2.8%	48
2	1426	3.5%	22	31.7	2.1%	0.3	3959	3.3%	58
3	1335	1.8%	11	37.1	3.4%	0.6	3719	6.1%	101
4	1721	1.2%	9	34.8	1.8%	0.3	3411	2.8%	43
5	1200	2.0%	11	29.9	1.5%	0.2	4848	0.7%	14
6	1254	3.2%	18	26.3	2.4%	0.3	4660	5.1%	106
7	1218	1.3%	7	[1]30.4	2.8%	0.4	4502	3.7%	75
8	1126	2.6%	13	22.3	3.6%	0.4	4683	4.7%	98
9	759	2.5%	9	26.1	1.2%	0.1	3784	2.4%	40
10	892	1.9%	8	23.6	3.8%	0.4	4338	4.8%	92
11	1454	1.6%	10	30.7	2.1%	0.3	4226	4.0%	76
12	918	3.2%	13	31.6	4.2%	0.6	5945	3.5%	93
13	1493	2.1%	14	35.1	1.3%	0.2	3893	2.5%	44
14	2010	2.6%	24	23.2	4.4%	0.5	3698	4.4%	73
15	867	1.2%	5	26.1	1.1%	0.1	4124	2.4%	44
16	870	0.9%	4	30.1	2.6%	0.4	3836	4.3%	75
17	973	1.8%	8	28.3	2.3%	0.3	4057	8.9%	161

[1]n=4

Table 3.59 Total elemental mass fractions of major groups in walnut samples- *continuous*

Analyte	Mg			Mn			P		
LOD	66	*mg/kg*		58	*µg/g*		12	*mg/kg*	
LOQ	153			97			28		
Sample Code (n=5)	Mass Fraction mg /kg	%RSD	s.u	Mass Fraction mg /kg	%RSD	s.u	Mass Fraction mg /kg	%RSD	s.u
1	1674	0.7%	5	18.0	0.6%	0.1	3482	1.5%	23
2	1641	1.4%	10	23.4	1.3%	0.1	4000	1.2%	21
3	1535	1.3%	9	26.8	1.9%	0.2	3636	1.1%	17
4	1581	1.4%	10	31.5	1.1%	0.2	3771	2.4%	40
5	1715	2.0%	15	86.0	1.5%	0.6	3978	1.6%	28
6	1620	1.0%	8	60.5	1.4%	0.4	3651	1.7%	28
7	1771	1.7%	13	69.9	1.5%	0.5	4110	1.3%	23
8	1859	1.6%	13	12.6	1.1%	0.1	3650	1.3%	20
9	1171	2.2%	11	79.2	1.9%	0.7	2774	2.0%	25
10	1412	2.0%	13	23.3	1.8%	0.2	3181	1.5%	21
11	1597	1.0%	7	33.6	1.4%	0.2	3955	1.1%	19
12	1570	1.8%	13	41.5	2.6%	0.5	3775	0.8%	13
13	1582	1.2%	8	32.1	1.8%	0.3	3796	1.0%	17
14	1489	1.1%	7	137	0.8%	0.5	3850	1.1%	18
15	1705	1.2%	9	23.0	1.7%	0.2	3567	0.7%	10
16	1546	1.4%	9	30	1.9%	0.3	3639	2.0%	32
17	1541	2.2%	15	33	1.9%	0.3	3675	1.7%	28

3.3.4 Evaluation of Digestion Procedures for Mineralization of Soil

Ideally, a complete digestion has to be performed for the determination of elemental mass fraction in soil samples. As each soil sample has unique composition, an optimum digestion procedure needs to be developed.

Four different digestion procedures were planned to be compared by using NIST SRM 2711a "Montana II Soil" and two different soil samples which were collected from Istanbul (No 5) and Bursa (No 6). Acid compositions and applied microwave digestion temperature programs are given in section 2.3.3.3.

As it is summarized in Table 3.60, at least 95±5% recovery except for chromium which has 79±3.4% recovery was obtained by Digestion Procedure 1 for mineralization of NIST SRM 2711a. Digestion Procedure 2 was also found to be successful for analysis of As, Cu, Ni, P, Pb, and Zn. However, as the recovery values for the rest of the parameters were in the range of 3-87%, it was concluded that Digestion Procedure 2 could not achieve complete digestion and found to be unsuccessful for digestion of NIST SRM 2711a. On the other hand, performances of Digestion Procedure 3 and of Digestion Procedure 4 based on NIST SRM 2711a measurements were found to be comparable with Digestion Procedure 1. As the recovery values of As, Cr, and Mg obtained by Digestion Procedure 3 were found to be 88.1±1.7%, 76.9±3.3%, and 68±23%, the recovery values for As, Ni, P, and Pb were found to be 87.2±1.0%, 86.5±3.1%, 87.2±0.6% and 87.4±5.0% obtained by Digestion Procedure 4, respectively.

Table 3.60 Measurement results of NIST 2711a using different digestion procedures

Analyte	Digestion Procedure 1					Digestion Procedure 2			Digestion Procedure 3			Digestion Procedure 4		
	Mass fraction	%RSD (*n=8*)	Recovery	U % *(k=2)*	U *(k=2)*	Mass fraction	RSD (*n=3*)	Recovery	Mass fraction	%RSD (*n=3*)	Recovery	Mass fraction	%RSD (*n=3*)	Recovery
As, mg/kg	101	3.2%	94.3%	13%	13.2	105	1.8%	98.1%	94.3	1.7%	88.1%	93.3	1.0%	87.2%
[3]**B**, mg/kg	48.2	7.0%	96.4%	13%	6.5	26.8	10.0%	53.7%	45.4	10%	90.9%			
Ba, mg/kg	718	[2]0.3%	98.4%	4.4%	32	259	5.1%	35.5%	679	[2]3.8%	93.0%	712	[2]1.5%	97.6%
Ca, %	2.43	[2]0.7%	100.3%	4.2%	0.10	1.72	1.6%	71.3%	2.22	[2]3.4%	91.8%	2.38	[2]1.9%	98.4%
Cd, mg/kg	54.9	3.7%	101.5%	4.8%	2.6	60.3	1.6%	111.4%	52.9	2.0%	97.7%	50.9	0.8%	94.0%
Co, mg/kg	9.47	2.9%	96.0%	9.4%	0.89	8.63	2.5%	87.3%	9.43	0.9%	95.3%	10.5	5.5%	106.0%
Cr, mg/kg	41.1	[1]3.4%	78.6%	14%	5.9	32.6	5.8%	62.2%	40.2	3.3%	76.9%	50.1	4.5%	95.8%
Cu, mg/kg	132	2.1%	94.4%	5.9%	8	137	3.5%	97.9%	131	2.0%	93.9%	145	5.0%	103.3%
Fe, %	2.81	[2]0.7%	99.7%	3.4%	0.10	2.42	2.3%	85.8%	2.70	[2]0.7%	95.8%	2.78	[2]1.3%	98.7%
K, %	2.51	[2]1.2%	103.5%	9.0%	0.23	0.83	10.9%	34.0%	2.38	[2]4.3%	98.1%	2.44	[2]1.6%	100.6%
Mg, %	1.02	[2]0.6%	95.3%	11%	0.11	0.80	1.4%	75.2%	0.73	[2]23%	68.3%	0.97	[2]1.6%	91.0%
Mn, mg/kg	660	0.6%	97.8%	6.2%	41	550	1.6%	81.5%	643	[2]1.1%	95.2%	648	[2]0.9%	96.1%
Na, %	1.15	1.3%	95.6%	9.6%	0.11	0.03	15%	2.9%	1.12	1.6%	93.6%	1.15	0.9%	95.9%
Ni, mg/kg	20.2	[1]1.5%	93.3%	19%	3.9	19.5	2.5%	89.9%	20.8	1.3%	95.7%	18.8	3.1%	86.5%
P, mg/kg	836	3.8%	99.3%	5.0%	42	864	2.3%	102.6%	778	1.3%	92.4%	734	0.6%	87.2%
Pb, %	0.139	1.7%	99.0%	9.4%	0.01	0.131	2.0%	93.6%	0.140	0.7%	100.0%	0.122	5.0%	87.4%
Sb, mg/kg	23.8	[1]4.7%	100.2%	8.4%	2.0	13.9	3.0%	58.2%	21.4	5.7%	90.0%	24.2	1.2%	101.8%
Sr, mg/kg	239	4.4%	98.7%	6.9%	16	135	1.4%	55.8%	234	1.4%	96.7%	227	0.8%	93.9%
Zn, mg/kg	392	3.0%	94.7%	5.1%	20	398	2.6%	96.0%	400	1.3%	88.1%	430	2.2%	103.8%

[1]n=7
[2]n=3
[3]Informative Value

The performances of procedures on NIST SRM 2711a was also evaluated by using ERM Application Note1 which also takes precision of measurement into account. As the uncertainty budgets of Digestion Procedure 2, Digestion Procedure 3 and Digestion Procedure 4 were not calculated due to absence of intermediate precision, standard deviation instead of combined uncertainty of the results were used in the calculations of ERM Application Note 1. As it is seen in Table 3.61, the performance of Digestion Procedure 1 for all parameters except for chromium were demonstrated as successful based on ERM Application Note 1 evaluation. On the other hand, only the results of Ba, Cd, K, Pb, Sb, Sr, and Zn obtained by Digestion Procedure 3 and the results of Ba, Ca, Co, Cr, Cu, Fe, K and Sb obtained by Digestion Procedure 4 were found to be successful based on this evaluation. However, as the evaluation was performed using the standard deviation of single day measurements for last three digestion procedures, the results for some parameters might be valid if combined uncertainties could be taken into account.

Table 3.61 Performances of digestion procedures based on NIST SRM 2711a

Analyte	NIST SRM 2711a Montana II Soil		ERM Application Note 1 Procedure 1			ERM Application Note 1 Procedure 2			ERM Application Note 1 Procedure 3			ERM Application Note 1 Procedure 4		
	Certified Value	U (k=2)	Δ	$U_Δ$	Result	Δ	$U_Δ$	Result	Δ	$U_Δ$	Result	Δ	$U_Δ$	Result
As, mg/kg	107	5	6	14	Passed	2	6	Passed	13	6	Failed	14	5	Failed
[1]**B***, mg/kg	50	-	2	-	-	23	-	-	4.6	-	-	-	-	-
Ba, mg/kg	730	15	12	35	Passed	471	30	Failed	51	53	Passed	18	26	Passed
Ca, %	2.42	0.06	0.01	0.12	Passed	0.70	0.08	Failed	0.20	0.16	Failed	0.04	0.11	Passed
Cd, mg/kg	54.1	0.5	0.8	2.7	Passed	6.2	1.9	Failed	1.2	2.1	Passed	3.2	0.9	Failed
Co, mg/kg	9.89	0.18	0.42	0.91	Passed	1.26	0.46	Failed	0.46	0.24	Failed	0.6	1.2	Passed
Cr, mg/kg	52.3	2.9	11.2	18.0	Failed	19.7	4.8	Failed	12.1	4.0	Failed	2.2	5.4	Passed
Cu, mg/kg	140	2	8	8	Passed	3	10	Passed	8.6	5.6	Failed	4.6	14.7	Passed
Fe, %	2.82	0.04	0.01	0.10	Passed	0.40	0.12	Failed	0.12	0.06	Failed	0.04	0.08	Passed
K, %	2.53	0.10	0.02	0.25	Passed	1.70	0.21	Failed	0.15	0.23	Passed	0.09	0.13	Passed
Mg, %	1.07	0.06	0.05	0.13	Passed	0.27	0.06	Failed	0.3	0.3	Failed	0.10	0.07	Failed
Mn, mg/kg	675	18	15	45	Passed	125	25	Failed	32	23	Failed	27	22	Failed
Na, %	1.20	0.01	0.05	0.11	Passed	1.17	0.01	Failed	0.08	0.04	Failed	0.05	0.02	Failed
Ni, mg/kg	21.7	0.7	1.5	3.9	Passed	2.2	1.2	Failed	0.9	0.9	Failed	2.9	1.4	Failed
P, mg/kg	842	11	6.0	43.2	Passed	22	41.2	Passed	64	23.5	Failed	108	14	Failed
Pb, %	0.140	0.001	0.001	0.013	Passed	0.009	0.005	Failed	0.0001	0.002	Passed	0.02	0.01	Failed
Sb,mg/kg	23.8	1.4	0.04	1.4	Passed	9.9	1.6	Failed	2.4	2.8	Passed	0.4	1.5	Passed
Sr,mg/kg	242	10	3	19	Passed	107	10	Failed	8	12	Passed	15	10	Failed
Zn, mg/kg	414	11	22	23	Passed	16	24	Passed	14	15	Passed	16	22	Failed

[1]Informative Value

The performances of digestion procedures were also compared by applying them to two real soil samples. As Digestion Procedure 1 performance was found to be optimum based on NIST SRM 2711a, performances of other three procedures on real samples (No 5 & No 6) were compared with respect to Digestion Procedure 1 by applying student t-test (Table 3.64 - Table 3.65) . The obtained mass fractions of all parameters for No 5 and No 6 are given in Table 3.62 and Table 3.63, respectively.

As it was also demonstrated in the evaluation of digestion performances on NIST SRM 2711a, the results of Digestion Procedure 2 were mostly reported as different from the results of Digestion Procedure 1 for both sample No 5 and Sample No 6. It should be emphasized that all the non-compliant results obtained by Digestion Procedure 2 are lower than the results obtained by Digestion Procedure 1 which is a good sign for incomplete digestion as also mentioned in the evaluation of results obtained for NIST SRM 2711a. Therefore, no further investigation was performed for Digestion Procedure 2.

In the results of Sample No 5, three parameters (Co, Fe, and P) obtained by Digestion Procedure 3 and eight parameters (Co, Cr, Cu, Ni, P, Sr and Zn) obtained by applying Digestion Procedure 4 were not compatible with the results of Digestion Procedure 1. Although the procedural blank levels for each procedure were comparable, the mass fraction of Co, Cr, Cu, Ni, and Zn was found to be higher than the values obtained by Digestion Procedure 1. The higher values for Co, Cr and Cu obtained by Digestion Procedure 4 were also compatible with recovery values as seen in Table 3.60. In contrast to this, higher mass fractions were determined for Fe, P and Sr by using Digestion Procedure 1 and the recovery values obtained for P and Sr show similar behavior in the results of NIST SRM 2711a as given in Table 3.60.

In the results of Sample No 6, eight parameters (As, Co, Cr, Cu, Ni, P, Pb and Zn) obtained by Digestion Procedure 3 and 14 parameters (Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, P, Pb, and Zn) obtained by Digestion Procedure 4 were not found to be compatible with the results of Digestion Procedure 1. The mass fraction of As, P and Pb determined by using Digestion Procedure 3 were found to be lower

than the amounts obtained by Digestion Procedure 1 and these lower results were also observed in NIST SRM 2711a measurements except for Pb. On the other hand, as the similar recovery values obtained for Co, Cr, Cu, Ni in NIST SRM 2711a by using both Digestion procedure 1 and Procedure 3, the higher mass fractions were determined for sample No 6 by applying Digestion Procedure 3. In contrast to this, the mass fraction of Zn in Sample No 6 was detected as 100.6±1.3 mg/kg and 94.7±2.2 mg/kg by using Digestion Procedure 3 and Digestion Procedure 1, respectively. These results are not compatible with the recovery values of the techniques given in Table 3.60.

As a conclusion, the detailed investigation of different digestion procedures on certified reference material and also two different real samples showed that unique digestion procedures for each soil matrix should be developed in order to succeed complete digestion for all analyte. However, development of unique digestion procedure for each soil to be applied in authentication studies is not feasible and applicable. Therefore, all the soil samples were mineralized by using Digestion Procedure 1 as the results of real samples and NIST SRM 2711a was found to be relatively comparable with digestion Procedure 3 and Procedure 4 for most of the target analytes.

Table 3.62 Measurement results of sample No 5-İstanbul using different digestion procedures

Sample No 5	Digestion Procedure 1				Digestion Procedure 2				Digestion Procedure 3				Digestion Procedure 4			
Analyte	Mass Fraction	s (n=4)	s.u	%RSD	Mass Fraction	s (n=3)	s.u	%RSD	Mass Fraction	s (n=3)	s.u	%RSD	Mass Fraction	s (n=3)	s.u	%RSD
As, mg/kg	5.17	0.39	0.18	7.6%	3.16	0.17	0.10	5.2%	4.32	0.33	0.19	7.5%	5.52	0.28	0.16	5.1%
B, mg/kg	29.0	[3]1.5	0.7	5.3%	17.3	2.2	1.3	13%	28.1	1.3	0.8	4.8%	-	-	-	-
Ba, mg/kg	630	[1]23	13	3.6%	425	[1]56	32	13%	598	35	20	5.8%	634	23	13	3.6%
Ca, %	0.44	[2]0.05	0.03	11%	0.432	[1]0.005	0.003	1.2%	0.37	0.07	0.04	18%	0.64	0.18	0.10	28%
Cd, mg/kg	1.22	[3]0.06	0.03	5.0%	0.94	0.03	0.02	3.7%	1.18	0.03	0.01	2.1%	1.25	0.03	0.02	2.6%
Co, mg/kg	16.0	0.4	0.2	2.6%	16.7	0.2	0.1	1.3%	17.0	0.3	0.1	1.5%	18.9	0.7	0.4	3.7%
Cr, mg/kg	57.0	2.4	1.2	4.1%	52.2	3.0	1.7	5.8%	59.4	0.7	0.4	1.2%	80.1	5.1	3.0	6.4%
Cu, mg/kg	37.3	0.7	0.4	2.0%	37.0	0.6	0.3	1.6%	37.5	0.1	0.1	0.4%	42.9	2.1	1.2	5.0%
Fe, %	5.10	[1]0.08	0.04	1.5%	4.51	0.06	0.04	1.4%	4.83	0.13	741	2.7%	5.06	0.08	482	1.6%
K, %	1.82	[1]0.23	0.13	12%	1.17	0.21	0.12	18%	1.79	0.23	0.13	13%	1.93	0.04	0.02	2.2%
Mg, %	0.61	[2]0.12	0.08	21%	0.56	0.03	0.02	5.4%	0.40	0.25	0.15	64%	0.66	0.03	0.02	4.9%
Mn, mg/kg	822	[1]19	11	2.3%	728	10	6	1.4%	801	8	5	1.0%	835	11	7	1.4%
Na, mg/kg	3147	[1]49	28	1.6%	386	41	24	11%	3086	90	52	2.9%	3457	263	152	7.6%
Ni, mg/kg	26.4	0.6	0.3	2.2%	26.9	0.4	0.2	1.5%	27.1	0.1	0.1	0.5%	31.2	2.1	1.2	6.8%
P, mg/kg	1019	[3]43	22	4.2%	913	38	19	4.1%	958	14	7	1.4%	902	5	2	0.5%
Pb, mg/kg	31.1	1.2	0.6	3.8%	28.3	0.4	0.2	1.5%	31.5	0.9	0.5	2.7%	29.7	0.9	0.5	2.9%
Sb, mg/kg	31.5	3.0	1.5	9.4%	23.9	1.0	0.5	4.2%	30.1	0.9	0.4	2.9%	29.9	0.8	0.4	2.8%
Sr, mg/kg	50.4	1.6	0.8	3.2%	37.7	1.0	0.5	2.7%	48.8	0.9	0.4	1.7%	46.7	0.3	0.2	0.7%
Zn, mg/kg	212	2.1	1.0	1.0%	207	7.1	4.1	3.4%	215	4.4	2.5	2.0%	232	4.3	2.5	1.9%

[1]n=3
[2]n=2
[3]n=5

Table 3.63 Measurement results of sample No 6-Bursa using different digestion procedures

Sample No 6	Digestion Procedure 1				Digestion Procedure 2				Digestion Procedure 3				Digestion Procedure 4			
Analyte	Mass Fraction	s (n=5)	s.u	%RSD	Mass Fraction	s (n=3)	s.u	%RSD	Mass Fraction	s (n=3)	s.u	%RSD	Mass Fraction	s (n=3)	s.u	%RSD
As, mg/kg	51.0	0.39	0.1	0.5%	46.3	2.6	1.3	5.7%	48.1	1.4	0.7	2.8%	51.7	0.9	0.5	1.8%
B, mg/kg	42.6	4.6	2.0	10.7%	26.8	2.3	1.3	9%	42.4	2.1	1.2	5%	-	-	-	-
Ba, mg/kg	319	[1]8	4	2.4%	228	9	5	4.2%	308	14	8	4.6%	338	6	3	1.8%
Ca, %	2.08	[1]0.03	0.02	1.6%	1.83	0.11	0.07	6.1%	2.02	0.11	0.06	5.4%	2.19	0.04	0.02	1.7%
Cd, mg/kg	1.17	0.01	0.004	0.7%	0.93	0.07	0.03	7.1%	1.16	0.07	0.03	7.1%	1.269	0.005	0.002	0.4%
Co, mg/kg	26.2	0.3	0.15	1.3%	29.2	0.3	0.19	1.1%	28.8	0.3	0.1	0.9%	31.2	0.4	0.2	1.2%
Cr, mg/kg	139	4	2	3.0%	150	9	5	6.2%	157	2	1	1.2%	193	4	2	2.0%
Cu, mg/kg	62.9	1.3	0.6	2.1%	72.6	1.5	0.9	2.1%	69.1	0.6	0.3	0.8%	75.1	0.4	0.2	0.5%
Fe, %	5.02	[1]0.08	0.04	1.5%	4.52	0.13	0.07	2.8%	4.93	0.26	0.15	5.3%	5.34	0.04	0.02	0.7%
K, %	1.292	[1]0.010	0.006	0.8%	0.94	0.07	0.04	7.8%	1.30	0.04	0.02	3.1%	1.37	0.03	0.02	2.1%
Mg, %	1.11	[1]0.03	0.02	3%	1.04	0.03	0.01	2.4%	1.00	0.08	0.04	8%	1.21	0.02	0.01	1.4%
Mn, mg/kg	1469	[1]10	6	0.7%	1284	41	24	3.2%	1476	57	33	3.9%	1619	53	31	3.3%
Na, %	0.540	[1]0.005	0.003	0.9%	0.031	0.004	0.003	13.9%	0.537	0.009	0.005	1.6%	0.539	0.002	0.001	0.4%
Ni, mg/kg	85.4	1.4	0.6	1.7%	97.5	3.2	1.8	3.3%	94.6	0.4	0.3	0.5%	104.4	0.6	0.3	0.5%
P, mg/kg	1070	6	3	0.6%	1056	78	39	7.4%	1029	28	14	2.7%	996	13	6	1.3%
Pb, mg/kg	26.2	0.4	0.2	1.7%	24.0	1.2	0.7	5.1%	24.8	0.3	0.2	1.2%	23.8	[2]0.2	0.1	0.6%
Sb, mg/kg	44.3	0.3	0.1	0.6%	37.3	2.1	1.0	5.6%	43.8	1.4	0.7	3.2%	45.5	1.3	0.6	2.8%
Sr, mg/kg	110.0	0.6	0.3	0.6%	89	12	6	13%	111.5	1.1	0.6	1.0%	109.9	2.5	1.2	2.2%
Zn, mg/kg	94.7	2.2	1.0	2.4%	96.3	1.9	1.1	2.0%	100.6	1.3	0.7	1.3%	106.4	1.5	0.9	1.5%

[1]n=3
[2]n=2

Table 3.64 Comparison of digestion performances of procedures on Sample No 5

			Digestion Procedure 2				Digestion Procedure 3				Digestion Procedure 4			
No 5	**Digestion Procedure 1**		**Student t-test**				**Student t-test**				**Student t-test**			
Analyte	Mass Fraction	sd	t value	df	$t_{critical}$	Result	t value	df	$t_{critical}$	Result	t value	df	$t_{critical}$	Result
As, mg/kg	5.17	0.30	9.19	5.0	2.57	Different	2.50	5.0	2.57	Identical	1.38	5.0	2.57	Identical
B, mg/kg	29.0	1.5	8.09	6.0	2.45	Different	0.93	6.0	2.45	Identical	-	-	-	
Ba, mg/kg	630	23	5.89	4.0	2.78	Different	1.35	4.0	2.78	Identical	0.20	4.0	2.78	Identical
Ca, %	4390	478	0.26	3.0	3.18	Identical	1.55	3.0	3.18	Identical	1.88	3.0	3.18	Identical
Cd, mg/kg	1.22	0.06	8.34	6.0	2.45	Different	1.26	6.0	2.45	Identical	0.87	6.0	2.45	Identical
Co, mg/kg	16.0	0.4	2.82	5.0	2.57	Different	4.14	5.0	2.57	Different	6.28	5.0	2.57	Different
Cr, mg/kg	57.0	2.4	2.30	5.0	2.57	Identical	1.91	5.0	2.57	Identical	7.22	5.0	2.57	Different
Cu, mg/kg	37.3	0.7	0.66	5.0	2.57	Identical	0.64	5.0	2.57	Identical	4.38	5.0	2.57	Different
Fe, %	5.10	0.08	10.2	4.0	2.78	Different	3.06	4.0	2.78	Different	0.61	4.0	2.78	Identical
K, %	1.82	0.23	3.61	4.0	2.78	Different	0.16	4.0	2.78	Identical	0.82	4.0	2.78	Identical
Mg, %	0.61	0.12	0.58	3.0	3.18	Identical	1.25	3.0	3.18	Identical	0.58	3.0	3.18	Identical
Mn, mg/kg	822	19	7.60	4.0	2.78	Different	1.79	4.0	2.78	Identical	1.02	4.0	2.78	Identical
Na, mg/kg	3147	49	74.4	4.0	2.78	Different	1.02	4.0	2.78	Identical	2.01	4.0	2.78	Identical
Ni, mg/kg	26.4	0.6	1.19	5.0	2.57	Identical	2.13	5.0	2.57	Identical	3.84	5.0	2.57	Different
P, mg/kg	1019	43	3.64	6.0	2.45	Different	2.91	6.0	2.45	Different	5.98	6.0	2.45	Different
Pb, mg/kg	31.1	1.2	4.47	5.0	2.57	Different	0.46	5.0	2.57	Identical	1.83	5.0	2.57	Identical
Sb, mg/kg	31.5	3.0	5.26	6.0	2.45	Different	1.00	6.0	2.45	Identical	1.15	6.0	2.45	Identical
Sr, mg/kg	50.4	1.6	13.6	6.0	2.45	Different	1.77	6.0	2.45	Identical	4.90	6.0	2.45	Different
Zn, mg/kg	212	2.1	1.08	5.0	2.57	Identical	1.05	5.0	2.57	Identical	7.33	5.0	2.57	Different

Table 3.65 Comparison of digestion performances of procedures on Sample No 6

			Digestion Procedure 2				Digestion Procedure 3				Digestion Procedure 4			
No 6	**Digestion Procedure 1**		**Student t-test**				**Student t-test**				**Student t-test**			
Analyte	Mass Fraction	sd (n=5)	t value	df	$t_{critical}$	Result	t value	df	$t_{critical}$	Result	t value	df	$t_{critical}$	Result
As, mg/kg	51.0	0.2	3.06	6.0	2.45	Different	3.73	6.0	2.45	Different	1.20	6.0	2.45	Identical
B, mg/kg	42.6	4.6	6.47	6.0	2.45	Different	0.06	6.0	2.45	Identical	-	-	-	-
Ba, mg/kg	319	8	13.0	4.0	2.78	Different	1.2	4.0	2.78	Identical	3.5	4.0	2.78	Different
Ca, %	2.08	0.03	3.80	4.0	2.78	Different	0.91	4.0	2.78	Identical	3.8	4.0	2.78	Different
Cd, mg/kg	1.17	0.01	6.30	6.0	2.45	Different	0.39	6.0	2.45	Identical	20.6	6.0	2.45	Different
Co, mg/kg	26.2	0.3	12.6	6.0	2.45	Different	12.2	6.0	2.57	Different	18.6	6.0	2.57	Different
Cr, mg/kg	139	4	1.87	6.0	2.45	Identical	8.31	6.0	2.57	Different	18.4	6.0	2.57	Different
Cu, mg/kg	62.9	1.3	9.04	6.0	2.45	Different	9.27	6.0	2.57	Different	19.6	6.0	2.57	Different
Fe, %	5.02	0.08	5.7	4.0	2.78	Different	0.6	4.0	2.78	Identical	6.2	4.0	2.78	Different
K, %	1.29	0.01	8.62	4.0	2.78	Different	0.3	4.0	2.78	Identical	4.3	4.0	2.78	Different
Mg, %	1.11	0.03	2.86	4.0	2.78	Different	2.2	4.0	2.78	Identical	4.8	4.0	2.78	Different
Mn, mg/kg	1469	10	7.58	4.0	2.78	Different	0.2	4.0	2.78	Identical	4.8	4.0	2.78	Different
Na, mg/kg	5396	47	137.8	4.0	2.78	Different	0.4	4.0	2.78	Identical	0.3	4.0	2.78	Identical
Ni, mg/kg	85.4	1.4	6.25	6.0	2.45	Different	13.5	6.0	2.57	Different	26.7	6.0	2.57	Different
P, mg/kg	1070	6	0.30	6.0	2.45	Identical	2.6	6.0	2.45	Different	9.3	6.0	2.45	Different
Pb, mg/kg	26.2	0.4	3.04	6.0	2.45	Different	5.1	6.0	2.57	Different	10.4	5.0	2.57	Different
Sb, mg/kg	44.3	0.3	5.80	6.0	2.45	Different	0.6	6.0	2.45	Identical	1.6	6.0	2.45	Identical
Sr, mg/kg	110.0	0.6	3.0	6.0	2.45	Different	2.2	6.0	2.45	Identical	0.1	6.0	2.45	Identical
Zn, mg/kg	94.7	2.2	1.08	6.0	2.45	Identical	4.8	6.0	2.57	Different	8.7	6.0	2.57	Different

3.3.5 Method Validation for Determination of Total Elemental Mass Fraction in Soil

Total elemental mass fraction in soil samples were performed by HR-ICP-MS and ICP-OES. Method validation of these two methods were performed by NIST SRM 2711a *Montana II Soil*. The samples were mineralized using pressure and temperature-controlled microwave digestion system using Digestion Procedure 1. Matrix matched external calibration technique with internal standard (^{115}In for ICP-MS, Nb for ICP-OES) was used in order to minimize matrix effects and variations in performances of the instruments during analysis period. All sample preparation was performed gravimetrically.

3.3.5.1 ICP-MS Analysis in Soil Matrix

Sample preparation and determination of Ba, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn were performed by using HR-ICP-MS. These analytes were subdivided in two groups as major (Ba, Ca, Fe, K, Mg, Mn and Na) and minor (Co, Cr, Cu, Ni, Pb, Zn). Digested samples were diluted by a factor of 200 and 30 further before introducing them to ICP-MS for determination of major and minor group analytes, respectively.

Limit of detection, limit of quantification, repeatability (within day precision), intermediate precision (between days precision), trueness, linearity, working range and selectivity have been investigated in the method validation.

The working range of the method for each analyte is reported in Table 3.66. These ranges were calculated using the obtained linear calibration plots and taking the dilution factor of samples into account. Linearity which is described as correlation coefficient within the working range was always found to be >0.999.

The sensitivity of the method was also reported in terms of limit of detection ($3s+C_{blank}$) and quantification ($10s+C_{blank}$) in Table 3.67. Method precision was evaluated in terms of both intermediate precision and repeatability using the results obtained from two independent day analysis. The combined uncertainty for repeatability was varying in the range from 0.79 to 2.44% and intermediate precision of the method was reported in the range from 0.76 - 7.24 %.

Table 3.66 Analytes and working range of the method for multi elemental determination in soil by HR-ICP-MS

Groups	Analyte	Isotope	Resolution Mode	Working Range of Method
MINOR	Co, *mg/kg*	^{59}Co	MR	2 - 340
	Cr, *mg/kg*	^{52}Cr, ^{53}Cr	MR	10 -1425
	Cu, *mg/kg*	^{63}Cu, ^{65}Cu	MR	3 - 465
	Ni, *mg/kg*	^{60}Ni, ^{62}Ni	MR	1.8 - 285
	Pb, *mg/kg*	^{206}Pb, ^{207}Pb, ^{208}Pb	MR	9-1460
	Zn, *mg/kg*	^{66}Zn, ^{68}Zn	MR	26-3470
MAJOR	Ba, *mg/kg*	^{135}Ba, ^{137}Ba, ^{138}Ba	MR	26- 1600
	Ca, %	^{42}Ca,^{44}Ca	MR	0.3 - 19
	Fe, %	^{56}Fe, ^{57}Fe	MR	778 - 3111
	K, %	^{39}K	HR	0.15 - 9.4
	Mg, %	^{24}Mg, ^{25}Mg, ^{26}Mg	HR	0.08 - 4.7
	Mn, *mg/kg*	^{55}Mn	MR	28 - 2325
	Na, %	^{23}Na	MR	0.07 - 4.3

Table 3.67 Sensitivity and precision of the method for multi elemental analysis in soil by HR-ICP-MS

		Sensitivity		Precision	
		LOD (n=10)	*LOQ (n=10)*	*u(w)*[2] *%*	*u(b)*[3] *%*
Minor Group	Co, *ng/g*	8.2	21	1.64	3.93
	Cr, *mg/kg*	0.21	0.47	2.44	6.03
	Cu, *mg/kg*	0.24	0.44	1.45	1.85
	Ni, *mg/kg*	0.29	0.58	1.41	7.24
	Pb, *mg/kg*	0.07	0.15	1.09	4.57
	Zn, *mg/kg*	3.2	7.7	1.59	1.11
Major Group,	Ba, *mg/kg*	0.7	1.6	0.58	0.95
	[1]Ca, *mg/kg*	61	119	0.83	1.35
	Fe, *mg/kg*	8.2	19	1.28	0.76
	K, *mg/kg*	74	140	1.61	3.66
	Mg, *mg/kg*	62	143	0.79	$MS_{between} < MS_{within}$
	Mn, *ng/g*	1.0	2.8	1.11	2.17
	Na, *mg/kg*	52	130	1.75	$MS_{between} < MS_{within}$

[1]n=6
[2]Combined uncertainty on repeatability
[3]Combined uncertainty on intermediate precision

Trueness of the method for the determination of total elemental mass fraction in soil samples by HR-ICP-MS are summarized in the Table 3.68. As it was already discussed in section 3.3.4, the recovery rates of NIST SRM 2711a for all analytes except Cr was in the range of 94.7-100.5%.

Table 3.68 Trueness of the method for multi elemental analysis in soil by HR-ICP-MS

	Certified value U (*k=2*)	Measured Value *(n=8)*	Recovery %
Ba, mg/kg	730 ± 15	718 ± 2	98.4 ± 0.3
Ca, %	2.42± 0.06	2.43 ± 0.02	100.3 ± 0.7
Co, mg/kg	9.89 ± 0.18	9.47 ± 0.27	96.0 ± 2.9
Cr, mg/kg	52.3 ± 2.9	41.1 ± 1.4	78.6 ± 3.4
Cu, mg/kg	140 ± 2	132 ± 3	94.4 ± 5.9
Fe, %	2.82 ± 0.04	2.81 ± 0.02	99.7 ± 0.7
K, %	2.53 ± 0.10	2.51 ± 0.03	103.5% ± 1.2%
Mg, %	1.07 ± 0.06	1.02 ± 0.01	95.3% ± 0.6%
Mn, mg/kg	675 ± 18	660 ± 4	97.8% ± 0.6%
Na, %	1.2 ± 0.01	1.15 ± 0.02	95.6% ± 1.3%
Ni, mg/kg	21.7 ± 0.7	20.2 ± 0.3	93.3% ± 1.5%
Pb, %	0.140 ± 0.001	0.139 ± 0.02	99.0% ± 1.7%
Zn, mg/kg	414 ± 11	392 ± 12	94.7% ± 3.0%

Measurement uncertainty for multi element determination in soil by HR-ICP-MS was calculated using top down approach according to EURACHEM/CITAC Quantifying Uncertainty in Analytical Measurement [264] and also Evaluation of measurement data – Guide to the expression of uncertainty JCGM 100:2008 [265]. Precision of method (repeatability and intermediate precision), trueness (Bias) based on NIST SRM 2711a and uncertainty of the SRM were taken into account in the calculation of measurement uncertainty. The measurement uncertainty for the proposed method of multi element determination in soil samples by HR-ICP-MS are given in Table 3.69. As significant biases appeared in the results of Ba, Cr, Mg, Na, Ni with the certified value of the SRM, this bias had

a significant contribution on their measurement uncertainty budgets. On the other hand, intermediate precision of the method for K, Ni and Pb had also a significant contribution on the budgets.

Table 3.69 Estimated measurement uncertainty values for the determination of total elemental content in soil by HR-ICP-MS

	Mass Fraction	u *(k=1)*	U *(k=2)*	% U *(k=2)*
Ba, *mg/kg*	712	16	31	4.4
Ca, *%*	2.39	0.05	0.10	4.1
Co, *ng/g*	9.8	0.5	0.9	9.4
Cr, *ng/g*	39	9	17	43
Cu, *mg/kg*	130	3.8	7.6	5.9
Fe, *%*	2.78	0.05	0.09	3.4
K, *%*	2.44	0.11	0.22	9.0
Mg, *mg/kg*	1.02	0.06	0.11	11
Mn, *mg/kg*	673	21	41	6.2
Na, *%*	1.15	0.06	0.11	9.6
Ni, *mg/kg*	19.1	1.9	3.7	19
Pb, *%*	0.144	0.007	0.014	9.4
Zn, *mg/kg*	397	10	20	5.1

3.3.5.2 ICP-OES Analysis of Soil Matrix

During the validation of multi element determination in soil by HR-ICP-MS, some technical struggles appeared like instability which could not be compensated by using internal standard during the period of sequence, heavy memory effects. Therefore, determination of As, B, Cd, P and Sr was performed by ICP-OES. Digested samples were diluted by a factor of 1.25 further before introducing them to ICP-OES. Limit of detection, limit of quantification, repeatability (within day precision), intermediate precision (between days precision), trueness, linearity, working range and selectivity have been investigated in the method validation.

The working range and selected line for each analyte are given in Table 3.70. These ranges were calculated using the obtained linear calibration curves and taking the dilution factor of samples into account. Linearity which is described as correlation coefficient within the working range was always found be >0.999 for As, Cd, P, Sb and Sr and >0.99 for B.

Table 3.70 Analytes and working range of the method for multi elemental analysis in soil by ICP-OES

Analyte	λ (nm)	Working Range of Method
As, *mg/kg*	189.042	0.9 - 185
B, *mg/kg*	249.677	10 - 345
Cd, *mg/kg*	214.438	0.3 -70
P, *mg/kg*	177.495	93 - 5418
Sb, *mg/kg*	231.147	1.7 - 350
Sr, *mg/kg*	421.552	16 - 1870

The sensitivity of the method was also reported in terms of limit of detection ($3s+C_{blank}$) and quantification ($10s+C_{blank}$) in Table 3.71. Method precision was evaluated in terms of both intermediate precision and repeatability using the results obtained from two independent day analysis. The combined uncertainty for repeatability was found as in the range from 1.8 to 2.4% and combined uncertainty on intermediate precision was reported as 0.52% for As, 6.42% for B and found as smaller than the within day repeatability for Cd, P, Sb and Sr.

Table 3.71 Sensitivity and precision of the method for multi elemental determination in soil by ICP-OES

	Sensitivity		Precision	
	LOD (n=10)	*LOQ (n=10)*	*u(w)[1] %*	*u(b)[2] %*
As, *mg/kg*	0.7	2.0	1.83	0.52
B, *mg/kg*	1.9	5.3	1.82	6.42
Cd, *mg/kg*	0.05	0.14	1.96	$MS_{between}<MS_{within}$
P, *mg/kg*	1.3	2.4	1.99	$MS_{between}<MS_{within}$
Sb, *mg/kg*	0.6	1.4	2.39	$MS_{between}<MS_{within}$
Sr, *mg/kg*	0.01	0.03	2.30	$MS_{between}<MS_{within}$

[1]Combined uncertainty on repeatability
[2]Combined uncertainty on intermediate precision

Trueness of the method for the determination of total elemental mass fraction in soil samples by ICP-OES were studied by using NIST SRM 2711a and results are summarized in the Table 3.72. The recovery rates of NIST SRM 2711a were found in the range of 95 ± 5% for all analytes.

Table 3.72 Trueness of the method for multi elemental analysis in soil by ICP-OES

	Certified value U (*k=2*)	Measured Value (*n=8*)	Recovery %
As, mg/kg	107 ± 5	101 ± 3	94.3 ± 3.0
[1]**B**, mg/kg	50	48.2 ± 3.4	96.4 ± 7.0
Cd, mg/kg	54.1 ± 0.5	54.9 ± 2.0	101.5 ± 3.8
P, mg/kg	842 ± 11	836 ± 32	99.3 ± 3.8
[2]**Sb**, mg/kg	23.8 ± 1.4	23.8 ± 1.1	100 ± 5
Sr, mg/kg	242 ± 10	239 ± 11	98.7 ± 4.3

[1]Informative value
[2]n=7

Measurement uncertainty for multi element determination in soil by ICP-OES was calculated as done for the method by HR-ICP-MS and summarized in Table 3.73. The expanded measurement uncertainty for As and B were calculated bigger than 10% due to the presence of significant bias and relatively higher intermediate precision, respectively.

Table 3.73 Estimated measurement uncertainty values for the determination of total elemental content in soil by ICP-OES

	Mass Fraction	u (*k=1*)	U (*k=2*)	% U (*k=2*)
As, mg/kg	102.2	6.5	13.4	13.1
B, mg/kg	51.7	3.5	6.9	13.4
Cd, mg/kg	54.9	1.4	2.7	4.8
P, mg/kg	844	21	42	5.0
Sb, mg/kg	23.7	1.0	2.0	8.4
Sr, mg/kg	237	8	16	6.9

3.3.6 Total Elemental Mass Fractions of Soil Samples

Five independent subsamples from each dried soil samples with procedural blanks were digested using temperature and pressure-controlled microwave digestion system. Analysis of digested soil samples were performed as described in the section 4.3.5. Moisture content of each soils was determined by keeping them at 110 °C for two hours cycles (4 cycle) and moisture content was found to be stable

after first two hours for each soil. Summary of dry-mass basis mass fractions of the samples is given in Table 3.74.

Table 3.74 Total elemental mass fractions of target analytes in soil samples

Analyte	Ca			Ba			Fe		
LOD	61	*mg/kg*		0.7	*mg/kg*		8	*mg/kg*	
LOQ	119			1.6			19		
Sample Code (n=5)	Mass Fraction %	%RSD	s.u	Mass Fraction mg /kg	%RSD	s.u	Mass Fraction %	%RSD	s.u
1	4.93	4.2%	0.09	125	2.8%	2	4.16	8.7%	0.16
2									
3	4.80	1.4%	0.03	160	4.2%	3	7.09	1.4%	0.04
4	9.07	2.2%	0.09	231	0.9%	1	4.81	2.0%	0.04
5	0.484	2.4%	0.005	669	2.3%	7	5.27	2.1%	0.05
6	2.19	1.5%	0.01	331	1.4%	2	5.29	1.6%	0.04
7	10.4	1.1%	0.05	100	6.1%	3	5.94	4.4%	0.12
8	7.44	1.6%	0.05	142	2.0%	1	4.45	1.3%	0.03
9									
10									
11	10.5	0.8%	0.04	310	0.9%	1	6.03	1.6%	0.04
12	13.0	3.7%	0.2	345	3.2%	5	2.93	0.8%	0.01
13	1.97	5.0%	0.04	1068	2.8%	13	3.77	2.1%	0.04
14	14.1	0.6%	0.04	174	1.8%	1	3.18	1.4%	0.02
15	4.32	1.5%	0.03	[1]277	3.6%	15	4.37	2.1%	0.04
16	7.62	1.6%	0.06	188	5.5%	5	2.26	2.7%	0.03
17									

[1]n=4

Table 3.74 Total elemental mass fractions of target analytes in soil samples - *continuous*

Analyte	K			Mg			Mn			Na		
LOD	74	*mg/kg*		62	*mg/kg*		1.0	*mg/kg*		52	*mg/kg*	
LOQ	140			143			2.8			130		
Sample Code (n=5)	Mass Fraction %	%RSD	s.u	Mass Fraction %	%RSD	s.u	Mass Fraction mg /kg	%RSD	s.u	Mass Fraction %	%RSD	s.u
1	0.84	3.3%	0.01	[1]46,7	5.1%	6.0	1017	2.5%	11	4.65	7.1%	0.15
2												
3	0.59	6.2%	0.02	15.6	4.6%	0.3	1300	4.1%	24	19.1	10.6%	0.9
4	1.69	1.6%	0.01	22.8	2.5%	0.3	794	1.8%	6	5.88	2.2%	0.06
5	1.85	3.7%	0.03	7.07	7.7%	0.24	913	3.1%	13	3.40	2.9%	0.04
6	1.27	3.6%	0.02	12.3	0.9%	0.0	1644	3.6%	27	5.78	2.1%	0.06
7	0.83	2.1%	0.01	10.9	11.8%	0.6	846	1.5%	6	0.366	2.9%	0.005
8	0.83	2.5%	0.01	40.1	1.9%	0.3	994	1.0%	4	3.90	1.7%	0.03
9												
10												
11	1.25	5.9%	0.03	26.0	1.4%	0.2	1079	1.7%	8	11.0	1.2%	0.1
12	1.12	2.9%	0.01	14.2	1.5%	0.1	1067	1.8%	9	2.20	1.6%	0.02
13	2.11	16.3%	0.15	6.72	49.7%	1.49	781	3.6%	12	15.0	1.2%	0.1
14	0.77	0.5%	0.00	16.3	0.8%	0.1	764	1.9%	7	6.82	0.9%	0.03
15	0.99	3.3%	0.01	15.6	5.2%	0.4	1146	1.7%	9	11.3	1.9%	0.10
16	0.88	2.7%	0.01	21.5	4.9%	0.5	619	1.1%	3	6.26	1.9%	0.05
17												

[1]n=3

Table 3.74 Total elemental mass fractions of target analytes in soil samples-- *continuous*

Analyte	As			B			Cd		
LOD	0.74	mg/kg		1.8	mg/kg		0.05	mg/kg	
LOQ	2.05			5.6			0.14		
Sample Code (n=5)	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction mg/kg	%RSD	s.u
1	[1]9.76	3.9%	0.19	43.4	3.0%	0.6	1.42	2.8%	0.02
2									
3	2.84	3.9%	0.05	10.7	9.4%	0.5	1.36	4.8%	0.03
4	7.72	2.0%	0.07	63.5	0.8%	0.2	1.11	0.4%	0.00
5	5.02	5.4%	0.12	26.5	2.8%	0.3	1.22	4.6%	0.03
6	51.0	0.5%	0.12	40.9	11.1%	2.0	1.17	0.9%	0.00
7	[1]5.06	8.2%	0.21	26.7	2.6%	0.3	[1]0.95	2.6%	0.01
8	2.96	5.8%	0.08	34.9	1.5%	0.2	0.92	7.2%	0.03
9									
10									
11	18.2	7.6%	0.6	34.9	5.2%	0.7	1.12	0.8%	0.00
12	41.2	4.7%	0.9	97.0	3.9%	1.7	1.34	2.9%	0.02
13	[1]22.6	3.1%	0.4	[1]26.3	7.2%	2.7	1.12	12%	0.06
14	5.79	2.7%	0.07	20.9	4.9%	0.5	0.93	8.3%	0.03
15	12.1	5.2%	0.3	28.3	7.9%	1.0	1.15	5.6%	0.03
16	[1]4.29	16%	0.3	44.9	4.3%	0.87	0.88	13%	0.05
17									

[1]n=4

Table 3.74 Total elemental mass fractions of target analytes in soil samples - *continuous*

Analyte	Co			Cr			Cu		
LOD	0.008	mg/kg		0.21	mg/kg		0.24	mg/kg	
LOQ	0.021			0.47			0.44		
Sample Code (n=5)	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction mg/kg	%RSD	s.u
1	57.5	1.2%	0.3	809	5.5%	20	[1]40.5	1.7%	0.4
2									
3	38.1	2.5%	0.4	190	7.6%	6	43.9	3.1%	0.6
4	24.6	2.5%	0.3	151	8.4%	6	45.4	4.5%	0.9
5	[1]16.0	2.6%	0.2	57.0	4.1%	1.2	[1]37.3	2.0%	0.4
6	26.2	1.3%	0.2	139	3.0%	1.9	62.9	2.1%	0.6
7	34.7	1.8%	0.3	188	6.4%	5	34.7	2.7%	0.4
8	40.1	2.3%	0.4	690	49%	151	102	1.6%	1
9									
10									
11	28.7	3.4%	0.4	161	6.3%	5	52.0	4.0%	0.9
12	23.0	2.7%	0.3	211	7.5%	7	406	6.4%	12
13	14.4	2.3%	0.2	41.3	6.6%	1.2	28.5	3.3%	0.4
14	14.2	1.6%	0.1	117	28%	15	24.6	1.7%	0.2
15	16.0	0.8%	0.1	34.4	6.5%	1.0	41.5	1.0%	0.2
16	14.3	2.2%	0.2	[1]393	40%	79	[1]94.6	2.1%	0.7
17									

[1]n=4

Table 3. 74 Total elemental mass fractions of target analytes in soil samples - *continuous*

Analyte	Ni			P			Pb		
LOD	0.29	mg/kg		1.3	mg/kg		0.07	mg/kg	
LOQ	0.58			2.4			0.15		
Sample Code (n=5)	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction mg/kg	%RSD	s.u
1	959	1.1%	5	1482	3.8%	25	16	1.2%	0
2									
3	89.7	5.4%	2.2	1172	4.6%	24	[1]8.4	1.1%	0.05
4	77.8	7.2%	2.5	1307	1.1%	6	18.1	2.7%	0.2
5	[1]26.4	2.2%	0.3	1019	3.7%	17	31.1	3.8%	0.6
6	85.4	1.7%	0.6	1070	0.6%	3	26.2	1.7%	0.4
7	151	3.6%	2	[1]1862	2.6%	24	14	2.3%	0.1
8	585	2.0%	5	1817	1.5%	13	[1]15.1	0.9%	0.1
9									
10									
11	118	2.9%	2	1795	1.8%	14	21.9	2.7%	0
12	324	1.7%	2	1793	5.4%	43	25.4	1.9%	0
13	18.6	4.2%	0.3	[1]1011	1.4%	7	58.8	2.3%	0.6
14	62.3	1.3%	0.4	771	8.9%	31	10.3	4.7%	0.2
15	28.0	0.8%	0.1	702	4.9%	15	21.2	4.1%	0.4
16	[1]166	1.6%	1	1845	1.8%	15	[1]11.8	4.2%	0.2
17									

[1]n=4

Table 3. 74 Total elemental mass fractions of target analytes in soil samples - *continuous*

Analyte	Sb			Sr			Zn		
LOD	0.6	mg/kg			mg/kg		3.2	mg/kg	
LOQ	1.4						7.7		
Sample Code (n=5)	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction mg/kg	%RSD	s.u	Mass Fraction mg/kg	%RSD	s.u
1	90.4	2.5%	1.02	186	6.0%	5	134	2.1%	1
2									
3	60.5	5.5%	1.49	305	4.8%	7	91.4	3.7%	1.5
4	36.4	0.4%	0.07	169	1.3%	1	102	1.7%	1
5	[1]30.3	8.9%	1.35	50.4	2.8%	0.6	[1]212	1.0%	1
6	44.3	0.6%	0.11	110	0.7%	0.3	94.7	2.4%	1.0
7	[1]45.1	6.0%	1.35	[1]97	4.9%	2	141	2.3%	1
8	67.3	0.5%	0.14	92.9	1.8%	0.7	85.0	3.3%	1.2
9									
10									
11	38.6	0.9%	0.16	220	1.5%	1	119	2.3%	1
12	39.7	3.7%	0.65	446	12%	24	136	2.4%	1
13	[1]30.1	2.2%	0.34	[1]558	5.0%	11	74.4	3.5%	1.2
14	29.5	7.7%	1.02	239	4.4%	5	64.8	1.5%	0.4
15	[1]32.9	5.3%	0.87	232	5.7%	6	77.2	2.1%	0.7
16	31.5	6.6%	0.93	194	1.2%	1	[1]94.6	1.7%	0.8
17									

[1]n=4

3.3.7 Evaluating Elemental Composition Relation of Walnuts

As the number of walnut samples was limited, Spearman's Rho correlation test was performed to identify possible relationships between the element contents. Among 17 elements, only 5 of them (As, Ba, Na, Ni and Pb) were not in a relationship with any others. In general, 14 moderate positive/negative correlations and one high negative correlation (between Sr and Mg) was reported for pairwise correlation between elements. Moreover, Sr has also moderate negative correlation with B and K at 99% and 95% confidence level, respectively. As it is seen in Table 3.75, except Sr, the remaining elements, B, Ca, Cd, Co, Cu, Fe, K, P and Zn showed positive moderate correlations. On the other hand, Sr was in a negative relationship between B, K and Mg. Among all the elements, Zn was the most correlated element with others (Ca, Cu, Co and P). The estimated correlation coefficients were all positive and statistically significant at both 95% and 99% confidence level.

Table 3.75 Correlation matrix for element concentration in walnuts

	As	B	Ba	Ca	Cd	Co	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Sr	Zn
As	1.00	-0.21	0.400	-0.176	-0.208	-0.053	-0.062	-0.019	0.256	-0.223	0.236	-0.114	-0.107	-0.057	-0.161	0.109	-0.191
B		1.00	0.054	0.238	-0.186	-0.028	-0.213	0.085	0.238	0.647**	-0.176	-0.14	0.123	0.451	0.292	-0.623**	0.069
Ba			1.000	0.243	0.178	0.411	0.284	-0.157	0.132	-0.108	0.404	-0.455	0.059	0.218	-0.243	0.189	-0.186
Ca				1.000	0.005	0.417	0.520*	0.428	-0.275	0.108	0.228	-0.405	-0.395	0.642**	-0.174	-0.061	0.586*
Cd					1.000	0.690**	0.08	-0.098	-0.128	-0.356	0.367	-0.004	0.436	-0.266	0.219	0.302	0.193
Co						1.000	0.348	0.067	-0.131	-0.106	0.675**	0.053	0.069	0.233	0.219	0.141	0.530*
Cu							1.000	0.385	0.098	0.054	0.292	-0.151	-0.294	0.539*	-0.47	0.154	0.502*
Fe								1.000	-0.147	-0.077	0.137	-0.136	-0.46	0.457	-0.166	-0.001	0.632**
K									1.000	0.527*	0.051	0.033	0.299	0.25	-0.115	-0.586*	-0.145
Mg										1.000	-0.235	0.181	-0.076	0.402	-0.121	-0.743**	0.022
Mn											1.000	0.153	0.007	0.466	0.122	0.132	0.365
Na												1.000	-0.173	-0.098	0.377	-0.048	0.168
Ni													1.000	-0.23	0.18	-0.056	-0.169
P														1.000	-0.092	-0.346	0.554*
Pb															1.000	-0.088	0.119
Sr																1.000	-0.02
Zn																	1.000

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

3.3.8 The Multi-element Composition Relation Between Walnuts and Soils

Spearman's Rho test was applied for evaluating relationships of multi-element composition between walnuts and provenance soil. Spearman's Rho correlation coefficient between two measurement variables were calculated for all element pairs and those of the significant element pairs are listed in Table 3.76. The contents of As, B and Sr in walnuts were significantly and positively correlated with those in soils while a negative correlation was detected for Cu. On the other hand, the relationship between different element pairs (walnuts/soil) were also statistically evaluated. It was noted that As/Ba, As/Pb and B/Zn have a positive correlation which means as the content of Ba and Pb increase in soil the amount of As in walnut also increase. Moreover, B/Na, B/Sr, Ba/Co, Ba/Sb, Co/Ni, Cu/B, Cu/Cr, Cu/Mg, Cu/Ni, Cu/P, Cu/Sb, K/Na, Mg/Sr, Ni/Na , Ni/Zn, Sr/Co, Sr/Na, Sr/Sb, Sr/Zn showed a negative correlation in walnut and soil samples.

Table 3.76 Correlation coefficients of elemental composition between walnuts and soil

Element $_{Walnut/Soil}$	Correlation Coefficient	P value
As/As	0.805**	0.001
As/Ba	0.660*	0.014
As/Pb	0.560*	0.047
B/B	0.580*	0.038
B/Na	-0.582*	0.037
B/Sr	-0.571*	0.041
B/Zn	0.714**	0.06
Ba/Co	-0.613*	0.026
Ba/Sb	-0.588*	0.035
Co/Ni	-0.601*	0.030
Cu/B	-0.726**	0.005
Cu/Cr	-0.731**	0.005
Cu/Cu	-0.632*	0.021
Cu/Mg	-0.845**	0.000
Cu/Ni	-0.797**	0.001
Cu/P	-0.593*	0.033
Cu/Sb	-0.555*	0.049
K/Na	-0.615*	0.025
Mg/Sr	-0.703**	0.007
Ni/Na	-0.626*	0.022
Ni/Zn	-0.560*	0.046
Sr/Co	-0.696**	0.008
Sr/Na	-0.659*	0.014
Sr/Sb	-0.714**	0.006
Sr/Sr	0.560*	0.046
Sr/Zn	-0.555*	0.049

*Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

3.4 Conclusion

The main purpose of this thesis was to develop highly sensitive and accurate analytical techniques by mass spectrometry to be used in certification measurements in a candidate certified reference material, investigation, understanding the plant metabolism and also make a preliminary investigation for authentication analysis.

Herein, SI traceable TEA/$Mg(OH)_2$ assisted ID^3MS was developed and fully validated. This technique has provided very high trueness as well as very high precision for Cd, Cr, Cu, Fe, Ni, Pb and Zn and it was used in the whole certification measurements including homogenization, short term stability, long term stability and characterization. As the certified value has been determined via applying primary technique by a single laboratory which is one of the defined options for the characterization of a candidate CRM in ISO 17034 guide, the uncertainty of each 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 certificate was prepared as follows [288].

Name of the Material	:	Elements in Sea Water
Material Code	:	UME CRM 1206
Issue Date	:	12.02.2021
Revision Date	:	29.09.2021 (Revision history can be found on the last page)
Validity Period of the Certificate	:	12 months from the sales date
Certified Values	:	

Element	Mass Fraction[1,3] µg/kg	Uncertainty[2,3] µg/kg
Cd	0.433	0.010
Cr	2.44	0.20
Cu	1.019	0.023
Fe	12.7	1.4
Ni	4.568	0.043
Pb	1.068	0.017
Zn	8.52	0.42

[1] Certified values have been assigned by using ID-ICP-MS method.
[2] The expanded uncertainty of certified value includes characterization, homogeneity, stability components and is stated as the standard uncertainty multiplied by the coverage factor k = 2, which for a normal distribution corresponds to a coverage probability of approximately 95 %. The standard uncertainty of measurement has been determined in accordance with GUM "Guide to the Expression of Uncertainty in Measurement".
[3] The certified values and the uncertainties are traceable to the International System of Units (SI).

Figure 3.50 Certificate of UME CRM 1206 Elements in Sea Water

As arsenic is monoisotopic element, application of primary measurement technique could not be option during the characterization measurements. Instead, matrix matched external calibration technique was applied for the determination of As by ICP-MS/MS. However, if the characterization of a parameter cannot be measured with more than one method or primary method as a TÜBİTAK UME strategy, the value is given as an information with some exceptions. Therefore, arsenic has been provided as informative value in the certificate of UME CRM 1206.

In the second chapter of the thesis, mass spectrometry was used for understanding the metabolization of inorganic selenium species uptaken by leek samples which were exposed to hydroponic cultivation in climatic chamber. Total selenium determination was performed using oxygen mass shift mode of ICP-MS/MS which provides ultra-trace LOD values (2.2 ng/g) for the determination of Se in digested samples. Moreover, two speciation methods have been developed using strong anion exchange column and reverse phase column for the determination of

selenium species in enzymatic digested and gastrointestinal digested samples by ICP-MS/MS. In this research, mainly the following conclusions have been made:

- Even at the highest concentration (450 μM Se(IV); 325 μM Se(VI)), fortification of leek with inorganic species had no effect on growth.
- It has been demonstrated that leeks may accumulate up to 1800 mg/kg Se when fortified with selenite and up to 600 mg/kg Se when fortified with selenate.
- Biotransformation rate of selenite and selenate into organo-selenium species was approximately 90% and in the rage of 30%-60%, respectively.
- In selenite fortified leek, MeSeCys and SeMet were determined to be the most prevalent selenium species
- In gastrointestinal digest of Se(IV) and Se(VI) fortified samples, recovery rates were 76% and 93%, respectively; nevertheless, MeSeCys was shown to be the most prevalent species in leaves and stems of Se(IV) fortified leeks
- It is preferable to use of Se(IV) as a fertilizer in the growing of Se enriched leeks.

In the last chapter, multi element profiling technique using mainly a sector field inductively coupled plasma mass spectrometry were developed and applied for analysis of walnuts and their soils of origin to figure out potential markers that could be used in the authentication of walnuts in an extensive studies. In this pre-research, nineteen element were determined for multi-element profiling in walnuts and provenance soils. Since the composition of soil in the earth shows significant difference in their compositions, mineralization efficiency was evaluated by using four different digestion procedures and optimum digestion program was applied to all soil sources. This pre-investigation study showed:

- 15 pairwise correlation between elements (B/Mg, B/Sr, Ca/Cu, Ca/P, Ca/Zn, Cd/Co, Co/Mn, Co/Zn, Cu/P, Cu/Zn, Fe/Zn, K/Mg, K/Sr, Mg/Sr, P/Zn,) were detected in walnuts.
- Sr was found to be in moderate negative correlation with B, K and high negative correlation with Mg in walnut samples.
- Zn was found to be the most correlated element in walnut samples.

- The amount of As, B and Sr in walnuts and soil were found to be significantly and positively correlated.
- Two positive correlation between different element pairs (walnuts/soil), As/Ba, As/Pb, were detected
- Fifteen negative pairwise correlation in walnuts/soil (B/Na, B/Sr, B/Zn, Ba/Co, Ba/Sb, Co/Ni, Cu/B, Cu/Cr, Cu/Mg, Cu/Ni, Cu/P, Cu/Sb, K/Na, Mg/Sr and Ni/Na) were observed.

REFERENCES

[1] H. Matusiewicz and E. Bulska, *Inorganic trace analytics: trace element analysis and speciation*. De Gruyter, 2017.

[2] J. H. Martin, "Glacial-Interglacial CO2 Change: The iron hypothesis," *Paleoceanography*, vol. 5, no. 1, pp. 1–13, 1990.

[3] P. G. Falkowski, "The role of phytoplankton photosynthesis in global biogeochemical cycles," *Photosynth. Res.*, vol. 39, no. 3, pp. 235–258, 1994, doi: 10.1007/bf00014586.

[4]. R Mukhopadhyay *et al.*, "The biogeochemical cycles of trace metals in the oceans," Dekker, 2002. Accessed: Mar. 29, 2020. [Online]. Available: www.unizh.ch/microeco/uni/kurs/mikoek/results/project2/arsen.html.

[5] R. C. Xie *et al.*, "The cadmium-phosphate relationship in the western South Atlantic - The importance of mode and intermediate waters on the global systematics," *Mar. Chem.*, vol. 177, pp. 110–123, Nov. 2014, doi: 10.1016/j.marchem.2015.06.011.

[6] P. J. Lam and R. F. Anderson, "GEOTRACES: The marine biogeochemical cycle of trace elements and their ısotopes," *Elements*, vol. 14, no. 6, pp. 377–378, 2018, doi: 10.2138/gselements.14.6.377.

[7] Y. Sohrin and K. W. Bruland, "Global status of trace elements in the ocean," *TrAC - Trends Anal. Chem.*, vol. 30, no. 8, pp. 1291–1307, 2011, doi: 10.1016/j.trac.2011.03.006.

[8] W. G. Sunda, "Feedback interactions between trace metal nutrients and phytoplankton in the ocean," *Frontiers in Microbiology*, vol. 3, no. JUN. Frontiers in mocrobiology, pp. 1–22, 2012, doi: 10.3389/fmicb.2012.00204.

[9] S. W. G. Ã, "GEOTRACES - An international study of the global marine biogeochemical cycles of trace elements and their isotopes," *Chemie der Erde*, vol. 67, no. 2, pp. 85–131, 2007, doi: 10.1016/j.chemer.2007.02.001.

[10] U. Muller, *Inorganic structural chemistry.*, Second. John Wiley & Sons, Ltd, 2006.

[11] K.-J. Appenroth, *Soil heavy metals*. Springer, 2010.

[12] J. H. Duffus, "'heavy metals' - A meaningless term? (IUPAC technical report)," *Pure Appl. Chem.*, vol. 74, no. 5, pp. 793–807, 2002, doi: 10.1351/pac200274050793.

[13] Vanloon W. Gary and Duffy J. Stephen, *Environmental chemistry: A global perspective - Gary W. VanLoon, Stephen J. Duffy - Google Kitaplar*. Oxford: Oxford University Press, 2000.

[14] D. A. Phipps, "Chemistry and Biochemistry of trace metals in biological systems," *Eff. Heavy Met. Pollut. Plants*, pp. 1–54, 1981, doi: 10.1007/978-94-011-7339-1_1.

[15] E. Nieboer and D. H. S. Richardson, "The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions," *Environ. Pollution. Ser. B, Chem. Phys.*, vol. 1, no. 1, pp. 3–26, 1980, doi: 10.1016/0143-148X(80)90017-8.

[16] B. Kacar, A. K. Garcia, and A. D. Anbar, "Evolutionary history of bioessential elements can guide the search for life in the universe," *ChemBioChem*, vol. 22, no. 1, pp. 114–119, Jan. 2021, doi: 10.1002/CBIC.202000500.

[17] R. Aversa, V. Petrescu, A. Apicella, I. T. Petrescu, F. Ion, and T. Petrescu, “The basic elements of life’s,” *Am. J. Eng. Appl. Sci. Orig. Res. Pap.*, 2016, doi: 10.3844/ajeassp.2016.1189.1197.

[18] P. Olmedo, A. F. Hernández, A. Pla, P. Femia, A. Navas-Acien, and F. Gil, “Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples. Risk and nutritional assessment and mercury–selenium balance,” *Food Chem. Toxicol.*, vol. 62, pp. 299–307, Dec. 2013, doi: 10.1016/j.fct.2013.08.076.

[19] N. Jalbani, F. Ahmed, T. G. kazi, U. Rashid, A. B. Munshi, and A. Kandhro, “Determination of essential elements (Cu, Fe and Zn) in juices of commercially available in Pakistan,” *Food Chem. Toxicol.*, vol. 48, no. 10, pp. 2737–2740, Oct. 2010, doi: 10.1016/j.fct.2010.06.048.

[20] A. Szymczycha-Madeja, M. Welna, and P. Pohl, “Determination of essential and non-essential elements in green and black teas by FAAS and ICP OES simplified – multivariate classification of different tea products,” *Microchem. J.*, vol. 121, pp. 122–129, Jul. 2015, doi: 10.1016/j.microc.2015.02.009.

[21] F. G. Antes *et al.*, “Determination of toxic elements in coal by ICP-MS after digestion using microwave-induced combustion,” *Talanta*, vol. 83, no. 2, pp. 364–369, Dec. 2010, doi: 10.1016/j.talanta.2010.09.030.

[22] P. Olmedo, A. Pla, A. F. Hernández, F. Barbier, L. Ayouni, and F. Gil, “Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers,” *Environ. Int.*, vol. 59, pp. 63–72, Sep. 2013, doi: 10.1016/j.envınt.2013.05.005.

[23] A. Londonio, E. Morzán, and P. Smichowski, “Determination of toxic and potentially toxic elements in rice and rice-based products by inductively coupled plasma-mass spectrometry,” *Food Chem.*, vol. 284, pp. 149–154, Jun. 2019, doi: 10.1016/j.foodchem.2019.01.104.

[24] T. Oymak, Ş. Tokalioğlu, V. Yilmaz, Ş. Kartal, and D. Aydin, “Determination of lead and cadmium in food samples by the coprecipitation method,” *Food Chem.*, vol. 113, no. 4, pp. 1314–1317, Apr. 2009, doi: 10.1016/j.foodchem.2008.08.064.

[25] M. D. G. A. Korn *et al.*, “Separation and preconcentration procedures for the determination of lead using spectrometric techniques: A review,” *Talanta*, vol. 69, no. 1, pp. 16–24, Mar. 2006, doi: 10.1016/j.talanta.2005.10.043.

[26] T. Oymak, Ş. Tokalioğlu, V. Yilmaz, Ş. Kartal, and D. Aydin, “Determination of lead and cadmium in food samples by the coprecipitation method,” *Food Chem.*, vol. 113, no. 4, pp. 1314–1317, Apr. 2009, doi: 10.1016/j.foodchem.2008.08.064.

[27] J. E. Marcovecchio, S. E. Botté, C. E. Domini, and R. H. Freije, “Heavy metals, major metals, trace elements,” in *Handbook of Water Analysis*, 3rd ed., CRC Press, 2013, p. 50.

[28] Parlamento Europeo, “Directive 2008/50/CE of the European Union and Council of 21 May 2008 on ambient air quality and cleaner air for Europe,” *Official Journal of the European Union*, 2008. https://www.boe.es/doue/2002/189/L00012-00025.pdf (accessed Sep. 05, 2021).

[29] EU Parliament, “Directive 2004/107/EC of the European Parliament and of

the Council of 15 December 2004 relating to arsenic, cadmium, mercury, nicker and polyciclic aromatic hydrocarbons in ambient air.," *Off. J. Eur. Communities.*, vol. 23, pp. 3–16, 2005, Accessed: Sep. 05, 2021. [Online]. Available: https://eur-lex.europa.eu/legal-content/en/txt/pdf/?uri=celex:32004l0107&from=en.

[30] European Commission, "Dırectıve 2013/39/eu of the european parlıament and of the councıl of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy," *Official Journal of the European Union*, 2013. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex:32013L0039 (accessed Sep. 05, 2021).

[31] The Council of the European Communities, "Council Directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture (86/278/EEC)," *Off. J. Eur. Communities*, vol. L, no. 181/6, p. 7, 1986, Accessed: Sep. 05, 2021. [Online].Available:https://eur-lex.europa.eu/legal-content/en/txt/pdf/?uri=celex:31986l0278&from=en.

[32] "RASFF - food and feed safety alerts." https://ec.europa.eu/food/safety/rasff-food-and-feed-safety-alerts_en (accessed Sep. 05, 2021).

[33] C. R. Commission Regulation (EC) No 1881/2006, "Comission Regulation EC No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs," *Official Journal of the European Union*, 2006. http://www.efsa.europa.eu/etc/medialib/efsa/ (accessed Sep. 05, 2021).

[34] S. L. C. Ferreira *et al.*, "Atomic absorption spectrometry – A multi element technique," *TrAC - Trends in Analytical Chemistry*, vol. 100. Elsevier B.V., pp. 1–6, Mar. 01, 2018, doi: 10.1016/j.trac.2017.12.012.

[35] P. Wu, S. He, B. Luo, and X. Hou, "Flame furnace atomic absorption spectrometry: A review," *Appl. Spectrosc. Rev.*, vol. 44, no. 5, pp. 411–437, 2009, doi: 10.1080/05704920903018585.

[36] O. Y. Ataman, "Vapor generation and atom traps: Atomic absorption spectrometry at the ng/L level," *Spectrochimica Acta - Part B Atomic Spectroscopy*, vol. 63, no. 8. Elsevier, pp. 825–834, Aug. 01, 2008, doi: 10.1016/j.sab.2008.03.013.

[37] G. L. Moore, *Analytical spectroscopy library*, vol. 3. Elsevier, 1989.

[38] D. J. Butcher, "Atomic fluorescence spectrometry: A review of advances in instrumentation and novel applications," *Appl. Spectrosc. Rev.*, vol. 51, no. 5, pp. 397–416, 2016, doi: 10.1080/05704928.2016.1141099.

[39] D. Sánchez-Rodas, W. T. Corns, B. Chen, and P. B. Stockwell, "Atomic fluorescence spectrometry: A suitable detection technique in speciation studies for arsenic, selenium, antimony and mercury," *J. Anal. At. Spectrom.*, vol. 25, no. 7, pp. 933–946, Jul. 2010, doi: 10.1039/b917755h.

[40] L. Liu, H. Deng, L. Wu, C. Zheng, and X. Hou, "UV-induced carbonyl generation with formic acid for sensitive determination of nickel by atomic fluorescence spectrometry," *Talanta*, vol. 80, no. 3, pp. 1239–1244, Jan. 2010, doi: 10.1016/j.talanta.2009.09.016.

[41] Y. Wang, K. Xu, X. Jiang, X. Hou, and C. Zheng, "Dual-mode chemical vapor generation for simultaneous determination of hydride-forming and non-

hydride-forming elements by atomic fluorescence spectrometry," *Analyst*, vol. 139, no. 10, pp. 2538–2544, Apr. 2014, doi: 10.1039/c4an00066h.

[42] H. Deng, C. Zheng, L. Liu, L. Wu, X. Hou, and Y. Lv, "Photochemical vapor generation of carbonyl for ultrasensitive atomic fluorescence spectrometric determination of cobalt," *Microchem. J.*, vol. 96, no. 2, pp. 277–282, Nov. 2010, doi: 10.1016/j.microc.2010.04.002.

[43] O. Butler *et al.*, "Atomic Spectrometry Updates: A 25-year retrospective," *J. Anal. At. Spectrom.*, vol. 25, no. 10, pp. 1546–1566, 2010, doi: 10.1039/c005518m.

[44] M. Sargent *et al.*, "The role of ICP-MS in inorganic chemical metrology," *Metrologia*, vol. 56, no. 3, p. 034005, Apr. 2019, doi: 10.1088/1681-7575/ab0eac.

[45] A. R. Date and A. L. Gray, "Plasma source mass spectrometry using an inductively coupled plasma and a high resolution quadrupole mass filter," *Analyst*, vol. 106, no. 1269, pp. 1255–1267, Jan. 1981, doi: 10.1039/an9810601255.

[46] K. Sakata and K. Kawabata, "Reduction of fundamental polyatomic ions in inductively coupled plasma mass spectrometry," *Spectrochim. Acta Part B At. Spectrosc.*, vol. 49, no. 10, pp. 1027–1038, 1994, doi: 10.1016/0584-8547(94)80088-X.

[47] M. G. Minnich and R. S. Houk, "Comparison of cryogenic and membrane desolvation for attenuation of oxide, hydride and hydroxide ions and ions containing chlorine in inductively coupled plasma mass spectrometry," *J. Anal. At. Spectrom.*, vol. 13, no. 3, pp. 167–174, 1998, doi: 10.1039/a704274d.

[48] I. Sakata and K. Kawabata, "Reduction of fundamental polyatomic ions in inductively coupled plasma mass spectrometry," *Spectrochim. Acta*, vol. 49B, no. 10, pp. 1027–1038, Sep 1994, doi:10.1016/0584-8547(94)80088-X.

[49] M. G. Minnich and R. S. Houk, "Comparison of cryogenic and membrane desolvation for attenuation of oxide, hydride and hydroxide ions and ions containing chlorine in inductively coupled plasma mass spectrometry," *J. Anal. At. Spectrom.*, vol. 13, no. 3, pp. 167–174, 1998, doi: 10.1039/a704274d.

[50] T.-S. Lum and S.-Y. K. Leung, "Strategies to overcome spectral interference in ICP-MS detection," *J. Anal. At. Spectrom.*, vol. 31, no. 5, pp. 1051–1162, 2016, doi:10.1039/c5ja00497g.

[51] E. Hywel Evans, J. Pisonero, C. M. M. Smith, and R. N. Taylor, "Atomic spectrometry update:review of advances in atomic spectrometry and related techniques," *J. Anal. At. Spectrom.*, vol. 30, pp. 1017–1037, 2015, doi: 10.1039/c4ja90019g.

[52] S. D. Tanner, "Characterization of ionization and matrix suppression in inductively coupled 'cold' plasma mass spectrometry," *J. Anal. At. Spectrom.*, vol. 10, no. 11, pp. 905–921, 1995, doi: 10.1039/ja9951000905.

[53] E. Bolea Fernandez, L. Balcaen, M. Resano, and F. Vanhaecke, "Overcoming spectral overlap via inductively coupled plasma – tandem mass spectrometry (ICP-MS/MS). A tutorial review.," *J. Anal. At. Spectrom.*, 2017, doi: 10.1039/c7ja00010c.

[54] N. Jakubowski, L. Moens, and F. Vanhaecke, “Sector field mass spectrometers in ICP-MS,” *Spectrochim. Acta Part B At. Spectrosc.*, vol. 53, no. 13, pp. 1739–1763, 1998, doi: 10.1016/S0584-8547(98)00222-5.
[55] P. Turner, T. Merren, J. Speakman, and C. Haines, *Interface studies in the ICP-mass spectrometer, in: G. Holland, S.D. Tanner (Eds.), Plasma source mass spectrometry: developments and applications*. Cambridge: Royal Society of Chemistry, 1997.
[56] S. D’Ilio, N. Violante, C. Majorani, and F. Petrucci, “Dynamic reaction cell ICP-MS for determination of total As, Cr, Se and V in complex matrices: Still a challenge? A review,” *Anal. Chim. Acta*, vol. 698, no. 1–2, pp. 6–13, 2011, doi: 10.1016/j.aca.2011.04.052.
[57] L. Balcaen, E. Bolea-Fernandez, M. Resano, and F. Vanhaecke, “Inductively coupled plasma - Tandem mass spectrometry (ICP-MS/MS): A powerful and universal tool for the interference-free determination of (ultra)trace elements - A tutorial review,” *Anal. Chim. Acta*, vol. 894, pp. 7–19, 2015, doi: 10.1016/j.aca.2015.08.053.
[58] E. Bolea-Fernandez, L. Balcaen, M. Resano, and F. Vanhaecke, “Potential of methyl fluoride as a universal reaction gas to overcome spectral interference in the determination of ultratrace concentrations of metals in biofluids using inductively coupled plasma-tandem mass spectrometry,” *Anal. Chem.*, vol. 86, no. 15, pp. 7969–7977, 2014, doi: 10.1021/ac502023h.
[59] “ISO GUIDE 35 Reference Materials - Guidance for characterization and assessment of homogeneity and stability,” 2017.
[60] “ISO 17034:2016 General requirements for the competence of reference material producers,” 2016.
[61] J. I. García Alonso and P. Rodríguez-González, *Isotope dilution mass spectrometry*. Royal Society of Chemistry, 2013.
[62] P. De Bièvre, “SAC 92. Isotope dilution mass spectrometry as a primary method of analysis,” *Anal. Proc.*, vol. 30, no. 8, pp. 328–333, 1993, doi: 10.1039/AP9933000328.
[63] J. Meija and Z. Mester, “Paradigms in isotope dilution mass spectrometry for elemental speciation analysis,” *Anal. Chim. Acta*, vol. 607, no. 2, pp. 115–125, Jan. 2008, doi: 10.1016/j.aca.2007.11.050.
[64] J. Vogl and W. Pritzkow, “Isotope dilution mass spectrometry — A primary method of measurement and its role for RM certification,” *MAPAN*, vol. 25, no. 3, pp. 135–164, Sep. 2010, doi: 10.1007/s12647-010-0017-7.
[65] C. G. J. Petersen, “The yearly immigration of young plaice into the Limfjord from the German Sea,” 1896.
[66] H. H. Ussing, “Analysis of protein by means of deterium-containing amino-acids,” *Nature*, vol. 144, p. 977, 1939.
[67] D. Rittenberg and G. L. Foster, “A new procedure for quantitative analysis by ısotope dilution, with application to the determination of amino acids and fatty acids” 1940, doi: 10.1016/S0021-9258(18)73304-8.
[68] J. F. Pickup and K. McPherson, “Theoretical considerations in stable isotope dilution mass spectrometry for organic analysis,” *Anal. Chem.*, vol. 48, no. 13, pp. 1885–1890, 1976.
[69] Bureau International des Poids et Mesures, “JCGM 200 : 2012 International vocabulary of metrology – Basic and general concepts and associated

terms," *(VIM) Vocab. Int. métrologie*, no. Vim, 2012.
[70] M. J. T. Milton and T. J. Quinn, "Primary methods for the measurement of amount of substance," *Metrologia*, vol. 38, no. 4, pp. 289–296, Aug. 2001, doi: 10.1088/0026-1394/38/4/1.
[71] J. Vogl, "Characterisation of reference materials by isotope dilution mass spectrometry," *J. Anal. At. Spectrom.*, vol. 22, no. 5, p. 475, May 2007, doi: 10.1039/b614612k.
[72] P. Rodríguez-González and J. Ignacio García Alonso, "Mass spectrometry | Isotope dilution mass spectrometry," *Encycl. Anal. Sci.*, pp. 411–420, Jan. 2019, doi: 10.1016/B978-0-12-409547-2.14387-2.
[73] J. Vogl, "Measurement uncertainty in single, double and triple isotope dilution mass spectrometry," *Rapid Commun. Mass Spectrom.*, vol. 26, no. 3, pp. 275–281, Feb. 2012, doi: 10.1002/rcm.5306.
[74] E. Pagliano, Z. Mester, and J. Meija, "Reduction of measurement uncertainty by experimental design in high-order (double, triple, and quadruple) isotope dilution mass spectrometry: application to GC-MS measurement of bromide," *Anal. Bioanal. Chem.*, vol. 405, no. 9, pp. 2879–2887, Mar. 2013, doi: 10.1007/s00216-013-6724-5.
[75] M. J. T. Milton and J. Wang, "High accuracy method for isotope dilution mass spectrometry with application to the measurement of carbon dioxide," *Int. J. Mass Spectrom.*, vol. 218, no. 1, pp. 63–73, Jun. 2002, doi: 10.1016/S1387-3806(02)00663-2.
[76] M. J. T. Milton and J. Wang, "High accuracy method for the application of isotope dilution to gas chromatography/mass spectrometric analysis of gases," *Rapid Commun. Mass Spectrom.*, vol. 17, no. 23, pp. 2621–2625, Dec. 2003, doi: 10.1002/rcm.1238.
[77] P. Rodríguez-González, J. M. Marchante-Gayón, J. I. García Alonso, and A. Sanz-Medel, "Isotope dilution analysis for elemental speciation: a tutorial review," *Spectrochim. Acta Part B At. Spectrosc.*, vol. 60, no. 2, pp. 151–207, Feb. 2005, doi: 10.1016/j.sab.2005.01.005.
[78] S. M. Nelms, C. R. Quétel, T. Prohaska, J. Vogl, and P. D. P. Taylor, "Evaluation of detector dead time calculation models for ICP-MS," *J. Anal. At. Spectrom.*, vol. 16, no. 4, pp. 333–338, 2001, doi: 10.1039/b007913h.
[79] K. G. Heumann, S. M. Gallus, G. Rädlinger, and J. Vogl, "Precision and accuracy in isotope ratio measurements by plasma source mass spectrometry," *J. Anal. At. Spectrom.*, vol. 13, no. 9, p. 1001, Jan. 1998, doi: 10.1039/a801965g.
[80] J. Meija *et al.*, "Isotopic compositions of the elements 2013 (IUPAC Technical Report)," *Pure Appl. Chem.*, vol. 88, no. 3, pp. 293–306, 2016, doi: 10.1515/pac-2015-0503.
[81] C. P. Ingle, B. L. Sharp, M. S. A. Horstwood, R. R. Parrish, and D. J. Lewis, "Instrument response functions, mass bias and matrix effects in isotope ratio measurements and semi-quantitative analysis by single and multi-collector ICP-MS," *J. Anal. At. Spectrom.*, vol. 18, no. 3, pp. 219–229, 2003, doi: 10.1039/b211527a.
[82] E. C. Jrc *et al.*, "Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches (IUPAC Recommendations 2000)," *Pure Appl.*

Chem., vol. 72, no. 8, pp. 1453–1470, 2000.
[83] M. Nordberg, D. M. Templeton, O. Andersen, and J. H. Duffus, "Glossary of terms used in ecotoxicology (IUPAC Recommendations 2009)," *Pure Appl. Chem.*, vol. 81, no. 5, pp. 829–970, 2009, doi: 10.1351/PAC-REC-08-07-09.
[84] P. Bermejo, J. L. Capelo, A. Mota, Y. Madrid, and C. C. Amara, "Enzymatic digestion and ultrasonication: a powerful combination in analytical chemistry," doi: 10.1016/j.trac.2004.06.007.
[85] M. Marcinkowska and D. Barałkiewicz, "Multielemental speciation analysis by advanced hyphenated technique – HPLC/ICP-MS: A review," *Talanta*, vol. 161. Elsevier B.V., pp. 177–204, Dec. 01, 2016, doi: 10.1016/j.talanta.2016.08.034.
[86] B. Markiewicz, I. Komorowicz, A. Sajnóg, M. Belter, and D. Barałkiewicz, "Chromium and its speciation in water samples by HPLC/ICP-MS - Technique establishing metrological traceability: A review since 2000," *Talanta*, vol. 132, pp. 814–828, 2015, doi: 10.1016/j.talanta.2014.10.002.
[87] S. Mädler *et al.*, "Ultra-trace level speciated isotope dilution measurement of Cr(VI) using ion chromatography tandem mass spectrometry in environmental waters," *Talanta*, vol. 156–157, pp. 104–111, 2016, doi: 10.1016/j.talanta.2016.04.064.
[88] M. S. Reid *et al.*, "Arsenic speciation analysis: A review with an emphasis on chromatographic separations," *TrAC - Trends in Analytical Chemistry*, vol. 123. Elsevier B.V., p. 115770, Feb. 01, 2020, doi: 10.1016/j.trac.2019.115770.
[89] M. L. Chen, L. Y. Ma, and X. W. Chen, "New procedures for arsenic speciation: A review," *Talanta*, vol. 125. Elsevier B.V., pp. 78–86, Jul. 01, 2014, doi: 10.1016/j.talanta.2014.02.037.
[90] B. Sadee, M. E. Foulkes, and S. J. Hill, "Coupled techniques for arsenic speciation in food and drinking water: A review," *Journal of Analytical Atomic Spectrometry*, vol. 30, no. 1. Royal Society of Chemistry, pp. 102–118, Jan. 01, 2015, doi: 10.1039/c4ja00269e.
[91] J. Delafiori, G. Ring, and A. Furey, "Clinical applications of HPLC-ICP-MS element speciation: A review," *Talanta*, vol. 153. Elsevier B.V., pp. 306–331, Jun. 01, 2016, doi: 10.1016/j.talanta.2016.02.035.
[92] N. Unceta, F. Séby, J. Malherbe, and O. F. X. Donard, "Chromium speciation in solid matrices and regulation: A review," *Analytical and Bioanalytical Chemistry*, vol. 397, no. 3. Springer, pp. 1097–1111, Jun. 23, 2010, doi: 10.1007/s00216-009-3417-1.
[93] M. P. Rayman, "Selenium intake, status, and health: a complex relationship," *Hormones*, no. 19, pp. 9–14, 2020, doi: 10.1007/s42000-019-00125-5.
[94] Y. Hu, W. Zhang, H. Cheng, and S. Tao, "Public health risk of arsenic species in chicken tissues from live poultry markets of guangdong province, china," *Environ. Sci. Technol.*, vol. 51, no. 6, pp. 3508–3517, Mar. 2017, doi: 10.1021/acs.est.6b06258.
[95] Z. Yang *et al.*, "Arsenic metabolites, ıncluding n-acetyl-4-hydroxy-m-arsanilic acid, in chicken litter from a roxarsone-feeding study ınvolving 1600 chickens," *Environ. Sci. Technol.*, vol. 50, no. 13, pp. 6737–6743, Jul. 2016, doi: 10.1021/acs.est.5b05619.

[96] H. Peng *et al.*, “Liquid chromatography combined with atomic and molecular mass spectrometry for speciation of arsenic in chicken liver,” *J. Chromatogr. A*, vol. 1370, pp. 40–49, Nov. 2014, doi: 10.1016/j.chroma.2014.10.012.
[97] Q. Liu, H. Peng, X. Lu, and X. C. Le, “Enzyme-assisted extraction and liquid chromatography mass spectrometry for the determination of arsenic species in chicken meat,” *Anal. Chim. Acta*, vol. 888, pp. 1–9, Aug. 2015, doi: 10.1016/j.aca.2015.05.001.
[98] H. Peng *et al.*, “Methylated phenylarsenical metabolites discovered in chicken liver,” *Angew. Chemie*, vol. 129, no. 24, pp. 6877–6881, Jun. 2017, doi: 10.1002/ange.201700736.
[99] A. C. Schmidt, K. Kutschera, J. Mattusch, and M. Otto, “Analysis of accumulation, extractability, and metabolization of five different phenylarsenic compounds in plants by ion chromatography with mass spectrometric detection and by atomic emission spectroscopy,” *Chemosphere*, vol. 73, no. 11, pp. 1781–1787, Dec. 2008, doi: 10.1016/j.chemosphere.2008.09.001.
[100] R. P. Monasterio, J. A. Londonio, S. S. Farias, P. Smichowski, and R. G. Wuilloud, “Organic solvent-free reversed-phase ion-pairing liquid chromatography coupled to atomic fluorescence spectrometry for organoarsenic species determination in several matrices,” *J. Agric. Food Chem.*, vol. 59, no. 8, pp. 3566–3574, Apr. 2011, doi: 10.1021/jf104654y.
[101] K. Kinoshita, A. Noguchi, K. Ishii, A. Tamaoka, T. Ochi, and T. Kaise, “Urine analysis of patients exposed to phenylarsenic compounds via accidental pollution,” *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, vol. 867, no. 2, pp. 179–188, May 2008, doi: 10.1016/j.jchromb.2008.03.033.
[102] J. Cui, Y. bing Xiao, L. Dai, X. hong Zhao, and Y. Wang, “Speciation of organoarsenic species in food of animal origin using accelerated solvent extraction (ASE) with determination by HPLC-hydride generation-atomic fluorescence spectrometry (HG-AFS),” *Food Anal. Methods*, vol. 6, no. 2, pp. 370–379, Apr. 2013, doi: 10.1007/s12161-012-9533-0.
[103] Y. K. Tsoi and K. Sze-Yin Leung, “Simultaneous determination of seven elemental species in estuarine waters by LC-ICP-DRC-MS,” *J. Anal. At. Spectrom.*, vol. 25, no. 6, pp. 880–885, May 2010, doi: 10.1039/b923343a.
[104] Y. A. Lin, S. J. Jiang, A. C. Sahayam, and Y. L. Huang, “Speciation of chromium in edible animal oils after microwave extraction and liquid chromatography inductively coupled plasma mass spectrometry,” *Microchemical Journal*, vol. 128, pp. 274-278, Sep 2016, doi:10.1016/j.microc.2016.05.001.
[105] H. Gürleyük and D. Wallschläger, “Determination of chromium(III) and chromium(VI) using suppressed ion chromatography inductively coupled plasma mass spectrometry,” *J. Anal. At. Spectrom.*, vol. 16, no. 9, pp. 926–930, Jan. 2001, doi: 10.1039/b102740a.
[106] S. Abbasi and A. Bahiraei, “Ultra trace quantification of chromium(VI) in food and water samples by highly sensitive catalytic adsorptive stripping voltammetry with rubeanic acid,” *Food Chemistry*, vol. 133, no. 3, pp. 1075-1080, Agu 2012, doi:10.1016j.foodchem.2012.02.003.
[107] E. M. Hamilton, S. D. Young, E. H. Bailey, and M. J. Watts, “Chromium

speciation in foodstuffs: A review," *Food Chemistry*, vol. 250. Elsevier Ltd, pp. 105–112, Jun. 01, 2018, doi: 10.1016/j.foodchem.2018.01.016.
[108] R. Boyd, "Selenium stories," 2011. doi: 10.1038/nchem.1076.
[109] J. R. De Laeter *et al.*, "Atomic weights of the elements: Review 2000 (IUPAC Technical Report)," *Pure Appl. Chem.*, vol. 75, no. 6, pp. 683–800, 2003, doi: 10.1351/pac200375060683.
[110] S. M. Taylor, S R; McLennan, *The continental crust: Its composition and evolution*. 1985.
[111] G. D. Gebreeyessus and F. Zewge, "A review on environmental selenium issues," *SN Appl. Sci.*, vol. 1, no. 1, 2019, doi: 10.1007/s42452-018-0032-9.
[112] J. Fernández-Lodeiro *et al.*, "Synthesis and biological properties of selenium- and tellurium-containing dyes," *Dye. Pigment.*, vol. 110, pp. 28–48, 2014, doi: 10.1016/j.dyepig.2014.04.044.
[113] F. M. Fordyce, "Selenium deficiency and toxicity in the environment," in *Essentials of Medical Geology: Revised Edition*, Springer Netherlands, 2013, pp. 375–416.
[114] K. Pyrzy Âska, "Determination of Selenium Species in Environmental Samples," *Microchim. Acta*, vol. 140, pp. 55–62, 2002, doi: 10.1007/s00604-001-0899-8.
[115] C. C. Johnson, F. M. Fordyce, and M. P. Rayman, "Conference on 'Over-and undernutrition: challenges and approaches,'" in *Symposium on "Geographical and geological influences on nutrition" Factors controlling the distribution of selenium in the environment and their impact on health and nutrition*, 2009, pp. 119–132, doi: 10.1017/S0029665109991807.
[116] P. Susantha Perera, K. Tennakone, R. A. Kumara, I. R. M. Kottegoda, V. P. S. Perera, and L. P. Aponsu, "Nanoporous n-TiO_2/selenium/p-CuCNS photovoltaic cell Nanoporous n-TiO 2 /selenium/p-CuCNS photovoltaic cell," *Artic. J. Phys. D Appl. Phys.*, vol. 31, pp. 2326–2330, 1999, doi: 10.1088/0022-3727/31/18/019.
[117] M. Panahi-Kalamuei, M. Salavati-Niasari, and S. M. Hosseinpour-Mashkani, "Facile microwave synthesis, characterization, and solar cell application of selenium nanoparticles," *J. Alloys Compd.*, vol. 617, pp. 627–632, Dec. 2014, doi: 10.1016/j.jallcom.2014.07.174.
[118] G. H. Lin, M. Z. He, and J. O. M. Bockris, "An initial investigation of amorphous silicon-selenium solar cells," *Sol. Energy Mater. Sol. Cells*, vol. 44, no. 2, pp. 157–164, Nov. 1996, doi: 10.1016/0927-0248(96)00037-2.
[119] M. Lenz and P. N. L. Lens, "The essential toxin: The changing perception of selenium in environmental sciences," *Sci. Total Environ.*, vol. 407, pp. 3620–3633, 2008, doi: 10.1016/j.scitotenv.2008.07.056.
[120] F. K. W., "A new toxicant occurring naturally in certain samples of plant foodstuffs. 1. Results obtained in preliminary feeding trials.," *Journal of Nutrition*, 1934. https://www.cabdirect.org/cabdirect/abstract/19341404172 (accessed May 16, 2021).
[121] K. Schwarz and C. Foltz, "Selenium as an integral part of factor 3 against dietary necrosis liver degeneration," *J. Am. Chem. Soc.*, vol. 79, p. 3292-3293, 1957.

[122] E. Dumont, “Hyphenated techniques for speciation of Se in biological matrices,” Universiteit Gent, 2006.
[123] J. T. Rotruck, A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, and W. G. Hoekstra, “Selenium: Biochemical role as a component of glatathione peroxidase,” *Science (80-.).*, vol. 179, no. 4073, pp. 588–590, Feb. 1973, doi: 10.1126/science.179.4073.588.
[124] N. Terry, A. M. Zayed, M. P. De Souza, and A. S. Tarun, “Selenium in higher plants,” *Annu. Rev. Plant Biol.*, vol. 51, pp. 401–432, 2000, doi: 10.1146/annurev.arplant.51.1.401.
[125] X. Zhang, H. He, J. Xiang, H. Yin, and T. Hou, “Selenium-containing proteins/peptides from plants: A review on the structures and functions,” *J. Agric. Food Chem.*, vol. 68, no. 51, pp. 15061–15073, 2020, doi: 10.1021/acs.jafc.0c05594.
[126] M. P. Rayman, “The importance of selenium to human health,” *Lancet*, vol. 356, no. 9225, pp. 233–241, 2000, doi: 10.1016/S0140-6736(00)02490-9.
[127] M. Kieliszek and S. Błazejak, “Selenium: Significance, and outlook for supplementation,” *Nutrition*, vol. 29, no. 5, pp. 713–718, 2013, doi: 10.1016/j.nut.2012.11.012.
[128] J. C. Avery and P. R. Hoffmann, “Selenium, selenoproteins, and immunity,” *Nutrients*, vol. 10, no. 9, 2018, doi: 10.3390/nu10091203.
[129] Z. Huang, A. H. Rose, and P. R. Hoffmann, “The role of selenium in ınflammation and imunity: from molecular mechanisms to therapeutic opportunities,” *Antioxid. Redox Signal.*, vol. 16, no. 7, pp. 705–743, 2012, doi: 10.1089/ars.2011.4145.
[130] L. C. Clark *et al.*, “Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: A randomized controlled trial,” *J. Am. Med. Assoc.*, vol. 276, no. 24, pp. 1957–1963, Dec. 1996, doi: 10.1001/jama.276.24.1957.
[131] Y. M. Xiong *et al.*, “Association study between polymorphisms in selenoprotein genes and susceptibility to Kashin-Beck disease,” *Osteoarthr. Cartil.*, vol. 18, no. 6, pp. 817–824, Jun. 2010, doi: 10.1016/j.joca.2010.02.004.
[132] M. A. Beck, J. Handy, and O. A. Levander, “Host nutritional status: The neglected virulence factor,” *Trends Microbiol.*, vol. 12, no. 9, pp. 417–423, 2004, doi: 10.1016/j.tim.2004.07.007.
[133] J. Bleys, A. Navas-Acien, and E. Guallar, “Serum selenium levels and all-cause, cancer, and cardiovascular mortality among US adults,” *Arch. Intern. Med.*, vol. 168, no. 4, pp. 404–410, Feb. 2008, doi: 10.1001/archinternmed.2007.74.
[134] M. P. Rayman, “Selenium and human health,” *Lancet*, vol. 379, pp. 1256–68, 2012, doi: 10.1016/S0140.
[135] Q. Wu *et al.*, “Low population selenium status is associated with increased prevalence of thyroid disease,” *J Clin Endocrinol Metab*, vol. 100, no. 11, pp. 4037–4047, 2015, doi: 10.1210/jc.2015-2222.
[136] M. P. Rayman and S. Stranges, “Epidemiology of selenium and type 2 diabetes: Can we make sense of it?,” *Free Radic. Biol. Med.*, vol. 65, pp. 1557–1564, 2013, doi: 10.1016/j.freeradbiomed.2013.04.003.
[137] M. Vinceti, C. Malagoli, M. Bergomi, G. Vivoli, J. R. Marshall, and M. Reid,

"Correspondence re: Duffield-Lillico et al. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: A summary report of the nutritional prevention of cancer trial," *Cancer Epidemiol. Biomarkers Prev.*, vol. 12, no. 1, pp. 77–78, 2003.

[138] D. J. Hughes *et al.*, "Selenium status is associated with colorectal cancer risk in the European prospective investigation of cancer and nutrition cohort," *Int. J. Cancer*, vol. 136, no. 5, pp. 1149–1161, Mar. 2015, doi: 10.1002/ijc.29071.

[139] G. Izydorczyk, B. Ligas, K. Mikula, A. Witek-Krowiak, K. Moustakas, and K. Chojnacka, "Biofortification of edible plants with selenium and iodine-A systematic literature review," *Sci. Total Environ.*, vol. 754, p. 141983, 2021, doi: 10.1016/j.scitotenv.2020.141983.

[140] P. J. White *et al.*, "Interactions between selenium and sulphur nutrition in Arabidopsis thaliana," *J. Exp. Bot.*, vol. 55, no. 404, pp. 1927–1937, 2004, doi: 10.1093/jxb/erh192.

[141] Y. G. Zhu, E. A. H. Pilon-Smits, F. J. Zhao, P. N. Williams, and A. A. Meharg, "Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation," *Trends Plant Sci.*, vol. 14, no. 8, pp. 436–442, 2009, doi: 10.1016/j.tplants.2009.06.006.

[142] M. P. Arvy, "Selenate and selenite uptake and translocation in bean plants (Phaseolus vulgaris)," *J. Exp. Bot.*, vol. 44, no. 6, pp. 1083–1087, Jun. 1993, doi: 10.1093/jxb/44.6.1083.

[143] J. L. Hopper and D. R. Parker, "Plant availability of selenite and selenate as influenced by the competing ions phosphate and sulfate," *Plant Soil*, vol. 210, pp. 199–207, 1999.

[144] L. Zhang *et al.*, "OsPT2, a phosphate transporter, is involved in the active uptake of selenite in rice," *New Phytol.*, vol. 201, no. 4, pp. 1183–1191, Mar. 2014, doi: 10.1111/nph.12596.

[145] H.-F. Li, S. P. McGrath, and F.-J. Zhao, "Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite," *New Phytol.*, vol. 178, no. 1, pp. 92–102, Apr. 2008, doi: 10.1111/j.1469-8137.2007.02343.x.

[146] T. G. Sors, D. R. Ellis, and D. E. Salt, "Selenium uptake, translocation, assimilation and metabolic fate in plants," *Photosynth. Res.*, vol. 86, no. 3, pp. 373–389, 2005, doi: 10.1007/s11120-005-5222-9.

[147] P. D. Whanger, "Selenium and its relationship to cancer: an update," *Br. J. Nutr.*, vol. 91, no. 1, pp. 11–28, 2004, doi: 10.1079/bjn20031015.

[148] A. Zayed, C. M. Lytle, and N. Terry, "Accumulation and volatilization of different chemical species of selenium by plants," *Planta*, vol. 206, no. 2, pp. 284–292, 1998, doi: 10.1007/s004250050402.

[149] B. Welz and P. Stauss, "Interferences from hydride-forming elements on selenium in hydride-generation atomic absorption spectrometry with a heated quartz tube atomizer," *Spectrochim. Acta Part B At. Spectrosc.*, vol. 48, no. 8, pp. 951–976, Jul. 1993, doi: 10.1016/S0584-8547(05)80002-3.

[150] R. Allabashi, J. Rendl, and M. Grasserbauer, "Validation of three atomic absorption spectrometric methods for the determination of selenium - A comparative evaluation of performance characteristics," *Fresenius. J. Anal. Chem.*, vol. 357, no. 8, pp. 1024–1028, 1997, doi:

10.1007/s002160050298.
[151] P. Borella *et al.*, “Selenium determination in biological matrices,” *Microchem. J.*, vol. 58, no. 3, pp. 325–336, Mar. 1998, doi: 10.1006/mchj.1997.1565.
[152] L. Schloske, H. Waldner, and F. Marx, “Optimisation of sample pre-treatment in the HG-AAS selenium analysis,” *Anal. Bioanal. Chem.*, vol. 372, no. 5–6, pp. 700–704, Feb. 2002, doi: 10.1007/s00216-001-1229-z.
[153] L. Zhang, Y. Morita, A. Sakuragawa, and A. Isozaki, “Inorganic speciation of As(III, V), Se(IV, VI) and Sb(III, V) in natural water with GF-AAS using solid phase extraction technology,” *Talanta*, vol. 72, no. 2, pp. 723–729, Apr. 2007, doi: 10.1016/j.talanta.2006.12.001.
[154] A. Vongkul and S. Dejmanee, “Determination of selenium in milk by graphite furnace atomic absorption spectrometry,” 2013. doi: 10.14233/ajchem.2013.15982.
[155] A. Bidari, E. Zeini Jahromi, Y. Assadi, and M. R. Milani Hosseini, “Monitoring of selenium in water samples using dispersive liquid-liquid microextraction followed by iridium-modified tube graphite furnace atomic absorption spectrometry,” *Microchem. J.*, vol. 87, no. 1, pp. 6–12, Oct. 2007, doi: 10.1016/j.microc.2007.04.003.
[156] M. G. Ventura, V. Stibilj, M. do C. Freitas, and A. M. G. Pacheco, “Determination of ultratrace levels of selenium in fruit and vegetable samples grown and consumed in Portugal,” *Food Chem.*, vol. 115, no. 1, pp. 200–206, 2009, doi: 10.1016/j.foodchem.2008.10.089.
[157] P. Smrkolj and V. Stibilj, “Determination of selenium in vegetables by hydride generation atomic fluorescence spectrometry,” *Anal. Chim. Acta*, vol. 512, pp. 11–17, 2004, doi: 10.1016/j.aca.2004.02.033.
[158] L. Rahman, W. T. Corns, D. W. Bryce, and P. B. Stockwell, “Determination of mercury, selenium, bismuth, arsenic and antimony in human hair by microwave digestion atomic fluorescence spectrometry,” *Talanta*, vol. 52, no. 5, pp. 833–843, Aug. 2000, doi: 10.1016/S0039-9140(00)00436-7.
[159] G. Souza Valasques *et al.*, “Extraction induced by emulsion breaking for As, Se and Hg determination in crude palm oil by vapor generation-AFS,” *Food Chem.*, vol. 318, 2020, doi: 10.1016/j.foodchem.2020.126473.
[160] P. Smrkolj, L. Pograjc, C. Hlastan-Ribič, and V. Stibilj, “Selenium content in selected Slovenian foodstuffs and estimated daily intakes of selenium,” *Food Chem.*, vol. 90, no. 4, pp. 691–697, May 2005, doi: 10.1016/j.foodchem.2004.04.028.
[161] Z. Zou, Y. Deng, J. Hu, X. Jiang, and X. Hou, “Recent trends in atomic fluorescence spectrometry towards miniaturized instrumentation-A review,” *Analytica Chimica Acta*, vol. 1019. Elsevier B.V., pp. 25–37, Aug. 17, 2018, doi: 10.1016/j.aca.2018.01.061.
[162] M. N. Matos Reyes, M. L. Cervera, and M. De La Guardia, “Determination of total Sb, Se, Te, and Bi and evaluation of their inorganic species in garlic by hydride-generation-atomic-fluorescence spectrometry,” *Anal. Bioanal. Chem.*, vol. 394, no. 6, pp. 1557–1562, Jul. 2009, doi: 10.1007/s00216-009-2713-0.
[163] H. Li, Y. Luo, Z. Li, L. Yang, and Q. Wang, “Nanosemiconductor-based photocatalytic vapor generation systems for subsequent selenium

determination and speciation with atomic fluorescence spectrometry and inductively coupled plasma mass spectrometry," *Anal. Chem.*, vol. 84, no. 6, pp. 2974–2981, Mar. 2012, doi: 10.1021/ac3001995.

[164] P. Devi *et al.*, "A systematic review and meta-analysis of voltammetric and optical techniques for inorganic selenium determination in water," *TrAC - Trends in Analytical Chemistry*, vol. 95. Elsevier B.V., pp. 69–85, Oct. 01, 2017, doi: 10.1016/j.trac.2017.07.012.

[165] M. Pettine, T. J. McDonald, M. Sohn, G. A. K. Anquandah, R. Zboril, and V. K. Sharma, "A critical review of selenium analysis in natural water samples," *Trends in Environmental Analytical Chemistry*, vol. 5. Elsevier, pp. 1–7, Feb. 01, 2015, doi: 10.1016/j.teac.2015.01.001.

[166] R. Segura *et al.*, "Development of electrochemical sensors for the determination of selenium using gold nanoparticles modified electrodes," *Sensors Actuators, B Chem.*, vol. 220, pp. 263–269, Jun. 2015, doi: 10.1016/j.snb.2015.05.016.

[167] M. F. Cabral, V. A. Pedrosa, and S. A. S. Machado, "Deposition of selenium thin layers on gold surfaces from sulphuric acid media: Studies using electrochemical quartz crystal microbalance, cyclic voltammetry and AFM," *Electrochim. Acta*, vol. 55, no. 3, pp. 1184–1192, Jan. 2010, doi: 10.1016/j.electacta.2009.10.008.

[168] M. Ashournia and A. Aliakbar, "Determination of Se(IV) in natural waters by adsorptive stripping voltammetry of 5-nitropiazselenol," *J. Hazard. Mater.*, vol. 174, no. 1–3, pp. 788–794, Feb. 2010, doi: 10.1016/j.jhazmat.2009.09.121.

[169] T. Ferri and P. Sangiorgio, "Determination of selenium speciation in river waters by adsorption on iron(III)-Chelex-100 resin and differential pulse cathodic stripping voltammetry," *Anal. Chim. Acta*, vol. 321, no. 2–3, pp. 185–193, Mar. 1996, doi: 10.1016/0003-2670(95)00569-2.

[170] V. Stará and M. Kopanica, "Cathodic stripping voltammetry and adsorptive stripping voltammetry of selenium(IV)," *Anal. Chim. Acta*, vol. 208, no. C, pp. 231–236, Jan. 1988, doi: 10.1016/S0003-2670(00)80750-3.

[171] F. A. Bertolino, A. A. J. Torriero, E. Salinas, R. Olsina, L. D. Martinez, and J. Raba, "Speciation analysis of selenium in natural water using square-wave voltammetry after preconcentration on activated carbon," *Anal. Chim. Acta*, vol. 572, no. 1, pp. 32–38, Jul. 2006, doi: 10.1016/j.aca.2006.05.021.

[172] Z. H. Fernández *et al.*, "Application of cold vapor-atomic absorption (CVAAS) spectrophotometry and inductively coupled plasma-atomic emission spectrometry methods for cadmium, mercury and lead analyses of fish samples. Validation of the method of CVAAS," *Food Control*, vol. 48, pp. 37–42, Feb. 2015, doi: 10.1016/j.foodcont.2014.05.056.

[173] X. Peng and Z. Wang, "Ultrasensitive determination of selenium and arsenic by modified helium atmospheric pressure glow discharge optical emission spectrometry coupled with hydride generation," *Anal. Chem.*, vol. 91, no. 15, pp. 10073–10080, Aug. 2019, doi: 10.1021/acs.analchem.9b02006.

[174] B. Jackson, A. Liba, and J. Nelson, "Advantages of reaction cell ICP-MS on doubly charged interferences for arsenic and selenium analysis in foods.," *J. Anal. At. Spectrom.*, vol. 2015, pp. 1–5, 2014, doi: 10.1039/c4ja00310a.

[175] S. Stürup, R. B. Hayes, and U. Peters, "Development and application of a

simple routine method for the determination of selenium in serum by octopole reaction system ICPMS," *Anal. Bioanal. Chem.*, vol. 381, no. 3, pp. 686–694, 2005, doi: 10.1007/s00216-004-2946-x.
[176] N. Elwaer and H. Hintelmann, "Comparing the precision of selenium isotope ratio measurements using collision cell and sector field inductively coupled plasma mass spectrometry," *Talanta*, vol. 75, no. 1, pp. 205–214, 2008, doi: 10.1016/j.talanta.2007.10.046.
[177] D. P. Bishop *et al.*, "Determination of selenium in serum in the presence of gadolinium with ICP-QQQ-MS," *Analyst*, vol. 140, no. 8, pp. 2842–2846, 2015, doi: 10.1039/c4an02283a.
[178] L. Bamonti, S. Theiner, N. Rohr-Udilova, B. K. Keppler, and G. Koellensperger, "Accurate high throughput quantification of selenium in biological samples – the potential of combining isotope dilution ICP-tandem mass spectrometry with flow injection," *J. Anal. At. Spectrom.*, vol. 31, no. 11, pp. 2227–2232, 2016, doi: 10.1039/c6ja00209a.
[179] A. M. Featherstone, A. T. Townsend, G. A. Jacobson, and G. M. Peterson, "Comparison of methods for the determination of total selenium in plasma by magnetic sector inductively coupled plasma mass spectrometry," *Anal. Chim. Acta*, vol. 512, no. 2, pp. 319–327, 2004, doi: 10.1016/j.aca.2004.02.058.
[180] B. Hattendorf and D. Günther, "Strategies for method development for an inductively coupled plasma mass spectrometer with bandpass reaction cell. Approaches with different reaction gases for the determination of selenium," *Spectrochim. Acta Part B*, vol. 58, pp. 1–13, Jan 2003, doi: 10.1016/S0584-8547(02)00168-4.
[181] D. Schaumlöffel, K. Bierła, and R. ŁobińSki, "Accurate determination of selenium in blood serum by isotope dilution analysis using inductively coupled plasma collision cell mass spectrometry with xenon as collision gas," *J. Anal. At. Spectrom.*, vol. 22, no. 3, p. 318, 2007, doi: 10.1039/b611324a.
[182] L. Hinojosa Reyes, J. M. Marchante Gayon, J. I. Garcia Alonso, and A. Sanz-Medel, "Determination of selenium in biological materials by isotope dilution analysis with an octapole reaction system ICP-MS," *J. Anal. At. Spectrom.*, vol. 18, pp. 11–16, 2003, doi: 10.1039/b209213a.
[183] S. F. Boulyga and J. S. Becker, "ICP-MS with hexapole collision cell for isotope ratio measurements of Ca, Fe, and Se.," *Fresenius. J. Anal. Chem.*, vol. 370, pp. 618–623, 2001, doi: 10.1007/s002160100851.
[184] L. Balcaen, E. Bolea-Fernandez, M. Resano, and F. Vanhaecke, "Inductively coupled plasma e Tandem mass spectrometry (ICP-MS/ MS): A powerful and universal tool for the interference-free determination of (ultra)trace elements e A tutorial review," *Anal. Chim. Acta*, vol. 894, pp. 7–19, 2015, doi: 10.1016/j.aca.2015.08.053.
[185] Z. Pedrero and Y. Madrid, "Review novel approaches for selenium speciation in foodstuffs and biological specimens: A review," *Anal. Chim. Acta*, vol. 634, pp. 135–152, 2009, doi: 10.1016/j.aca.2008.12.026.
[186] X. Dauchy, M. Potin-Gautier, A. Astruc, and M. Astruc, "Analytical methods for the speciation of selenium compounds: a review," Springer-Verlag, 1994.
[187] P. C. Uden, "Modern trends in the speciation of selenium by hyphenated

techniques," *Analytical and Bioanalytical Chemistry*, vol. 373, no. 6. Springer, pp. 422–431, Jul. 02, 2002, doi: 10.1007/s00216-002-1405-9.

[188] C. B'Hymer and J. A. Caruso, "Selenium speciation analysis using inductively coupled plasma-mass spectrometry," *Journal of Chromatography A*, vol. 1114, no. 1. Elsevier, pp. 1–20, May 05, 2006, doi: 10.1016/j.chroma.2006.02.063.

[189] K. Pyrzynska and A. Sentkowska, "Selenium in plant foods: speciation analysis, bioavailability, and factors affecting composition," *Crit. Rev. Food Sci. Nutr.*, vol. 61, no. 8, pp. 1340–1352, 2021, doi: 10.1080/10408398.2020.1758027.

[190] A. P. Vonderheide *et al.*, "Characterization of selenium species in Brazil nuts by HPLC-ICP-MS and ES-MS," *J. Agric. Food Chem.*, vol. 50, no. 20, pp. 5722–5728, 2002, doi: 10.1021/jf0256541.

[191] J. L. Capelo, P. Ximénez-Embún, Y. Madrid-Albarrán, and C. Cámara, "Enzymatic probe sonication: Enhancement of protease-catalyzed hydrolysis of selenium bound to proteins in yeast," *Anal. Chem.*, vol. 76, no. 1, pp. 233–237, 2004, doi: 10.1021/ac034871d.

[192] O. Cankur, S. K. V Yathavakilla, and J. A. Caruso, "Selenium speciation in dill (Anethum graveolens L.) by ion pairing reversed phase and cation exchange HPLC with ICP-MS detection," *Talanta*, vol. 70, no. 4, pp. 784–790, 2006, doi: 10.1016/j.talanta.2006.01.040.

[193] M. Montes-Bayón, M. J. Díaz, E. Blanco González, and A. Sanz-Medel, "Evaluation of different sample extraction strategies for selenium determination in selenium-enriched plants (Allium sativum and Brassica juncea) and Se speciation by HPLC-ICP-MS," *Talanta*, vol. 68, pp. 1287–1293, 2006, doi: 10.1016/j.talanta.2005.07.040.

[194] E. Kápolna, M. Shah, J. A. Caruso, and P. Fodor, "Selenium speciation studies in Se-enriched chives (Allium schoenoprasum) by HPLC-ICP-MS," *Food Chem.*, vol. 101, no. 4, pp. 1398–1406, Jan. 2007, doi: 10.1016/j.foodchem.2006.03.048.

[195] R. V. S. Lavu *et al.*, "Fertilizing soil with selenium fertilizers: Impact on concentration, speciation, and bioaccessibility of selenium in leek (Allium ampeloprasum)," *J. Agric. Food Chem.*, vol. 60, no. 44, pp. 10930–10935, Nov. 2012, doi: 10.1021/jf302931z.

[196] M. Longchamp, M. Castrec-Rouelle, P. Biron, and T. Bariac, "Variations in the accumulation, localization and rate of metabolization of selenium in mature Zea mays plants supplied with selenite or selenate," *Food Chem.*, vol. 182, pp. 128–135, 2015, doi: 10.1016/j.foodchem.2015.02.137.

[197] D. Kremer, G. Ilgen, and J. Feldmann, "GC-ICP-MS determination of dimethylselenide in human breath after ingestion of 77Se-enriched selenite: Monitoring of in-vivo methylation of selenium," *Anal. Bioanal. Chem.*, vol. 383, no. 3, pp. 509–515, 2005, doi: 10.1007/s00216-005-0001-1.

[198] J. Meija, M. Montes-Bayón, D. L. Le Duc, N. Terry, and J. A. Caruso, "Simultaneous monitoring of volatile selenium and sulfur species from se accumulating plants (wild type and genetically modified) by GC/MS and GC/ICPMS using solid-phase microextraction for sample introduction," *Anal. Chem.*, vol. 74, no. 22, pp. 5837–5844, 2002, doi: 10.1021/ac020285t.

[199] B. Chen, M. He, X. Mao, R. Cui, D. Pang, and B. Hu, “Ionic liquids improved reversed-phase HPLC on-line coupled with ICP-MS for selenium speciation,” *Talanta*, vol. 83, pp. 724–731, 2011, doi: 10.1016/j.talanta.2010.10.023.
[200] K. M. Kubachka *et al.*, “Evaluation of selenium in dietary supplements using elemental speciation,” *Food Chem.*, vol. 218, pp. 313–320, 2017, doi: 10.1016/j.foodchem.2016.08.086.
[201] E. B. Both *et al.*, “Selenolanthionine is the major water-soluble selenium compound in the selenium tolerant plant Cardamine violifolia,” *Biochim. Biophys. Acta - Gen. Subj.*, vol. 1862, no. 11, pp. 2354–2362, 2018, doi: 10.1016/j.bbagen.2018.01.006.
[202] S. Bakirdere, M. Volkan, and O. Y. Ataman, “Speciation of selenium in supplements by high performance liquid chromatography-inductively coupled plasma-mass spectrometry,” *Anal. Lett.*, vol. 48, no. 9, pp. 1511–1523, 2015, doi: 10.1080/00032719.2014.986679.
[203] T. Guerin, A. Astruc, and M. Astruc, “Speciation of arsenic and selenium compounds by HPLC hyphenated to specific detectors: a review of the main separation techniques,” *Talanta*, vol. 50, pp. 1–24, Aug. 1999, doi:10.1016/S0039-9140(99)00140-X.
[204] G. Vinci, R. Preti, A. Tieri, and S. Vieri, “Authenticity and quality of animal origin food investigated by stable-isotope ratio analysis,” *J. Sci. Food Agric.*, vol. 93, no. 3, pp. 439–448, Feb. 2013, doi: 10.1002/JSFA.5970.
[205] S. A. Wadood, G. Boli, Z. Xiaowen, I. Hussain, and W. Yimin, “Recent development in the application of analytical techniques for the traceability and authenticity of food of plant origin,” *Microchem. J.*, vol. 152, p. 104295, Jan. 2020, doi: 10.1016/j.mıcroc.2019.104295.
[206] H. Zhao, S. Zhang, and Z. Zhang, “Relationship between multi-element composition in tea leaves and in provenance soils for geographical traceability,” *Food Control*, vol. 76, pp. 82–87, 2017, doi: 10.1016/j.foodcont.2017.01.006.
[207] S. A. Wadood, G. Boli, and W. Yimin, “Geographical traceability of wheat and its products using multielement light stable isotopes coupled with chemometrics,” *J. Mass Spectrom.*, vol. 54, no. 2, pp. 178–188, Feb. 2019, doi: 10.1002/jms.4312.
[208] S. Behkami, S. M. Zain, M. Gholami, and S. Bakirdere, “Isotopic ratio analysis of cattle tail hair: A potential tool in building the database for cow milk geographical traceability,” *Food Chem.*, vol. 217, pp. 438–444, 2017, doi: 10.1016/j.foodchem.2016.08.130.
[209] R. Petrini, L. Sansone, F. F. Slejko, A. Buccianti, P. Marcuzzo, and D. Tomasi, “The 87Sr/86Sr strontium isotopic systematics applied to Glera vineyards: A tracer for the geographical origin of the Prosecco,” *Food Chem.*, vol. 170, pp. 138–144, 2015, doi: 10.1016/j.foodchem.2014.08.051.
[210] H. C. Liu, C. F. You, C. Y. Chen, Y. C. Liu, and M. T. Chung, “Geographic determination of coffee beans using multi-element analysis and isotope ratios of boron and strontium,” *Food Chem.*, vol. 142, pp. 439–445, 2014, doi: 10.1016/j.foodchem.2013.07.082.
[211] S. A. Drivelos and C. A. Georgiou, “Multi-element and multi-isotope-ratio analysis to determine the geographical origin of foods in the European Union,” *TrAC - Trends Anal. Chem.*, vol. 40, pp. 38–51, Nov. 2012, doi:

10.1016/j.trac.2012.08.003.
[212] "Quality schemes explained | European Commission," 2020. https://ec.europa.eu/info/food-farming-fisheries/food-safety-and-quality/certification/quality-labels/quality-schemes-explained_en (accessed Sep. 07, 2021).
[213] F. Galgano, F. Favati, M. Caruso, T. Scarpa, and A. Palma, "Analysis of trace elements in southern Italian wines and their classification according to provenance," *LWT - Food Sci. Technol.*, vol. 41, no. 10, pp. 1808–1815, Dec. 2008, doi: 10.1016/j.lwt.2008.01.015.
[214] S. Lohumi, S. Lee, H. Lee, and B. K. Cho, "A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration," *Trends Food Sci. Technol.*, vol. 46, no. 1, pp. 85–98, Nov. 2015, doi: 10.1016/j.tıfs.2015.08.003.
[215] V. Lakshmi, "Food alteration," *Int. J. Sci. Invent. Today*, vol. 1, no. 2, pp. 106–103, 2012, Accessed: Sep. 07, 2021. [Online]. Available: http://www.ijsit.com/admin/ijsit_files/food adulteratıon_1.2.4.pdf.
[216] K. Everstine, "Economically motivated adulteration: Implications for food protection and alternate approaches to detection," University of Minnesota, 2013.
[217] "Asian Honey, Banned in Europe, Is Flooding U.S. Grocery Shelves | Food Safety News." http://www.foodsafetynews.com/2011/08/honey-laundering/#.Ui7md19OLGh (accessed Sep. 07, 2021).
[218] D. Scarano and R. Rao, "DNA markers for food products authentication," *Divers. 2014, Vol. 6, Pages 579-596*, vol. 6, no. 3, pp. 579–596, Sep. 2014, doi: 10.3390/D6030579.
[219] M. Labra, M. Miele, B. Ledda, F. Grassi, M. Mazzei, and F. Sala, "Morphological characterization, essential oil composition and DNA genotyping of Ocimum basilicum L. cultivars," *Plant Sci.*, vol. 167, pp. 725–731, 2004, doi: 10.1016/j.plantsci.2004.04.026.
[220] S. M. Budge, S. J. Iverson, W. D. Bowen, and R. G. Ackman, "Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence," *Can. J. Fish. Aquat. Sci.*, vol. 59, no. 5, pp. 886–898, 2002, doi: 10.1139/F02-062.
[221] I. M. Chung *et al.*, "Discrimination of organic milk by stable isotope ratio, vitamin E, and fatty acid profiling combined with multivariate analysis: A case study of monthly and seasonal variation in Korea for 2016–2017," *Food Chem.*, vol. 261, pp. 112–123, Sep. 2018, doi: 10.1016/j.foodchem.2018.04.017.
[222] E. Capuano, R. Gravink, R. Boerrigter-Eenling, and S. M. van Ruth, "Fatty acid and triglycerides profiling of retail organic, conventional and pasture milk: Implications for health and authenticity," *Int. Dairy J.*, vol. 42, pp. 58–63, 2015, doi: 10.1016/j.idairyj.2014.11.002.
[223] Z. Piravi-Vanak, M. Ghavami, H. Ezzatpanah, J. Arab, H. Safafar, and J. B. Ghasemi, "Evaluation of Authenticity of ıranian olive oil by fatty acid and triacylglycerol profiles," *J. Am. Oil Chem. Soc. 2009 869*, vol. 86, no. 9, pp. 827–833, Jun. 2009, doi: 10.1007/S11746-009-1419-Y.
[224] F. Ricardo *et al.*, "Potential use of fatty acid profiles of the adductor muscle

of cockles (Cerastoderma edule) for traceability of collection site," *Nat. Publ. Gr.*, vol. 5, p. 11125, 2015, doi: 10.1038/srep11125.
[225] M. Torres-Moreno, E. Torrescasana, J. Salas-Salvadó, and C. Blanch, "Nutritional composition and fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions," *Food Chem.*, vol. 166, pp. 125–132, 2015, doi: 10.1016/j.foodchem.2014.05.141.
[226] K. B. Bat *et al.*, "Primary and secondary metabolites as a tool for differentiation of apple juice according to cultivar and geographical origin," *LWT*, vol. 90, pp. 238–245, Apr. 2018, doi: 10.1016/j.lwt.2017.12.026.
[227] N. Fayeulle *et al.*, "Fast discrimination of chocolate quality based on average-mass-spectra fingerprints of cocoa polyphenols," *J. Agric. Food Chem.*, vol. 67, no. 9, pp. 2723–2731, 2019, doi: 10.1021/acs.jafc.8b06456.
[228] R. N. D'Souza, S. Grimbs, B. Behrends, H. Bernaert, M. S. Ullrich, and N. Kuhnert, "Origin-based polyphenolic fingerprinting of Theobroma cacao in unfermented and fermented beans," *Food Res. Int.*, vol. 99, pp. 550–559, Sep. 2017, doi: 10.1016/j.foodres.2017.06.007.
[229] H. E. Tahir *et al.*, "The use of analytical techniques coupled with chemometrics for tracing the geographical origin of oils: A systematic review (2013–2020)," *Food Chemistry*, vol. 366. Elsevier, p. 130633, Jan. 01, 2022, doi: 10.1016/j.foodchem.2021.130633.
[230] A. Cambrai, C. Marcic, S. Phane Morville, P. Sae Houer, F. Bindler, and E. Marchioni, "Differentiation of chocolates according to the cocoa's geographical origin using chemometrics," *J. Agric. Food Chem*, vol. 58, pp. 1478–1483, 2010, doi: 10.1021/jf903471e.
[231] B. Krajnc *et al.*, "Selective methods to ınvestigate authenticity and geographical origin of mediterranean food products," *Food Rev. Int.*, vol. 37, no. 6, pp. 656–682, 2021, doi: 10.1080/87559129.2020.1717521.
[232] K. Gopi, D. Mazumder, J. Sammut, and N. Saintilan, "Determining the provenance and authenticity of seafood: A review of current methodologies," *Trends Food Sci. Technol.*, vol. 91, pp. 294–304, 2019, doi: 10.1016/j.tifs.2019.07.010.
[233] J. R. Ehleringer, L. A. Chesson, L. O. Valenzuela, B. J. Tipple, and L. A. Martinelli, "Stable isotopes trace the truth: from adulterated foods to crime scenes," *Elements*, vol. 11, no. 4, pp. 259–264, Aug. 2015, doi: 10.2113/gselements.11.4.259.
[234] L. Li, C. E. Boyd, and Z. Sun, "Authentication of fishery and aquaculture products by multi-element and stable isotope analysis," *Food Chem.*, vol. 194, pp. 1238–1244, 2016, doi: 10.1016/j.foodchem.2015.08.123.
[235] R. J. S. Teixeira, S. Gomes, V. Malheiro, L. Pereira, and J. R. Fernandes, "A multidisciplinary fingerprinting approach for authenticity and geographical traceability of portuguese wines," *Foods*, vol. 10, no. 1044, pp. 1–16, 2021.
[236] E. J. Won *et al.*, "A multi-elements isotope approach to assess the geographic provenance of manila clams (Ruditapes philippinarum) via recombining appropriate elements," *Foods*, vol. 10, no. 3, 2021, doi: 10.3390/foods10030646.
[237] H. C. Liu, C. F. You, C. Y. Chen, Y. C. Liu, and M. T. Chung, "Geographic determination of coffee beans using multi-element analysis and isotope

ratios of boron and strontium," *Food Chem.*, vol. 142, pp. 439–445, 2014, doi: 10.1016/j.foodchem.2013.07.082.
[238] S. Geilert, J. Vogl, M. Rosner, S. Voerkelius, and T. Eichert, "Isotope fractionation in bell pepper," *Mass Spectrom. Open Access*, vol. 1, no. 1, pp. 1–6, 2015, doi: 10.4172/mso.1000101.
[239] I. Coelho, I. Castanheira, J. M. Bordado, O. Donard, and J. A. L. Silva, "Recent developments and trends in the application of strontium and its isotopes in biological related fields," *TrAC Trends Anal. Chem.*, vol. 90, pp. 45–61, May 2017, doi: 10.1016/j.trac.2017.02.005.
[240] S. García-Ruiz, M. Moldovan, G. Fortunato, S. Wunderli, and J. I. García Alonso, "Evaluation of strontium isotope abundance ratios in combination with multi-elemental analysis as a possible tool to study the geographical origin of ciders," *Anal. Chim. Acta*, vol. 590, pp. 55–66, 2007, doi: 10.1016/j.aca.2007.03.016.
[241] T. S. Pilgrim, R. J. Watling, and K. Grice, "Application of trace element and stable isotope signatures to determine the provenance of tea (Camellia sinensis) samples," *Food Chem.*, vol. 118, no. 4, pp. 921–926, Feb. 2010, doi: 10.1016/j.foodchem.2008.08.077.
[242] P. P. Coetzee, F. E. Steffens, R. J. Eiselen, O. P. Augustyn, L. Balcaen, and F. Vanhaecke, "Multi-element analysis of south african wines by ICP-MS and their classification according to geographical origin," *J. Agric. Food Chem.*, vol. 53, no. 13, pp. 5060–5066, 2005, doi: 10.1021/jf048268n.
[243] K. D. Gabriela Thiel, Georg Geisler, Ines Blechshmidt, "Determination of trace elements in wines and classification according to their provenance," *Anal Bioanal Chem*, vol. 378, pp. 1630–1636, 2004, doi: 10.1007/s00216-003-2444-6.
[244] M. D. M. Castiñeira Gómez, I. Feldmann, N. Jakubowski, and J. T. Andersson, "Classification of german white wines with certified brand of origin by multielement quantitation and pattern recognition techniques," *J. Agric. Food Chem.*, vol. 52, no. 10, pp. 2962–2974, 2004, doi: 10.1021/jf035120f.
[245] S. Kelly, K. Heaton, and J. Hoogewerff, "Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis," *Trends Food Sci. Technol.*, vol. 16, no. 12, pp. 555–567, Dec. 2005, doi: 10.1016/j.tıfs.2005.08.008.
[246] F. D. Bora, A. Donici, T. Rusu, A. Bunea, D. Popescu, and C. I. Bunea, "Elemental profile and 207Pb/206Pb, 208Pb/206Pb, 204Pb/206Pb, 87Sr/86Sr isotope ratio as fingerprints for geographical traceability of Romanian wines," *Not. Bot. Horti Agrobot. Cluj-Napoca*, vol. 46, no. 1, pp. 223–239, 2018, doi: 10.15835/nbha46110853.
[247] I. Coelho *et al.*, "Combining multielement analysis and chemometrics to trace the geographical origin of Rocha pear," *J. Food Compos. Anal.*, vol. 77, pp. 1–8, Apr. 2019, doi: 10.1016/j.jfca.2018.12.005.
[248] C. M. R. Almeida and M. T. S. D. Vasconcelos, "Multielement composition of wines and their precursors including provenance soil and their potentialities as fingerprints of wine origin," *J. Agric. Food Chem.*, vol. 51, no. 16, pp. 4788–4798, 2003, doi: 10.1021/jf034145b.
[249] E. Dumont, F. Vanhaecke, and R. Cornelis, "Selenium speciation from food

source to metabolites: A critical review," *Anal. Bioanal. Chem.*, vol. 385, no. 7, pp. 1304–1323, 2006, doi: 10.1007/s00216-006-0529-8.
[250] N. Golubkina, T. Seredin, T. Kriachko, and G. Caruso, "Nutritional features of leek cultivars and effect of selenium-enriched leaves from goliath variety on bread physical, quality and antioxidant attributes," *Ital. J. Food Sci.*, vol. 31, no. 2, pp. 288–300, 2019, doi: 10.14674/IJFS-1277.
[251] C. Ip *et al.*, "Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention," *J. Agric. Food Chem.*, vol. 48, no. 6, pp. 2062–2070, 2000, doi: 10.1021/jf000051f.
[252] J. Wu, "Determination of picomolar iron in seawater by double Mg(OH)2 precipitation isotope dilution high-resolution ICPMS," *Mar. Chem.*, vol. 103, no. 3–4, pp. 370–381, 2007, doi: 10.1016/j.marchem.2006.10.006.
[253] I. Petrov, C. R. Quétel, and P. D. P. Taylor, "Investigation on sources of contamination, for an accurate analytical blank estimation, during measurements of the Fe content in seawater by isotope dilution inductively coupled plasma mass spectrometry," *J. Anal. At. Spectrom.*, vol. 22, no. 6, pp. 608–615, May 2007, doi: 10.1039/b700540g.
[254] Z. Arslan, T. Oymak, and J. White, "Triethylamine-assisted $Mg(OH)_2$ coprecipitation/preconcentration for determination of trace metals and rare earth elements in seawater by inductively coupled plasma mass spectrometry (ICP-MS)," *Anal. Chim. Acta*, vol. 1008, pp. 18–28, 2018, doi: 10.1016/j.aca.2018.01.017.
[255] H. Sawatari, T. Hayashi, E. Fujimori, A. Hirose, and H. Haraguchi, "Multielement determination of trace elements in coastal seawater by ICP-MS and ICP-AES after aluminum coprecipitation associated with magnesium," *Bull. Chem. Soc. Jpn.*, vol. 69, no. 7, pp. 1925–1931, Jul. 1996, doi: 10.1246/bcsj.69.1925.
[256] Y. Sun and M. Sun, "Determination of 42 trace elements in seawater by ınductively coupled plasma mass spectrometry after apdc chelate co-precipitation combined with iron," *Anal. Lett.*, vol. 40, no. 12, pp. 2391–2404, Oct. 2007, doi: 10.1080/00032710701576056.
[257] F. Ardini, E. Magi, and M. Grotti, "Determination of ultratrace levels of dissolved metals in seawater by reaction cell inductively coupled plasma mass spectrometry after ammonia induced magnesium hydroxide coprecipitation," *Anal. Chim. Acta*, vol. 706, no. 1, pp. 84–88, Nov. 2011, doi: 10.1016/j.aca.2011.07.046.
[258] J. Wu and E. A. Boyle, "Determination of iron in seawater by high-resolution isotope dilution inductively coupled plasma mass spectrometry after Mg(OH)2 coprecipitation," *Anal. Chim. Acta*, vol. 367, no. 1–3, pp. 183–191, Jul. 1998, doi: 10.1016/S0003-2670(98)00145-7.
[259] Y. Z. Peng, Y. M. Huang, D. X. Yuan, Y. Li, and Z. Bin Gong, "Rapid analysis of heavy metals in coastal seawater using preconcentration with precipitation/co-precipitation on membrane and detection with X-ray fluorescence," *Fenxi Huaxue/ Chinese J. Anal. Chem.*, 2012, doi: 10.1016/S1872-2040(11)60554-9.
[260] B. Arı and S. Bakırdere, "A primary reference method for the characterization of Cd, Cr, Cu, Ni, Pb and Zn in a candidate certified

reference seawater material: TEA/$Mg(OH)_2$ assisted ID^3MS by triple quadrupole ICP-MS/MS," *Anal. Chim. Acta*, vol. 1140, pp. 178–189, Dec. 2020, doi: 10.1016/j.aca.2020.10.004.

[261] B. Arı, S. Z. Can, and S. Bakırdere, "Traceable and accurate quantification of iron in seawater using isotope dilution calibration strategies by triple quadrupole ICP-MS/MS: Characterization measurements of iron in a candidate seawater CRM," *Talanta*, vol. 209, no. October 2019, p. 120503, 2020, doi: 10.1016/j.talanta.2019.120503.

[262] F. Vanhaecke, L. Balcaen, I. Deconinck, I. De Schrijver, C. Marisa Almeida, and L. Moens, "Mass discrimination in dynamic reaction cell (DRC)-ICP-mass spectrometry," *J. Anal. At. Spectrom.*, vol. 18, no. 9, p. 1060, Sep. 2003, doi: 10.1039/b303528j.

[263] F. Vanhaecke, L. Balcaen, G. De Wannemacker, and L. Moens, "Capabilities of inductively coupled plasma mass spectrometry for the measurement of Fe isotope ratios," *J. Anal. At. Spectrom.*, vol. 17, no. 8, pp. 933–943, Aug. 2002, doi: 10.1039/b202409h.

[264] S. Ellison, M. Rosslein, and A. Williams, "EURACHEM/CITAC - Quantifying uncertainty in analytical measurement," 2012.

[265] GUM-2008, "Evaluation of measurement data — Guide to the expression of uncertainty in measurement," 2008. doi: 10.1373/clinchem.2003.030528.

[266] "GUM Workbench Professional Version 2 . 4," *Metrodata GmbH*, 2018. http://www.metrodata.de.

[267] P. Bhatia *et al.*, "Selenium bioaccessibility and speciation in biofortified Pleurotus mushrooms grown on selenium-rich agricultural residues," *Food Chem.*, vol. 140, no. 1–2, pp. 225–230, 2013, doi: 10.1016/j.foodchem.2013.02.054.

[268] J. Wu and E. A. Boyle, "Low blank preconcentration technique for the determination of lead, copper, and cadmium in small-volume seawater samples by isotope dilution ICPMS," *Anal. Chem.*, vol. 69, no. 13, pp. 2464–2470, 1997, doi: 10.1021/ac961204u.

[269] J. Wu and E. A. Boyle, "Determination of iron in seawater by high-resolution isotope dilution inductively coupled plasma mass spectrometry after $Mg(OH)_2$ coprecipitation," *Anal. Chim. Acta*, vol. 367, no. 1–3, pp. 183–191, Jul. 1998, doi: 10.1016/S0003-2670(98)00145-7.

[270] S. L. Jackson, J. Spence, D. J. Janssen, A. R. S. Ross, and J. T. Cullen, "Determination of Mn, Fe, Ni, Cu, Zn, Cd and Pb in seawater using offline extraction and triple quadrupole ICP-MS/MS," *J. Anal. At. Spectrom.*, vol. 00, pp. 1–10, 2018, doi: 10.1039/C7JA00237H.

[271] E. Vassileva, I. Wysocka, A. M. Orani, and C. Quétel, "Off-line preconcentration and inductively coupled plasma sector field mass spectrometry simultaneous determination of Cd, Co, Cu, Mn, Ni, Pb and Zn mass fractions in seawater: Procedure validation," *Spectrochim. Acta Part B At. Spectrosc.*, vol. 153, pp. 19–27, Mar. 2019, doi: 10.1016/j.sab.2019.01.001.

[272] D. V. Biller and K. W. Bruland, "Analysis of Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb in seawater using the Nobias-chelate PA1 resin and magnetic sector inductively coupled plasma mass spectrometry (ICP-MS)," *Mar. Chem.*, vol. 130–131, pp. 12–20, Feb. 2012, doi: 10.1016/J.MARCHEM.2011.12.001.

[273] Y. Sohrin *et al.*, “Multielemental determination of geotraces key trace metals in seawater by ICPMS after preconcentration using an ethylenediaminetriacetic acid chelating resin,” *Anal. Chem.*, vol. 80, no. 16, pp. 6267–6273, Aug. 2008, doi: 10.1021/ac800500f.
[274] M. E. Lagerström, M. P. Field, M. Séguret, L. Fischer, S. Hann, and R. M. Sherrell, “Automated on-line flow-injection ICP-MS determination of trace metals (Mn, Fe, Co, Ni, Cu and Zn) in open ocean seawater: Application to the GEOTRACES program,” *Mar. Chem.*, vol. 155, pp. 71–80, Sep. 2013, doi: 10.1016/j.marchem.2013.06.001.
[275] T. Minami *et al.*, “An off-line automated preconcentration system with ethylenediaminetriacetate chelating resin for the determination of trace metals in seawater by high-resolution inductively coupled plasma mass spectrometry,” *Anal. Chim. Acta*, vol. 854, pp. 183–190, 2015, doi: 10.1016/j.aca.2014.11.016.
[276] T. Linsinger, K. Europejska, C. Badawcze, I. Materia, and B. Email, “Application note 1,” 2010.
[277] A. Lamberty, H. Schimmel, and J. Pauwels, “The study of the stability of reference materials by isochronous measurements,” in *Fresenius' Journal of Analytical Chemistry*, 1998, vol. 360, no. 3–4, pp. 359–361, doi: 10.1007/s002160050711.
[278] T. P. J. Linsinger, J. Pauwels, A. Lamberty, H. G. Schimmel, A. M. H. Van Der Veen, and L. Siekmann, “Estimating the uncertainty of stability for matrix CRMs,” *Fresenius. J. Anal. Chem.*, vol. 370, pp. 183–188, 2001.
[279] B. Arı, E. Öz, S. Z. Can, and S. Bakırdere, “Bioaccessibility and bioavailability of selenium species in se-enriched leeks (Allium Porrum) cultivated by hydroponically,” *Food Chem.*, vol. 372, no. October 2021, p. 131314, 2021, doi: 10.1016/j.foodchem.2021.131314.
[280] K. Wrobel, M. Guerrero Esperanza, E. Yanez Barrientos, A. Rosa Corrales Escobosa, K. Wrobel, and K. Wrobel katarzyn, “Different approaches in metabolomic analysis of plants exposed to selenium: a comprehensive review,” *Acta Physiol. Plant.*, vol. 42, p. 125, 2020, doi: 10.1007/s11738-020-03113-0.
[281] E. Kápolna and P. Fodor, “Bioavailability of selenium from selenium-enriched green onions (*Allium fistulosum*) and chives (*Allium schoenoprasum*) after ‘ *in vitro* ’ gastrointestinal digestion,” *Int. J. Food Sci. Nutr.*, vol. 58, no. 4, pp. 282–296, Jan. 2007, doi: 10.1080/09637480601154335.
[282] Emrah Yildirim, “Multielement speciation using HPLC-ICPMS,” Middle East Technical University, 2015.
[283] E. Do Nascimento Da Silva, F. Aureli, M. D'Amato, A. Raggi, S. Cadore, and F. Cubadda, “Correction to: Selenium bioaccessibility and speciation in selenium-enriched lettuce: Investigation of the selenocompounds liberated after in vitro simulated human digestion using two-dimensional HPLC-ICP-MS,” *J. Agric. Food Chem.*, vol. 65, no. 43, p. 9568, 2017, doi: 10.1021/acs.jafc.7b04800.
[284] Y. Ogra, K. Ishiwata, Y. Iwashita, and K. T. Suzuki, “Simultaneous speciation of selenium and sulfur species in selenized odorless garlic (Allium sativum L. Shiro) and shallot (Allium ascalonicum) by HPLC-inductively coupled

plasma-(octopole reaction system)-mass spectrometry and electrospray ionization-tand," *J. Chromatogr. A*, vol. 1093, no. 1–2, pp. 118–125, Nov. 2005, doi: 10.1016/j.chroma.2005.07.081.

[285] Y. Shiobara, T. Yoshida, and K. T. Suzuki, "Effects of dietary selenium species on Se concentrations in hair, blood, and urine," *Toxicol. Appl. Pharmacol.*, vol. 152, no. 2, pp. 309–314, 1998, doi: 10.1006/taap.1998.8537.

[286] K. Wróbel *et al.*, "HPLC-ICP-MS speciation of selenium in enriched onion leaves - A potential dietary source of Se-methylselenocysteine," *Food Chem.*, vol. 86, no. 4, pp. 617–623, Aug. 2004, doi: 10.1016/j.foodchem.2003.11.005.

[287] E. Bulska, I. A. Wysocka, M. H. Wierzbicka, K. Proost, K. Janssens, and G. Falkenberg, "In vivo investigation of the distribution and the local speciation of selenium in Allium cepa L. by means of microscopic X-ray absorption near-edge structure spectroscopy and confocal microscopic X-ray fluorescence analysis," *Anal. Chem.*, vol. 78, no. 22, pp. 7616–7624, Nov. 2006, doi: 10.1021/ac060380s.

[288] "TÜBİTAK UME Referans Malzemeler." https://rm.ume.tubitak.gov.tr/urun.aspx?u=62 (Accessed: Sep. 05, 2021). [Online].

PUBLICATIONS FROM THE THESIS

Papers

1. Arı, B., Can, S.Z., Bakırdere, S**.,** Traceable and accurate quantification of iron in seawater using isotope dilution calibration strategies by triple quadrupole ICP-MS/MS: Characterization measurements of iron in a candidate seawater CRM, *Talanta***,** 2020, 209, 120503

2. Arı, B., Bakırdere**,** S., A primary reference method for the characterization of Cd, Cr, Cu, Ni, Pb and Zn in a candidate certified reference seawater material: TEA/Mg (OH) 2 assisted ID3MS by triple quadrupole ICP-MS/MS, *Analytica Chimica Acta*, 2020, 1140, 178-189.

3. Arı, B. Öz E. Can Z. S, Bakırdere S., Bioaccessibility and bioavailability of selenium species in Se-enriched leeks (*Allium Porrum*) cultivated hydroponically, *Food Chemistry, 2022,* 372, 131314.

Conference Papers

1. Arı, B., Can, S.Z., Bakırdere, S., Measurements of iron in a candidate seawater CRM by triple quadrupole ICPMS/MS with isotope dilution calibration strategies, 2nd International Congress on Analytical and Bioanalytical Chemistry (2nd ICABC 2020), 11-14 March 2020, Antalya, Turkey