View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Duquesne University: Digital Commons

Duquesne University
Duquesne Scholarship Collection

Electronic Theses and Dissertations

Summer 2004

Development, Research and Validation of Environmental Speciation Methods; and Evaluation by Speciated Isotope Dilution Mass Spectrometry in Mercury and Chromium Speciation Analysis

G. M. Mizanur Rahman

Follow this and additional works at: https://dsc.duq.edu/etd

Recommended Citation

Rahman, G. (2004). Development, Research and Validation of Environmental Speciation Methods; and Evaluation by Speciated Isotope Dilution Mass Spectrometry in Mercury and Chromium Speciation Analysis (Doctoral dissertation, Duquesne University). Retrieved from https://dsc.duq.edu/etd/1081

This Immediate Access is brought to you for free and open access by Duquesne Scholarship Collection. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Duquesne Scholarship Collection. For more information, please contact phillipsg@duq.edu.

Development, Research and Validation of Environmental Speciation Methods: Evaluation by Speciated Isotope Dilution Mass Spectrometry in Mercury and Chromium Speciation Analysis

A Dissertation Presented to the Bayer School of Natural and Environmental Sciences of Duquesne University

In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

By:

G. M. Mizanur Rahman

August 19, 2004

All Rights Reserved ©

2004

G. M. Mizanur Rahman

Name: **G. M. Mizanur Rahman**

Dissertation Title: **Development, Research and Validation of Environmental Speciation Methods: Evaluation by Speciated Isotope Dilution Mass Spectrometry in Mercury and Chromium Speciation Analysis**

Degree: **Doctor of Philosophy**

Date: **August 19, 2004**

Approved: _____
Dr. H. M. "Skip" Kingston
Dissertation Director
Professor of Chemistry and Biochemistry

Approved: _____
Dr. Omar W. Steward
Professor of Chemistry and Biochemistry

Approved: _____
Dr. Jeffrey D. Evanseck
Associate Professor of Chemistry and Biochemistry

Approved: _____
Dr. John C. Kern
Assistant Professor of Mathematics and Computer Science

Approved: _____
Dr. Jeffry D. Madura
Chairman, Department of Chemistry and Biochemistry
Professor of Chemistry and Biochemistry

Approved: _____
Dr. David W. Seybert
Dean, Bayer School of Natural and Environmental Sciences
Professor of Chemistry and Biochemistry

This work is dedicated to my loving family, brothers, sisters, parents and in-laws for their unconditional and endless love

Acknowledgments

I would like to manifest my deepest consciousness of gratitude and appreciation to my revered dissertation advisor, Dr. H. M. “Skip” Kingston, who is not only my academic advisor but has also been my personal mentor during the past few years. He taught me analytical chemistry and helped me to prosper academically and gain maturity. I would also like to thank “Skip” and Ms. Mary Lynn Kingston for their constant observations, constructive advices, reviewing and emendation of manuscripts.

I would like to take the opportunity of expressing my deepest sense of gratitude to other members of my dissertation committee, Dr. Omar W. Steward, Dr. Jeffrey D. Evanseck and Dr. John C. Kern for their continuous help, guidance, comments and suggestions throughout my research. I would also like to thank Dr. Thomas L. Isenhour and Dr. Bret Larget for their helpful discussion on mathematics and statistics during my second rotation research.

Throughout my graduate study, I have been blessed with a number of very good fellow students and colleagues. I would like to thank Dr. Theodore G. Towns, Dr. Robert C. Richter, Dr. Dirk D. Link, Dr. Helen M. Boylan, Dr. Ye Han, Ms. Sejal Iyer, Mr. David Lineman, Mr. David Ionadi, Ms. Pallavi Deshpande and Mr. Sandeep Bhandari for their assistance in my research. I would like to specially thank to Dr. Theodore G. Towns and Mr. Matt Pamuku for their help in editing my dissertation.

I am also grateful to all the teaching and non-teaching staffs of the Department of Chemistry and Biochemistry for their help in every possible way during the entire period of my research. Thanks to Dan Bodnar, Dave Hardesty and Andrew Venanzio for their

help in fixing instruments and keep them running. Thanks to Mary Ann Quinn, Kathy Woodburn, Kathy Land and Dianne Robertson for administrative support.

I would like to acknowledge the Bayer School of Natural and Environmental Sciences and the Department of Chemistry and Biochemistry for the assistantships that I received during the course of my graduate studies. I would like to acknowledge Milestone Inc. and Agilent Technologies for instrumentation support.

I would like to acknowledge Ms. Shen-yi Yang, US EPA and Ms. Sara W. Hartwell, SAIC, for funding interlaboratory validation study for EPA draft Method 3200. Thanks to Mr. Rock Vitale and Mr. Kyle Clay, Environmental Standard, Inc. for funding hexavalent chromium study.

Finally, I would like to offer my special thanks to my wife Tamanna, to my sons Safwan and Rakin, to my parents, brothers, sisters and in-laws for their endless love, continuous encouragement, prolific support and sacrifice throughout this dissertation.

G. M. Mizanur Rahman (Ph.D., Chemistry)

Development, Research and Validation of Environmental Speciation Methods: Evaluation by Speciated Isotope Dilution Mass Spectrometry in Mercury and Chromium Speciation Analysis

Abstract of a Dissertation at Duquesne University

Dissertation supervised by Professor H. M. 'Skip' Kingston

The toxicity of an element depends upon its chemical forms, which also gives vital information for samples and their relevance. A sequential extraction method for mercury speciation in soil and sediments was established based on the mobility and toxicity of different mercury species and was proposed as a draft US EPA Method 3200. In order to finalize the method, an interlaboratory validation study was performed, and the final results verified the applicability of the method. Some of the widely used mercury speciation methods were evaluated during this study and the results were compared with those obtained from EPA draft Method 3200. The performances of these methods were also tested by using SIDMS technique (EPA Method 6800) as a diagnostic tool. A highly pure isotopically labeled methylmercury ($CH_3{}^{201}Hg^+$) was synthesized from commercially available isotopically enriched inorganic mercury (^{201}HgO) and tetramethyltin with a yield of more than 90% in a synthesis procedure lasting less than 1.5 h at 60 °C; the product was characterized using ICP-MS during this study.

A simple and fast closed-vessel microwave-assisted extraction method based on acidic extractant has been developed for mercury speciation from soils and sediments. The optimized sample preparation was achieved in a closed-vessel system by heating 1.0

g of sample in 10.0 mL of 4.0 M HNO_3 for 10 min at 100 °C with magnetic stirring. During this study, not only the SIDMS technique was established and validated for the first time for mercury speciation analysis but also a set of generic equations for one, two and three species systems in terms of unidirectional and bidirectional transformations have been developed. The modified SIDMS technique was used for the speciation of chromium from road construction materials, soils and sediments by applying microwave-assisted alkaline extraction instead of hot-plate extraction, and was observed that these matrices contain 30-70 times more Cr(VI) compared to the US EPA threshold limit (5 mg/L). This dissertation investigates and provides solution to some difficulties associated with the analyses of mercury and chromium species. The techniques developed and tested here are being adopted worldwide to assist with species related problems.

Table of Contents

Chapter 1: Chemistry, Toxicology, and Environmental Aspects of Mercury

1.1 Introduction 1

1.2 Current Research Objectives 4

1.3 Chronology of Mercury Investigations 4

1.4 Mercury Species Levels and Pathways in the Environment 5

1.5 Toxicology of Mercury Compounds 12

1.6 Mercury Analysis 17

1.6.1 Total Mercury 17

1.6.2 Mercury Speciation 20

1.6.3 History of Mercury Analysis at Duquesne University 23

1.7 Conclusions 23

1.8 References 25

Chapter 2: Synthesis and Characterization of Isotopically Enriched Methylmercury

2.1 Introduction 30

2.2 Experimental 35

2.2.1 Instrumentation 35

2.2.2 Reagents and Standards 36

2.2.3 Synthesis Procedure 38

2.2.3.1 Synthesis of ^{201}Hg enriched methylmercury 38

2.2.3.2 Purification procedure 39

2.2.4 Availability of Isotopically Enriched Methylmercury 39

2.3 Results and Discussion 39

2.3.1 Optimization of Synthesis Conditions 39

2.3.2 Characterization of Isotopically Enriched Methylmercury Synthesized 44

2.4 Conclusions 46

2.5 References 47

Chapter 3: Interlaboratory Validation of EPA Draft Method 3200

3.1 Introduction 50

3.2 Experimental 51

3.2.1 Preparation of the Reference Soil 51

3.2.2 Participating Laboratories 53

3.2.3 Strategy of Method Performance Study 54

3.3 Results and Discussions 55

3.3.1 Validation with Speciated Isotope Dilution Mass Spectrometry 55

3.3.2 Statistical Evaluations of the Data Obtained from Participating Labs 58

3.3.2.1 Extractable Inorganic Mercury 62

3.3.2.2 Extractable Organic Mercury 65

3.3.2.3 Total Extractable Mercury 65

3.3.2.4 Total Mercury 66

3.4 Conclusions 69

3.5 References 72

Chapter 4: Application of EPA Method 6800 to Evaluate Different Mercury Speciation Extraction Methods in Soils and Sediments

4.1 Introduction 74

4.2 Experimental 76

4.2.1 Instrumentation 76

4.2.2 Reagents and Chemicals 77

4.2.3 Samples and Standards 77

4.2.4 Extraction Procedure 78

4.2.4.1 Draft EPA Method 3200 (M-3200) 78

4.2.4.2 Sonication (SONI-1) 79

4.2.4.3 Sonication (SONI-2) 80

4.2.4.4 Focused Microwave-Assisted Extraction (FMAE) 80

4.2.4.5 Cold Acid Extraction (CAE) 81

4.2.4.6 EPA Method 3051A 81

4.2.4.7 Hot Alkaline Extraction 81
4.2.4.8 Extraction Procedure for SIDMS 82
4.2.5 SIDMS Detection 82
4.2.5.1 Algorithms, Assumptions and Calculations 82
4.2.5.2 Dead-Time 86
4.2.5.3 Mass Bias 87
4.3 Results and Discussions 88
4.3.1 Total Mercury with EPA Method 7473 88
4.3.2 Evaluation of Selected Mercury Speciation Methods using Conventional Procedure 88
4.3.3 Evaluation of Selected Mercury Speciation Methods using SIDMS 96
4.4 Conclusions 101
4.5 References 103

Chapter 5: Development of Microwave-Assisted Extraction Method for Mercury Speciation in Soils and Sediments

5.1 Introduction 106
5.2 Experimental 110
5.2.1 Instrumentation 110
5.2.2 Reagents and Chemicals 111
5.2.3 Standard Solutions and Certified Reference Materials 111
5.2.4 Optimized Analytical Procedure 113
5.3 Results and Discussions 113
5.3.1 Stability of Mercury Species under Microwave Irradiation 113
5.3.2 Optimization of HNO_3 Concentration 115
5.3.3 Optimization of Sample Weight 118
5.3.4 Optimization of Irradiation Temperature 121
5.3.5 Optimization of Irradiation Time 123
5.3.6 Validation of the Development and Optimized Method using Reference Soils and Standard Reference Materials (SRMs) 124

5.3.7 Application of EPA Method 6800 in the Validation of the Current Extraction Method under Study 128
5.4 Conclusions 130
5.5 References 131

Chapter 6: Derivation of Generic Equations for SIDMS Analysis and Correction of 1, 2 and 3 Species Conversion Simultaneously

6.1 Introduction 134
6.2 Bi-directional Transformation 136
6.2.1 Algorithms, Assumptions and Calculations 136
6.2.1.1 Two Species 136
6.2.1.2 Three Species 141
6.2.2 Calculation of Total Number of Moles and New Isotopic Abundances after Double Spiking 152
6.2.3 Calculation of Total Number of Moles and New Isotopic Abundances after Triple Spiking 154
6.2.4 Construction of EXCEL® Spreadsheet 156
6.2.5 Validation of SIDMS Equations 156
6.2.5.1 Validation using Chromium (Two Species) 157
6.2.5.2 Validation using Mercury (Two and Three Species) 158
6.3 Unidirectional Transformation 162
6.3.1 Algorithms, Assumptions and Calculations 162
6.3.1.1 Conversion from K to L 163
6.3.1.2 Conversion from L to K 165
6.3.2 Construction of EXCEL® Spreadsheet 168
6.3.3 Validation of SIDMS Equations 169
6.3.3.1 Validation using Chromium 169
6.3.3.2 Validation using Mercury 170
6.4 Conclusions 171
6.5 References 171

Chapter 7: Application of Modified SIDMS Analysis for Cr(VI) in Selected Soil and Sludge Samples

7.1 Introduction 174

7.2 Experimental 178

7.2.1 Instrumentation 178

7.2.2 Reagents, Solutions and Samples 179

7.2.3 Sample Preparation Procedures for Cr(VI) 183

7.2.3.1 Total Digestion Procedure: EPA Method 3052 183

7.2.3.2 Extraction: SIDMS Procedure 184

7.2.3.3 Extraction: Method 3060A for Method 6800 and IDMS 184

7.2.3.4 Algorithms, Assumptions and Calculations 185

7.3 Results and Discussions 188

7.3.1 Total Elemental Analysis 188

7.3.2 Cr(VI) Speciated Analysis 190

7.3.2.1 Isotope Dilution Mass Spectrometry (IDMS) 195

7.3.2.2 SIDMS Detection 196

7.3.2.3 Method 6800 Detection 201

7.3.2.4 Method 3060A (External Calibration) 202

7.4 Conclusions 203

7.5 References 205

Appendices

Appendix-A: Publications 207

Appendix-B: Poster Presentations 208

Appendix-C: Oral Presentations 208

Appendix-D: 36 Possible Transformations for a Three Species System. 210

List of Figures

Figure Description Page

FIGURE 1-1 Biogeochemical cycle of mercury in the environment. 11

FIGURE 1-2 E_h-pH plot of the various methylmercury species in an aquatic system. 11

FIGURE 2-1 Survey of analytical abstracts for the word "speciation" or "species" in the titles of published papers since 1980. 31

FIGURE 2-2 Typical Chromatogram for separation of inorganic mercury and methylmercury. 36

FIGURE 2-3 Chromatogram for synthesized isotope enriched methylmercury ($CH_3{}^{201}Hg^+$). Chromatograms for different masses (^{202}Hg, ^{201}Hg, and ^{199}Hg) were shifted from the baseline by adding 300, 200 and 100 CPS, respectively, with the original counts for clarity. 41

FIGURE 2-4 Chromatogram for a mixture of $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ in isopropanol. The mixture was kept on bench-top at room temperature for 6 h for equilibration. Chromatograms for different masses (^{202}Hg and ^{201}Hg) were shifted from the baseline by adding 200 and 100 CPS with the original counts for clarity. 42

FIGURE 2-5 Chromatogram for a mixture of $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ in 1% $Na_2S_2O_3$. The mixture was kept on bench-top at room temperature for 6 h for equilibration. Chromatograms for different masses (^{202}Hg and ^{201}Hg) were shifted from the baseline by adding 200 and 100 CPS with the original counts for clarity. 43

FIGURE 2-6 Chromatogram for a mixture of naturally abundant and isotopically enriched methylmercury. Chromatograms for different masses (^{202}Hg and ^{201}Hg) were shifted from the baseline by adding 100 and 50 CPS with the original counts for clarity. 44

FIGURE 3-1 Percent recovery of extractable inorganic mercury in a) Material-1 and b) Material-2. 64

FIGURE 3-2 Percent recovery of extractable organic mercury in a) Material-1 and b) Material-2. 66

FIGURE 3-3 Percent recovery of total extractable mercury in a) Material-1 and b) Material-2. 67

FIGURE 3-4 Percent recovery of total mercury in a) Material-1 and b) Material-2. 68

FIGURE 4-1 Chromatogram for mercury species in Material-1 after double spiking with $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^{+}$, and application of cold acid extraction method. 102

FIGURE 4-2 Chromatogram for mercury species in Material-1 after double spiking with $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^{+}$, and extraction with proposed EPA Method 3200. 103

FIGURE 5-1 The effects of optimization of nitric acid concentration on the efficiency of extraction and stability of mercury species for (a) spiked blank soil and (b) SRM 2711 (spiked with methylmercury). 118

FIGURE 5-2 The effects of optimization of the sample weight on extraction efficiency for (a) spiked blank soil and (b) SRM 2711 (spiked with methylmercury). 120

FIGURE 5-3 The effects of optimization of the irradiation temperature on extraction efficiency and stability of mercury species in (a) spiked blank soil and (b) SRM 2711 (spiked with methylmercury). 123

FIGURE 5-4 The effects of optimization of the irradiation time on extraction efficiency and stability of mercury species in (a) spiked blank soil and (b) SRM 2711 (spiked with methylmercury). 126

FIGURE 6-1 Schematic for bidirectional transformation for two species system. 136

FIGURE 6-2 Bidirectional transformation for three species system. 142

FIGURE 7-1 pH-E_h diagram of Cr species. 175

FIGURE 7-2 Typical chromatogram for separation of Cr(III) and Cr(VI). 179

FIGURE 7-3 Reduction of Cr(VI) during measurement. Injection 5 and 15 min after acidification of extract to pH between 1 and 2 with concentrated HNO_3. 197

FIGURE 7-4 Cr(VI) measurements. Injection 5 and 15 min after acidification of extract to pH between 5 and 6. 198

FIGURE 7-5 External calibration graph for determination of Cr(VI). 203

List of Tables

Table Description Page

TABLE 1-I Species and Fields of Interest in Speciation Analysis. 2

TABLE 1-II Use of Organomercury Compounds. 6

TABLE 1-III Some Bioconcentration Factors for Total Mercury. 13

TABLE 1-IV Summary of Toxic Properties of Different Forms of Mercury in Humans. 14

TABLE 2-I HPLC-ICP-MS Operating Conditions. 37

TABLE 2-II Results for Characterization of Naturally Abundant and Synthesized Isotopically Enriched Methylmercury with ICP-MS. 38

TABLE 2-III Results for the Preliminary and Final Synthesis of Isotopically Enriched Methylmercury. Analysis by DMA-80 and HPLC-ICP-MS. 40

TABLE 3-I Summary of Reference Materials Used in the Validation Study. 53

TABLE 3-II The Deconvoluted Concentration and Percent Transformation of Mercury Species in Material-1 and Material-2 Using SIDMS Calculations. 57

TABLE 3-III Final Results from Different Participating Laboratories for the Validation of EPA Draft Method 3200: Percent Recovery with 95% CL. 59

TABLE 3-IV Total Percent Recovery from Interlaboratory Validation Study. 61

TABLE 3-V Mean and Median Recoveries for Different Mercury Fractions Across Laboratories for Each Reference Material. 69

TABLE 4-I Isotope Abundances for Naturally Abundant Mercury and Isotopically Enriched ^{199}HgO and ^{201}HgO. 79

TABLE 4-II Determination of Total Mercury Concentration by EPA Method 7473. Uncertainties are at 95% CL, n = 4. 88

TABLE 4-III EPA Draft Method 3200 Performances Reported as Percent Recovery. Uncertainties are reported as 95% CL with n = 4. 91

TABLE 4-IV Ultrasound Assisted Extraction (SONI-1) Method Performances Reported as Percent Recovery. Uncertainties are reported as 95% CL with n = 4. 92

TABLE 4-V Ultrasound Assisted Extraction (SONI-2) Method Performances Reported as Percent Recovery. Uncertainties are reported as 95% CL with n = 4. 92

TABLE 4-VI Focused Microwave Assisted Extraction (FMAE) Method Performances Reported as Percent Recovery. Uncertainties are reported as 95% CL with n = 4. 93

TABLE 4-VII Cold Acid Extraction (CAE) Method Performances Reported as Percent Recovery. Uncertainties are reported as 95% CL with n = 4. 93

TABLE 4-VIII EPA Method 3051A Performances Reported as Percent Recovery. Uncertainties are reported as 95% CL with n = 4. 96

TABLE 4-IX Hot Alkaline Extraction Method Performances Reported as Percent Recovery. Uncertainties are reported as 95% CL with n = 4. 97

TABLE 4-X The Deconvoluted Concentration and Percent Transformation of Mercury Species in Material-1 and Material-2 using SIDMS Calculations. 100

TABLE 5-I Stability of Mercury Species Under Microwave Irradiation. 115

TABLE 5-II Percent Recovery Results for Optimization of HNO_3 Concentration. 116

TABLE 5-III Percent Recovery Results for Optimization of Sample Weight. 119

TABLE 5-IV Percent Recovery Results for Optimization of Irradiation Temperature. 122

TABLE 5-V Percent Recovery Results for Optimization of Irradiation Time. 125

TABLE 5-VI Comparison of Different Analysis Methods for the Validation of the Microwave-Assisted Extraction Results. 127

TABLE 5-VII The Deconvoluted Concentration and Transformation of Mercury Species in Material-1 using SIDMS Calculations. 129

TABLE 6-I Chromium(VI) Found in DOS-SC-10G Sample. 158

TABLE 6-II Chromium (VI) Found in DOS-SC-10G Sample by Spiking with Impure Spikes. 158

TABLE 6-III Mercury Speciation Results for Two Species Bidirectional Transformation. 159

TABLE 6-IV Isotope Abundance of Different Mercury Species. 161

TABLE 6-V Theoretical Isotope Ratios for Synthetic Samples. 162

TABLE 6-VI Deconvoluted Concentration and Percent Transformation for Synthetic Sample. 162

TABLE 6-VII Deconvoluted Cr(III) and Cr(VI) Concentrations and Percent Conversions Calculated by using Unidirectional SIDMS Equations and Spreadsheet. 170

TABLE 6-VIII Deconvoluted Hg^{2+} and CH_3Hg^+ Concentrations and Percent Conversions Calculated by using Unidirectional SIDMS Equations and Spreadsheet. 170

TABLE 7-I HPLC-ICP-MS Operating Conditions. 180

TABLE 7-II Isotope Abundances and Average Atomic Weight of Standards and Spike. 182

TABLE 7-III Information Regarding Sample Processing. 183

TABLE 7-IV Summary for Total Digestion Analysis at 95% CL. 189

TABLE 7-V Cr(VI) Results in CA Samples: Extracted using EPA Method 3060A 191

TABLE 7-VI Cr(VI) Results in CA Samples: Double Spiked Before Extraction with EPA Method 3060A. 192

TABLE 7-VII Method 6800 (SIDMS): Double Spiked Before Extraction (Sample - LOV-SC-7F-1). 193

TABLE 7-VIII Method 6800 (SIDMS): Double Spiked After Extraction (Sample - LOV-SC-7F-1). 194

Chapter 1

Chemistry, Toxicology and Environmental Aspects of Mercury

1.1 Introduction

According to the International Union of Pure and Applied Chemistry (IUPAC), the term 'speciation' can be defined as a form of an element specified as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure (*1*). On this basis, two broad approaches can be distinguished: (i) 'organometallic speciation' involving metals whose inorganic and organic forms are characterized based on different toxicity, mobility, etc. (e.g. Hg, Pb and Sn); and (ii) 'inorganic speciation' involving metals whose different oxidation states are characterized by different toxicity, mobility, etc. (e.g. Cr, As, Se, and Sb) (*2*).

It is widely established in biochemistry that trace elements play important roles in various cellular, genomic functions and metabolic pathways within living organisms. While some metals (e.g. Hg, Pb) and metalloids (As) are highly toxic whereas others (e.g. Mo, Mn, Fe, Co, Cu, Zn, Se, Si, B), are considered essential in numerous life processes. A number of other elements (e.g. V, Cr, Ni) are considered to be beneficial to living organisms (*3*).

The variety of major target chemical species in different disciplines is shown in Table 1-I (*4*). Basically, they can be divided into well-known organometallic environmental pollutants which have created great environmental concern in past few decades (e.g. butyltins, phenyltins and alkyllead), products resulting from transformation of toxic elements (e.g. methylmercury, organoarsenic), and complexes of essential and

toxic metals and non-metals with biomolecules (e.g. selenomethionine, selenocystine). The assessment of oxidation state of some elements such as As(III) and As(V), Se(IV) and Se(VI), or Cr(III) and Cr(VI) are also of great interest (*5*).

TABLE 1-I. Species and Fields of Interest in Speciation Analysis (*4*).

Area of Interest	Species
Plant and animal biochemistry, ecotoxicology, nutrition	*Organometalloid species*: arsenobetaine, arsenocholine, selenoamino acids *Metallothioneines*: Cd, Cu, Zn *Phytochelatins*: Cd, Cu, Zn, Co, As *Polysaccharides*: Pb, Sr, Ba, Ca, Mg *Macrocycles*: chlorophyll derivatives, cobalamines
Environmental (aquatic and atmospheric chemistry)	*Redox states*: As(III)/As(V), Cr(III)/Cr(VI), Se(IV)/Se(VI), Sb(III)/Sb(V), Fe(II)/Fe(III) *Alkylmetals*: $(CH_3)_nSn^{(3-n)+}$, $(C_4H_9)_nSn^{(3-n)+}$, $(C_6H_5)_nSn^{(3-n)+}$, CH_3Hg^+, $(CH_3)_2Hg$, $C_2H_5Hg^+$, $(C_2H_5)_2Hg$, $(CH_3)_n(C_2H_5)_mPb^{(4-m-n)+}$, CH_3Cd^+, $(CH_3)_2Cd$
Industrial chemistry	*Metalloporphyrins*: Ni, V, Fe, Ga *Catalytic mixtures*: Ni, Ru, Rh *Organomercury and organoarsenic*: shale oil, gasoline, natural gas condensate
Clinical biochemistry	*Metalloenzymes*: Zn, Mo, Co *Metallodrugs*: Pt, Ru, Ti *Nucleic acids*: Cr, Ni, Pt, Ru *Transport proteins*: Al, Cu, Zn, Fe

The need to measure individual chemical species occur especially where these species are known to be very toxic, mobile and bioavailable to humans and biota.

Toxicity of some elements depends on their oxidation state, e.g. Cr(III) is a nutrient whereas Cr(VI) is a carcinogen. The degree of alkylation is another important cause of toxicity, e.g. tributyltins are more toxic than dibutyltins or monobutyltins, and dimethylmercury is more toxic than methylmercury. Sometime alkylation reduces the element toxicity, e.g. As(III) is toxic whereas arsenobetaine is non-toxic. Generally, metal alkylation helps the molecule to pass across the biological membrane and results therefore in accumulation in the food chain. In other cases, toxicity is caused by the volatility of the organometallic species and easy absorption through the lungs, as in the case of mercury (*5*).

According to the United States Environmental Protection Agency (US EPA), mercury is one of the "most significant environmental pollutants" of continuous concern on the global scale (*6*). Numerous national and international agencies and organizations have targeted mercury for emission control because of its tendency to highly bioconcentrate in the human food chain. The biogeochemical cycle of mercury has also received considerable attention due to the toxicity of methylmercury, the bioaccumulation of mercury in biota, and its biomagnification in the aquatic food chain. Consumption of mercury-contaminated fish is the principal pathway for human exposure and, from recent studies, it is found that the majority of mercury that bioaccumulates via the food chain is as methylmercury. Therefore, accurate information and understanding regarding the concentrations, transport, and transformation of mercury species in aquatic ecosystems is needed to predict potential impact on both human and aquatic life.

1.2 Current Research Objectives

The seven major objectives of the current study include: i) interlaboratory validation of draft EPA Method 3200; ii) evaluation of different widely used mercury speciation methods published in the literature and comparison with draft EPA Method 3200; iii) synthesis and characterization of isotopically labeled methylmercury; iv) validation of draft EPA Method 3200 and other literature methods using speciated isotope dilution mass spectrometry (SIDMS) (*75-77*) methodology; v) development of a highly efficient mercury speciation method based on closed-vessel microwave-assisted acid extraction; vi) development of generic, fundamental SIDMS analysis equations for one, two and three species systems in terms of bidirectional and unidirectional transformations, and their application to the validation of other more conventional speciation methods; and vii) application of SIDMS equations to environmental chromium speciation analysis.

1.3 Chronology of Mercury Investigations

The toxicity of mercury to humans was reported in ancient times. However, the present environmental and health interest started in the mid-1950s. The first epidemic caused by methylmercury poisoning occurred due to the consumption of large amounts of fish and/or shellfish contaminated by industrial waste containing methylmercury in Minamata, Japan. The symptoms become known as the Minamata disease and included numbness, and constriction of the visual field; in many cases, death occurred. Teratogenic effect, a characteristic of brain damage, was also noticed in children born during this time period (7). A second epidemic occurred for the same reason in Niigata, Japan. It should be noted that in both cases, mercury was discharged from local plastic processing plants in the form of methylmercury, which was formed from inorganic

mercury during production of acetaldehyde (8). The methylmercury release was very extensive, 200 μg/g of mercury was discovered in silt near the plant and the mercury content in the contaminated fish varied from 5-20 μg/g. Both epidemics left several hundred dead and hundreds of victims (*9*).

The third epidemic disaster was reported in Iraq in the early 1970s; the cause was the ingestion of bread and other grain products obtained from seeds treated with alkylmercury fungicide. Over 450 people died and 6,530 people were permanently affected (10). A positive result of this disaster is the world-wide ban on the application of alkylmercury compounds in fungicide (*11*).

In the 1960s, accumulation of mercury in wildlife and fish was noted in Sweden; similar observations were noted in the Great Lakes region of the United States and Canada. Both countries have since banned selling fish containing mercury levels above 500 μg/kg fresh weight (*12*).

1.4 Mercury Species Levels and Pathways in the Environment

In order to understand the role of different mercury species in the environment, the biogeochemical cycle of mercury must be known. Mercury exists naturally in mineral form, usually associated with ores or other geological materials. Mercury enters into the environment from a variety of sources, both natural and anthropogenic. Natural sources include volcanic emissions, degassing from soils, and volatilization from the oceans. Rains and other weather activity help to solubilize the mercury, while the extensive heat of volcanic eruptions helps to volatilize mercury. It is reported that 55,000 to 180,000 tons of mercury/year enter into the environment from the natural sources and 8,000 to 38,000 tons/year enter from anthropogenic sources (*9*). It is impossible to identify current

levels of mercury in the environment as either anthropogenic or natural, but several experts have estimated that humans have doubled or tripled the amount of mercury released into the environment (*6*).

Anthropogenic emissions of mercury are from the use of fossil fuels (especially coal), and other extracted, treated, or recycled mineral materials, as well as from mercury used intentionally in thousands of products and industrial processes. These include chlorine and caustic soda manufacture, laboratory use, paint manufacture (before 1991), electronic uses [such lighting (e.g. fluorescent lamps), wiring devices, switches and batteries], thermometers, thermostats, barometers (and other related instruments), dental supplies (e.g., dental amalgam fillings), and medical equipment (*13*). The commercial uses of organomercury compounds are given in Table 1-II.

TABLE 1-II. Use of Organomercury Compounds (*10*).

Compound	Use	Comments
CH_3HgX	Agricultural seed dressing, fungicide	Banned Sweden 1966, USA 1970 as seed disinfectant. Used in laboratories.
C_2H_5HgX	Cereal seed treatment	Banned USA, Canada 1970, Used in UK
RHgX	Catalyst for urethane, vinyl acetate production	
C_6H_5HgX	Seed dressings, fungicide, slimicide, and bactericide. For pulp, paper, paints	Banned as slimicide USA 1970. Banned as rice seed dressing Japan 1970. Used in UK.
p-$CH_3C_6H_4HgX$	Spermicide	
$ROCH_2CH_2HgX$	Seed dressing, fungicides	Banned Japan 1968, Used in UK
$ClCH_2CH(OCH_3)$-CH_2HgX	Fungicide, pesticide	

X = anionic group. Wide range of X known, e.g. OAc^-, PO_4^{3-}, Cl^-, $NHC(NH)NHCN^-$, etc.

Anthropogenic mercury releases are mainly from industrial processes and combustion sources. EPA estimates that combustion point sources account for 85% of anthropogenic mercury emissions. Four specific combustion source categories make up the majority of emissions: municipal and medical waste incineration (25% each), utility boilers (21%), and commercial/industrial boilers (12%) (*14*).

A small fraction of organomercury is released into the environment from anthropogenic sources, mostly from emissions as either vapors (elemental or oxidized mercury) or particles (oxidized compounds). Natural emissions are mainly in elemental mercury form. Mercury may reside in the atmosphere for years, allowing global circulation systems to transport elemental mercury emissions from the source of emission to anywhere on earth before transformation and deposition take place. The residence time is entirely dependent on the mercury species. For example, Hg^0, a volatile form, will enter into the atmosphere and, due to its very low solubility in water, will travel unchanged through the upper atmosphere for up to two years (*15*). On the other hand, Hg^{2+} is highly soluble in water. Therefore, when released in atmosphere, it deposits near the point source via wet deposition and acts as a local pollutant. When mercury salt is released into the atmosphere, it solubilizes with precipitation and reaches the water column.

The majority of mercury in surface soil exists as oxidized mercury complexes/compounds; however, a small fraction is methylmercury and elemental mercury. Mercury complexes deposited in soils can be transformed back into gaseous mercury by light and humic substances, and then re-enter the atmosphere. Studies have

consistently shown that plant uptake is negligible and consequently, animals foraging on plants accumulate little mercury (*6*).

In addition to direct deposition, mercury can also reach water from soil runoff, although the amount partitioning to runoff is expected to be small since mercury binds to soil; runoff is probably in the form of suspended sediments. It is in the aquatic ecosystem where the mercury cycle plays a major role. Once in water, mercury can either enters the food chain, settle into sediment, or volatilize back into the atmosphere. Entrance into the food chain begins with bacteria in water, which can take up mercury in its inorganic form and metabolize it to methylmercury. The methylmercury-containing bacteria may be consumed by the next level in the food chain, or they may excrete the methylmercury into the water where it can adsorb to plankton, which are also consumed by the next level in the food chain. Even small environmental concentrations of mercury in water can readily accumulate to potentially harmful concentrations in fish and fish-eating people; the concentration ratio of methylmercury in fish tissue to that in water is usually between 10,000 and 100,000. Fish at the top of the food chain, such as sharks and swordfish, have much higher mercury concentrations than fish lower on the food chain (*16*). According to EPA, forty-one states have advisories for mercury in one or more water bodies, and eleven states have issued statewide mercury advisories. The biogeochemical cycle of mercury is shown in Figure 1-1.

All possible pathways for interconversion of elemental mercury (Hg^0), inorganic mercury (Hg^{2+}) and methylmercury (CH_3Hg^+) are illustrated in detail in Figure 1-1. It can be seen from the illustration that both Hg^0 and Hg^{2+} enter the aquatic system via dry or wet deposition. They ultimately settle down to the sediment by sedimentation process.

Hg^0 is then oxidized to Hg^{2+}, and follows the same path as Hg^{2+}. In an alternative mechanism, Hg^0 is converted into methylmercury through oxidative methylation. Hg^{2+} converts into methylmercury through bacterial activity and enters the water column where they both are absorbed by aquatic life through digestion, adsorption and/or respiration (*10*). The mechanisms of synthesis/decomposition of methylmercury are not well understood. Once methylmercury is formed, it enters the food chain by rapid diffusion and tight binding to proteins in aquatic biota, and attains its highest concentration in the tissues of fish at the top of the food chain due to bioconcentration through the trophic levels. The main factors that affect the levels of methylmercury in fish are the dietary trophic level of the species, the age of the fish, microbial activity and the mercury concentration in the upper layer of the sediment, dissolved organic carbon content, salinity, pH and redox potential.

In natural environment, methylation of inorganic mercury may occur in sediments, in the water column, and in soils by humic and fulvic materials. It can occur under both aerobic and anaerobic conditions, but the maximum rate is observed in presence of strong oxidizing anaerobic environment and where several microorganisms exist. The pH of the environment also contributes to the formation of different methylmercury species, e.g. monomethylmercury forms under most acid or neutral conditions, whereas dimethylmercury forms under basic conditions (*10*). Other factors that affect methylation include: total inorganic mercury concentration, organic content of the sediment, pH, redox potential (E_h), temperature, the nature of microorganisms present and sulfide levels. High sulfide concentrations enhance the formation of least soluble mercuric sulfide, which is not bioavailable. Methylmercury does not build to more than

1.5% of total mercury in sediments following methylation (*17*). Demethylation may occur to Hg^0 and methane, but due to the bioaccumulation of methylmercury, methylation is more prevalent than the demethylation.

Figure 1-2 shows the E_h-pH stability diagram for methylmercury species. Note that the sulfide content and increasing pH results in the formation of mercury sulfide. The pH also affects the concentration of the methylmercury present in water column. It is found that the rate of mercury methylation increases with a decrease in pH (*18*). In real life, a decrease in pH enhances the release of mercury from sediment (*10*). Decreasing pH also affects inorganic mercury by increasing the amount of solubilized Hg^{2+} (*18*).

Besides these methylation processes, there are numerous biotic methylation processes where methylation of mercury results from the detoxification mechanisms of several bacteria. For example, methylcobalamin (CH_3CoB_{12}) reacts with mercury (II) in aqueous solution, as shown below, and produces methylmercury (*10*).

$$CH_3CoB_{12} + Hg^{2+} \xrightarrow{H_2O} CH_3Hg^+ + H_2OCoB_{12}$$

Dimethylmercury may also form by further methylation of methylmercury by methylcobalamin, but this step is very slow (approximately 600 times slower). Once the dimethylmercury is formed, it diffuses through the water column to the atmosphere and decomposes there in presence of light. It has been suggested that in aquatic system, approximately 30% of the total mercury is in the form of methylmercury, and therefore, marine systems can produce at least 500 tons of methylmercury/year (*10*).

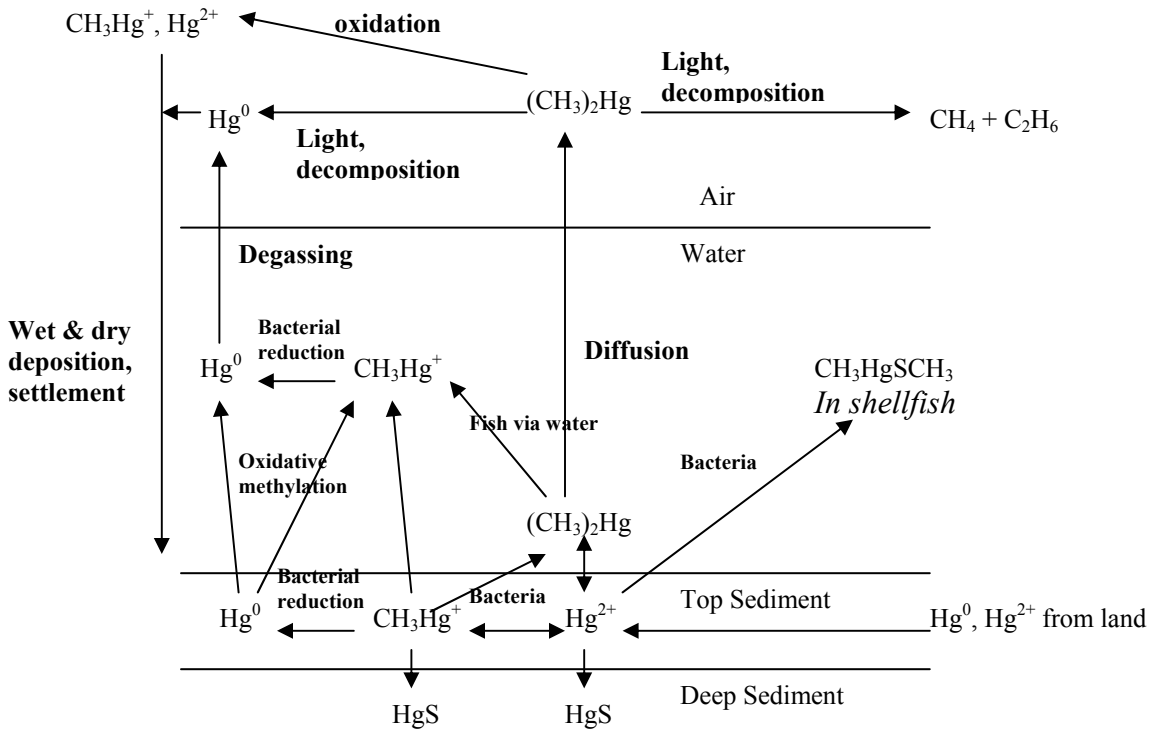


FIGURE 1-1. Biogeochemical cycle of mercury in the environment (*10*).

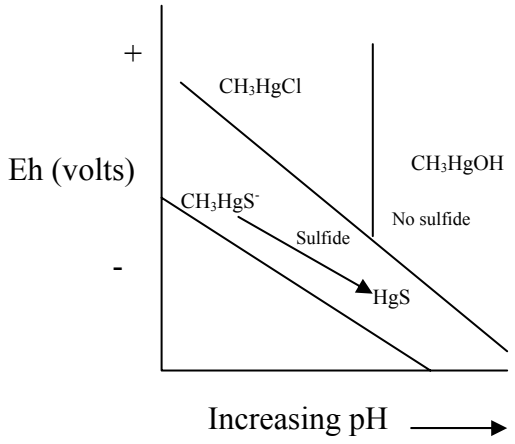


FIGURE 1-2. E_h-pH plot of the various methylmercury species in an aquatic system.

During the past three decades, levels of total mercury and methylmercury have been measured in different sectors of the environment, but the concentrations of methylmercury have not been determined separately as the total mercury concentrations are very low. From a literature survey, it is found that the concentration of total mercury in air is 2.0-6.0 ng/m^3, and in certain mineralized or industrialized areas the concentrations are in the ng/m^3 level. In industrialized, urban or mineralized areas, the levels of total mercury and methylmercury in sediments are usually measured in the range of 0-100 μg/g and 0-100 ng/g, respectively. Less than 2.0 ng/g of total mercury has been measured in most of the drinking water sources; in natural soil it is in the range of 0.02 to 0.4 μg/g. According to the United States Food and Drug Administration (US FDA), quantitative amounts of mercury are present in meal, fish and poultry groups on a regular basis (*19*). The potential for bioconcentration of mercury and methylmercury in different species is shown in Table 1-III.

From the above discussions, it can be seen that the serious risk of methylmercury poisoning in humans is likely to arise from either consumption of seed treated with methylmercury or consumption of fish containing higher amounts of methylmercury.

1.5 Toxicology of Mercury Compounds

All forms of mercury are toxic at some level; the toxicity depends entirely on the chemical form. It can be defined into three basic groups: mercury vapor (Hg^0), inorganic mercury (Hg^{2+}), and organomercury. The exposure, metabolism, toxic effects and symptoms are different for each form (*20*). A summary of toxic properties of different mercury species in humans is shown in Table 1-IV.

TABLE 1-III. Some Bioconcentration Factors for Total Mercury (*10*).

Matrix	Concentration range
Freshwater, seawater	1
Algae	10^3
Macrophytes	10^3
Seaweeds	10^4
Fish	10^4–10^5
Invertebrates	10^5
Oysters	10^4-10^5
Marine mammals	10^5–10^6
Seabirds	10^5–10^6

Mercury can exist in the vapor phase as Hg^0 because of its high vapor pressure, and in addition to the ingestion of mercury compounds, intoxication can thus take place by inhalation of the vapor. The short chain organomercury compounds, especially methylmercury, are the most important toxicologically because of their chemical stability. On the other hand, phenyl mercurials are rapidly degraded to inorganic mercury compounds upon entering mammalian tissues and therefore are similar to inorganic mercury in terms of their toxic effects. The interaction of mercury with biological ligands is based on the high affinity of mercury cations (Hg^{2+} or R-Hg^+) towards sulfur.

It is assumed that the selective toxicity of different mercury species results from a selective distribution to the various organs. It is well established that the distribution and the permeation of biological barriers (e.g. cell membrane, blood-brain barrier), depend on the lipophilicity of different mercury species. The lipophilicity is highest for Hg^0 and methylmercury, but low for Hg^{2+} and for organic compounds with polar groups (*21*).

TABLE 1-IV. Summary of Toxic Properties of Different Forms of Mercury in Humans (*12*).

Species	Exposure	Effects	Biological Indication
Hg^0	*Occupational*: Chlor-alkali industry, production of thermometer, thermostats and fluorescent bulbs, mercury mining, dentistry *Non-occupational*: Dental amalgam filling	*Severe exposure*: Tremor, gingivitis, erythrism, loss of memory, emotional and psychological disturbance, damage to kidneys *Lower exposure*: Cognitive deficits, mild proteinuria, insomnia, loss of attetite, immunological disturbances Damage is reversible	*Hg in urine*: chronic exposure, indication of Hg levels in kidney *Hg in blood*: indicator of short term exposure *Exhaled air*: short term exposure No good indicator for brain
Hg^{2+}	Antiseptic, leather industry, production of batteries, fungicides, use in bleaching soaps and creams	Chronic toxicity: neurological disorders similar to the effects of Hg^0. Repeated exposure of low doses affects the immune systems. Acute exposure may cause irreversible damage of kidney and indirectly cardiovascular collapse	Urine
CH_3Hg^+	Fungicide, slimicide, food – mainly fish and other marine products	Immediate damage of neuronal cells and delayed symptoms of sensory disturbance, constriction of visual field, deafness, motor aberrations, mental disorders, cramps, paralysis	Blood and hair

Occupational exposure to Hg vapor occurs in some specific industries and occupations, such as chlor-alkali electrolysis, production and recycling of fluorescent lamps and batteries and in dentistry offices. Some background exposure may also result from the release of minute amounts of mercury vapor and inorganic mercury in dental fillings. Exposure to organomercury may also occur from the chemical industry, whereas nonoccupational exposure to methylmercury occurs almost exclusively from the diet,

mainly fish, and remains without effects at background levels. But subacute intoxication may occur after ingestion of contaminated food.

The exposure route for mercury vapor is mainly through inhalation. Approximately 80% of inhaled mercury vapor is absorbed from the lungs into the bloodstream. Since mercury vapor is lipid soluble, it easily crosses the cell membrane. The dissolved vapor is then oxidized rapidly to Hg^{2+}, partly in the erythrocytes and partly after diffusion into other tissues. The oxidation is catalyzed by the enzyme catalase in the presence of trace hydrogen peroxide (*11*).

$$\text{Cat-OH} + H_2O_2 \rightarrow \text{Cat-OOH} + H_2O$$

$$\text{Cat-OOH} + Hg^0 \rightarrow \text{Cat-OH} + HgO$$

After formation of the oxidized form, considered as toxic species, it follows the same toxicological path as Hg^{2+}. After exposure to Hg^0, the mercury content ratio in red blood cell to plasma is between 1 and 2, which accounts for the initial neurological reaction observed by those exposed to high levels of mercury vapor. Based on the data, it is found that 50–90% of the body burden is located in the kidneys, where a large portion is bound to metallothionein, a low molecular weight, cysteine-rich protein (*22*). The rate of excretion for mercury vapor depends on exposure time. It is demonstrated that almost 50% of the absorbed mercury is excreted through feces and much less through the urine. But after a long time exposure, the urinary route (60%) predominates over the fecal route (40%), which reflects the renal accumulation (*23*). After exposure to mercury vapor, the elimination half-life for the whole body is 58 days. Upon short exposure to mercury vapor, about 90% of the mercury in blood is cleared with a half-life of 2-4 days, followed by a second phase with a half-life of 15-30 days (*11*).

Inorganic mercury can enter the human body through a number of routes, e.g. ingestion, absorption through skin and adsorption of mercuric salt aerosols in the lungs. The amount of absorption/adsorption depends on the solubility of the inorganic mercury species or the aerosol particle size. For humans, the absorption of inorganic mercury from food was estimated in the range of 5-10% of the dose (*23*).

A small fraction of inorganic mercury can penetrate the placental barriers. Kidneys are the potential target for inorganic mercury. A small portion of the absorbed ionic mercury is exhaled as vapor after reduction in tissues. Approximately 75-92% of the administered dose excretes through fecal excretion. The half-life of inorganic mercury compounds in the body is 30-60 days.

Methylmercury is one of the most toxic organomercury species and there is more concern about this species because of the extent of exposure. Nonoccupational exposure of methylmercury occurs almost exclusively through diet. Exposure to dimethylmercury (the most toxic organomercury species) appears to result in a very high absorption through lung and skin. More than 95% of the methylmercury in the diet is absorbed into the bloodstream through gastrointestinal tract. About 90% of the absorbed methylmercury in whole blood is bound to the red blood cells; further distribution to the organs is completed within four days. Like mercury vapor, methylmercury can also penetrate blood-brain and placental barriers.

In mammalian organisms, methylmercury slowly degrades into inorganic mercury, and is excreted through feces as inorganic mercury. Methylmercury is so mobile in the body that excretion is the rate limiting step for elimination. Other major deposition organs are the liver and kidneys. Of particular interest is the binding of methylmercury in

growing scalp hair, which makes hair a simple biomonitor for organomercury exposure. The half-life of organomercury is between 40-70 days (*11*).

1.6 Mercury Analysis

1.6.1 Total Mercury

A number of analytical methods can be found in the literature for quantitative determination of total mercury from different types of samples. A broad classification can be made to distinguish these methods as noninstrumental (i.e. gravimetric and titrimetric methods) and instrumental methods (i.e. atomic absorption or fluorescence spectrometry, neutron activation analysis, electrochemical methods, etc.) (*24*). Gravimetric technique is the oldest analytical method for mercury analysis. Distillation of mercury from sample followed by weighing is reported in an article published in 1931 by Stock (*25*). Other gravimetric methods were based on the precipitation of mercuric sulfide by the reaction of Hg^{2+} with either H_2S or CH_3CSNH_2 followed by accurate weighing (*24*). Titrimetric methods are better than the gravimetric methods. Sodium tetraphenylboron, ethylenediaminetetraacetic acid (EDTA), and ascorbic acid with bromosuccinimide are usually used for titrimetric analysis (*24*). These noninstrumental methods are not popular at present because of their limited sensitivity and/or selectivity.

In order to increase the sensitivity and selectivity towards mercury, spectrophotometric methods were developed and utilized in the 1950s and 1960s. During that time, dithizone (diphenylthiocabazone) was used to produce a colored complex with mercury, and was measured spectrophotometrically (*24*). Dithizone and its complex with mercury are both insoluble in water, but are easily soluble in organic solvents. Dithizone

acts as a dibasic acid and is highly selective to Hg^{2+} in the presence of a suitable masking agent (excess EDTA or SCN^-).

Atomic absorption spectrometry (AAS) is still a widely used analysis method for mercury determination from all types of environmental, biological and geological samples (*26*). The absorption of radiation by mercury was discovered by Wood (*27*) in 1939. Since then a number of modifications have been performed to increase the sensitivity. The two major developments have been "flameless atomic absorption" and the "cold vapor" technique, each capable of detecting around 0.05 ng.

The cold vapor atomic absorption spectrometry (CV-AAS) was developed by Hatch and Ott (*28*), and requires that the mercury first be brought into solution as Hg^{2+} and then reduced to the metallic vapor (Hg^0) and carried into a spectrophotometric cell by a stream of air or inert gas passing through the solution (*29*). Tin (II) chloride ($SnCl_2$) or sodium tetrahydroborate ($NaBH_4$) are the most commonly used reducing agents; ascorbic acid is also effective. The reduction of Hg^{2+} takes place according to the following reactions (*30*):

$$Hg^{2+} + Sn^{2+} \rightarrow Hg^0 + Sn^{4+}$$

$$Hg^{2+} + 2NaBH_4 + 6H_2O \rightarrow Hg^0 + 7H_2 + 2H_3BO_3 + 2Na^+$$

CV-AAS is the most accepted method by the EPA (*31*). The detection limit of CV-AAS can be improved by the addition of an amalgamation/deamalgamation preconcentration step to the conventional procedure. The development of flow injection technique and automation of the CV-AAS system has improved sample throughput, keeping the technique competitive with new technologies. The drawback of this technique is that it can only measure the elemental mercury. Therefore, in order to

determine any other mercury species, those species must first be converted to elemental mercury (*24*). The flameless atomic absorption covers procedures where the mercury is released as elemental vapor, either by combustion or thermal decomposition of the sample (*28*).

Various other instrumental methods are also available for the determination of total mercury. They include atomic fluorescence spectrometry (AFS), neutron activation analysis (NAA), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), and X-ray fluorescence spectrometry (XFS) (*24*). NAA and XFS are excellent methods for nondestructive analysis of mercury from samples. ICP-AES and ICP-MS are multielement determination methods, but the ICP-AES suffers from background interferences and is not suitable for routine analysis. On the other hand, although ICP-MS suffers from memory effects, it is a widely used element-selective method for the determination of trace elements from different samples. AFS is the only single elemental detection method that competes with the CV-AAS method. The overall setup of the cold vapor atomic fluorescence spectrometry (CV-AFS) is similar to that of the CV-AAS. The basis of AFS determination of mercury is the determination of the emitted radiation at a perpendicular angle to the incident light beam. The AFS method detection limit is 0.2 ng/L and can be further reduced by incorporation of the amalgamation/deamalgamation step. Because of the high sensitivity provided by this technique, gold trap CV-AFS is currently widely used in environmental analysis (*26*). But the draw backs of using the CV-AFS method is that mercury present in the reagents and carrier gas also preconcentrates and gives positively biased results.

Isotope dilution mass spectrometry is also a current method of choice for the determination of total mercury from different samples (*32-33*).

1.6.2 Mercury Speciation

Significant advancements in the selectivity and sensitivity of analytical methods for mercury speciation analysis have been observed in recent years. These improvements eventually allow for the determination of total and major mercury species from different environmental samples. Particular emphasis has been placed on the development of new analytical methods and future needs for environmental, biological, botanical and geological matrices (*24*). Analytical methods are usually selected on the basis of the nature of the matrices and the mercury concentration (*34*).

The scientific community realized the necessity for analysis of different forms of mercury just after the methylmercury disaster in Minamata, Japan. These analyses are still some of the "hot" topics in analytical chemistry today. Over the last forty three years, hundreds of papers have been published in the literature regarding determination of mercury and organomercury species from environmental, biological, botanical and geological matrices. The first method for analysis of inorganic mercury and organomercury was published in 1961 by Gage (*35*). The organomercury was extracted into benzene and measured spectrophotometrically as a dithizone complex, while leaving the inorganic mercury fraction in the aqueous phase. But this method was not well regarded by scientists because of its poor selectivity and sensitivity for mercury species (DL = 1 μg/g). After that, the original methods for the determination of methylmercury from biota and sediments were developed by Westöö (*36*). This method involves extraction of acidified sample with an organic solvent, e.g. benzene or toluene, and then

back-extraction into an aqueous solution of cysteine, followed by the detection with gas chromatography with electron capture detector (GC-ECD). The draw back of this method was that it was applicable for tissue and sediment samples, but not for water and air samples (*37*).

In 1971, Magos (*38*) developed a mercury speciation method based on CV-AAS detection. In this method, the mercury species were reduced to Hg^0 by using a mixture of reagents containing stannous chloride and cadmium chloride. Bloom and Fitzgerland (*39*) were the first to develop a method for the determination of organomercury species from air by using cryo-trapping, chromatographic separation, and CV-AFS detection. According to this method, the nonvolatile ionic mercury species are ethylated into volatile nonionic mercury species. Sodium tetraethylborate, $NaB(C_2H_5)_4$, is used as an ethylating agent to form methylethylmercury and diethylmercury from CH_3Hg^+ and Hg^{2+} according to the following reaction.

$$CH_3Hg^+ + Hg^{2+} + 3NaH(C_2H_5)_4 \rightarrow CH_3HgC_2H_5 + Hg(C_2H_5)_2 + 3Na^+ + 3B(C_2H_5)_3$$

These volatile species are then purged from solution at room temperature and then collected on suitable adsorbent materials, such as Carbotrap Tenax®, before analysis. GC mass spectrometry is routinely used for detection of the ethylated mercury species, although some other detection techniques can be also used as alternatives (*37*). Connection of the derivatization or degassing systems to an ICP-MS has also allowed determination of the concentration of different mercury species simultaneously (*40*). Unfortunately, it was found that the ethylation process suffers from interferences in terms of pH effects, inhibition from matrices containing high amounts of chloride (*41*), and dissolved organic matter (*42*). Therefore, a preconcentration method, namely

codistillation with water, was used (*43-44*) followed by extraction into organic solvents with back-extraction into water (*41*).

Many analytical techniques have been developed for the speciation of mercury, usually combining a powerful separation technique, such as gas chromatography (GC) [packed (*45*), capillary (*46*), or multicapillary column (*47*)], liquid chromatography (LC) (*48*), capillary electrophoresis (CE) (*49*) or sulphydrylated cotton fiber (SCF) (*50*) with a suitable detection technique, e.g. atomic absorption spectrometry (AAS) (*45*), atomic fluorescence spectrometry (AFS) (*30*), or inductively coupled plasma mass spectrometry (ICP-MS) (*51*). Despite improvements in the instrumentations, the quantitative mercury speciation analysis may be affected by the traditional problems related to nonquantitative recoveries and by the artifact formation. It is found from the literature that artifact formation of methylmercury from inorganic mercury may occur during the distillation step (*52-53*). It is now well established in the literature that methylation may also occur for other widely adapted extraction methods like acid and alkaline extractions (*54-58*). This methylation process is enhanced by the presence of higher inorganic mercury and organic content. On the other hand, demethylation may also take place during acid (*50*) or alkaline (*59*) extraction. The potential artifact formation can be checked by spiking the sample with appropriate stable mercury isotope.

Mercury has a number of stable isotopes that can be used in the study of methylation and demethylation at natural levels by simultaneously using inorganic mercury and methylmercury enriched in different isotopes. ICP-MS can be used for the determination of mercury isotope abundances. From the literature survey, it is found that the application of isotopically enriched inorganic mercury and/or isotopically enriched

methylmercury for tracing mercury species transformation and/or for determination of the species concentration is a growing field of interest (*51-52, 60-69*). But this technique, known as "isotope dilution mass spectrometry (IDMS)" or "species specific IDMS" is not well established due to the commercial absence of isotopically labeled methylmercury. Most of the research laboratories usually synthesize the isotopically labeled methylmercury in house on a microscale basis to perform the IDMS analysis.

1.6.3 History of Mercury Analysis at Duquesne University

Helen M. Boylan was the first member of this research group to determine mercury from coal and coal combustion by-products (*70*). She established an EPA Method (Method 7473) for analysis of total mercury from environmental samples (*71*). This method is highly efficient in on-site mercury analysis, which in turn reduces the sample analysis time and cost (*72-73*).

Ye Han was the second member of this group to work with mercury speciation (*50*). He also established an EPA method (Method 3200) (*74*) for speciation of mercury from soils and sediments by using selective solvent extraction and sulfydrylated-cotton-fiber aided separation, and detection with direct mercury analyzer-80 (DMA-80) and/or with ICP-MS.

1.7 Conclusions

The purpose of this chapter is to introduce the chemistry and toxicity of mercury species. From the historical review, it is found that anthropogenic sources are the major route for introduction of mercury into different sectors of the environment. The biogeochemical cycle of mercury species and their impact on the environment is also briefly reviewed.

The mercury toxicity helps not only to explain the biochemistry behind the mercury-related disasters but also the biochemistry related to the low level mercury exposure. This chapter also helps to illuminate why the analysis of total mercury is not meaningful anymore, and why there is a growing demand for speciation analysis.

From the historical background of the mercury speciation analysis techniques, it is found that most of the widely used methods induce formation of either methylmercury or inorganic mercury. Although some scientists are trying to overcome these obstacles by using the IDMS technique, it is not capable of tracing species conversion and making corrections simultaneously. Therefore, one objective of this study is to apply SIDMS as a diagnostic tool for mercury speciation analysis for the first time. Chapter 2 describes in detail the synthesis and characterization of isotopically enriched methylmercury, an essential species for IDMS and SIDMS analysis, from inorganic mercury and tetramethyltin. Different mercury speciation methods published in the literature were evaluated both conventionally and using SIDMS technique; and the results are discussed in Chapter 4.

An inter-laboratory validation study for EPA draft Method 3200 was conducted by the United States Environmental Protection Agency (US EPA) and Science Applications International Corporation (SAIC); the findings from this study are discussed in Chapter 3. During the method development and validation stage, it is found that Method 3200 is less efficient for inorganic mercury extraction. Therefore, another objective of this study was to develop a highly-efficient new mercury speciation method. A new method has been developed based on closed-vessel microwave-assisted acid extraction and is discussed in detail in Chapter 5.

Another objective of the present study was to develop generic fundamental equations for one, two and three species systems for SIDMS analysis in terms of bidirectional and unidirectional transformations, and apply those for the validation of other more conventional speciation methods. The detail calculation schemes and algorithms are reported in Chapter 6. Chapter 6 will help the scientific community better understand the species conversion fundamentals and calculations. Chapter 7 describes the application of the SIDMS technique in environmental chromium speciation analysis based on modified extraction methodologies by applying microwave energy instead of hot-plate technique.

1.8 References

(1) Templeton, D.; Ariese, F.; Cornelis, R.; Danielsson, L. G.; Muntau, H.; van Leeuven, H. P.; Lobinski, R. *Pure Appl. Chem*. **2000**, *72*, 1453.

(2) Morabito, R. *Fresenius' J. Anal. Chem*. **1995**, *351*, 378-385.

(3) Kot, A.; Namiesnik, J. *Trends Anal. Chem*. **2000**, *19 (2+3)*, 69-79.

(4) *Hyphenated Techniques in Speciation Analysis*; Szpunar, J.; Lobinski, R. Eds.; The Royal Society of Chemistry: London, UK, **2004**; pp 2-13.

(5) Gomez-Ariza, J. L.; Morales, E.; Giraldez, I.; Sanchez-Rodas, D. In *Trace Element Speciation: For Environment, Food and Health;* Ebdon, L.; Pitts, L.; Cornelis, R.; Crews, H.; Donard, O. F. X.; Quevauviller, Ph., Eds.; Royal Society of Chemistry: Cambridge, UK. **2001**; pp 50-80.

(6) *US EPA: Mercury Study Report to the Congress*; Office of Air Quality Planning and Standards and Office of Research and Development: Washington, DC, December **1997**

(7) Montague, P.; Mantague, K. *Mercury*; Sierra Club: San Francisco, CA, **1971**.

(8) Takeuchi, T.; Eto, K. *The Pathology of Minamata Disease: A Tragic Story of Water Pollution*; Kyushu University Press Inc.: Okinawa, Yamaguchi, Japan, **1999**.

(9) *Comprehensive Organometallic Chemistry;* Wilkinson, G., Ed.; Pergamon Press: Oxford, **1982**.

(10) *Organometallic Compounds in the Environment: Principles and Reactions*; Craig, P.J., Ed.; John Wiley & Sons: New York, NY, **1986**; pp 65-110.

(11) Clarkson, R. W. *Crit. Rev. Clin. Lab. Sci.* **1997**, *34*, 369-403.

(12) Horvat, M. In *Trace Element Speciation: For Environment, Food and Health;* Ebdon, L.; Pitts, L.; Cornelis, R.; Crews, H.; Donard, O. F. X.; Quevauviller, Ph., Eds.; Royal Society of Chemistry: Cambridge, UK. **2001**; pp 127-141.

(13) *Ullmann's Encyclopedia of Industrial Chemistry;* 6th Edition, John Wiley & Sons: New York, **2003**.

(14) USEPA, http://www.epa.gov/grtlakes/bnsdocs/mercsrce/

(15) Davis, S. *Center for Clean Air Policy;* Washington, DC., **1998**; pp 1-77.

(16) *World Health Organization. Methylmercury;* Geneva, **1990**; p. 13.

(17) Craig, P. J.; Bartlett, P. D. *Nature* **1978**, *275*, 635-638.

(18) *Metal Speciation: Theory, Analysis, and Application*; Kramer, J. R.; Allen, H. E., Eds.; Lewis: New York, **1990**.

(19) DeCarlo, V. J. *US EPA Report No. 560/6-77-031;* Batelle Columbus Laboratories: Columbus, OH, **1977**.

(20) Suzuki, T.; Imura, N.; Clarkson, T. W. *Advances in Mercury Toxicology;* Plenum Press: New York, NY, **1991**.

(21) Hughes, W. L.; Ann, N. Y. *Acad. Sci*. **1957**, *65*, 454.

(22) Cherian, M. G.; Clarkson, T. W. *Chem. Biol. Interact*. **1976**, *12*, 109.

(23) Clarkson, T. W.; Hursh, J. B.; Sager, P. R.; Syversen, T. L. M. In *Bilogical Monitoring of Toxic Metals;* Clarkson, T. W.; Friberg, L.; Nordberg, G. F.; Sager, P. R., Eds.; Plenum Press: New York, NY, **1988**.

(24) *Encyclopedia of Analytical Science*; Townshend, A., Ed.; Academic Press: New York, NY, **1995**; Vol. 5, pp 3050-3070.

(25) Stock, A.; Lux, F.; Cucuel, F.; Gerstner, F. *Z. Angew. Chem*. **1931**, *44*, 200.

(26) Bloom, N. S.; Preus, E.; Katon, J.; Hiltner, M. *Anal. Chim. Acta* **2003**, *479*, 233-248.

(27) Woodson, T. T. *Rev. Sci. Instrum*. **1939**, *10*, 308-311.

(28) Hatch, W. R.; Ott, W. L. *Anal. Chem*. **1968**, *40*, 2085-2087.

(29) Chilov, S. *Talanta* **1975**, *22*, 205-232.

(30) Cai, Y. *Trends Anal. Chem*. **2000**, *19 (1)*, 62-66.

(31) Crock, J. G. In *Methods of Soil Analysis. Part 3. Chemical Method;* Soil Science Society of America and America Society of Agronomy: Madison, WI, **1996**; Vol. Series No. 5, pp 769-791.

(32) Smith, R. G. *Anal. Chem*. **1993**, *65*, 2485-2488.

(33) Christopher, S. J.; Long, S. E.; Rearick, M. S.; Fassett, J. D. *Anal. Chem*. **2001**, *73*, 2190-2199.

(34) Horvat, M. In *NATO ASI Series, Series 2: Environment, Vol. 21(Global and Regional Mercury Cycles: Sources, Fluxes and Mass Balances);* Baeyens, W.; Ebinghaus, R.; Vasiliev, O., Eds.; Kluwer Academic Publishers: **1997**; Chapter 1.

(35) Gage, J. C. *Analyst* **1961**, *86*, 457.

(36) Westöö, G. *Acta Chem. Scand*. **1967**, *21*, 1790-1800.

(37) Mason, R. P.; Benoit, J. M. In *Organometallic Compounds in the Environment,* 2nd Edition; Craig, P. J., Ed.; John Willey & Sons: England, UK, **2003**; pp 57-99.

(38) Magos, L. *Analyst* **1971**, *96*, 847-853.

(39) Bloom, N. S.; Fitzgerland, W. F. *Anal. Chim. Acta* **1988**, *209*, 151-161.

(40) Amouroux, D.; Tessier, E.; Pecheyran, C.; Donard, O. F. X. *Anal. Chim. Acta* **1998**, *377 (2-3)*, 241-254.

(41) Bloom, N. S. *Can. J. Fish. Aquat. Sci*. **1989**, *46*, 1131.

(42) Bloom, N. S.; Von Der Geest, E. J. *Water, Air Soil Pollut*. **1995**, *80 (1-4)*, 1319-1323.

(43) Horvat, M.; Bloom, N. S.; Liang, L. *Anal. Chim. Acta* **1993**, *281 (1)*, 135-152.

(44) Horvat, M.; Bloom, N. S.; Liang, L. *Anal. Chim. Acta* **1993**, *282 (1)*, 153-168.

(45) Tseng, C. M.; de Diego, A. Pinaly, H.; Amouroux, D.; Donard, O. F. X. *J. Anal. At. Spectrom*. **1998**, *13*, 755-764.

(46) Bulska, E.; Baxter, D.; Frech, W. *Anal. Chim. Acta* **1991**, *249*, 545-554.

(47) Rodriguez, I.; Mounicou, S.; Lobinski, R.; Sidelnikov, V.; Partushev, Y.; Yamanaka, M. *Anal. Chem*. **1999**, *71*, 4534-4543.

(48) Martinez, R.; Tagle, M.; Sanchez, J. E.; Sanz-Medel, A. *Anal. Chim. Acta* **2000**, *419*, 137-144.

(49) Silva da Rocha, M.; Soldada, A. B.; Blanco-Gonzalez, E.; Sanz-Medel, A. *J. Anal. At. Spectrom*. **2000**, *15*, 513-518.

(50) Han, Y.; Kingston, H. M.; Boylan, H. M.; Rahman, G. M. M.; Shah, S.; Richter, R. C.; Link, D. D.; Bhandari, S. *Anal. Bioanal. Chem*. **2003**, *375*, 428-436.

(51) Yang, L.; Mester, Z.; Sturgeon, R. E. *J. Anal. At. Spectrom*. **2003**, *18*, 431-436.

(52) Hintelmann, H.; Falter, R.; Ilgen, G.; Evans, R. D. *Fresenius' J. Anal. Chem*. **1997**, *358 (3)*, 363-370.

(53) Bloom, N. S.; Colman, J. A.; Barber, L. *Fresenius' J. Anal. Chem*. **1997**, *358 (3)*, 371-377.

(54) Hintelmann, H. *Chemosphere* **1999**, *39 (7)*, 1093-1105.

(55) Falter, R. *Chemosphere* **1999**, *39 (7),* 1051-1073.

(56) Falter, R. *Chemosphere* **1999**, *39 (7),* 1075-1091.

(57) Tseng, C. M.; De Diego, A.; Wasserman, J. C.; Amouroux, D.; Donard, O. F. X. *Chemosphere* **1999**, *39*, 1119-1136.

(58) Hammerschmidt, C. R.; Fitzgerland, W. F. *Anal. Chem*. **2001**, *73*, 5930-5936.

(59) Gaona, X.; Valiente, M. *Anal. Chim. Acta* **2003**, *480*, 219-230.

(60) Yang, L.; Colombini, V.; Maxwell, P.; Mester, Z.; Sturgeon, R. E. *J. Chromatogr., A*. **2003**, *1011*, 135-142.

(61) Wilken, R. D.; Falter, R. *Appl. Orgnomet. Chem*. **1998,** *12*, 551-557.

(62) Snell, J. P.; Stewart, I. I.; Sturgeon, R. E.; Frech, W. *J. Anal. At. Spectrom*. **2000**, *15*, 1540-1545.

(63) Qvarnstrom, J.; Lambertsson, L.; Havarinasab, S.; Hultman, P.; Frech, W. *Anal. Chem*. **2003**, *75*, 4120-4124.

(64) Monperrus, M.; Rodriguez Martin-Doimeadios, R. C.; Scancar, J.; Amouroux, D.; Donard, O. F. X. *Anal. Chem*. **2003**, *75*, 4095-4102.

(65) Lambertsson, L.; Lundberg, E.; Nilsson, M.; Frech, W. *J. Anal. At. Spectrom*. **2001**, *16*, 1296-1301.

(66) Hintelmann, H.; Evans, R. D. *Fresenius' J. Anal. Chem*. **1997**, *358*, 378-385.

(67) Hintelmann, H.; Evans, R. D.; Villeneuve, J. Y. *J. Anal. At. Spectrom*. **1995**, *10*, 619-624.

(68) Rodriguez Martin-Doimeadios, R. C.; Monperrus, M.; Krupp, E.; Amouroux, D.; Donard, O. F. X. *Anal. Chem*. **2003**, *75*, 3202-3211.

(69) Rodriguez Martin-Doimeadios, R. C.; Krupp, E.; Amouroux, D.; Donard, O. F. X. *Anal. Chem*. **2002**, *74*, 2505-2512.

(70) Boylan, H. M.; Cain, R. D.; Kingston, H. M. *J. Air Waste Manage. Assoc.* **2003**, *53 (11)*, 1318-11325.

(71) US EPA Method 7473. In: *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*; SW-846, Update IVA, Washington, DC, **2000**.

(72) Boylan, H. M.; Richter, R. C.; Kingston, H. M.; Angela, R. *Water, Air, Soil Pollut.* **2001**, *127 (1-4)*, 255-270.

(73) Boylan, H. M.; Ronning, T. A.; DeGroot, R. L.; Kingston, H. M. *Environ. Test. Anal.* **2000**, *9 (4),* 8-10, 13.

(74) US EPA Draft Method 3200, Mercury Species by Selective Solvent Extraction and Acid Digestion. June **2000**.

(75) US EPA Method 6800. In *Solid Waste Manual-846, Update IV*, U. S. Government Printing Office (GPO): Washington, DC, **2000**.

(76) Kingston, H. M. *U. S. Patent 5,414,259*, **1995**.

(77) Kingston, H. M. *PCT Int. Appl., Patent No. WO 9939198,* **1999**.

Chapter 2

Synthesis and Characterization of Isotopically Enriched Methylmercury

2.1 Introduction

Over the past decades, the interest in speciation analysis has increased significantly due to the growing awareness that many organometallic compounds are more toxic than their corresponding free metals (*1*). This is reflected in the increasing number of published papers (based on the survey of *Analytical Abstracts*) for the subject "speciation" or "species" since 1980 (Figure 2-1). The number of published papers was relatively constant from 1981 to 1990, at an average of 75 papers per year. It then increased significantly from 118 papers in 1991 to 259 in 2003, at an average of 245 papers per year. Mercury is one of the most dangerous contaminants in the environment. This is due to its accumulation in aquatic organisms and the "bioamplification" phenomena through the trophic chain. The determination of total mercury is frequently not sufficient for understanding the toxicological impact and pathway of mercury species in the environment. The toxicity, bioaccumulation and environmental mobility of mercury are highly dependent on its chemical forms. The organometallic compounds, especially methylmercury, are considered more toxic than the inorganic mercury compounds because of their high affinity for thiol groups (*2*). The environmental methylmercury originates largely from the methylation of inorganic mercury; major non-commercial sources of inorganic mercury are degassing of the earth's crust, emissions from volcanoes, and evaporation from natural bodies of water (*3*). One large anthropogenic source of inorganic mercury is the thermal conversion and volatilization of mercury compounds in coal used

world-wide in massive quantities in unremediated coal-fired power plants. Natural emission of methylmercury can be produced by biological activity on inorganic mercury in bottom sediments, decomposed fish and biological activity in soil (*4-5*). Methylmercury formed in these ways is introduced into the food chain and humans ingest it mainly through diet. The main target of methylmercury in humans is the central nervous system – especially the sensory, visual and auditory areas involved in coordination. The most severe effects lead to widespread brain damage, resulting in mental derangement, coma, and death (*6*). Therefore, it is essential to determine the exact concentration of inorganic mercury and methylmercury present in environmental, biological and food samples.

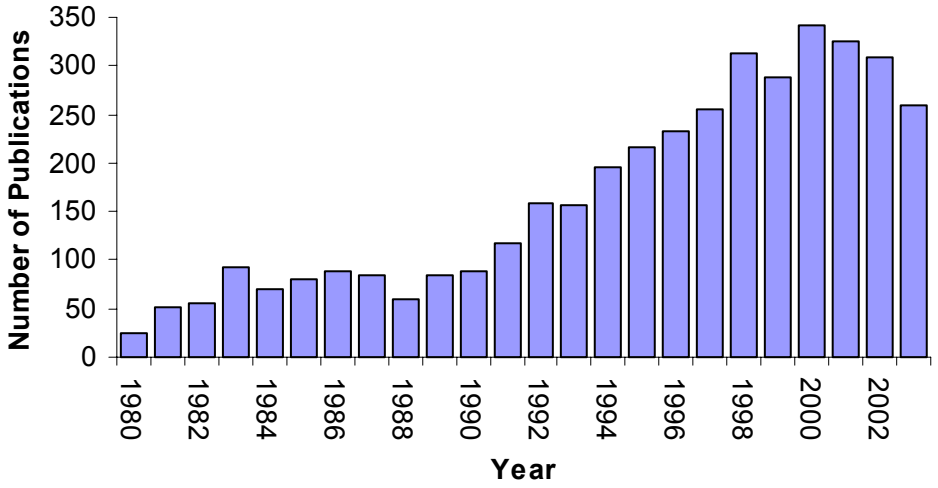


FIGURE 2-1. Survey of analytical abstracts for the word "speciation" or "species" in the titles of published papers since 1980.

Most of the published methods for mercury speciation in environmental samples are based on the Westöö procedure (*7*) (an acid leaching method), solvent extraction (*8-11*), distillation (*8,12,13*), or modification of Westöö methodology (*14*) (alkaline based

leaching) and supercritical fluid extraction (*15*). The most widely used separation techniques are: gas chromatography (GC), high performance liquid chromatography (HPLC) coupled with an element-selective detection technique such as atomic emission spectrometry (AES), atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS) or cold vapor atomic absorption spectrometry (CV-AAS). As all of the extraction methods use either acid or base with organic solvents, and after extraction most of them go through some kind of preconcentration steps (e.g. ethylation or reduction with $SnCl_2$, or hydride generation with $NaBH_4$), there is a possibility of interconversion or unidirectional transformation of inorganic mercury to organic mercury or vice versa during sample storage, shipment, extraction, preconcentration or analysis steps. Therefore, the results obtained using these procedures frequently introduce biases for either inorganic mercury or methylmercury, or both. In the literature, it was found that some of the researchers used isotope dilution mass spectrometry (IDMS) to determine the concentration of methylmercury from environmental samples by labeling methylmercury with isotopically enriched methylmercury (*16-19*). By using this technique, it is possible to determine the amount of methylmercury present in sample during extraction. However, the data do not reveal anything about the source of methylmercury; that is, whether this methylmercury is from the sample or is a product of methylation of inorganic mercury during extraction, preconcentration and/or analysis. In order to obtain true results from the extraction or analysis of environmental samples, it is required to label both the methylmercury and inorganic mercury with isotopically enriched methylmercury and inorganic mercury. This can be achieved by using EPA Method 6800 (*Elemental and Speciated Isotope Dilution*

Mass Spectrometry, SIDMS) (*20*). SIDMS maintains the advantages of IDMS while facilitating the tracing of the species-conversions after spiking and providing the ability to make corrections. In SIDMS, each species is "labeled" with a different isotopically enriched spike in the corresponding species form. Therefore, the interconversion and degradation that occur after spiking are traceable and can be corrected (*21-22*). However, in spite of the benefits of SIDMS, it is not being used widely as a method of analysis because of the commercial absence of isotopically enriched methylmercury. According to the US EPA (*23*), the method 6800 "is currently the only available means to make accurate and defensible speciated measurements" and "will serve as the reference method to define the species present in waste and environmental samples".

According to the literature survey, it is found that there are some proposed methods for the production of organomercury compounds, e.g. the reaction of tetramethyltin with inorganic mercury (*16,24*), the reaction between inorganic mercury and dimethylmercury (*25*), and the reaction of methylcobalamin (CH_3CoB_{12}, a vitamin B_{12} analog) with inorganic mercury (*26-30*). In most cases, dimethylmercury was produced along with monomethylmercury in the first step; the dimethylmercury was then converted to monomethylmercury. The production of dimethylmercury mainly depends on the reaction time, temperature and the ratio of inorganic mercury to methylcobalamin used. The principal focus of most of these studies (*24,25,28-30*) was the reaction product of the tetramethyltin or methylcobalamin with inorganic mercury, but not the synthesis of methylmercury with high purity and higher yield in order to use it as a standard compound. Only a few studies (*16,26,27*) were for the synthesis of isotopically enriched methylmercury. Rouleau and Block (*27*) carried out the synthesis using inorganic $^{203}Hg^{2+}$

and methylcobalamin with single step isolation with hexane/benzene (1:1) and the final solution was prepared into Na_2CO_3. The yield was 90% and time required was less than 4 h. Hintelmann and Evans (*16*) carried out the synthesis by reacting inorganic $^{201}Hg^{2+}$ and tetramethyltin with six steps of extraction and purification: i) extraction with toluene; ii) wash the extract with double deionized (DDI) water; iii) extract into 1 mM $Na_2S_2O_3$; iv) wash with toluene; v) add $CuSO_4$ and NaCl into the $Na_2S_2O_3$ extract; and vi) final extraction of methylmercury in toluene. No data was available for the percent yield, however it was reported that the required time was less than 4 h to complete the procedure. Reaction conditions were not provided for either of these methods. On the other hand, Martín-Doimeadios et. al. (*26*) synthesized isotopically enriched monomethylmercury using inorganic $^{201}Hg^{2+}$ with methylcobalamin with single step extraction and purification. The required time reported was less than 2 h and yield was about 90%. This method studied several parameters: pH, temperature, reaction time, and methylcobalamin to inorganic mercury ratio. Some of the methods suffer from disadvantages such as low yield (50-70%), long reaction time (1 day) and multistep purification.

Therefore, the purpose of this study is to investigate and optimize the synthesis of isotopically enriched methylmercury by using inorganic $^{201}Hg^{2+}$ and tetramethyltin as the starting material so as to achieve higher yield, shorter reaction time and fewer purification steps, and to evaluate the isotopic composition, purity and stability of the product over a practical shelf-life (for example, six months) by using high performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS).

2.2 Experimental

2.2.1 Instrumentation

A ConstaMetric 4100Bio/MS polymeric inert pump (Thermo Separation Products, Riviera Beach, FL, USA) and a 5 μm Supelcosil LC-18 HPLC column with a Pelliguard LC-18 guard column (Supelco, PA, USA) were used in this study to separate inorganic and methylmercury. A six-port injection valve (Valco Vicci) was used between the pump and column. Because no special interface is required between the LC-18 column and the ICP-MS, one outlet of the column is directly interfaced to the nebulizer of the ICP-MS with a piece of perfluoroalkoxy (PFA) tubing, and the other end is connected to a 50 μL TEFZEL™ sample loop (CETAC Technologies, Omaha, NE). Figure 2-2 shows a typical separation of inorganic and methylmercury using this system at a flow rate of 1.0 mL/min. The mobile phase was buffered 30% methanol (refer to Reagent Section).

An HP 4500 ICP-MS (Agilent Technologies, Palo Alto, CA, USA and Yokogawa Analytical System Inc., Tokyo, Japan) was used in this study. The sample delivery system consisted of a peristaltic pump and quartz spray chamber with concentric nebulizer and quartz torch. The instrument was fitted with platinum sampler and skimmer cones and optimized daily using 10 ppb tuning solution (Agilent Technologies, Palo Alto, CA, USA) containing Li, Y, Ce and Tl in 30% methanol. Time resolved analysis (TRA) mode was engaged. The operating conditions for the HPLC-ICP-MS set up are given in Table 2-I.

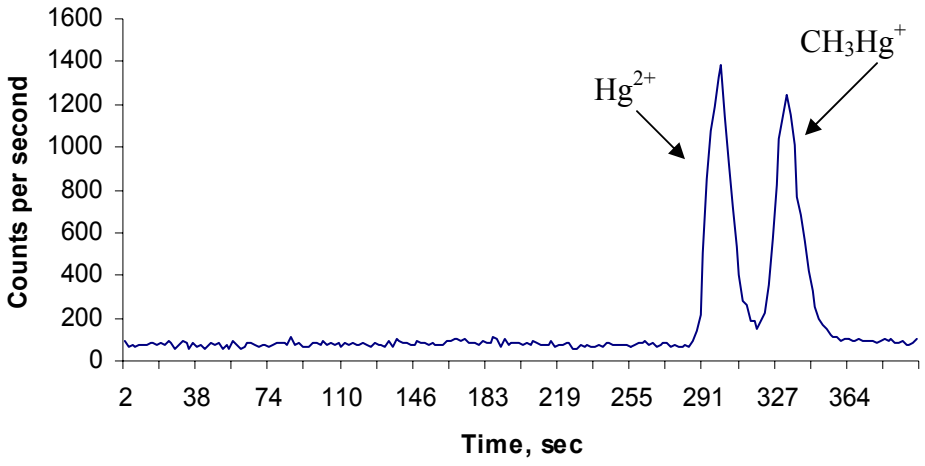


FIGURE 2-2. Typical chromatogram for separation of inorganic mercury and methyl mercury. [*Flow rate*: 1 mL/min; *Eluent*: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol/L ammonium acetate; *Column*: 5 μm Supelcosil LC-18 HPLC column].

A direct mercury analyzer (DMA-80, Milestone, Monroe, CT, USA) was used in this study to determine the total mercury content in each of the extraction and purification steps. The operation conditions for DMA-80 used throughout this work were based on the guidelines provided in EPA Method 7473 protocol (*31-32*).

2.2.2 Reagents and Standards

Double deionized (DDI) water (18 MΩ/cm), prepared from a Barnstead NANOpure Ultrapure Water System (Dubuque, Iowa, USA), was used in the preparation of all solutions throughout this study. Reagent grade HCl, Na_2SO_4, $Na_2S_2O_3$, toluene, isopropanol, ammonium acetate, 2-mercaptoethanol (98%), and optima grade methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA). The reagent grade tetramethyltin (98%) was obtained from Alfa Aesar (Ward Hill, MA, USA).

Standard solutions containing 1 mg/mL of $HgCl_2$ in 5% HNO_3 and CH_3HgCl in water were commercially available from Alfa Aesar (Ward Hill, MA, USA). ^{201}HgO, Lot # VX3060, was obtained from Isotech Inc. (Miamisburg, OH, USA). The natural and enriched isotope abundance of mercury standards are listed in Table 2-II.

TABLE 2-I. HPLC-ICP-MS Operating Conditions.

Plasma	
Plasma flow rate (L/min)	15.0
Auxiliary gas flow rate (L/min)	1.0
Radio frequency power (W)	1450
Sample cone	Platinum, 1.1 mm orifice
Skimmer cone	Platinum, 0.89 mm orifice
Measurement Parameters	
Analysis mode	Time resolved analysis (TRA)
Analysis isotopes[a]	^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg and ^{202}Hg
Nebulizer gas flow rate (L/min)	0.93–1.00
Peristaltic pump rate (rpm)	0.25
Integration time per point (s)	0.5
Total analysis time (s)	400
Eluent flow rate (mL/min)	1.0

[a] ^{204}Hg was not analyzed because of interference from ^{204}Pb.

HPLC speciation mobile phase, [30% (v/v) methanol + 0.005% 2-mercaptoethanol + 0.06 mol/L ammonium acetate), modified from Wilken's procedure (*33*), was prepared by diluting 300 mL of methanol, 50 μL of 2-mercaptoethanol and 4.8 g of ammonium acetate in 700 mL of DDI water.

2.2.3 Synthesis Procedure

2.2.3.1 Synthesis of ^{201}Hg enriched methylmercury

In order to prepare $^{201}HgCl_2$, 6 mL of $^{201}Hg^{2+}$ solution (11 μg/mL) was mixed with 2 mL of 6.0 M HCl in a 20 mL amber glass vial and stirred for 5 min. A 0.93 M methanolic solution of $(CH_3)_4Sn$ was prepared by mixing 0.340 g of $(CH_3)_4Sn$ into 2 mL methanol and then the mixture was quantitatively transferred into the $^{201}HgCl_2$ solution and the glass vial cap was put back on. The resulting reaction mixture was then stirred for 1 h at 60 °C in a water bath. The reaction mixture was cooled to room temperature and extracted 3 times with toluene (4 + 3 + 3 mL).

TABLE 2-II. Results for Characterization of Naturally Abundant and Synthesized Isotopically Enriched Methylmercury with ICP-MS.

Mass	Natural Abundance		Enriched ^{201}HgO		Enriched $CH_3{}^{201}Hg^+$
	Reported	Determined	Certified	Determined	Determined
196	0.15	0.179 ± 0.020	< 0.05	0.012 ± 0.001	0.025 ± 0.004
198	9.97	10.049 ± 0.035	0.08	0.108 ± 0.033	0.132 ± 0.040
199	16.87	16.966 ± 0.034	0.10	0.155 ± 0.061	0.200 ± 0.080
200	23.10	23.049 ± 0.106	0.45	0.637 ± 0.096	0.658 ± 0.094
201	13.18	13.381 ± 0.205	98.11	97.707 ± 0.316	97.530 ± 0.352
202	29.86	29.569 ± 0.078	1.18	1.270 ± 0.100	1.316 ± 0.117
204	6.87	6.809 ± 0.027	0.08	0.111 ± 0.027	0.139 ± 0.026
Total	100.00	100.000 ± 0.251	100.00	100.000 ± 0.353	100.000 ± 0.394

Uncertainties are at 95% CL, n = 4.

2.2.3.2 Purification Procedure

The synthesized methylmercury (in toluene) was then washed with DDI water 3 times (4 + 3 + 3 mL). 2.5 mL of the toluene extract was then dried over Na_2SO_4 and diluted with isopropanol (1:1, v/v). Another 2.0 mL of the toluene extract was taken and extracted twice with 2.5 mL of 1% $Na_2S_2O_3$. All of the extracts were stored in amber glass vials in a cold room at 4 °C until analysis.

2.2.4 Availability of Isotopically Enriched Methylmercury

To assist in the use of SIDMS, some isotopically labeled species will be provided for academic research upon request from this research group at Duquesne University (*34*), and will be available as a commercial product from *Applied Isotope Technologies (35)*.

2.3 Results and Discussion

2.3.1 Optimization of Synthesis Conditions

A total of five methylmercury syntheses were performed during this study. Hintelmann and Evans' (*16*) procedure for synthesis and purification of isotopically enriched methylmercury was followed step by step at the beginning of this study. The preliminary study was done using naturally abundant HgO and tetramethyltin. The effect of HCl concentration, temperature, reaction time, inorganic mercury to tetramethyltin ratio, and number of purification steps required were studied. Mercury present in the reaction mixture (remaining after toluene extraction), in water wash, in first toluene extract, in toluene wash, in 1% $Na_2S_2O_3$ extract, in NaCl + $CuSO_4$ fraction, and in the final toluene extract were all analyzed as total mercury using DMA-80. Only the methylmercury present in first toluene extract, in 1% $Na_2S_2O_3$ extract and final toluene extract from

preliminary studies were analyzed with HPLC-ICP-MS. The results from the DMA-80 and HPLC-ICP-MS analysis agree with each other. Final results and the respective synthesis conditions are reported in Table 2-III. The results are presented as percent recovery in parentheses and mercury content in each fraction in microgram units.

TABLE 2-III. Results for the Preliminary and Final Synthesis of Isotopically Enriched Methylmercury. Analysis by DMA-80 and HPLC-ICP-MS.

Hg content in different steps	Trial-1 µg (%)	Trial-2 µg (%)	Trial-3 µg (%)	Trial-4 µg (%)	Trial-5 µg (%)
Reaction mixture	5,990 (40.4)	5,168 (31.0)	379 (2.9)	3.4 (3.6)	2.5 (3.8)
Water wash	791 (5.3)	34 (0.2)	275 (2.1)	0.2 (0.2)	1.5 (2.3)
1st Toluene extract	8,031 (54.2)	11,470 (68.8)	12,355 (94.6)	91.2 (96.0)	61.8 (93.7)
Toluene wash	139 (0.9)	85 (0.5)	157 (1.2)	3.3 (3.5)	0.5 (0.8)
$Na_2S_2O_3$ extract	7,885 (53.2)	11,350 (68.1)	12,130 (92.9)	87.8 (92.4)	61.2 (92.7)
$NaCl/CuSO_4$ fraction	768 (5.2)	10 (0.1)	15 (0.1)	1.1 (1.2)	---
Final Toluene Extract	7,105 (47.9)	11,325 (67.9)	12,010 (92.0)	86 (90.5)	---
Total	14,793 (99.8)	16,622 (99.7)	12,836 (98.3)	94 (98.9)	65.7 (99.6)

Synthesis conditions.

Trial-1: 16 mg HgO, 2 mL 0.1 M HCl, 5 min., 0.385 g $(CH_3)_4Sn$, 3 h, room temperature; **Trial-2**: 18.0 mg HgO, 2 mL 6.0 M HCl, 5 min., 0.385 g $(CH_3)_4Sn$, 3 h, room temperature; **Tial-3**: 14.1mg HgO, 2 mL 6.0 M HCl, 5 min., 0.385 g $(CH_3)_4Sn$, 3 h, 60 °C; **Trial-4**: 95 µg $^{201}Hg^{2+}$, 2 mL 6.0 M HCl, 5 min., 0.385 g $(CH_3)_4Sn$, 3 h, 60 °C; **Trial-5**: 66 µg $^{201}Hg^{2+}$, 2 mL 6.0 M HCl, 5 min., 0.340 g $(CH_3)_4Sn$, 1 h, 60 °C.

From Table 2-III, it is found that the percent yield increased from 47.9% (synthesis 1) to 67.9% (synthesis 2) with the increase of the HCl concentration from 0.1 M to 6.0 M. Therefore, 6.0 M HCl was used during the rest of the study. The percent yield increased

from 67.9% (synthesis 2) to 92% (synthesis 3) by increasing the temperature from 20 °C (room temperature) to 60 °C. Therefore, the final synthesis was performed at 60 °C. By studying the reaction time it was found that the percent yield does not depend significantly on reaction time. Therefore, 1 h is selected for the final synthesis procedure. As shown in Table 2-III, it was also found that the ratio of inorganic mercury to tetramethyltin has no effect on percent yield.

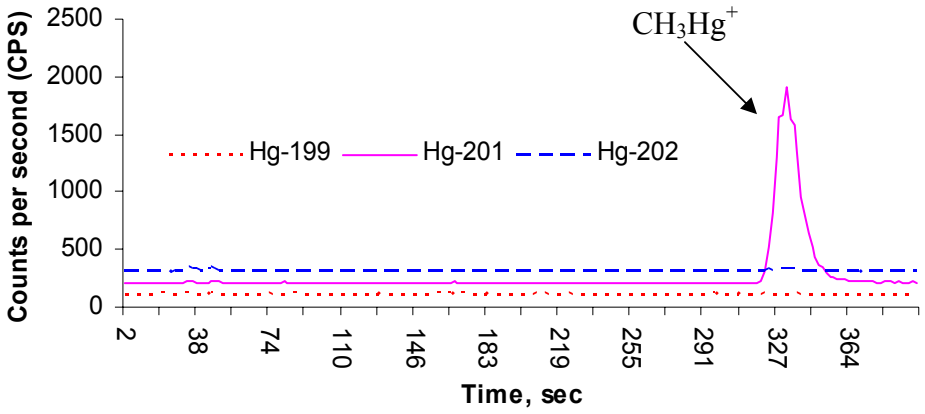


FIGURE 2-3. Chromatogram for synthesized isotope enriched methylmercury ($CH_3{}^{201}Hg^+$). Chromatograms for different masses (^{202}Hg, ^{201}Hg, and ^{199}Hg) were shifted from the baseline by adding 300, 200 and 100 CPS respectively with the original counts for clarity. [*Flow rate*: 1 mL/min; *Eluent*: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol/L ammonium acetate; *Column*: 5 µm Supelcosil LC-18 HPLC column].

Only methylmercury was detected during HPLC-ICP-MS analysis of the first toluene extract; no unreacted inorganic mercury or dimethylmercury was found (see Figure 2-3). Also from data presented in Table 2-III, it is found that the percent yield of methylmercury does not change significantly from the first toluene extract to the final

toluene extract. In all of the cases, the values were less than 4%. However, there are three steps between first toluene extract and the final toluene extract. It was decided to purify the synthesized methylmercury by washing the first toluene extract with DDI water and then drying over Na_2SO_4, then diluting with isopropanol to prepare the working standard. Unfortunately, during application of the synthesized isotopically enriched methylmercury (in isopropanol or in the toluene extract) in SIDMS analysis, it was found that the synthesized product induced both the sample inorganic mercury and the isotope enriched $^{199}Hg^{2+}$ to convert to methylmercury.

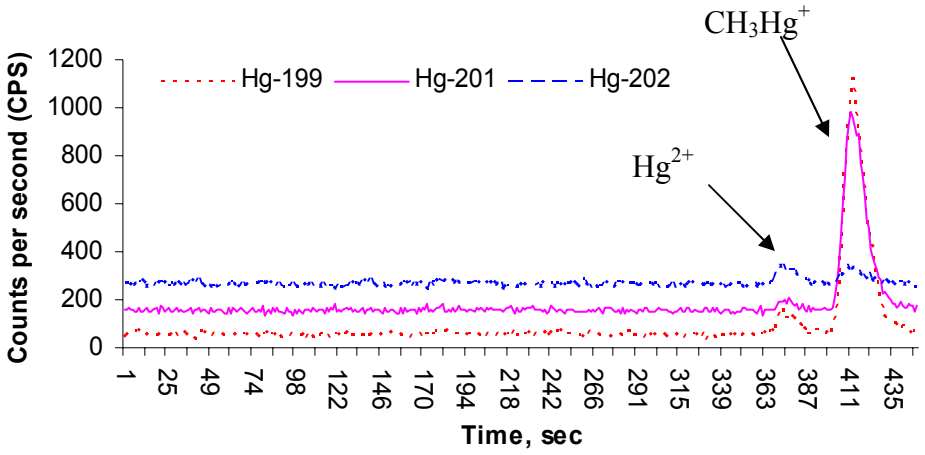


FIGURE 2-4. Chromatogram for a mixture of $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^{+}$ in isopropanol. The mixture was kept on bench-top at room temperature for 6 h for equilibration. Chromatograms for different masses (^{202}Hg and ^{201}Hg) were shifted from the baseline by adding 200 and 100 CPS with the original counts for clarity. [*Flow rate*: 0.8 mL/min; *Eluent*: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol/L ammonium acetate; *Column*: 5 μm Supelcosil LC-18 HPLC column].

The chromatogram shown in Figure 2-4 was obtained from a blank analysis with HPLC-ICP-MS. The blank was prepared by spiking equal amounts of $^{199}Hg^{2+}$ and

$CH_3{}^{201}Hg^+$ in DDI water and keeping on bench-top at room temperature for 6 h. This chromatogram shows that inorganic mercury has converted to methylmercury more than 90% within 6 h of equilibration without any treatment. Therefore, it was decided to include one more step in to the purification procedure by washing the first toluene extract with DDI water, and then extracting it into 1% $Na_2S_2O_3$(aq.). A blank was then prepared by spiking $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ in DDI water and keeping it on bench-top at room temperature for 6 h. The blank was then analyzed with HPLC-ICP-MS. No transformations between inorganic mercury and methylmercury were observed for $CH_3{}^{201}Hg^+$ extracted into 1% $Na_2S_2O_3$(aq.) (Figure 2-5).

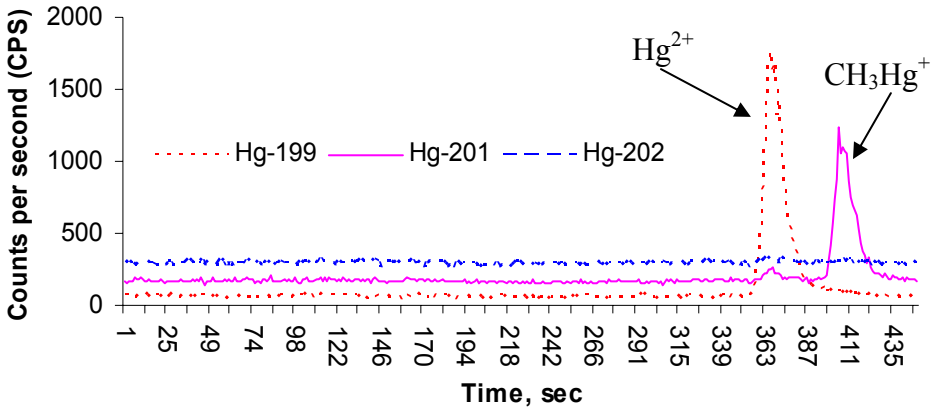


FIGURE 2-5. Chromatogram for a mixture of $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ in 1% $Na_2S_2O_3$. The mixture was kept on bench-top at room temperature for 6 h for equilibration. Chromatograms for different masses (^{202}Hg and ^{201}Hg) were shifted from the baseline by adding 200 and 100 CPS with the original counts for clarity. [*Flow rate*: 0.8 mL/min; *Eluent*: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol/L ammonium acetate; *Column*: 5 μm Supelcosil LC-18 HPLC column].

2.3.2 Characterization of the Synthesized Isotopically Enriched Methylmercury

After successful optimization of the synthesis procedure, an isotopically enriched methylmercury ($CH_3{}^{201}Hg^+$) was synthesized using ^{201}HgO and $(CH_3)_4Sn$, and analyzed using HPLC-ICP-MS (Figure 2-3). It is found that the chromatogram does not contain any inorganic mercury or any other mercury peaks but the methylmercury peak. In order to compare the peak position of the synthesized methylmercury with the naturally abundant methylmercury, these two standards were mixed at 1:10 ratio and analyzed with HPLC-ICP-MS (Figure 2-6). This chromatogram shows that both preparations overlapped and appeared as a single peak at similar elution times, confirming that the synthesized product is the isotopically enriched methylmercury.

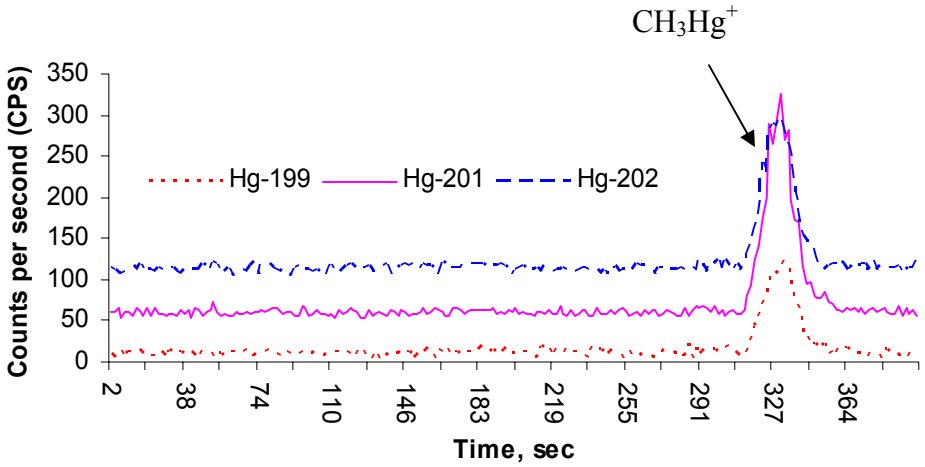


FIGURE 2-6. Chromatogram for a mixture of naturally abundant and isotopically enriched methylmercury. Chromatograms for different masses (^{202}Hg and ^{201}Hg) were shifted from the baseline by adding 100 and 50 CPS with the original counts for clarity. [*Flow rate*: 1 mL/min; *Eluent*: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol/L ammonium acetate; *Column*: 5 μm Supelcosil LC-18 HPLC column].

The isotopic abundances of the naturally abundant methylmercury (CH_3Hg^+) and the isotopically enriched ^{201}HgO were evaluated in order to compare the true measured isotope abundances with the reported natural abundance (*36*) and the isotope supplier's certified value. This study was done by using ICP-MS. The standard solutions were aspirated in direct mode and all isotope ratios were calculated for each species, and then the respective abundance of each isotope was calculated for each species. The results are reported in Table 2-II with 95% confidence level. The determined values agree with the reported and certified values in most cases, and as expected, the most enriched isotope in ^{201}HgO is ^{201}Hg compared to the natural abundance of methylmercury.

After synthesis of the isotopically enriched methylmercury, its isotope abundances were also determined using the same procedure as described previously, and are also reported in Table 2-II with 95% confidence level. The measured values correspond nicely with the certified values in most cases.

The concentration of the synthesized isotopically enriched methylmercury in 1% $Na_2S_2O_3$ was determined by reverse isotope dilution mass spectrometry (RIDMS) in two different approaches. First, the synthesized isotopically enriched methylmercury was mixed with naturally abundant methylmercury in 1:10 ratio, aspirated in direct mode to the ICP-MS five times, and measured in five replicates for each introduction. The isotope ratio of $^{201}Hg/^{202}Hg$ was determined with and without deadtime (*37*) and mass bias correction (*38*). From the obtained isotope ratios, the concentration of $CH_3{}^{201}Hg^+$ was calculated by using RIDMS equations and found to be 2.41 ± 0.01 μg/g and 2.52 ± 0.01 μg/g, respectively. The concentration indicates the yield is 91.3 ± 0.4%. Second, the mixture of the synthesized isotopically enriched methylmercury and the naturally

abundant methylmercury was analyzed by using HPLC-ICP-MS for four times. The isotope ratio of $^{201}Hg/^{202}Hg$ was determined with deadtime and mass bias correction. The concentration of $CH_3{}^{201}Hg^+$ was calculated by using RIDMS equations and was found to be 2.54 ± 0.21 μg/g. The concentration values obtained from both of these analyses correspond to each other at 95% confidence level. Also from HPLC-ICP-MS analysis, it was found that the product is 100% pure in methylmercury.

The concentration of the synthesized $CH_3{}^{201}Hg^+$ standard in 1% $Na_2S_2O_3$ was determined by RIDMS on October 02, 2002 as 2.41 ± 0.01 μg/g, on November 10, 2002 as 2.32 ± 0.23 μg/g and again on March 30, 2003 as 2.40 ± 0.01 μg/g. The concentrations of the synthesized standard over 180 days are not statistically distinguishable at the 95% confidence level. The concentration of the standard will continue to be checked over time for stability. The method developed, evaluated and documented in this chapter has been published in the peer reviewed literature in 2003 (*39*).The synthesized standard has successfully been used for the validation of EPA draft Method 3200 (*Mercury species separation by selective solvent extraction and acid digestion*) (*40*).

2.4 Conclusions

A highly pure isotopically enriched methylmercury, $CH_3{}^{201}Hg^+$, has been synthesized from commercially available ^{201}HgO and tetramethyltin with a yield of more than 90% in less than 1.5 h synthesis procedure at 60 °C. This procedure increases the efficiency of the previous synthesis (*16*) by ~1.8 times while providing for stability and purity. The synthesized and purified product is stable and does not induce transformation of the inorganic mercury to methylmercury during SIDMS or IDMS analysis of environmental

samples. Also during the synthesis procedure, the health hazard dimethylmercury is eliminated. This synthesis procedure is a safe and environmentally green protocol. Isotopically labeled species are necessary for application of SIDMS and must be made or obtained to use this method. Some of these species are now available for use in speciated analysis.

Epilogue: The developed synthesized procedure was further optimized for 1% $Na_2S_2O_3$ in July 2004 and greater than 99% yield was achieved. The procedure was then applied to synthesize a larger amount of isotopically enriched methylmercury (120 mg) for NIST. A highly pure isotopically enriched methylmercury was synthesized with a yield of more than 99%. This material (50 mg) was shipped to NIST and Professor David Owens of the College of Charleston, SC, USA for a toxicity study now underway.

2.5 References

(1) Leenaers, J.; Mol, W. V.; Infante, H. G.; Adams, F. C. *J. Anal. At. Spectrom*. **2002,** *17 (11)*, 1492-1497.

(2) Horvat, M. In *Trace Element Speciation: for environment, food and health;* Ebdon, L.; Pitts, L.; Cornelis, R.; Crews, H.; Donard, O. F. X.; Quevauviller, Ph., Eds.; Royal Society of Chemistry: Cambridge, UK, **2001**; pp 127-141.

(3) WHO; Environmental Health Criteria 101. *Methylmercury;* Geneva, Switzerland: World Health Organization. International Programme on Chemical Safety, **1990**.

(4) Jenson, S.; Jernelov, A. *Nature* **1969,** *223*, 753.

(5) Surma-Aho, K. *Chemosphere* **1986**, *14*, 353.

(6) U.S. Department of the Interior, *Mercury Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review;* **1987**.

(7) Westöö, G. *Acta Chem. Scand*. **1968**, *22*, 2277-2280.

(8) Han, Y.; Kingston, H. M.; Boylan, H. M.; Rahman, G. M. M.; Shah, S.; Richter, R. C.; Link, D. D.; Bhandari, S. *Anal. Bioanal. Chem*. **2003,** *375 (3)*, 428-438.

(9) Bloom, N. S.; Colman, J. A.; Barber, L. *Fresenius' J. Anal. Chem*. **1997,** *358 (3)*, 371-377.

(10) Hintelmann, H.; Hempel, M.; Wilken, R. D. *Environ. Sci. Technol*. **1995,** *29 (7)*, 1845-1850.

(11) Rogers, R. D. *J. Environ. Qual.* **1977,** *6*, 463.

(12) Horvat, M.; Bloom, N. S.; Liang, L. *Anal. Chim. Acta* **1993,** *281 (1)*, 135-152.

(13) Horvat, M.; Liang, L.; Bloom, N. S. *Anal. Chim. Acta* **1993,** *282 (1)*, 153-168.

(14) Sánchez Uría, J. E.; Sanz-Medel, A. *Talanta* **1998,** *47*, 509-524.

(15) Emteborg, H.; Björklund, E.; Ödman, F.; Karlsson, L.; Mathiasson, L.; Frech, W.; Baxter, D. C. *Analyst* **1996,** *121 (1)*, 19-29.

(16) Hintelmann, H.; Evans, R. D. *Fresenius' J. Anal. Chem*. **1997,** *358 (3)*, 378-385.

(17) Qvarnström, J.; Frech, W. *J. Anal. At. Spectrom*. **2002,** *17*, 1486-1491.

(18) Lambertsson, L.; Lundberg, E.; Nilsson, M.; Frech, W. *J. Anal. At. Spectrom*. **2001,** *16*, 1296-1301.

(19) Rogdriguez Martin-Doimeadios, R. C.; Krupp, E.; Amouroux, D.; Donard, O. F. X. *Anal. Chem*. **2002**, *74*, 2505-2512.

(20) US EPA Method 6800. In *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods SW–846, Update IVA,* US Government Printing Office (GPO): Washington DC, **2000**.

(21) Huo, D.; Kingston, H. M.; Larget, B. In *Comprehensive Analytical Chemistry;* Caruso, J. A.; Sutton, K. L.; Ackley, K. L., Eds.; *Elemental Speciation: New Approaches for Trace Element Analysis*, Vol. 33; Elsevier: New York, **2000**; pp 277-313.

(22) Kingston, H. M. US Patent Number: 5,414,259; **1995**.

(23) http://www.epa.gov/epaoswer/hazwaste/test/news2.htm accessed on July 8, **2004**.

(24) Cappon, C. J.; Smith, J. C. *Anal. Chem*. **1977,** *49 (3)*, 365-369.

(25) Wardell, J. L. In *Comprehensive Organometallic Chemistry;* Abel, E. W., Ed.; Pergamon Press: Oxford, **1995**; Vol. 3, p135.

(26) Rogdriguez Martín-Doimeadios, R. C.; Stoichev, T.; Krupp, E.; Amouroux, D.; Holeman, M.; Donard, O. F. X. *Appl. Organomet. Chem*. **2002,** *16 (10)*, 610-615.

(27) Rouleau, C.; Block, M. *Appl. Organomet. Chem*. **1997,** *11 (9)*, 751-753.

(28) Imura, N.; Sukegawa, E.; Pan, S. K.; Nagao, K.; Kim, J. U.; Kwan, T.; Ukita, T. *Science* **1971,** *172*, 1248.

(29) Bertilsson, L.; Neujahr, H. *Biochemistry* **1971,** *10*, 2805.

(30) Filippelli, M.; Baldi, F. *Appl. Organomet. Chem*. **1993,** *7 (7)*, 487-492.

(31) US EPA Method 7473. In *Test Method for Evaluating Solid Waste, Physical/Chemical Methods SW 846*, *Update IVA*. US GPO: Washington DC. **2000**.

(32) Boylan, H. M.; Kingston, H. M. S. *Am. Lab*. **1998,** *30 (19)*, 26.

(33) Wilken, R. D.; Hintelmann, H. *Water, Air, Soil Pollut.* **1991**, *56*, 427-437.

(34) http://www.sampleprep.duq.edu, referenced on July 14, **2004**.

(35) Applied Isotope Technologies. E-mail: AppliedIsotopes@Comcast.net

(36) Lide, D. R. *CRC Handbook of Chemistry and Physics*; CRC Press LLC, Florida, USA, **81st Edition**, **2000**; pp 1-17 and pp 11-172.

(37) Taylor, H. E. *Inductively Coupled Plasma-Mass Spectrometry: Practices and Techniques*; Academic Press: San Diego, CA, USA, **2001**; pp 48.

(38) Jarvis, K. E.; Gray, A. L.; Houk, R. S. *Handbook of Inductively Coupled Plasma Mass Spectrometry*; Blackie Academic and Professional: London, UK, **1992**; pp 315-316.

(39) Rahman, G. M. M.; Kingston, H. M. S.; Bhandari, S. *Appl. Organomet. Chem*. **2003**, *17 (12)*, 913-920.

(40) Rahman, G. M. M.; Kingston, H. M. S. *Anal. Chem*. **2004**, *76 (13)*, 3548-3555.

Chapter 3

Interlaboratory Validation of EPA Draft Method 3200

3.1 Introduction

The interest in determining the concentration of an individual chemical species, as opposed to determining the total elemental concentration, has increased significantly in recent years. This is true especially where these species are known to be very toxic to humans and biota (*1*). The toxicity, bioavailability, and environmental mobility of mercury in soil, sediments and water are very dependent on its chemical species. Methylmercury in many matrices can be an order of magnitude more mobile than the corresponding inorganic mercury species and, thus, more toxic and more readily bioaccumulated (*2*). The toxic impact of methylmercury on human was observed for the first time in Minamata, Japan in 1955 when the ingestion of fish contaminated with methylmercury resulted in hundreds of poisonings and one hundred fatalities. During the 1970s, the ingestion of wheat flour produced from seeds treated with organic mercury also led to large-scale poisoning and many deaths in Iraq (*3*). Therefore, it is essential to be able to determine the exact concentration of inorganic mercury and methylmercury from environmental, biological and food samples.

A new extraction procedure using acidic-ethanol solution has been developed to extract alkylmercury and soluble inorganic mercury from soil and sediment matrices (*4*). Heretofore, the technique for determining the speciation of mercury in soil and sediment samples was a succession of analytical steps: extraction, separation, and detection. The results obtained have been "operationally defined" using a given procedure. Therefore,

the significance of the analytical results was highly dependent on the extraction procedure, separation, and detection techniques, as well as on the stability of the species in these methods. Results are useful only if they correspond to well defined and accepted procedures. In other words, the only means to achieve sound interpretation of, and a basis for, decisions is when results are comparable using the same method in a similar matrix. The prerequisites for comparability are agreement of the procedures to be used, their testing and validation, and their possible implementation as a standard procedure. A study for evaluating the performance of the EPA draft Method 3200 has therefore been organized by the US EPA. It was recognized that, in order to arrive at sound conclusions on the analytical performance of a method, there was a strong need to use a similar matrix in the study. Therefore, two different types of reference soils were prepared and distributed for analysis among the participating laboratories.

3.2 Experimental

3.2.1 Preparation of the Reference Soil

Environmental Resource Associates® (ERA) (Arvada, CO, USA) prepared one set of three soil samples (labeled as Lot No. 0313-01-01-1: Inorganic mercury, Lot No. 0313-01-01-2: Organic mercury, and Lot No. 0313-01-01-3: Inorganic mercury and Organic mercury) by spiking HgO, CH_3HgCl and a mixture of the two in 100% processed topsoil. The three soil samples were shipped to Duquesne University (DU) on March 30, 2001 for evaluation, and concentration verification. In these identical soil matrices, inorganic mercury (HgO) was found to be approximately 50 μg/kg; organic mercury (CH_3HgCl) was approximately 50 μg/kg; and, in the mixed mercury sample, total

mercury was approximately 100 μg/kg, which is very small and difficult to detect by most of the available instruments after extraction. Therefore, it was decided not to use those samples for the interlaboratory validation study, but to prepare instead a new set of samples with higher concentrations.

ERA then prepared a new set of samples (Lot No. 0501-01-09) by spiking higher amounts of the different mercury species as described in the previous paragraph. These were shipped to DU for evaluation on May 10, 2001. And while the analysis found that these samples indeed contained higher concentrations of the different mercury species, the measured values – Inorganic mercury: 4.00 mg/kg; Organic mercury: 4.00 mg/kg; and Inorganic and Organic mercury: 6.00 mg/kg – were 1,000 times smaller than their purported values. ERA at that point rechecked the samples and revised their certificates on May 23, 2001. It was decided to use these samples for the inter-laboratory validation study. ERA was asked also to prepare another set of soil samples by adding a certain percent of silica and higher mercury species concentrations. ERA then prepared two sets of soil samples and labeled them as Material-1 (100% processed topsoil) and Material-2 (75% processed topsoil and 25% Ottawa sand), Lot No. 0611-01-02. These soil samples were shipped to DU for evaluation on July 11, 2001 and analyzed. These soil samples (Material-1 and Material-2) containing only the mixture of inorganic mercury and organic mercury were then distributed to three participating laboratories (including DU) on August 09, 2001 for validation of the EPA draft Method 3200. ERA also shipped the same two materials [but from a different Lot (Lot No. 0416-03-01)] to three other participating labs on April 16, 2003 for validation study (see Table 3-I).

TABLE 3-I. Summary of Reference Materials Used in the Validation Study.

Material	Sample Name	Lot No.	Date Shipped	Mercury Species	Made-to Value Concentration
Test Material (Later Material-1)	Inorganic Mercury	0313-01-01.1	3/30/2001	HgO	49.8 μg/kg
	Organic Mercury	0313-01-01.2	3/30/2001	CH_3HgCl	50.0 μg/kg
	Inorganic Mercury	0313-01-01.3	3/30/2001	HgO	49.8 μg/kg
	Organic Mercury			CH_3HgCl	50.0 μg/kg
	Inorganic Mercury	0501-01-09	5/10/2001	HgO	4,000 mg/kg
	Organic Mercury	0501-01-09	5/10/2001	CH_3HgCl	4,000 mg/kg
	Inorganic Mercury	0501-01-09	5/10/2001	HgO	3,000 mg/kg
	Organic Mercury			CH_3HgCl	3,000 mg/kg
	Inorganic Mercury	0501-01-09	5/10/2001	HgO	4,000 μg/kg*
	Organic Mercury	0501-01-09	5/10/2001	CH_3HgCl	4,000 μg/kg*
	Inorganic Mercury	0501-01-09	5/10/2001	HgO	3,000 μg/kg*
	Organic Mercury			CH_3HgCl	3,000 μg/kg*
Material-1	Inorganic Mercury	0611-01-02	7/11/2001	HgO	4,000 μg/kg
	Organic Mercury	0611-01-02	7/11/2001	CH_3HgCl	4,000 μg/kg
	Inorganic Mercury	0611-01-02	7/11/2001	HgO	3,000 μg/kg
	Organic Mercury			CH_3HgCl	3,000 μg/kg
Material-2	Inorganic Mercury	0611-01-02	7/11/2001	HgO	6,000 μg/kg
	Organic Mercury	0611-01-02	7/11/2001	CH_3HgCl	6,000 μg/kg
	Inorganic Mercury	0611-01-02	7/11/2001	HgO	4,500 μg/kg
	Organic Mercury			CH_3HgCl	4,500 μg/kg
Material-1	Inorganic Mercury	0416-03-01	4/16/2003	HgO	3,000 μg/kg
	Organic Mercury			CH_3HgCl	3,000 μg/kg
Material-2	Inorganic Mercury	0416-03-01	4/16/2003	HgO	4,500 μg/kg
	Organic Mercury			CH_3HgCl	4,500 μg/kg

* Made-to value was revised by ERA on 5/23/2001. Material-1: 100% processed topsoil; and Material-2: a mixture of processed topsoil plus Ottawa sand in a ratio of 75:25, respectively.

3.2.2 Participating Laboratories

The following laboratories participated in the interlaboratory studies: Center for Microwave and Analytical Chemistry at Duquesne University (DU); PDC Laboratories,

Inc. (PDC); APPL Inc. (APPL); Severn Trent Laboratories, Inc. (STL); Brooks Rand LLC (BRLLC); and Environmental and Occupational Health Sciences Institute at Rutgers University (EOHSI/RU).

3.2.3 Strategy of Method Performance Study

As the developing laboratory of the proposed method, DU compared the extraction efficiency of the proposed method with most of the published methylmercury extraction methods and mercury speciation methods. A brief description of each method, along with the final results, is reported elsewhere (*5*).

It is observed that most mercury speciation methods available in the literature are based on either a chromatographic separation technique, or they obtain the amount of inorganic mercury from the difference of total mercury and methylmercury. Similarly, some obtain the amount of methylmercury from the difference of total mercury and inorganic mercury. These are often analytically unreliable methods of speciation. Also, there is a chance to obtain biased results, positively or negatively, from this kind of analysis. These techniques do not provide any information about the source of methylmercury or inorganic mercury; that is, whether a reported amount of methylmercury or inorganic mercury is actually present in the analyzed sample, or merely a result of species transformation or interconversion. Therefore, it was decided to apply EPA Method 6800 (*Elemental and Speciated Isotope Dilution Mass Spectrometry*) (*6*), originally developed by Kingston research group at Duquesne University, as a diagnostic tool and for validation of the EPA draft method 3200. The fundamental theory of SIDMS in environmental systems is established and documented

(*6-11*). The primary requirement of EPA Method 6800 for the present application is the availability of isotopically labeled methylmercury and inorganic mercury. Isotopically labeled inorganic mercury is available commercially; however, isotopically labeled methylmercury is not at this time. Therefore, isotopically labeled methylmercury was synthesized in the DU lab by the author. The detailed synthesis procedure and characterization of the synthesized isotopically labeled methylmercury is described elsewhere (*12*).

3.3 Results and Discussions

3.3.1 Validation with Speciated Isotope Dilution Mass Spectrometry (SIDMS)

The shelf-life and the species composition in the specifically prepared soil samples were evaluated periodically using SIDMS protocol along with extraction by the EPA draft Method 3200. The SIDMS analysis procedure is described in detail elsewhere (*5*). The amount of inorganic mercury and methylmercury determined in Material-1 was 2.68 ± 0.34 μg/g and 2.20 ± 0.29 μg/g, respectively, on October 28, 2002 and was 2.85 ± 0.47 μg/g and 2.25 ± 0.10 μg/g, respectively, on November 03, 2003. The amount of inorganic mercury found to be converted to methylmercury in Material-1 during extraction or analysis at the mentioned dates was 0 ± 3% and 3 ± 1%, respectively. The amount of methylmercury found to be converted to inorganic mercury in Material-1, during extraction or analysis for the same period, was 0 ± 9% and 0 ± 5%, respectively. The analysis shows that the concentrations of both methylmercury and inorganic mercury in Material-1 were stable and the amounts of their interconversion were statistically indistinguishable over time. In the case of Material-2, only the SIDMS data

for October 28, 2002 is available; this material was unavailable for testing on November 3, 2003. The amount of inorganic mercury and methylmercury determined in Material-2 by the SIDMS method was 3.55 ± 0.61 μg/g and 2.79 ± 0.29 μg/g, respectively; the amount of inorganic mercury converted to methylmercury was 2 ± 2%; and that for methylmercury to inorganic mercury was 6 ± 5%. The concentration in Material-2 determined by SIDMS (spiking occurring before extraction) was less than its 'made-to-value' (4.5 μg inorganic mercury per gram of Material-2 and 4.5 μg methylmercury per gram of Material-2) due to the incomplete extraction from the sample of each species.

An evaluation of the EPA draft Method 3200 (*4*) (M-3200), along with other literature methods, was also performed using EPA Method 6800. The methods evaluated in this study are based on sonication [SONI-1 (*13*) and SONI-2 (*14*)], focused microwave-assisted extraction (FMAE) (*15*), and cold acid extraction (CAE) (*16*). The sample preparation with each of these methods is discussed elsewhere (*5*). The final concentrations of inorganic mercury and methylmercury, and their percent transformation during extraction or analysis for Material-1 and Material-2 using different extraction methods with SIDMS protocol are summarized in Table 3-II. When comparing sample matrices, Table 3-II demonstrates with 95% confidence level (CL) that statistically indistinguishable percent recovery of inorganic mercury and methylmercury was achieved from Material-1 and Material-2 using the extraction methods studied [except for methylmercury from Material-2 by FMAE (50 ± 3%) and by SONI-2 (51 ± 5%)]. When comparing method performance for methylmercury, statistically indistinguishable percent recovery in Material-1 was achieved with 95% CL by all the methods studied, whereas in the case of Material-2, a bimodal distribution was

observed by grouping M-3200 and SONI-1 in one group (approximately 60%) and SONI-2 and FMAE in another group (approximately 50%). When comparing method performance for inorganic mercury, the percent recovery with 95% CL in both Materials showed a similar bimodal distribution by grouping M-3200 and SONI-1 in one group showing approximately 85% recovery, and SONI-2 and FMAE in another group showing approximately 65% recovery. The overall percent recoveries of both inorganic mercury and methylmercury obtained by SIDMS analysis of Material-1 and Material-2 were less than the 'made-to' values, which is due to incomplete equilibration between the sample and the spike species.

TABLE 3-II. The Deconvoluted Concentration and Percent Transformation of Mercury Species in Material-1 and Material-2 Using SIDMS Calculations.

Sample	Extraction Method	Deconvoluted Concentration (μg/g)		% Recovery		Interconversion (%)	
		Hg^{2+}	CH_3Hg^+	Hg^{2+}	CH_3Hg^+	Hg^{2+} to CH_3Hg^+	CH_3Hg^+ to Hg^{2+}
Material-1	M-3200	2.68 ± 0.34	2.20 ± 0.29	89 ± 12	73 ± 10	0 ± 3	0 ± 9
	SONI-1	2.49 ± 0.16	1.83 ± 0.13	83 ± 5	61 ± 4	5 ± 1	45 ± 4
	SONI-2	1.88 ± 0.20	1.96 ± 0.24	63 ± 7	65 ± 8	2 ± 3	10 ± 5
	FMAE	1.99 ± 0.14	2.01 ± 0.16	66 ± 8	67 ± 5	0 ± 3	7 ± 3
	CAE	NA	NA	NA	NA	NA	~ 100
Material-2	M-3200	4.09 ± 0.93	2.79 ± 0.29	91 ± 21	62 ± 6	2 ± 2	6 ± 5
	SONI-1	3.67 ± 0.16	2.65 ± 0.09	81 ± 3	59 ± 2	2 ± 1	44 ± 4
	SONI-2	3.09 ± 0.23	2.29 ± 0.20	67 ± 5	51 ± 5	1 ± 1	2 ± 2
	FMAE	3.09 ± 0.24	2.26 ± 0.13	69 ± 5	50 ± 3	2 ± 1	4 ± 3
	CAE	NA	NA	NA	NA	NA	~ 100

Uncertainties are expressed with 95% CL, n = 9. NA: analyzed but did not perform SIDMS calculations.

Our evaluation demonstrates that the M-3200 has better extraction capability than the other methods with little or no transformation between species. SONI-2 and FMAE also show little transformation, but these two methods extract less efficiently than M-3200. SONI-1 has better extraction efficiency than M-3200, but induces approximately 45% transformation of methylmercury to inorganic mercury for both Material-1 and Material-2. Thus, application of the SONI-1 extraction process for mercury speciation analysis will overestimate the inorganic mercury and, at the same time, underestimate the methylmercury. SIDMS calculations for CAE could not be performed since all the methylmercury, including the spiked $CH_3{}^{201}Hg^+$, in both materials was transformed into Hg^{2+} during extraction. This is one circumstance that SIDMS cannot correct, since all of the species of interest were destroyed. Hence, the application of CAE in mercury speciation analysis will provide completely inaccurate information about the sample.

3.3.2 Statistical Evaluations of the Data Obtained from Participating Laboratories

After obtaining the final data from the six participating laboratories, several observations were made. First, Labs 1, 4, 5 and 6, reported three separate mercury concentration measurements in Materials-1 and Material-2 for each of the following four categories: extractable inorganic; extractable organic; semi-mobile; and non-mobile mercury. From these measurements, the total extractable mercury and total mercury were calculated in straightforward fashion; and considered as the additional two categories. Lab 2 reported four separate mercury concentrations for Materials-1 and Mateiral-2 in only one

category (total extractable mercury), but no measurement for other categories. The remaining lab, Lab 3, reported their data in percent recovery (not in concentration) for both materials across all six categories. Lab 3 did not report individual mercury concentration measurements, nor how many times of each measurement was taken.

TABLE 3-III. Final Results from Different Participating Laboratories for the Validation of EPA Draft Method 3200: Percent Recovery with 95% CL.

Laboratory	Material	% Recovery for Extractable			% Recovery for Semi-Mobile Mercury	% Recovery for Non-Mobile Mercury
		Inorganic Mercury	Organic Mercury	Total Mercury		
Lab 1	Material-1	28.8 ± 5.4	102.7 ± 14.5	65.8 ± 8.5	34.2 ± 11.2	1.0 ± 0.3
	Material-2	43.9 ± 5.9	85.4 ± 6.3	64.7 ± 4.3	33.7 ± 2.6	0.5 ± 0.04
Lab 2	Material-1	NR	NR	52.7 ± 16.3	NR	NR
	Material-2	NR	NR	34.8 ± 8.9	NR	NR
Lab 3	Material-1	30.0	73.4	51.7	45.7	2.6
	Material-2	29.8	56.8	43.3	51.9	4.9
Lab 4	Material-1	NR	NR	82.8 ± 4.8	22.8 ± 4.8	0.5
	Material-2	NR	NR	71.9 ± 6.9	18.1 ± 6.4	0.3
	Material-1	7.6 ± 6.2	63.3 ± 14.3	35.4 ± 7.6	22.8 ± 4.8	0.5
	Material-2	14.4 ± 11.6	59.3 ± 6.4	36.9 ± 4.5	18.1 ± 6.4	0.3
Lab 5	Material-1	4.0 ± 6.8	54.6 ± 1.6	29.3 ± 3.5	40.8 ± 4.3	0.6 ± 0.1
	Material-2	9.4 ± 7.0	47.5 ± 4.2	28.4 ± 4.1	30.8 ± 4.3	0.6 ± 0.1
Lab 6	Material-1	17.7 ± 9.0	94.7 ± 10.0	56.2 ± 6.7	33.0 ± 9.4	0.3 ± 0.2
	Material-2	12.9 ± 9.2	76.1 ± 6.9	44.5 ± 5.7	20.0 ± 4.2	0.2

NR = Not reported by the lab.

Table 3-III summarizes the percent recovery results of various mercury fractions in Material-1 and Material-2 that are calculated by the author based on the

average reported concentration measurements. Margins of error given for each percent recovery are calculated using a standard 95% CL for an unknown mean. Without knowledge of individual measurements, no margins of error can be given for the percent recoveries reported by Lab 3. Note in Table 3-III the additional percent recovery results for Lab 4. Lab 4 measured mercury concentration with and without separation (all other labs measured with separation only). The first set of percent recoveries (82.8 ± 4.8% for total extractable mercury, 22.8 ± 4.8% for semi-mobile mercury, and 0.5% for non-mobile mercury in Material-1; 71.9 ± 6.9% for total extractable mercury; 18.1 ± 6.4% for semi-mobile mercury, and 0.3% for non-mobile mercury in Material-2) for Lab 4 represent measurements taken without separation. The second set of percent recoveries (7.6 ± 6.2% for inorganic extractable mercury, 63.3 ± 14.3% for organic extractable mercury, 35.4 ± 7.6% for total extractable mercury, 22.8 ± 4.8% for semi-mobile mercury, and 0.5% for non-mobile mercury in Material-1; 14.4 ± 11.6% for inorganic extractable mercury; 59.3 ± 6.4% for organic extractable mercury, 36.9 ± 4.5% for total extractable mercury, 18.1 ± 6.4% for semi-mobile mercury, and 0.3% for non-mobile mercury in Material-2), were based on the mercury measurements taken with separation.

Table 3-IV displays percent recoveries of total mercury for the five reporting labs (Lab 2 did not report the results of non-extractable mercury in Materials-1 and Material-2 that would permit calculation of percent recovery of total mercury). Lab 1 and Lab 3 obtained 100% total mercury for both materials after mass balance. Lab 6 obtained 100% recovery for Material-1, 65% recovery for Material-2. Lab 5 did not obtain 100% recovery and lost 29% and 40% mercury for Material-1 and Material-2, respectively. For Lab 4, if the reported value for the total extractable mercury is

considered, then they managed to get 100% recovery from both the studied materials. But if the speciation value is used then Lab 4 obtained approximately 60% recovery for both materials and lost almost 40%. The reason for these losses is probably the poor recovery of mercury during their sulphydrylated cotton fiber (SCF) aided solid phase separation steps.

TABLE 3-IV. Total Percent Recovery from Interlaboratory Validation Study.

Laboratory	Lot No.	Material	Made-to Value (µg /g)	Total Percent Recovery* (%)
Lab 1	0611-01-02	Material-1	6.0	101.0 ± 14.1
		Material-2	9.0	98.9 ± 5.0
Lab 3	0611-01-02	Material-1	6.0	100
		Material-2	9.0	100.1
Lab 4	0416-03-01	Material-1	6.0	58.7 ± 8.9 (106.1 ± 6.8)
		Material-2	9.0	55.3 ± 7.8 (90.3 ± 9.4)
Lab 5	0416-03-01	Material-1	6.0	70.7 ± 5.5
		Material-2	9.0	59.9 ± 5.9
Lab 6	0416-03-01	Material-1	6.0	89.4 ± 11.6
		Material-2	9.0	64.7 ± 7.1

*values in parentheses represent the total percent recovery considering the total extractable results obtained from direct analysis.

Uncertainties are expressed with 95% CL, n = 3.

Another observation made upon data examination is that the detection technique varied among the six laboratories. Two labs, Lab 1 and Lab 6, used liquid chromatography coupled with inductively coupled plasma mass spectrometry (LC-ICP-MS), while three other labs, Lab 2, Lab 4, and Lab 5 used cold vapor atomic absorption spectrometry (CV-AAS). Lab 3 did not specify the detection technique it utilized. Close inspection of the data summarized in Tables 3-III and 3-IV reveal higher percent recoveries on average for labs using LC-ICP-MS technology. In the following subsections, data is analyzed in the categories of extractable organic, extractable inorganic, total extractable and total mercury, to determine whether a significant difference exists between measurements from the two detection techniques. Direct analysis of semi-mobile and non-mobile mercury measurements are of lesser interest, due to their low solubility and toxicity. These categories were not considered explicitly in the analyses. Other investigations of interest, including an examination of differences between labs sharing the same detection technique, are also included in the following subsections.

3.3.2.1 Extractable Inorganic Mercury

The measurements of extractable inorganic mercury were separated into two groups: those obtained from LC-ICP-MS and those obtained from CV-AAS. Six LC-ICP-MS measurements (three from Lab 1 and three from Lab 6) and six CV-AAS measurements (three from Lab 4 and three from Lab 5) for Material-1 and Material-2 were analyzed with two-sample *t*-tests. The comparison of Material-1 concentrations yielded a *p*-value less than 0.0004. This indicates the average LC-ICP-MS measurement is significantly

higher than CV-AAS Material-1 measurement. Applying the same test to the Material-2 data gives a *p*-value of 0.0331. This is further evidence (although not as strong as with Material-1) suggesting a difference between detection techniques. Noticing that Lab 6 consistently reports lower concentration measurements than Lab 1, while Lab 5 consistently reports lower concentration measurements than Lab 4, a two-sample *t*-test comparing the lower LC-ICP-MS measurements (Lab 6) with the higher CV-AAS measurements (Lab 4) was conducted. The LC-ICP-MS technique for Material-1 was again found to yield a significantly higher measurement (*p*-value = 0.0102) than CV-AAS. For Material-2, however, the LC-ICP-MS technique did not yield significantly greater measurements than CV-AAS (*p*-value = 0.6619). Table 3-III provides support for these results; the difference in percent recovery between Lab 6 and Lab 4 is much greater for Material-1 than for Material-2.

It is worthwhile to recognize the measurement variation between labs using the same detection technique. Measurements from Lab 1 and Lab 6, although based on the same detection technique, suggest the average Lab 1 measurement is significantly higher than that of Lab 6 (*p*-value = 0.0085 for Material-1, 0.0003 for Material-2). The analogous comparison between Lab 4 and Lab 5 is not significant (*p*-value = 0.0850 for Material-1, 0.0990 for Material-2). These results are reflected in Table 3-III, where the percent recovery differences between Lab 1 and Lab 6 are larger than those between Lab 4 and Lab 5. One explanation for any between-lab, within-detection-technique variation could be the difference in laboratory chemist's familiarity with Method 3200. Similar analyses for data from categories that follow show that Lab 1 is not always significantly greater than Lab 6, while Lab 4 is sometimes significantly greater than Lab 5. We omit

these analyses and attribute any significant difference between Lab 1 and Lab 6, and between Lab 4 and Lab 5, to the variability of laboratory chemist's experience with Method 3200. The bar graphs produced based on the performance of various labs for extractable inorganic mercury from both Material-1 and Material-2 are shown also in Figure 3-1. The overall percent recovery data from participating labs averaged 17.6 ± 3.8% for Material-1 and 22.1 ± 4.4% for Material-2.

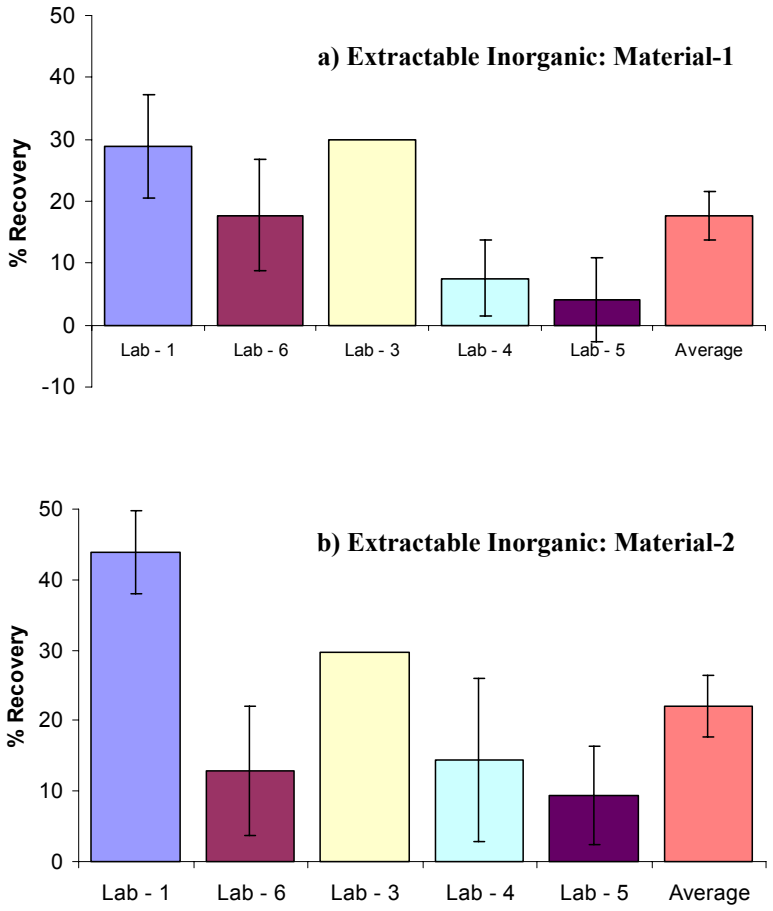


FIGURE 3-1. Percent recovery of extractable inorganic mercury in a) Material-1 and b) Material-2. (Uncertainties, 95% CL, n = 3).

3.3.2.2 Extractable Organic Mercury

The measurements of extractable organic mercury were separated into two groups according to detection technique (in analogous fashion to the previous extractable inorganic mercury analysis). The test for a difference in mean Material-1 concentrations was significant (p-value < 0.0001), as was the test for Material-2 (p-value < 0.0001). These results are consistent with those from the inorganic analysis: LC-ICP-MS technique yields a higher average concentration measurement than CV-AAS technique. When comparing Lab 6 with Lab 4 (lowest average from LC-ICP-MS with highest average from CV-AAS), the analysis of measurements from both materials reveals significant results (p-values of 0.0012 and 0.0007 for Materials-1 and Material-2, respectively). Extractable organic mercury data completely support the superiority of LC-ICP-MS over CV-AAS. The bar graphs produced from the extractable organic mercury results are shown in Figure 3-2. The overall recovery data from participating labs averaged 77.7 ± 5.7% for Material-1 and 65.0 ± 3.3% for Material-2.

3.3.2.3 Total Extractable Mercury

Recall that Lab 2 reported four total extractable mercury measurements for Material-1 and for Material-2. These measurements are grouped with those from the two CV-AAS labs (Lab 4 and Lab 5), and compared with the resulting sample of ten measurements with the six measurements from LC-ICP-MS labs (Lab 1 and Lab 6). When examining Material-1, the total extractable mercury measurements based on LC-ICP-MS showed a significantly higher average than measurements based on CV-AAS (p-value = 0.0003). This result was also obtained when comparing Material-2 measurements (p-value =

0.0021). The total extractable mercury fraction results for both materials are shown in Figure 3-3. The overall recovery data from participating labs averaged 56.4 ± 2.5% for Material-1 and 47.9 ± 2.1% for Material-2.

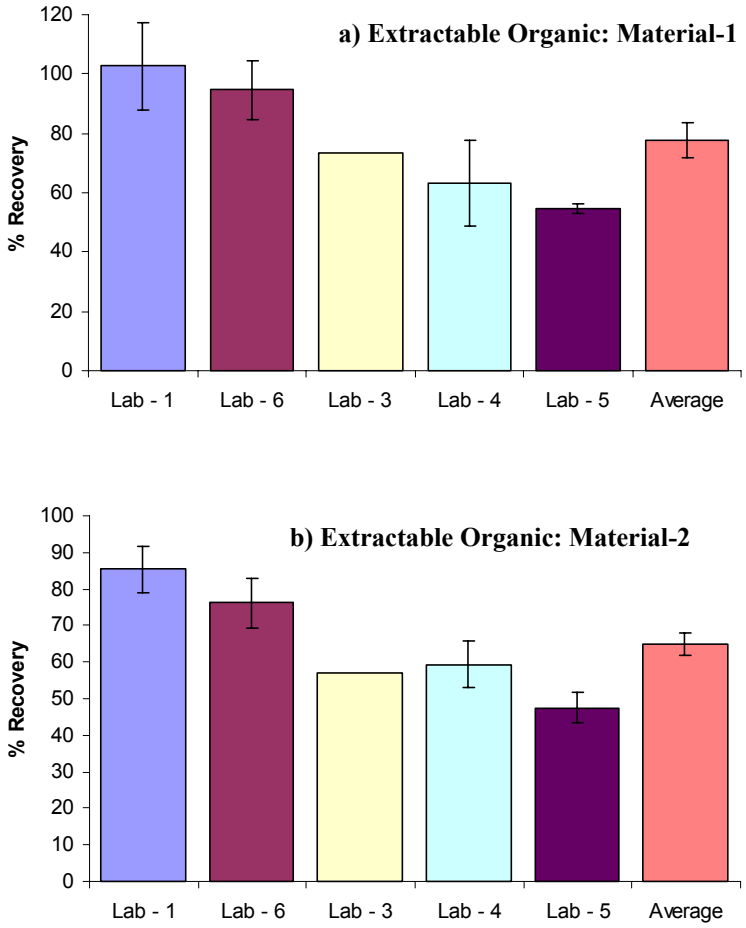


FIGURE 3-2. Percent recovery of extractable organic mercury in a) Material-1; and b) Material-2. (Uncertainties, 95% CL, n=3).

3.3.2.4 Total Mercury

Total mercury measurements (summarized in terms of percent recovery in Table 3-IV) were grouped according to detection technique and analyzed for significant differences between group means. Since Lab 2 did not report total mercury, the sample size for both

detection-technique groups is again six. A test of Material-1 measurements reveals a significantly higher average measurement from LC-ICP-MS labs as compared to CV-AAS labs (*p*-value = 0.0006). The average LC-ICP-MS lab measurement for Material-2 was also found to be greater than that for CV-AAS labs with a moderately significant *p*-value of 0.0480. The total mercury results for both materials are shown in Figure 3-4. The overall recovery data from participating labs averaged 93.4 ± 5.0% for Material-1 and 82.8 ± 3.5% for Material-2.

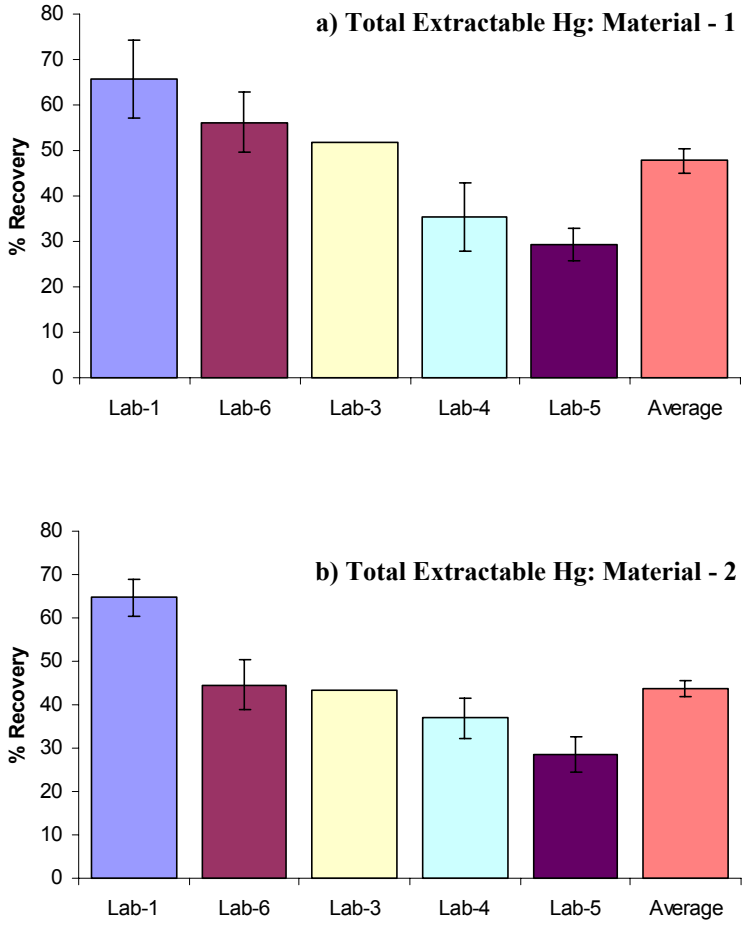


FIGURE 3-3. Percent recovery of total extractable mercury in a) Material-1; and b) Material-2. (Uncertainties, 95% CL, n=3).

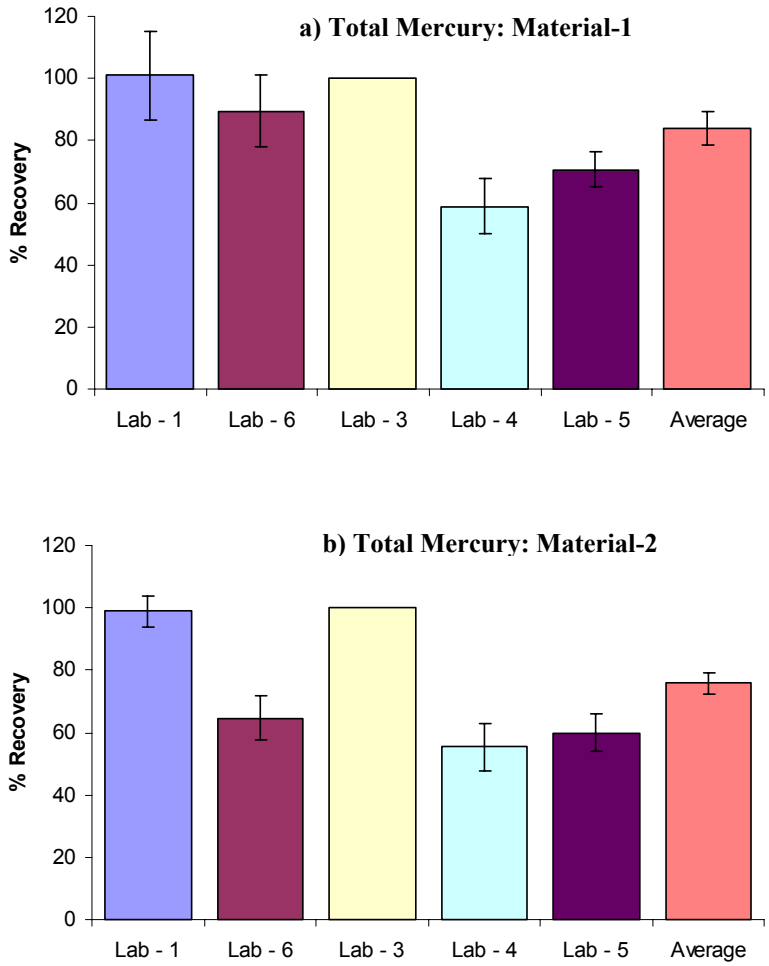


FIGURE 3-4. Percent recovery of total mercury in a) Material-1; and b) Material-2. (Uncertainties, 95% CL, n = 3).

The analysis demonstrates that data for the various mercury fractions reported by different labs are widely spread around their arithmetic mean and are not distributed normally. Also, the number of participating labs is very small (only six). In this type of asymmetric data set, the presence of any extreme value (larger or smaller) will unduly influence the arithmetic mean. Therefore, it is more appropriate to use the median instead of the mean to find the most probable value and its confidence limits.

Comparison of the calculated arithmetic mean and median, along with the 95% CL, is displayed in Table 3-V. It is found that in most cases, the mean and median are statistically indistinguishable at their 95% CL.

TABLE 3-V. Mean and Median Recoveries for Different Mercury Fractions Across Laboratories for Each Reference Materials.

Mercury Fraction	Minimum Value (%)	Maximum Value (%)	Mean (%)	Median (%)	95% CL*
EIM1	4.0	30.0	17.6	17.7	3.8
EIM2	9.4	43.9	22.1	14.4	4.4
EOM1	54.6	102.7	77.7	73.4	5.7
EOM2	47.5	85.4	65.0	59.3	3.0
ETM1	29.3	82.8	56.4	54.4	2.5
ETM2	28.4	71.9	47.9	43.9	2.1
TM1	70.7	106.1	93.4	100.0	5.0
TM2	59.9	100.1	82.8	90.3	3.5

*These uncertainty values (95% CL) are calculated for the pooled standard deviation for each species fraction reported by all participating labs.

EIM1 = Extractable Inorganic Mercury in Material-1; **EIM2** = Extractable Inorganic Mercury in Material-2; **EOM1** = Extractable Organic Mercury in Material-1; **EOM2** = Extractable Organic Mercury in Material-2; **ETM1** = Extractable Total Mercury in Material-1; **ETM2** = Extractable Total Mercury in Material-2; **TM1** = Total Mercury in Material-1; **TM2** = Total Mercury in Material-2.

3.4 Conclusions

From the statistical analyses it is observed that 1) measurements from labs using LC-ICP-MS technique have, in most cases, a significantly higher mean mercury measurement compared to labs using CV-AAS technology (for all categories); 2) the

measurements from Lab 1 are NOT always responsible for this significant difference (in 1). Comparing the "worst" performing LC-ICP-MS lab with the "best" performing CV-AAS lab yields a significantly higher LC-ICP-MS mean in one or both of the materials for each category; never is the CV-AAS mean significantly higher than that from LC-ICP-MS; 3) the detection technique (LC-ICP-MS) seems to be the only reasonable explanation for the significant results in 1) and 2); and 4) mean mercury measurements between labs sharing a common detection technique are sometimes significantly different, and sometimes not, with no pattern to support the hypothesis of material (1 or 2) or category (extractable inorganic, etc.) causality. Such significant differences are attributed to variation in lab chemists and equipment.

EPA draft Method 3200 (for mercury speciation) has been validated in a limited validation study using six laboratories. It performed successfully and was applied with relative success by all the participating laboratories. Most of the laboratories do not routinely perform speciated measurements; this was reflected in the data. However, the laboratories without experience in this type of analysis and speciation protocol were able to perform Method 3200 adequately to obtain meaningful data. These data are limited to speciation of mercury in two types of reference soil materials; these samples do demonstrate that the method is practical and provides a meaningful speciation protocol for the various solubility and toxic forms of mercury. Evaluation of the reported data reveals that this method is highly efficient, as compared to the literature methods evaluated for extracting the highly toxic methylmercury species (a targeted environmentally significant species of concern) from soils. However, its extraction efficiency for inorganic mercury is not as high as it is for the methylmercury. In

comparison with other methods that also do not induce transformations of the mercury species, it had the highest extraction efficiency for both inorganic and organometallic fractions (*5*). The design of the method, including mass balance options, permits the inorganic mercury recovery in multiple ways, for example in later stages as non-extractable mercury, which is mainly the less mobile and less toxic inorganic mercury species.

In the summary compilations (such as in Table 3-V), the calculated mean and median for most of the mercury fractions were statistically indistinguishable at their 95% CL. In some cases, these two set of values did not overlap, which reflects two main factors: (1) detection method related bimodal distributions, and (2) the small number of participating laboratories. The average precision and accuracy would likely increase with a commensurate decrease in uncertainty, if the number of participating labs was greater. That some of the labs obtained lower recoveries of inorganic mercury and methylmercury might be due to the loss of these two species during SCF-SPE separation, or perhaps an indication of some other strategic analytical practice. Lab 1 and Lab 6 achieved consistently better recoveries, which reflect their more efficient detection systems. In these laboratories, the use of chromatographic techniques coupled with inductively coupled plasma mass spectrometry for speciated detection appears to be more efficient. In this case, the extract was directly analyzed by chromatographic and instrumental systems without the use of SCF-SPE separation. Therefore, there is little chance of losing mercury species and less chance of contamination. When Lab 4 analyzed their extractable mercury fraction directly with CV-AAS, they achieved more than double the recovery obtained from the SCF-SPE separation. Here the detection

system was also CV-AAS. Several of the laboratories used CV-AAS for detection followed by SCF-SPE separation.

The SCF-SPE is a method of choice when the concentration of any one of the species differs from the other species by several orders of magnitude, or if a laboratory does not have a chromatographic analysis system to use for speciation. In the latter situation, the mercury species should be separated based on the SCF-SPE technique. The advantage of this technique over the chromatographic technique is that it not only separates, but also preconcentrates the species. The recovery results obtained from different labs based on the SCF-SPE speciation technique demonstrate greater losses and differences among one another due to the lack of experience in employing this technique. It is a recommendation of the proposed method that SCF-SPE cartridges should be prepared *in-situ* by the user, if not available commercially. The surface area of the cartridges made in different labs, or in the same lab by different analysts, can vary. Consequently, the efficiency of speciation would be different and loss of species due to their retention in the cartridges would also vary from lab to lab. Therefore, if possible, the SCF-SPE cartridges should be acquired commercially to increase the uniformity of the technique. The interlaboratory validation study results reported in this chapter have been submitted for publication in a peer reviewed scientific journal (*17*).

3.5 References

(1) Quevauviller, P. *Spectrochim. Acta, Part B* **1998,** *53*, 1261-1279.

(2) Bloom, N. S.; Porcella, D. B. *Nature* **1994**, *357*, 695.

(3) Mason, R. P.; Benott, J. M. In *Organometallic Compounds in the Environment*; Craig, P. J. Ed.; John Wiley & Sons Ltd: England, **2003**; pp 57-99.

(4) Han, Y.; Kingston, H. M.; Boylan, H. M.; Rahman, G. M. M.; Shah, S.; Richter, R. C.; Link, D. D.; Bhandari, S. *Anal. Bioanal. Chem*. **2003,** *375 (3)*, 428-436.

(5) Rahman, G. M. M.; Kingston, H. M. S. *Anal. Chem*. **2004,** *76 (13)*, 3548-3555.

(6) US EPA Method 6800. In *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods SW-846, Update IVA*, US Government Printing Office (GPO): Washington DC, **2000**.

(7) Kingston, H. M. *US Patent* 5 414 259, **1995**.

(8) Huo, D.; Kingston, H. M. *Anal. Chem.* **2000,** *72 (20)*, 5047-5054.

(9) Huo, D.; Kingston, H. M.; Larget, B. In *Comprehensive Analytical Chemistry;* Caruso, J. A.; Sutton, K. L.; Ackley, K. L., Eds.; Elemental Speciation: New Approaches for Trace Element Analysis, Vol. 33. Elsevier: New York, **2000**; pp 277-313.

(10) Kingston, H. M.; Huo, D.; Lu, Y.; Chalk, S. *Spectrochim. Acta, Part B* **1998,** *53*, 299-309.

(11) Huo, D.; Lu, Y.; Kingston, H. M. *Environ. Sci. Technol*. **1998,** *32 (21)*, 3418-3423.

(12) Rahman, G. M. M.; Kingston, H. M. S.; Bhandari, S. *Appl. Organomet. Chem.* **2003,** *17 (12)*, 913-920.

(13) Rio-Segade, S.; Bendicho, C. *J. Anal. At. Spectrom*. **1999,** *14*, 263-268.

(14) Hammerschmidt, C. R.; Fitzgerald, W. F. *Anal. Chem*. **2001,** *73*, 5930-5936.

(15) Tseng, C. M.; Diego, A. D.; Martin, F. M.; Donard, O. F. X. *J. Anal. At. Spectrom*. **1997,** *12*, 629-635.

(16) Morton, J.; Carolan, V. A.; Gardiner, P. H. E. *J. Anal. At. Spectrom*. **2002**, *17*, 377-381.

(17) Rahman, G. M. M.; Kingston, H. M. S.; Kern, J. C.; Hartwell, S. W.; Anderson, R. F.; Yang, S. Y. *Appl. Organomet. Chem.* **2004** (Submitted for Publication).

Chapter 4

Application of EPA Method 6800 to Evaluate Different Mercury Speciation Extraction Methods in Soils and Sediments

4.1 Introduction

In most natural soils and sediments, mercury is present mainly as inorganic ions (Hg^{2+}) and as methylmercury ions (CH_3Hg^+). The methylmercury is frequently a bacterial transformation of inorganic mercury in aquatic, biologically-productive locations and, being more mobile and more toxic, results in higher health risks in the environment and the food chain. Similar analogies, such as the biological and natural production of tin, arsenic, lead and chromium species, demonstrate that mobile and more toxic species share this phenomenon of both increased toxicity and mobility causing increased risks from transformed species. It is essential to determine the exact concentration of different forms of mercury in soils and sediments. There is concern and agreement that the speciation of mercury in soils and sediments is important to understand its geochemistry and physiological pathways, leading to accumulation of mercury in higher trophic levels of organisms (*1*). Most widely used methods for the speciation of mercury are based on various distillations, alkaline extractions, supercritical fluid extractions, and hot or cold acid extractions, followed by one or two separation steps. The separation and detection techniques associated with these methods include gas chromatography (GC), high performance liquid chromatography (HPLC) coupled with element-selective detection techniques, e.g. atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS) or cold vapor atomic absorption spectrometry (CV-

AAS). Most of the previously mentioned methods use acids or bases with or without organic solvents for extraction, and after extraction most of them go through sample preconcentration steps (e.g. ethylation or reduction with $SnCl_2$, or hydride generation with $NaBH_4$). Therefore, the possibility of bidirectional or unidirectional transformation from inorganic mercury to methylmercury during the analysis has been documented to have occurred with some analysis protocols (*2-6*), or from methylmercury to inorganic mercury (*7*) during sample storage, shipment, extraction, preconcentration or analysis steps. As a result, the values obtained using these procedures frequently include positive or negative biases for either inorganic mercury or methylmercury, or for both.

The purpose of this study is the evaluation of different selective extraction methods for mercury speciation and the comparison of those results with the results obtained from the proposed EPA draft Method 3200 (*7*). The methods evaluated in this study are based on sonication (*6,8*), focused microwave-assisted extraction (*9*), and cold acid extraction (*10*). Sample preparation with each of these methods is discussed in section 4.2, *Experimental*. As none of these methods can correct for any interconversion or transformation of inorganic mercury to methylmercury, or vice versa, the EPA Method 6800 (*Elemental and Speciated Isotope Dilution Mass Spectrometry, SIDMS*) (*11*), originally invented by Dr. Kingston and developed by the Kingston research group at Duquesne University, was used as a diagnostic tool and determinative technique. EPA Method 6800 is uniquely capable of being used as a correction tool to evaluate species transformation and corrections of both species simultaneously, and can also be used as a protocol step evaluation tool, trapping errors from specific steps of procedures. Any interconversions that occur after spiking are traceable and can be quantitatively

corrected by monitoring isotopes in each species. Because SIDMS can measure species concentrations at the time of spiking (by spiking a sample both before and after its extraction), SIDMS can be used to identify a procedure that alters species distribution in a multistep protocol. The fundamental theory of SIDMS in environmental systems is established and documented (*11-16*).

4.2 Experimental

4.2.1 Instrumentation

A ConstaMetric 4100Bio/MS polymeric inert pump (Thermo Separation Products, Riviera Beach, FL, USA) and a 5 μm Supelcosil LC-18 HPLC column with a Pelliguard LC-18 guard column (Supelco, Bellefonte, PA, USA) were used in this study to separate inorganic mercury and methylmercury. A six-port injection valve (Valco Instrument Co. Inc., Houston, TX, USA) was used between the pump and column. Because no special interface is required between the LC-18 column and the ICP-MS, one outlet of the column is directly interfaced to the nebulizer of the ICP-MS with a piece of PFA tubing; the other end is connected to a 50 μL TEFZEL™ sample loop (CETAC Technologies, Omaha, NE, USA). The mobile phase was buffered 30% methanol (refer to Reagent Section).

An HP 4500 ICP-MS (Agilent Technologies, Palo Alto, CA, USA and Yokogawa Analytical System Inc., Tokyo, Japan) was used in this study. The sample delivery system consisted of a peristaltic pump and quartz spray chamber with concentric nebulizer and quartz torch. The instrument was fitted with platinum sampler and skimmer cones and optimized daily using 10 ppb tuning solution (Agilent

Technologies, Palo Alto, CA, USA) containing Li, Y, Ce and Tl in 30% methanol and in 2% HNO_3. The *Spectrum* mode was engaged for direct analysis; the *Time Resolved Analysis (TRA)* mode was engaged for speciation analysis.

A direct mercury analyzer (DMA-80, Milestone, Monroe, CT, USA) was used in this study to determine the total mercury content in each of the extracts. The operation conditions for the DMA-80 used throughout this work were based on the guidelines provided in the EPA Method 7473 protocol (developed by this research group at Duquesne University) (*17-18*).

4.2.2 Reagents and Chemicals

Double deionized (DDI) water (18 MΩ cm^{-1}) prepared from a Barnstead NANOpure Ultrapure Water System (Dubuque, IA, USA), was used in the preparation of all solutions throughout this study. Reagent grade HCl, HNO_3, H_2O_2, ammonium acetate, 2-mercaptoethanol (98%), ethanol and optima grade methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA).

HPLC speciation mobile phase [30% (v/v) methanol + 0.005% 2-mercaptoethanol + 0.06 mol/L ammonium acetate], modified from Wilken's procedure (*19*), was prepared by diluting 300 mL of methanol, 50 μL of 2-mercaptoethanol and 4.8 g of ammonium acetate in 700 mL of DDI water.

4.2.3 Samples and Standards

The samples were obtained from Environmental Resource Associates (ERA) (Arvada, CO, USA). ERA prepared three types of samples in two different soil matrices. The matrices were labeled as Material-1, 100% processed topsoil, and Material-2, a mixture

of 75% processed topsoil and 25% Ottawa sand. Three types of samples were prepared by spiking HgO (labeled as Inorganic mercury), CH_3HgCl (labeled as Organic mercury), and equal mixtures of HgO and CH_3HgCl (labeled as Mixed mercury). SRM 2704 (Buffalo River Sediment) and SRM 2711 (Montana Soil) obtained from NIST (Gaithersburg, MD, USA) were used in this study as method control.

Standard solutions containing 1 mg/mL of $HgCl_2$ in 5% HNO_3 and 1 mg/mL of CH_3HgCl in water were commercially available from Alfa Aesar (Ward Hill, MA, USA). All stock solutions were stored in a cold room at 4 °C. Working standards were made daily by proper dilution with DDI water. ^{201}HgO, Lot# VX3060, was obtained from Isotech Inc. (Miamisburg, OH, USA); ^{199}HgO, Batch# 168490, was obtained from Oak Ridge National Laboratories (Oak Ridge, TN, USA). Since the isotopically enriched methylmercury was not available commercially, it was synthesized in house from ^{201}HgO and tetramethyltin. The isotopic composition of the naturally abundant mercury (*20*), isotopically enriched ^{199}HgO and ^{201}HgO are listed in Table 4-I. The synthesis and characterization of isotopically labeled methylmercury are cited elsewhere (*21*).

4.2.4 Extraction Procedure

4.2.4.1 EPA Draft Method 3200 (M-3200) (*7*)

Approximately 1.0 g portions of the samples (Material-1, Material-2, SRM 2704, and SRM 2711) were weighed into disposable glass centrifuge tubes; 2.5 mL of extraction solvent (2% HCl + 10% Ethanol) was added in each tube. The mixture was then vortexed for 1 min and sonicated at 60 ± 2 °C for 7 min in a bath-type sonicator. The extracts were then centrifuged at 3200 rpm for 5 min. The cooled extracts were

quantitatively transferred into 50 mL sample vials and the extraction process was repeated three more times. All the extracts were added together and diluted to a certain volume with DDI water. The blanks were prepared with the same reagent and procedure.

TABLE 4-I. Isotope Abundances for Naturally Abundant Mercury and Isotopically Enriched ^{119}HgO and ^{201}HgO.

Mass	Natural Abundance	Enriched ^{199}HgO	Enriched ^{201}HgO
196	0.15	< 0.02	< 0.05
198	9.97	1.63	0.08
199	16.87	91.95	0.10
200	23.10	4.92	0.45
201	13.18	0.66	98.11
202	29.86	0.73	1.18
204	6.87	0.11	0.08
Total	**100.00**	**100.00**	**100.00**

4.2.4.2 Sonication-1 (SONI-1) (*8*)

Approximately 0.4 g portions of the samples (Material-1, Material-2, SRM 2704, and SRM 2711) were weighed into disposable glass centrifuge tubes; 5 mL of 5 M HCl was added in each tube. Tubes were vortexed for 1 min and then sonicated for 4 min in a bath-type sonicator. After extraction, the sample solution was centrifuged at 3500 rpm for 10 min. The cooled extract was quantitatively transferred into a 50 mL polypropylene graduated centrifuge tube. The extraction process was repeated two more times and all extracts were collected in the same sample vial and diluted to a certain

volume by adding DDI water. The blanks were prepared with the same reagent and procedure.

4.2.4.3 Sonication-2 (SONI-2) (*6*)

Approximately 0.25 g portions of the samples (Material-1, Material-2, SRM 2704, and SRM 2711) were weighed into disposable glass centrifuge tubes; 8 mL of 1.2 M HNO_3 was added in each tube. Tubes were vortexed for 1 min and then sonicated for 15 min at 55 °C in a bath-type sonicator. The centrifuge tubes were vortexed to mix the solvent with the sample and enhance extraction efficiency at 5 min intervals. After the extraction time is over, the extraction solutions were centrifuged at 3000 rpm for 5 min. The extract was transferred quantitatively to polypropylene graduated tubes and the extraction process was repeated one more time. The blanks were prepared with the same reagent and procedure.

4.2.4.4 Focused Microwave-Assisted Extraction (FMAE) (*9*)

Approximately 1.0 g portions of the sample (Material-1, Material-2, SRM 2704 and SRM 2711) were weighed into microwave vessels; 10 mL of 2.0 M HNO_3 was added in each vessel. The vessel lids were set back and the vessels were irradiated at 60 W for 4 min. The extracts were then filtered, diluted with DDI water to certain volume, and stored in 50 mL graduated sample vials until analyzed. The blanks were prepared with the same reagent and procedure.

4.2.4.5 Cold Acid Extraction (CAE) (*10*)

Approximately 0.1 g portions of the samples (Material-1, Material-2, SRM 2704 and SRM 2711) were weighed into 10 mL centrifuge tubes; 2 mL of concentrated HNO_3 and 1 mL of 30% H_2O_2 were added. The mixture was then vortexed for 1 min and kept on a bench-top overnight. The next day, the mixture was vortexed for 1 min and then centrifuged for 5 min at 3500 rpm. The extracts were transferred quantitatively into polypropylene sample vials, diluted with DDI water to a certain volume, and stored in a cold room at 4 °C until analyzed. Analyses were usually completed with three different instruments within 2-3 days of extraction. The blanks were prepared with the same reagent and procedure.

4.2.4.6 EPA Method 3051A (*22*)

Approximately 0.5 g portions of the samples (Material-1, Material-2, SRM 2704 and SRM 2711) were weighed into the high pressure microwave vessels; 9 mL of concentrated HNO_3 and 3 mL of concentrated HCl were added. Magnetic stirrer bars were added to each of the vessels. The vessel lids were set back and the vessels were irradiated at 175 °C for 5 min. A 5 min ramping time was used to reach the desire temperature. The extracts were then filtered, diluted with DDI water to certain volume, and stored in 50 mL graduated sample vials until analyzed. The blanks were prepared with the same reagent and procedure.

4.2.4.7 Hot Alkaline Extraction (HAE) (*23*)

Approximately 0.25 g portions of the samples (Material-1, Material-2, SRM 2704 and SRM 2711) were weighed into 10 mL centrifuge tubes; 2.5 mL of 10 M KOH was added

in each tubes. Tubes were vortexed for 1 min and then heated in a boiling water bath for 25 min. After the extraction time was completed, the extraction solutions were centrifuged at 3500 rpm for 10 min. The extract was transferred quantitatively to polypropylene graduated tubes and the extraction process was repeated three more times. The tubes were washed with 1% (w/v) NaCl solution, and then with 7.5 mL of concentrated HNO_3. 0.5 mL of 1% (w/v) $K_2Cr_2O_7$ solution was added as an oxidizing agent to each vial and kept in cold room until analysis. The blanks were prepared with the same reagent and procedure.

4.2.4.8 Extraction Procedure for SIDMS

In order to perform SIDMS analysis of each of the selected mercury speciation methods, the mixed mercury soil samples from Material-1 and Material-2 were weighed into either centrifuge tubes or into microwave vessels (based on the corresponding method requirement) and double spiked with $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^{+}$. The amount of isotope spike depends on the levels of inorganic mercury and methylmercury present in the sample. The samples were allowed to equilibrate for 1 h and then extracted according to the procedure discussed under each selected method. Extracts were analyzed using HPLC-ICP-MS.

4.2.5 SIDMS Detection

4.2.5.1 Algorithms, Assumptions and Calculations

For demonstration purpose, the equations for two species system (HgO and CH_3HgCl in aqueous sample) are shown below. The derivation is based on these assumptions: the spike isotope and the natural isotopes are equilibrated before species transformations;

there is no selective loss of the species; each isotopic spike has been converted to a complete one species form (in this case, all Hg in ^{199}HgO spike is in Hg^{2+} form; all Hg in $CH_3{}^{201}HgCl$ spike is in CH_3Hg^+ form).

Consider an aqueous sample containing Hg^{2+} and CH_3Hg^+ with concentrations of C_x^{Hg2+} (μmol/g) and $C_x^{CH_3Hg+}$ (μmol/g), respectively. Weigh W_x g of the sample, followed by the addition of W_s^{Hg2+} g of ^{199}HgO spike and $W_s^{CH_3Hg+}$ g of $CH_3{}^{201}HgCl$ spike into the sample. After spiking, the sample contains

$$^{199}A_x C_x^{Hg2+} W_x + {}^{199}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+} \text{ μmol of } ^{199}Hg \text{ as } Hg^{2+}$$

and $$^{199}A_x C_x^{CH_3Hg+} W_x + {}^{199}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+} \text{ μmol of } ^{199}Hg \text{ as } CH_3Hg^+$$

where "A" represents the isotopic abundance. Assuming these two species undergo bidirectional transformations after the spike isotopes equilibrate with the sample isotopes, the fraction of Hg^{2+} that converts to CH_3Hg^+ is α and the fraction of CH_3Hg^+ that converts to Hg^{2+} is β. The total amount of ^{199}Hg in Hg^{2+} form thus changes to

$$(^{199}A_x C_x^{Hg2+} W_x + {}^{199}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+})(1-\alpha) + (^{199}A_x C_x^{CH_3Hg+} W_x + {}^{199}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+})\beta$$

after the interconversions between CH_3Hg^+ and Hg^{2+}. Similarly, the total amount of ^{202}Hg in Hg^{2+} form changes to

$$(^{202}A_x C_x^{Hg2+} W_x + {}^{202}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+})(1-\alpha) + {}^{202}A_x C_x^{CH_3Hg+} W_x + {}^{202}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+})\beta.$$

Therefore, the expression for the isotope ratio of ^{199}Hg to ^{202}Hg in Hg^{2+}, $R_{199/202}^{Hg2+}$, can be constructed as Equation 4-1. Following a similar procedure, Equation 4-2 through Equation 4-4 can be constructed.

$$R_{199/202}^{Hg2+} = \frac{\left(^{199}A_x C_x^{Hg2+} W_x + {}^{199}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+}\right)(1-\alpha) + \left(^{199}A_x C_x^{CH_3Hg+} W_x + {}^{199}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+}\right)\beta}{\left(^{202}A_x C_x^{Hg2+} W_x + {}^{202}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+}\right)(1-\alpha) + \left(^{202}A_x C_x^{CH_3Hg+} W_x + {}^{202}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+}\right)\beta}$$

$$R_{201/202}^{Hg2+} = \frac{\left({}^{201}A_x C_x^{Hg2+} W_x + {}^{201}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+}\right)(1-\alpha) + \left({}^{201}A_x C_x^{CH_3Hg+} W_x + {}^{201}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+}\right)\beta}{\left({}^{202}A_x C_x^{Hg2+} W_x + {}^{202}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+}\right)(1-\alpha) + \left({}^{202}A_x C_x^{CH_3Hg+} W_x + {}^{202}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+}\right)\beta}$$

For CH_3Hg^+

$$R_{199/202}^{CH_3Hg+} = \frac{\left({}^{199}A_x C_x^{Hg2+} W_x + {}^{199}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+}\right)\alpha + \left({}^{199}A_x C_x^{CH_3Hg+} W_x + {}^{199}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+}\right)(1-\beta)}{\left({}^{202}A_x C_x^{Hg2+} W_x + {}^{202}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+}\right)\alpha + \left({}^{202}A_x C_x^{CH_3Hg+} W_x + {}^{202}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+}\right)(1-\beta)}$$

$$R_{201/202}^{CH_3Hg+} = \frac{\left({}^{201}A_x C_x^{Hg2+} W_x + {}^{201}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+}\right)\alpha + \left({}^{201}A_x C_x^{CH_3Hg+} W_x + {}^{201}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+}\right)(1-\beta)}{\left({}^{202}A_x C_x^{Hg2+} W_x + {}^{202}A_s^{Hg2+} C_s^{Hg2+} W_s^{Hg2+}\right)\alpha + \left({}^{202}A_x C_x^{CH_3Hg+} W_x + {}^{202}A_s^{CH_3Hg+} C_s^{CH_3Hg+} W_s^{CH_3Hg+}\right)(1-\beta)}$$

where,

$R_{199/202}^{Hg2+}$ is the isotope ratio of ^{199}Hg to ^{202}Hg of Hg^{2+} in the spiked sample (unknown)

$R_{201/202}^{Hg2+}$ is the isotope ratio of ^{201}Hg to ^{202}Hg of Hg^{2+} in the spiked sample (unknown)

$R_{199/202}^{CH_3Hg+}$ is the isotope ratio of ^{199}Hg to ^{202}Hg of CH_3Hg^+ in the spiked sample (unknown)

$R_{201/202}^{CH_3Hg+}$ is the isotope ratio of ^{201}Hg to ^{202}Hg of CH_3Hg^+ in the spiked sample (unknown)

${}^{199}A_x$ is the natural relative isotopic abundance of ^{199}Hg in the sample

${}^{202}A_x$ is the natural relative isotopic abundance of ^{202}Hg in the sample

${}^{201}A_x$ is the natural relative isotopic abundance of ^{201}Hg in the sample

${}^{199}A_s^{Hg2+}$ is the relative isotopic abundance of ^{199}Hg in the $^{199}Hg^{2+}$ spike

${}^{202}A_s^{Hg2+}$ is the relative isotopic abundance of ^{202}Hg in the $^{199}Hg^{2+}$ spike

${}^{201}A_s^{Hg2+}$ is the relative isotopic abundance of ^{201}Hg in the $^{199}Hg^{2+}$ spike

${}^{199}A_s^{CH_3Hg+}$ is the relative isotopic abundance of ^{199}Hg in the $CH_3{}^{201}Hg^+$ spike

${}^{202}A_s^{CH_3Hg+}$ is the relative isotopic abundance of ^{202}Hg in the $CH_3{}^{201}Hg^+$ spike

${}^{201}A_s^{CH_3Hg+}$	is the relative isotopic abundance of ^{201}Hg in the $CH_3{}^{201}Hg^+$ spike
C_x^{Hg2+}	is the concentration of Hg^{2+} in the sample (μmol/g, unknown)
$C_x^{CH_3Hg+}$	is the concentration of CH_3Hg^+ in the sample (μmole/g, unknown)
W_x	is the weight of the sample (g)
C_s^{Hg2+}	is the concentration of Hg^{2+} in the $^{199}Hg^{2+}$ spike (μmol/g)
W_s^{Hg2+}	is the weight of the $^{199}Hg^{2+}$ spike (g)
$C_s^{CH_3Hg+}$	is the concentration of CH_3Hg^+ in the $CH_3{}^{201}Hg^+$ spike (μmol/g)
$W_s^{CH_3Hg+}$	is the weight of the $CH_3{}^{201}Hg^+$ spike (g)
α	is the proportion of Hg^{2+} transformed to CH_3Hg^+ (unknown)
β	is the proportion of CH_3Hg^+ transformed to Hg^{2+} (unknown)

The eight unknown factors in these four equations are the isotope ratios of 199/202 and 201/202 for both Hg^{2+} and CH_3Hg^+, C_x^{Hg2+}, $C_x^{CH_3Hg+}$, α and β. The isotopic ratios of 199/202 and 201/202 for both Hg^{2+} and CH_3Hg^+ can be measured from the HPLC-ICP-MS analysis. Now the four equations contain four unknown factors and may be solved easily for the concentrations of inorganic mercury, C_x^{Hg2+}, and methylmercury, $C_x^{CH_3Hg+}$, present in sample and for the fraction of inorganic mercury transformed to methylmercury, α, and the fraction of methylmercury transformed into inorganic mercury, β.

4.2.5.2 Deadtime

Deadtime (τ) is the interval during which the detector and its associated counting electronics are unable to resolve successive pulses. If the true count rate (n) is much less than 1/τ, then

$$n_{corr} = \frac{n_{meas}}{1 - n_{meas}\tau} \qquad \text{Eq.4-5}$$

where n_{corr} is the deadtime corrected count rate, n_{meas} is the measured count rate, and τ is the detector deadtime (*24-25*).

The deadtime can be determined by measuring the isotope ratios of an element in solutions of different concentrations. A series of solutions containing 0, 5, 10, 20, 30, 40, 50 and 60 ng/g of Hg, respectively, were prepared by diluting 1000 μg/g $HgCl_2$ in 5% HNO_3 stock solution. The direct aspiration mode was used to measure masses of 199, 201, and 202. The total integration time for each measurement was 15 s. The instrument software integrates signals and calculates count rates (counts per second, CPS) for each mass. The counts were then exported to Microsoft Excel® for further processing.

The counts for the background, the Hg^{2+} peak, and the CH_3Hg^+ peak were integrated. Background signals were subtracted from the total counts for each mass. The background-subtracted signals were then used to calculate the isotope ratios for $^{199}Hg/^{202}Hg$ and $^{201}Hg/^{202}Hg$ in each solution

$$R_m = \frac{{}^{\text{Isotope1}}S_{\text{standard}} - {}^{\text{Isotope1}}S_{\text{background}}}{{}^{\text{Isotope2}}S_{\text{standard}} - {}^{\text{Isotope2}}S_{\text{background}}} \qquad \text{Eq. 4-6}$$

where, R_m is the measured isotope ratio; Isotope1 is either ^{199}Hg or ^{201}Hg and Isotope2 is ^{202}Hg; ${}^{Isotope1}S_{standard}$ and ${}^{Isotope2}S_{standard}$ are the count rates for Isotope1 and Isotope2 of the standard, respectively; ${}^{Isotope1}S_{background}$ and ${}^{Isotope2}S_{background}$ are the count rates for Isotope1 and Isotope2 of the background, respectively.

4.2.5.3 Mass Bias (*26*)

Instrumental mass discrimination or fractionation effects are changes induced in the "true" isotope ratios. Mass fractionations that occur in the ionization process and mass discriminations that occur in transmission and detection cause these effects. The mass bias effects must be individually measured as they depend on the instrument drift and cannot be modeled and quantitatively predicted. Therefore, using ICP-MS to measure isotope ratios requires the assumption that mass bias factors remain constant between calibration and sample measurements. To correct for the mass bias, mass bias factors must be determined (*24*).

$$\text{mass bias factor} = R_t/R_m \qquad \text{Eq.4-7}$$

where, R_t and R_m are the true and the measured isotope ratios of the standard material, respectively. The measured isotope ratios of the samples can be corrected by using:

$$R_c = \text{mass bias factor} \times R_m \qquad \text{Eqn.4-8}$$

where, R_c and R_m are the corrected and the measured isotope ratios of the sample, respectively.

4.3 Results and Discussions

4.3.1 Total Mercury with EPA Method 7473

At the beginning of this study, the combined species, total mercury, content in the six different samples and two SRMs was determined directly (without sample preparation) by using EPA Method 7473. Results are summarized in Table 4-II. The results obtained for different soil samples and SRMs from the direct mercury analyses, except for organic mercury in Material-1, are statistically indistinguishable from their corresponding "made-to" or certified value at 95% CL.

TABLE 4-II. Determination of Total Mercury Concentration by EPA Method 7473.

Sample	Sample Type	Certified / "Made-to" Value (μg/g)	Measured Value (μg/g)	Recovery (%)
Material-1	Inorganic Mercury	4.0	4.1 ± 0.2	102 ± 4
	Organic Mercury	4.0	3.6 ± 0.3	89 ± 7
	Mixed Mercury	(3.0 + 3.0) = 6.0	5.7 ± 0.6	95 ± 10
Material-2	Inorganic Mercury	6.0	6.7 ± 1.0	112 ± 17
	Organic Mercury	6.0	5.4 ± 0.6	91 ± 10
	Mixed Mercury	(4.5 + 4.5) = 9.0	9.0 ± 1.1	100 ± 12
SRM 2704	Buffalo River Sediment	1.40 ± 0.07	1.50 ± 0.09	104 ± 6
SRM 2711	Montana Soil	6.25 ± 0.19	6.37 ± 0.47	101 ± 7

Uncertainties are at 95% CL, n = 4.

4.3.2 Evaluation of Selected Mercury Speciation Methods using Conventional Procedure

After processing the samples with each of the extraction processes, the extracts were analyzed with three different instruments to compare the species content in various

fractions. There are differences in these methods of detection that were also evaluated as a necessary component of this study. The DMA-80 and ICP-MS were used for the determination of total elemental mercury, whereas the HPLC-ICP-MS was used for the determination of total and speciation analyses. The concentration of inorganic mercury and methylmercury in different samples was determined by using the external calibration curve method. In the case of HPLC-ICP-MS analysis, the total mercury concentration was determined by the summation of inorganic mercury and methylmercury concentrations measured from their corresponding peaks using an external calibration technique; these concentrations depend on the corresponding peak area. The percent recovery values for both inorganic mercury and methylmercury were calculated based on the certified or the "made-to" concentration of each species in the respective sample. The percent recovery for inorganic mercury and methylmercury for each of the samples are compiled in Tables 4-III through 4-IX, and are evaluated and discussed as they relate to the different methods of extraction. After careful evaluation of each result obtained for total mercury from different detection techniques, these results are found to be statistically indistinguishable at 95% confidence level.

It is found from data in Table 4-IV that the SONI-1 method extracted nearly 100% of inorganic mercury from Material-1, Material-2 and SRM 2711, and 62 ± 13 % from SRM 2704. The CAE method extracted approximately 100% and 70% of inorganic mercury from SRMs and spiked soil materials (1 and 2), respectively (Table 4-VII). The EPA Method 3051A extracted approximately 90% of inorganic mercury from Mateiral-1 and Material-2, and approximately 100% of inorganic mercury from SRMs (Table 4-VIII). Although these three methods have higher extraction efficiency for inorganic

mercury for all the samples studied, they also induced partial or complete transformation of methylmercury into inorganic mercury during extraction, accounting for these apparent efficiencies. It was notable that clean spiked soil samples containing only methylmercury should show no inorganic mercury peak during analysis using HPLC-ICP-MS (speciation). However, inorganic mercury peak was observed during analysis of the extracts from SONI-1, CAE and EPA Method 3051A extraction methods. In Table 4-IV, 4-VII, and 4-VIII, values reported in parentheses represent (i) the amount of methylmercury converted to inorganic mercury for samples initially containing only methylmercury; and (ii) the summation of inorganic mercury and the converted methylmercury for samples initially containing mixed mercury species. For example, after analysis of the SONI-1 data from Material-1 and Material-2 (Table 4-IV) containing only methylmercury it was found that the total mercury recovery was 86 ± 15% and 81 ± 18%, respectively, of which 68 ± 13% and 69 ± 17% of the initial methylmercury was converted to inorganic mercury and, as a result, the percent recovery of mercury as methylmercury for both materials was only 18 ± 7% and 12 ± 4%, respectively. The percent recovery for inorganic mercury with SONI-1 was found to be 181 ± 3% and 154 ± 3% for Material-1 and Material-2, respectively, initially containing mixed mercury species. At the same time, the percent recovery of mercury as methylmercury was found to be 31 ± 13% and 16 ± 3% for Material-1 and Material-2, respectively (Table 4-IV). After evaluation of the SONI-1 data in Table 4-IV it is found that the remaining amount of methylmercury after conversion to inorganic mercury was found to be statistically indistinguishable at 95% CL for both organic mercury and

mixed mercury-containing materials. The total mercury recovery with this method was also statistically indistinguishable at 95% CL for both materials.

TABLE 4-III. EPA Draft Method 3200 Performances Reported as Percent Recovery.

Sample		Certified/"Made-to" value (μg/g)		DMA-80 (%)	ICP-MS (%)	HPLC-ICP-MS (%)		
		Hg^{2+}	CH_3Hg^+			Hg^{2+}	CH_3Hg^+	Average*
Mateiral-1	Inorganic	4.0	---	33 ± 3	35 ± 3	31 ± 7	---	31 ± 7
	Organic	---	4.0	97 ± 3	95 ± 1	---	109 ± 15	109 ± 15
	Mixed	3.0	3.0	62 ± 1	63 ± 4	29 ± 5	103 ± 10	66 ± 5
Material-2	Inorganic	6.0	---	55 ± 4	56 ± 2	47 ± 18	---	47 ± 18
	Organic	---	6.0	94 ± 3	96 ± 1	---	93 ± 13	93 ± 13
	Mixed	4.5	4.5	64 ± 6	64 ± 5	44 ± 4	85 ± 4	65 ± 3
SRM 2704		1.40 ± 0.07	---	1.2 ± 0.1	1.3 ± 0.03	ND	ND	ND
SRM 2711		6.25 ± 0.19	---	2.9 ± 0.3	3.0 ± 0.3	ND	ND	ND

Uncertainties are reported as 95% CL with n = 4. ND – analyzed, but not detectable.
*Average = average of Hg^{2+} and CH_3Hg^+ data from HPLC-ICP-MS.

The materials containing organic mercury and mixed mercury were extracted with CAE; the total mercury recovery results were approximately 80% (Table 4-VII) and were statistically indistinguishable at 95% CL for all the samples and detection techniques studied. During HPLC-ICP-MS analysis, no methylmercury peak was observed for extracts from CAE from the 100% methylmercury and mixed mercury-spiked Material-1 and Material-2. For both materials, all the methylmercury was converted during extraction with CAE and detected as inorganic mercury in HPLC-ICP-MS analysis, indicating quantitative conversion of methylmercury to inorganic mercury.

TABLE 4-IV. Ultrasound Assisted Extraction (SONI-1) Method Performances Reported as Percent Recovery.

Sample		Certified/"Made-to" value (μg/g)		DMA-80 (%)	ICP-MS (%)	HPLC-ICP-MS (%)		
		Hg^{2+}	CH_3Hg^+			Hg^{2+}	CH_3Hg^+	Average*
Mateiral-1	Inorganic	4.0	---	96 ± 3	98 ± 2	98 ± 16	---	98 ± 16
	Organic	---	4.0	87 ± 4	94 ± 2	**(68 ± 13)**[a]	18 ± 7	86 ± 15
	Mixed	3.0	3.0	101 ± 3	102 ± 2	**(181 ± 3)**[b]	31 ± 13	106 ± 13
Material-2	Inorganic	6.0	---	92 ± 5	86 ± 2	93 ± 13	---	93 ± 13
	Organic	---	6.0	75 ± 4	79 ± 3	**(69 ± 17)**[a]	12 ± 4	81 ± 18
	Mixed	4.5	4.5	89 ± 2	87 ± 2	**(154 ± 3)**[b]	16 ± 3	85 ± 4
SRM 2704		1.40 ± 0.07	---	50 ± 3	50 ± 1	62 ± 13	---	62 ± 13
SRM 2711		6.25 ± 0.19	---	102 ± 2	105 ± 1	103 ± 10	---	103 ± 10

[a]the amount of methylmercury converted to inorganic mercury; [b]the summation of inorganic mercury and the converted methylmercury. Uncertainties are reported as 95% CL with n = 4.

TABLE 4-V. Ultrasound Assisted Extraction (SONI-2) Method Performances Reported as Percent Recovery.

Sample		Certified/"Made-to" value (μg/g)		DMA-80 (%)	ICP-MS (%)	HPLC-ICP-MS (%)		
		Hg^{2+}	CH_3Hg^+			Hg^{2+}	CH_3Hg^+	Average*
Mateiral-1	Inorganic	4.0	---	21 ± 3	18 ± 2	20 ± 9	---	20 ± 9
	Organic	---	4.0	87 ± 3	79 ± 2	---	87 ± 11	87 ± 11
	Mixed	3.0	3.0	58 ± 1	51 ± 1	19 ± 4	75 ± 11	47 ± 12
Material-2	Inorganic	6.0	---	24 ± 3	20 ± 2	22 ± 1	---	22 ± 1
	Organic	---	6.0	70 ± 7	73 ± 5	---	69 ± 1	69 ± 1
	Mixed	4.5	4.5	46 ± 4	48 ± 4	20 ± 6	72 ± 7	46 ± 10
SRM 2704		1.40 ± 0.07	---	27 ± 2	31 ± 2	22 ± 4	---	22 ± 4
SRM 2711		6.25 ± 0.19	---	6 ± 0.3	7 ± 0.3	6 ± 1	---	6 ± 1

Uncertainties are reported as 95% CL with n = 4. *Average = average of Hg^{2+} and CH_3Hg^+ data from HPLC-ICP-MS.

TABLE 4-VI. Focused Microwave Assisted Extraction (FMAE) Method Performances Reported as Percent Recovery.

Sample		Certified/ "Made-to" value (μg/g)		DMA-80 (%)	ICP-MS (%)	HPLC-ICP-MS (%)		
		Hg^{2+}	CH_3Hg^+			Hg^{2+}	CH_3Hg^+	Average*
Mateiral-1	Inorganic	4.0	---	24 ± 2	22 ± 2	27 ± 1	---	27 ± 1
	Organic	---	4.0	84 ± 3	79 ± 2	---	74 ± 2	74 ± 2
	Mixed	3.0	3.0	47 ± 1	49 ± 1	20 ± 1	72 ± 3	46 ± 2
Material-2	Inorganic	6.0	---	29 ± 4	31 ± 1	23 ± 2	---	23 ± 2
	Organic	---	6.0	47 ± 2	45 ± 1	---	37 ± 10	37 ± 10
	Mixed	4.5	4.5	33 ± 4	27 ± 3	18 ± 5	38 ± 12	27 ± 6
SRM 2704		1.40 ± 0.07	---	ND	ND	ND	ND	ND
SRM 2711		6.25 ± 0.19	---	ND	ND	ND	ND	ND

ND – analyzed, but not detectable. Uncertainties are reported as 95% CL with n = 4.

TABLE 4-VII. Cold Acid Extraction (CAE) Method Performances Reported as Percent Recovery.

Sample		Certified/ "Made-to" value (μg/g)		DMA-80 (%)	ICP-MS (%)	HPLC-ICP-MS (%)		
		Hg^{2+}	CH_3Hg^+			Hg^{2+}	CH_3Hg^+	Average*
Mateiral-1	Inorganic	4.0	---	73 ± 7	64 ± 4	64 ± 5	---	64 ± 5
	Organic	---	4.0	84 ± 4	77 ± 4	**(85 ± 3)**[a]	ND	85 ± 3
	Mixed	3.0	3.0	76 ± 2	75 ± 1	**(145 ± 14)**[b]	ND	73 ± 7
Material-2	Inorganic	6.0	---	66 ± 6	68 ± 4	75 ± 11	---	75 ± 11
	Organic	---	6.0	85 ± 8	89 ± 6	**(84 ± 12)**[a]	ND	84 ± 12
	Mixed	4.5	4.5	75 ± 6	73 ± 4	**(154 ± 18)**[b]	ND	77 ± 9
SRM 2704		1.40 ± 0.07	---	109 ± 1	113 ± 1	107 ± 13	---	107 ± 13
SRM 2711		6.25 ± 0.19	---	93 ± 5	91 ± 1	93 ± 6	---	93 ± 6

ND- analyzed, but not detectable. Uncertainties are reported as 95% CL with n = 4.
*Average = average of Hg^{2+} and CH_3Hg^+ data from HPLC-ICP-MS. [a]the amount of methylmercury converted to inorganic mercury; [b]the summation of inorganic mercury and the converted methylmercury.

The materials containing methylmercury and mixed mercury were extracted with EPA Method 3051A and the total mercury recovery was approximately 70% and 85%, respectively (Table 4-VIII), and are statistically indistinguishable at 95% CL for all the samples and detection technique studied. During HPLC-ICP-MS analysis, no methylmercury peak was observed for extracts from the EPA Method 3051A for the 100% methylmercury and mixed mercury spiked Material-1 and Material-2. For both materials, all the methylmercury was converted during extraction and detected as inorganic mercury in HPLC-ICP-MS analysis, indicating quantitative conversion of methylmercury to inorganic mercury.

Therefore, these three methods (SONI-1, CAE and EPA Method 3051A) appear not to be suitable for the mercury speciation analysis, as complete or significant conversion of methylmercury, for the test materials in this study, is caused by the protocol of these procedures.

The extraction efficiency of EPA Method 3200 for methylmercury was approximately 100% for samples containing only organic mercury in Material-1 and Material-2, and mixed mercury in Material-1 (Table 4-III). For mixed mercury in Material-2, the recovery was 85 ± 4%. The extraction efficiency of SONI-2 for methylmercury was also higher and found to be approximately 75% for all the materials studied (Table 4-V). For MAE, the extraction efficiency was ~ 40% for Material-2 and ~ 72% for Material-1 (Table 4-VI). It was also demonstrated that EPA Method 3200, SONI-2 and MAE methods do not induce organic mercury to transform into inorganic mercury. However, the extraction efficiency of SONI-2 and MAE methods for both inorganic and organic mercury in Material-1 and Material-2 is less than that of the EPA

Method 3200. The extraction efficiencies of these three methods (SONI-2, MAE, and EPA Method 3200), for inorganic mercury from samples containing only inorganic mercury and mixed mercury, were statistically indistinguishable at 95% CL within the same matrix type and same methods. On the other hand, the same kind of extraction efficiencies were observed for these three methods for samples containing only methylmercury and mixed mercury species within the same matrix type and same method, and statistically are not distinguishable at 95% CL. The microwave-assisted method considered in this study was based on a focused microwave method that used 60 W power with a single vessel and claimed to reach a temperature of 120 °C-125 °C. During this study, a closed vessel microwave system with 10 microwave vessels and 60 W power was used. A temperature of 25 °C was achieved, as the temperature profile of a microwave system depends on the applied power and which, in turn, depends on the number of samples or total mass of the extraction system used. Therefore, the extraction efficiency was less than the reported value by the original method. The extraction efficiency would have increased if higher temperature and/or higher power was used during this study. In these circumstances the stability or fate of the mercury species might be compromised.

The materials containing inorganic mercury, methylmercury and mixed mercury were extracted using the hot alkaline extraction method. The total recovery for inorganic mercury and mixed mercury was approximately 90% for Material-1 and Material-2. The recovery of inorganic mercury from SRM 2704 was 85 ± 3%; recovery for SRM 2711 was only 28 ± 4%. The recovery from samples containing methylmercury was 76 ± 3% for Mateiral-1 and 57 ± 4% for Material-2. The extracts could not be analyzed using

ICP-MS and HPLC-ICP-MS because of the higher concentration of KOH and NaCl. The extracts were neutralized using concentrated HNO_3, but very low response was observed from ICP-MS signal. The reason for this might be the deposition of KOH and salt on ICP-MS cones. Another possible cause of the low response is due to the formation of mercury nitrate, which has higher ionization potential than Hg^{2+}.

TABLE 4-VIII. EPA Method 3051A Performances Reported as Percent Recovery.

Sample		"Made-to" Mercury Content (μg/g)		DMA-80 (%)	ICP-MS (%)	HPLC-ICP-MS (%)		
		Hg^{2+}	CH_3Hg^+			Hg^{2+}	CH_3Hg^+	Average*
Mateiral-1	Inorganic	4.0	---	95 ± 7	89 ± 2	82 ± 6	---	82 ± 6
	Organic	---	4.0	71 ± 5	66 ± 3	**(72 ± 5)**	ND	72 ± 5
	Mixed	3.0	3.0	86 ± 2	82 ± 4	**(156 ± 17)**	ND	78 ± 9
Material-2	Inorganic	6.0	---	84 ± 3	89 ± 2	79 ± 7	---	79 ± 7
	Organic	---	6.0	63 ± 7	65 ± 4	**(66 ± 9)**	ND	66 ± 9
	Mixed	4.5	4.5	84 ± 4	78 ± 3	**(153 ± 13)**	ND	76 ± 6
SRM 2704		1.40 ± 0.07	---	100 ± 4	98 ± 2	106 ± 7	---	106 ± 7
SRM 2711		6.25 ± 0.19	---	102 ± 2	98 ± 1	86 ± 12	---	86 ± 12

ND – analyzed but not detectable. *Average = average of Hg^{2+} and CH_3Hg^+ data from HPLC-ICP-MS. Uncertainties are reported as 95% CL with n = 4.

4.3.3 Evaluation of Selected Mercury Speciation Methods using SIDMS

The SIDMS (application of EPA Method 6800) analysis depends on some fundamental operations: isotopic spike preparation and calibration, sample collection and sample spiking, sample species and spike species equilibration, sample extraction, species separation, isotope ratio measurements of each speciated component, and deconvolution of the species concentrations and species transformations.

TABLE 4-IX. Hot Alkaline Extraction Method Performances Reported as Percent Recovery.

Sample		Certified/ "Made-to" value (μg/g)		DMA-80 (%)
		Hg^{2+}	CH_3Hg^+	
Mateiral-1	Inorganic	4.0	---	91 ± 4
	Organic	---	4.0	76 ± 3
	Mixed	3.0	3.0	92 ± 4
Material-2	Inorganic	6.0	---	81 ± 6
	Organic	---	6.0	57 ± 4
	Mixed	4.5	4.5	89 ± 4
SRM 2704		1.40 ± 0.07	---	85 ± 3
SRM 2711		6.25 ± 0.19	---	28 ± 4

Uncertainties are reported as 95% CL with n = 4.

For SIDMS analysis, samples were double spiked with known amounts of isotopically enriched inorganic mercury ($^{199}Hg^{2+}$) and methylmercury ($CH_3{}^{201}Hg^+$) in such a way that the desired isotope ratio became approximately 1:1. After equilibration with the sample species, the samples were extracted and analyzed with HPLC-ICP-MS. In order to do measurements of isotope ratios of each speciated component, the raw data obtained from the HPLC-ICP-MS was processed offline in specific computer algorithms using the SIDMS equations and methods provided in the Experimental Section (4.2.5.1). To date, no commercial software is available or found suitable for processing data acquired with the HPLC-ICP-MS system used in this study. Experimental raw data were exported into Microsoft Excel® for the appropriate processing.

Following is an outline of the data acquisition and processing procedures.

General data acquisition:

- Set up and tune ICP-MS using direct aspiration mode;
- Perform experiments to determine deadtime (*24-25*);
- Connect the outlet of the chromatographic column to the ICP-MS and stabilize the system. Inject sample through sample introduction loop and collect data in Time Resolved Analysis mode;
- Determine mass bias factors every four hours (*24,26*);
- Export data in comma separated version (CSV) format for processing in Microsoft Excel®.

General data processing and measurement quality assurance:

- Calculate deadtime;
- Use the determined deadtime to correct the count rates point by point; integrate the counts for the background, the Hg^{2+} peak and the CH_3Hg^+ peak by summing the deadtime-corrected count rates;
- Subtract background and calculate isotope ratio;
- Calculate mass bias factors for each isotope pair: $^{199}Hg/^{202}Hg$ and $^{201}Hg/^{202}Hg$; correct mass biases in the sample isotope ratios.

After instrumental analyses, raw data were exported as a CSV file to Microsoft Excel®. Deadtime and mass bias corrected ratios for $^{199}Hg/^{202}Hg$ and $^{201}Hg/^{202}Hg$ were calculated for both inorganic mercury and methylmercury. The SIDMS calculations

were performed to calculate the concentration of inorganic mercury and methylmercury. The final concentrations of inorganic mercury and methylmercury in different samples and the percent transformation of inorganic mercury to methylmercury, and vice versa, during extraction are summarized in Table 4-X. It is demonstrated in Table 4-X that the percent recovery of both inorganic mercury and methylmercury from both materials was statistically indistinguishable at 95% CL, within the same species and extraction methods studied [except for methylmercury with MAE (50 ± 3%)]. The percent recovery of methylmercury from Material-1 was indistinguishable at 95% CL for all the extraction methods studied. The percent recovery of methylmercury for Material-2 formed a bimodal distribution by grouping EPA Method 3200 and SONI-1 in one group (approximately 60%), and SONI-2 and MAE in another group (approximately 50%). The percent recovery for inorganic mercury in both materials also showed a bimodal distribution by grouping EPA Method 3200 and SONI-1 in one group (approximately 85%), and SONI-2 and MAE in another group (approximately 65%).

The mercury species transformation results agree with those obtained from the preliminary extraction results by conventional extraction methods. However, the amounts of inorganic and methyl mercury obtained in SIDMS are less than the "made-to" value, which is probably due to the poor extraction efficiency of the different methods and/or lack of complete equilibration between the sample and spike species isotopes. It is confirmed (by validation) that EPA Method 3200 has better extraction capability with little or no transformation between species. SONI-2 and MAE methods also show less transformation, but have lower extraction efficiency when compared to EPA Method 3200. SONI-1 has better extraction efficiency, but it induces

methylmercury transformation into inorganic mercury. A 45 ± 4 Z% and 44 ± 4% transformation of methylmercury to inorganic mercury was observed for Material-1 and Material-2, respectively, with the SONI-1 method. Therefore, the application of this extraction process in mercury speciation analysis will overestimate the inorganic mercury and, at the same time, underestimate methylmercury.

TABLE 4-X. The Deconvoluted Concentration and Percent Transformation of Mercury Species in Material-1 and Material-2 using SIDMS Calculations.

Sample	Extraction Method	Deconvoluted Concentration (μg/g)		Recovery (%)		Interconversion (%)	
		Hg^{2+}	CH_3Hg^+	Hg^{2+}	CH_3Hg^+	Hg^{2+} to CH_3Hg^+	CH_3Hg^+ to Hg^{2+}
Material-1	M-3200	2.68 ± 0.34	2.20 ± 0.29	89 ± 12	73 ± 10	0 ± 3	0 ± 9
	SONI-1	2.49 ± 0.16	1.83 ± 0.13	83 ± 5	61 ± 4	5 ± 1	45 ± 4
	SONI-2	1.88 ± 0.20	1.96 ± 0.24	63 ± 7	65 ± 8	2 ± 3	10 ± 5
	MAE	1.99 ± 0.14	2.01 ± 0.16	66 ± 8	67 ± 5	0 ± 3	7 ± 3
	CAE	NA	NA	NA	NA	NA	~ 100
Material-2	M-3200	4.09 ± 0.93	2.79 ± 0.29	91 ± 21	62 ± 6	2 ± 2	6 ± 5
	SONI-1	3.67 ± 0.16	2.65 ± 0.09	81 ± 3	59 ± 2	2 ± 1	44 ± 4
	SONI-2	3.09 ± 0.23	2.29 ± 0.20	67 ± 5	51 ± 5	1 ± 1	2 ± 2
	MAE	3.09 ± 0.24	2.26 ± 0.13	69 ± 5	50 ± 3	2 ± 1	4 ± 3
	CAE	NA	NA	NA	NA	NA	~ 100

Uncertainties are expressed at 95% CL, n = 9. NA – analyzed but could not perform SIDMS calculations.

SIDMS calculations could not be performed for CAE because all of the methylmercury in both samples, including the spiked $CH_3{}^{201}Hg^+$, was transformed into

Hg^{2+} during the extraction process (see Figure 4-1). This is one of the times that SIDMS cannot correct, as all of the species of interest were destroyed. However, it is obvious that this occurred and is not as misleading as in other methods where it is not known that the species was transformed. Notice that the methylmercury peak is missing since 100% of the CH_3Hg^+ has been converted to Hg^{2+} species. The same type of transformation was also observed with Material-2 extract. Thus, the application of CAE in mercury speciation analysis will provide completely inaccurate information about the sample. For better comparison, a chromatogram obtained from the proposed EPA Method 3200 extract is shown in Figure 4-2 (Material-1). The first peak is for inorganic mercury and the second peak is for methylmercury at a flow rate of 0.8 mL/min. The same type of chromatogram was also obtained for Material-2 extract.

4.4 Conclusions

The evaluation of some selected mercury speciation methods has been performed successfully using both conventional and SIDMS techniques. Results for each of the selected extraction methods agreed in both techniques. EPA draft Method 3200, SONI-2 and MAE methods showed less extraction efficiency for inorganic mercury, but relatively higher extraction efficiency for methylmercury from soil materials tested. Of equal importance, these three methods did not induce interconversion of inorganic mercury to methylmercury, or vice versa. Of these three methods, proposed EPA draft Method 3200 demonstrated the best recovery for both inorganic mercury and methylmercury extraction. On the other hand, EPA Method 3051A, SONI-1 and CAE demonstrated higher efficiency in extraction of inorganic mercury, but induced

methylmercury transformation into inorganic mercury. These methods induced significant conversions of approximately 100% and 45% of the methylmercury to inorganic mercury during extractions with CAE and SONI-1, respectively. Therefore, these two methods are not suitable for mercury speciation analysis without isotopic correction using SIDMS to prevent relative precise bias from being interpreted as accurate specie measurement.

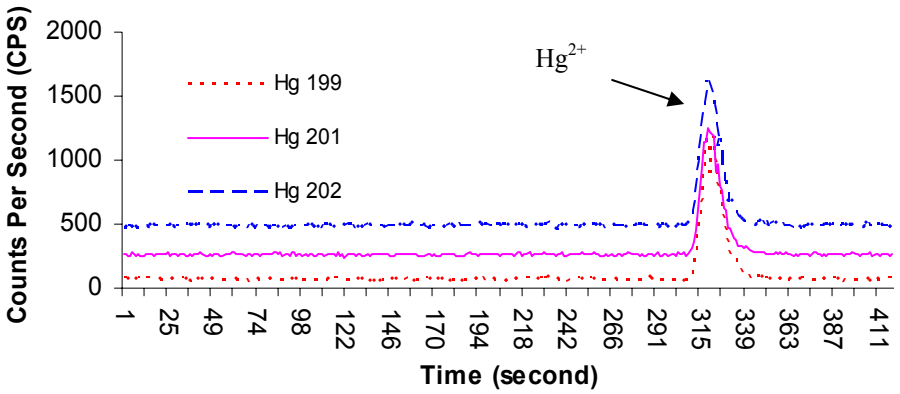


FIGURE 4-1: Chromatogram for mercury species in Material-1 after double spiking with $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^{+}$ and application of cold acid extraction (CAE) method; ^{201}Hg and ^{202}Hg baselines were shifted from the baseline by adding 200 CPS and 400 CPS, respectively, to the original counts for clarity. [*Flow rate*: 0.85 mL/min; *Eluent*: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol/L ammonium acetate; *Column*: 5 µm Supelcosil LC-18 HPLC column].

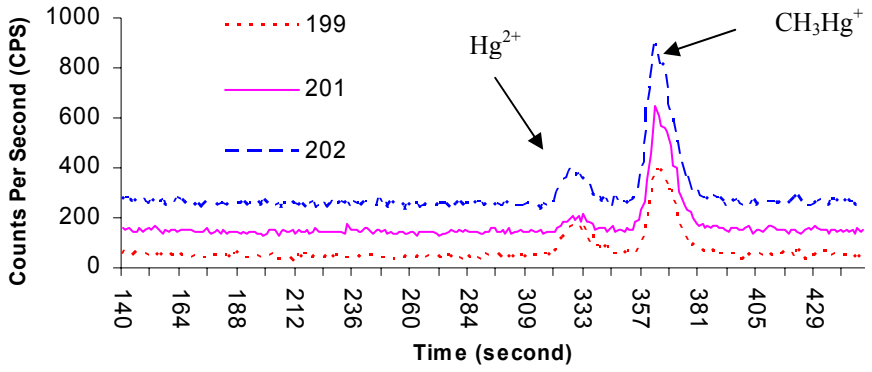


FIGURE 4-2: Chromatogram for mercury species in Material-1 after double spiking with $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^{+}$ and extraction with EPA draft Method 3200; ^{201}Hg and ^{202}Hg baselines were shifted from the baseline by adding 100 CPS and 200 CPS, respectively, to the original counts for clarity. [*Flow rate*: 0.80 mL/min; *Eluent*: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol/L ammonium acetate; *Column*: 5 µm Supelcosil LC-18 HPLC column].

4.5 References

(1) Qian, J.; Skyllberg, U.; Tu, Q.; Bleam, W. F.; Frech, W. *Fresenius' J. Anal. Chem*. **2000,** *367*, 467-473.

(2) Rodríguez Martín-Doimeadios, R. C.; Monperrus, M.; Krupp, E.; Amouroux, D.; Donard, O. F. X. *Anal. Chem*. **2003**, *75*, 3202-3211.

(3) Gaona, X.; Valiente, M. *Anal. Chim. Acta* **2003**, *480*, 219-230.

(4) Bloom, N. S.; Colman, J. A.; Barber, L.; *Fresenius' J. Anal. Chem*. **1997**, *358*, 371-377.

(5) Hintelmann, H.; Falter, R.; Ilgen, G.; Evans, R. D. *Fresenius' J. Anal. Chem*. **1997**, *358*, 363-370.

(6) Hammerschmidt, C. R.; Fitzgerald, W. F. *Anal. Chem*. **2001**, *73*, 5930-5936.

(7) Han, Y.; Kingston, H. M.; Boylan, H. M.; Rahman, G. M. M.; Shah, S.; Richter, R. C.; Link, D. D.; Bhandari, S. *Anal. Bioanal. Chem*. **2003,** *375*, 428-436.

(8) Rio-Segade, S.; Bendicho, C. *J. Anal. At. Spectrom*. **1999**, *14*, 263-268.

(9) Tseng, C. M.; Diego, A. D.; Martin, F. M.; Donard, O. F. X. *J. Anal. At. Spectrom*. **1997**, *12*, 629-635.

(10) Morton, J.; Carolan, V. A.; Gardiner, P. H. E. *J. Anal. At. Spectrom*. **2002**, *17*, 377-381.

(11) US EPA Method 6800. In: *Solid Waste Manual-846, Update IV*, US Government Printing Office (GPO): Washington DC, **2000**.

(12) Kingston, H. M. U.S. Patent, 5,414,259, **1995**.

(13) Huo, D.; Kingston, H.M. *Anal. Chem.* **2000**, *72(20)*, 5047-5054.

(14) Huo, D.; Kingston, H. M.; Larget, B. In *Comprehensive Analytical Chemistry*, Caruso, J. A.; Sutton, K. L.; Ackley, K. L., Eds.; Elemental Speciation: New Approaches for Trace Element Analysis, *Vol. 33*. Elsevier: New York, **2000**, pp 277-313.

(15) Kingston, H. M.; Huo, D.; Lu, Y.; Chalk, S. *Spectrochim. Acta, Part B* **1998**, *53*, 299-309.

(16) Huo, D.; Lu, Y.; Kingston, H. M. *Environ. Sci. Technol*. **1998**, *32*, 3418.

(17) US EPA Method 7473. In *Test Method for Evaluating Solid Waste, Physical/Chemical Methods SW 846*, *Update IVA*. US GPO, Washington, DC. **2000**.

(18) Boylan, H. M.; Kingston, H. M. S. *Am. Lab*. **1998**, *30(19)*, 26.

(19) Wilken, R. D.; Hintelmann, H. *Water, Air, Soil Pollut*. **1991**, *56*, 427.

(20) Lide, D. R. *CRC Handbook of Chemistry and Physics*, 81st edition, CRC Press LLC: Florida, USA, **2000**.

(21) Rahman, G. M. M.; Kingston, H. M. S.; Bhandari, S. *Appl. Organomet. Chem*. **2003**, *17(12)*, 913-920.

(22) US EPA Method 3051A. In *Test method for Evaluating Solid Waste, Physical/Chemical Methods SW 846, Update IVA*. US GPO, Washington, DC. **2000**.

(23) Ubillus, F.; Alegria, A.; Barbera, R.; Farre, R.; Lagarda, M. *J. Food Chem*. **2000**, *71*, 529-533.

(24) Huo, D. *Ph.D. Thesis*, Duquesne University, Pittsburgh, PA, **1999**.

(25) Taylor, H. E. *Inductively Coupled Plasma-Mass Spectrometry: Practices and Techniques*; Academic Press: San Diego, CA, USA, **2001**, pp 48.

(26) Jarvis, K. E.; Gray, A. L.; Houk, R. S. *Handbook of Inductively Coupled Plasma Mass Spectrometry*; Blackie Academic & Professional: London, UK, **1992**, pp 315-316.

Chapter 5

Development of Microwave-Assisted Extraction Method for Mercury Speciation in Soils and Sediments

5.1 Introduction

Mercury speciation has long been a field of continuous concern. Such interest is mainly due to toxicological impact, ecological problems and biogeochemical cycling of mercury involving distribution, accumulation, transformations and transport pathways in the natural environment (*1*). Mercury is a very toxic element. However, the toxicity of mercury is highly dependent on its chemical form. Methylmercury is one of the most toxic mercury species. To understand the toxicological impact and pathway of mercury species in the environment, the determination of total mercury is frequently not sufficient. Therefore, the assessment of inorganic mercury and methyl mercury concentrations, specifically in sediments and soils, is very important to the interpretation of biogeochemical cycles of mercury in aquatic environments (*2*).

Determination of different mercury species from various complex matrices, e.g. soils and sediments, is still considered a difficult task due to the frequently very low concentration of methylmercury in soils and sediments (less than 1.5% of the total mercury) (*3*). The quality of the results mainly depends on the sample pretreatment stages (sampling, storage and sample preparation), in spite of significant improvements of the instrumentation techniques. The most widely used methods for the extraction and separation of inorganic and methylmercury are the Westöö technique (*4-7*) (acidic leaching method), alkaline digestion (*8-10*), steam distillation (*9-11*), solvent extraction (*12-14*), a modified Westöö methodology (*15*) (alkaline leaching technique), and

supercritical fluid extraction (*16*), followed by one or two separation steps. The separation and detection techniques associated with these methods include gas chromatography (GC), high performance liquid chromatography (HPLC) coupled with element-selective detection techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), atomic emission spectrometry (AES), atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), or cold vapor atomic absorption spectrometry (CV-AAS). As all of the aforementioned sample preparation methods use either acid or base with/without organic solvents, and, after extraction, most of them implement sample preconcentration steps (e.g. ethylation or reduction with $SnCl_2$ or hydride generation with $NaBH_4$), there are possibilities of interconversion or unidirectional transformation of inorganic mercury to organic mercury (*13,17-18*) or vice versa (*19*) during sample storage, shipment, extraction, preconcentration or analyses steps. Therefore, the results obtained using these procedures frequently introduce positive or negative biases for either inorganic mercury or methylmercury, or both. Besides such drawbacks, these methods require much solvent, labor and time.

The efficiency of the less solvent, and time consuming microwave-assisted extraction (MAE) technique for sample preparation in environmental applications has been evaluated elsewhere in different matrices (soils, sediments, and biological tissues), in different applications (total digestion for elemental analysis, extraction of selected organic compounds), and in speciation analysis (organotin). Vazquez et. al. (*20-21*) used the focused microwave-assisted extraction (FMAE) technique to extract methylmercury with HCl and toluene, a modified method of Westöö (*4-5*), from sediment and biological tissue samples. Tseng et. al. (*22-24*) also implemented FMAE for extraction of

methylmercury, also from sediment and tissue samples. There are several drawbacks of FMAE: samples must be extracted at atmospheric pressure and below the boiling point of the solvent; simultaneous extraction of multiple samples is not possible; it is difficult to preset a constant temperature profile as this technique only allows control of the applied power, which in turn is directly dependent on the number of samples or the total mass, and; there is a high possibility of losing the volatile organomercury compounds during extraction. However, no one has yet tried the closed-vessel microwave-assisted extraction technique (which is free from the aforementioned drawbacks) for mercury speciation in soils or sediments.

Therefore, the purpose of this study was to develop a microwave-assisted extraction procedure capable of quantitative extraction with little or no transformation of inorganic mercury and methylmercury from soils and sediments in a closed-vessel microwave system. Careful optimization of the conditions for the microwave extraction procedure is required to stabilize the mercury species in the microwave field, prior to speciation analysis. Essential parameters, such as concentration of the extraction solvent, amount of solvent, amount of sample, temperature and time of exposure must be optimized. The literature (*22*) suggests that nitric acid (HNO_3) is a better solvent for microwave-assisted extraction because it introduces little or no interferences to the ICP-MS. Therefore, nitric acid has been evaluated as an extraction solvent. The irradiation power, one of the most useful parameters for microwave extraction, was not optimized during this study due to its dependency on the number of samples or the total mass of the extraction medium.

This chapter describes a fast and easy method for the quantitation of inorganic mercury and methylmercury using closed-vessel microwave-assisted extraction, followed by separation with HPLC and detection with ICP-MS. The stability of the mercury species in a microwave field and the optimization of different parameters are also described in detail. The developed method was then validated by using different standard reference materials and reference soils obtained from Environmental Resource Associates®. The developed method was also validated using EPA Method 6800 [*Elemental and Speciated Isotope Dilution Mass Spectrometry, (IDMS and SIDMS respectively*)] (*25*). EPA Method 6800 was used as a diagnostic tool to check whether any interconversion between inorganic mercury and methylmercury is taking place during or after extraction. One of the unique applications of SIDMS is to trap errors related to specific portions of a protocol. This is accomplished by using multiple spikings with multiple isotope-labeled species at specific method protocol points. The error of the specific steps may be discovered, and their contribution to the overall transformation of a species may be known. To perform these types of applications, inorganic mercury and methylmercury labeled with multiple isotopes are required. Inorganic mercury labeled with different isotopes is commercially available, but methylmercury is not. Therefore, methylmercury labeled with multiple isotopes must be synthesized in the laboratory (*26*).

5.2 Experimental

5.2.1 Instrumentation

A laboratory microwave system (Ethos 1600) (Milestone, Monroe, CT, USA), equipped with temperature and pressure feedback control and magnetic stirring capability was used in this study. This device extracts ten samples simultaneously. The high pressure closed digestion vessels used for extraction are made of high purity TFM (a thermally resistant form of fluoropolymer) and have a capacity of 100 mL.

Caution*: Safety guidelines regarding work with microwave fields in the laboratory must be observed (27).*

A ConstaMetric 4100Bio/MS polymeric inert pump (Thermo Separation Products, Riviera Beach, FL, USA) and a 5 μm Supelcosil LC-18 HPLC column with a Pelliguard LC-18 guard column (Supelco, Bellefonte, PA, USA) were used in this study to separate inorganic and methylmercury. A six-port injection valve (Valco Instrument Co. Inc., Houston, TX, USA) was placed between the pump and the column. Because no special interface is required between the LC-18 column and the ICP-MS, one outlet of the column is directly interfaced with the nebulizer of the ICP-MS using a piece of PFA tubing; the other end is connected to a 50 μL TEFZEL™ sample loop (CETAC Technologies, Omaha, NE, USA).

An HP 4500 ICP-MS (Agilent Technologies, Palo Alto, CA, USA and Yokogawa Analytical Systems Inc., Tokyo, Japan) was used as detector for the HPLC system in this study. The sample delivery system consisted of a peristaltic pump and quartz spray chamber with concentric nebulizer and quartz torch. The instrument was fitted with platinum sampler and skimmer cones, and optimized daily using 10 ppb

tuning solution (Agilent Technologies, Palo Alto, CA, USA) containing Li, Y, Ce and Tl in 30% methanol. Time resolved analysis (TRA) mode was engaged for speciation analysis.

A direct mercury analyzer (DMA-80, Milestone, Monroe, CT, USA) was used in this study to determine the total mercury content in each of the extraction and purification steps. The operation for the DMA-80 used throughout this work was based on the guidelines provided in EPA Method 7473 protocol (*28-29*).

5.2.2 Reagents and Chemicals

Analytical grade nitric acid (Fisher Scientific, Pittsburgh, PA, USA) and double deionized (DDI) water (18 MΩ cm^{-1}), prepared from a Barnstead NANOpure Ultrapure Water System (Dubuque, IA, USA) were used. Different concentrations of nitric acid were prepared by diluting an appropriate volume of nitric acid in DDI water. Reagent grade HCl, $Na_2S_2O_3$, toluene, ammonium acetate, 2-mercaptoethanol (98%), and optima grade methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA). The reagent grade tetramethyltin (98%) was obtained from Alfa Aesar (Ward Hill, MA, USA).

HPLC speciation mobile phase [30% (v/v) methanol + 0.06 M ammonium acetate + 0.005% 2-mercaptoethanol), modified from Wilken's procedure (*30*), was prepared by diluting 300 mL of methanol, 50 μL of 2-mercaptoethanol and 4.8 g ammonium acetate with 700 mL of DDI water.

5.2.3 Standard Solutions and Certified Reference Materials

A standard stock solution of 1000 μg/mL of $HgCl_2$ in 5% HNO_3 and 1000 μg/mL of CH_3HgCl in water were commercially available from Alfa Aesar (Ward Hill, MA,

USA). All stock solutions were stored in amber glass bottles in a cold room at 4 °C. Working standards were prepared daily by proper dilution with DDI water. ^{201}HgO and ^{199}HgO were obtained from Isotech Inc. (Miamisburg, OH, USA).

$CH_3{}^{201}Hg^+$ was synthesized from ^{201}HgO using tetramethyltin (*26*). To prepare $^{201}HgCl_2$, 6 mL of $^{201}Hg^{2+}$ solution (11 μg/mL) was mixed with 2 mL of 6.0 M HCl in a 20 mL amber glass vial and stirred for 5 min. A 0.93 M methanolic solution of $(CH_3)_4Sn$ was prepared by dissolving 0.340 g of $(CH_3)_4Sn$ in 2 mL methanol. This reagent was quantitatively transferred into the $^{201}HgCl_2$ solution and the glass vial cap was put back on. The resulting reaction mixture was then stirred for 1 h in a 60 °C water bath to complete the synthesis. The reaction mixture was cooled to room temperature and extracted 3 times with toluene (4 + 3 + 3 mL). The synthesized methylmercury (in toluene) was washed with DDI water 3 times (4 + 3 +3 mL). The toluene extract was then extracted twice with 2.5 mL of 1% $Na_2S_2O_3$. All of the extracts were stored in amber glass vials in a cold room.

Caution: *Mercury compounds, especially methylmercury, are highly toxic materials. Proper knowledge and safety guidelines regarding working with mercury compounds are required to handle these compounds.*

NIST SRMs 1941a (Organics in Marine Sediment), 2704 (Buffalo River Sediment), 2709 (San Joaquin Soil), and 2711 (Montana Soil), blank soil (100% processed topsoil, mercury free, < 5 ng Hg/g) and reference soils (Material-1: 100% processed topsoil, and Material-2: 75% processed topsoil and 25% Ottawa sand) from Environmental Resource Associates® (ERA) (Arvada, CO, USA) were used for method development and validation.

5.2.4 Optimized Analytical Procedure

A sample of approximately 1.0 g homogenized soil or sediment and 10 mL of 4.0 M HNO_3 was placed in the microwave extraction vessels. A magnetic stirrer bar was added to each vessel for thorough mixing of solvent with the sample. Microwave vessels were sealed and irradiated at 100 °C for 10 min. with magnetic stirring on. A 2 min ramping time was used to reach the desired temperature of 100 °C. After microwave irradiation, the vessels were cooled to room temperature and extracts were filtered through 0.22 μm glass fiber filter and stored in the cold room until analyzed (usually less than 2 days). Blanks were prepared along with the samples in each batch.

To evaluate the stability of mercury species in the microwave field, 10 mL of nitric acid solution at different concentrations was spiked with 100 μL of Hg^{2+} standard (100 μg Hg^{2+} per mL standard) and 100 μL of CH_3Hg^+ standard (100 μg CH_3Hg^+ per mL standard) and irradiated at different temperatures and for different irradiation time. The blank soil was spiked with the same concentrations of inorganic mercury and methylmercury; SRM 2711 was spiked only with methylmercury, to which the extractant was added to optimize the microwave-assisted extraction procedure. The samples were then irradiated by varying both irradiation time and irradiation temperature.

5.3 Results and Discussion

5.3.1 Stability of Mercury Species under Microwave Irradiation

The effects of *nitric acid concentration* on the stability of mercury species was studied at different HNO_3 concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 M HNO_3) at 45

°C for 5 min. A mixed mercury standard (100 μg/mL) was used for spiking. 10 mL of extraction solvent for each concentration level was measured and dispensed into microwave vessels. 100 μL of the mixed standard was spiked into each of the vessels and a magnetic stirring bar was added. The samples were irradiated for 5 min and analyzed with HPLC-ICP-MS. It was found that both mercury species were stable at that temperature for 5 min up to 4.0 M HNO_3 concentrations (Table 5-I).

The *temperature effect* on the stability of mercury species was studied at different levels (30, 40, 50, 60, 70, 80, 90, and 100 °C) using 2.0 M HNO_3 as the solvent. The solvent was spiked with 100 μL of mixed mercury standard and irradiated for 5 min at different temperatures and analyzed with HPLC-ICP-MS. It was found that both mercury species were stable within this temperature range (Table 5-I).

The *irradiation time effect* on the stability of mercury species was also studied at different irradiation times (2, 4, 6, 8, 10, 12, and 14 min) using 2.0 M HNO_3. The solvent was also spiked with the same concentration of mixed mercury standard and irradiated at 100 °C for different time periods. It was found that both mercury species were stable within the time range studied (Table 5-I).

After careful evaluation of all data sets, it was concluded that mercury species are stable in 2.0 M nitric acid at temperatures up to 100 °C and for at least 14 min of microwave irradiation. Results may vary due to temperature and time effects, or show different trends with a higher concentration of nitric acid. During this study, only the extraction solvent was spiked with mixed mercury standard. Results may also vary or show different trends with soil or sediment samples. Therefore, the next step was to use

soil and sediment samples or SRMs to develop a methodology for microwave-assisted extraction of mercury species.

TABLE 5-I. Stability of Mercury Species under Microwave Irradiation: Percent Recovery at 95% CL, n = 3.

HNO_3 Concentration Effects (at 45 °C for 5 minutes)			Temperature Effects (2.0 M HNO_3 and 5 min)			Time Effects (2.0 M HNO_3 at 45 °C)		
(M)	Hg^{2+}	CH_3Hg^+	(°C)	Hg^{2+}	CH_3Hg^+	(min.)	Hg^{2+}	CH_3Hg^+
0.0	101 ± 4	96 ± 4	30	93 ± 6	93 ± 13	2	93 ± 12	96 ± 17
0.5	97 ± 4	97 ± 4	40	88 ±	106 ± 8	4	100 ± 14	103 ± 13
1.0	90 ± 5	94 ± 8	50	92 ± 14	98 ± 7	6	97 ± 5	107 ± 9
1.5	90 ± 8	93 ± 11	60	96 ± 11	101 ± 7	8	100 ± 2	91 ± 5
2.0	92 ± 8	99 ± 8	70	96 ± 14	96 ± 13	10	89 ± 15	100 ± 6
2.5	91 ± 8	89 ± 8	80	101 ± 10	103 ± 8	12	89 ± 14	99 ± 3
3.0	93 ± 6	89 ± 7	90	97 ± 4	103 ± 4	14	91 ± 15	89 ± 8
3.5	89 3	95 ±	100	103 ± 9	99 ± 8			
4.0	97 ± 6	97 ± 8						

5.3.2 Optimization of HNO_3 Concentration

The nitric acid *concentration effects* on the extraction efficiency and stability of mercury species in soils and SRM 2711 were studied. Approximately 0.4 g of each soil sample and SRM 2711 were weighed directly in the microwave vessel. Blank soil was spiked with a known amount of 100 μg/mL mixed mercury standard. As SRM 2711 contains only inorganic mercury, it was spiked with known amount of only methylmercury standard. Samples were left for 1 h to equilibrate, and then 10 mL of extraction solvent

(1.0, 2.5, 4.0, 5.5, and 7.0 M HNO_3) was added into the microwave vessel. It was then extracted at 50 °C with the following microwave procedure.

Step 1: Time = 2 min (Ramping to 50 °C); Temperature = 50 °C; Power = 1000 W

Step 2: Time = 5 min (Hold at 50 °C); Temperature = 50 °C; Power = 1000 W

Note: Automated feedback control was engaged for both protocol steps; Venting Time = 3 min.

After each extraction cycle was completed, the samples were cooled to room temperature and extracts were filtered through 0.22 μm glass fiber filter and stored in the cold room at 4 °C until analyzed. The extracts were analyzed with the DMA-80 and the ICP-MS for total mercury, and with the HPLC-ICP-MS for total and mercury speciation. Results are shown in Table 5-II.

TABLE 5-II. Percent Recovery Results for Optimization of HNO_3 Concentration.

Sample	HNO_3 Concentration (M)	DMA-80 (%)	ICP-MS (%)	HPLC-ICP-MS (%)		
				Hg^{2+}	CH_3Hg^+	Average*
Blank Soil (Spiked)	1.0	95 ± 76	98 ± 2	101 ± 7	91 ± 6	96 ± 5
	2.5	105 ± 2	101 ± 3	106 ± 1	107 ± 11	106 ± 6
	4.0	103 ± 2	95 ± 2	103 ± 1	105 ± 5	104 ± 3
	5.5	100 ± 2	94 ± 2	111 ± 7	87 ± 17	99 ± 9
	7.0	107 ± 8	102 ± 2	128 ± 5	69 ± 3	99 ± 3
SRM 2711 (Spiked)	1.0	54 ± 8	57 ± 2	44 ± 8	81 ± 3	63 ± 4
	2.5	57 ± 5	61 ± 2	51 ± 7	77 ± 9	64 ± 6
	4.0	70 ± 1	68 ± 3	74 ± 8	83 ± 5	79 ± 5
	5.5	74 ± 2	77 ± 2	103 ± 7	59 ± 8	81± 5
	7.0	92 ± 5	88 ± 1	147 ± 19	47 ± 17	97 ± 13

Uncertainties are reported as 95% CL with n = 4. *Average = average of Hg^{2+} and CH_3Hg^+ data from HPLC-ICP-MS.

From Table 5-II, it is observed that the results from different analysis methods were statistically indistinguishable at 95% CL. It was found that almost 100% of total mercury is extractable from spiked blank soil using 1.0 to 7.0 M HNO_3. On the other hand, the SRM 2711 extraction efficiency is highly dependent on the concentration of the solvent used. The extraction efficiency increases from 55% to 95% by increasing the nitric acid concentration from 1.0 M to 7.0 M. It is evident that the sample matrix influences the extraction efficiency. In the case of blank soil, the spikes were freshly added and had very limited time to interact physically and/or chemically with soil particles, and were easy to extract with solvents at different concentrations. On the other hand, SRM 2711 is a natural soil and inorganic mercury is naturally tightly bound with the soil particles. Therefore, it was difficult to extract with less concentrated extraction solvents.

However, from the speciation data for concentration effect on extraction efficiency and stability of mercury species (Figure 5-1), it is observed that methylmercury was extracted nearly 100% and 80% for spiked blank soil and SRM 2711, respectively, and was stable up to 4.0 M HNO_3. After that concentration, methylmercury transformations into inorganic mercury increased and recovery was decreased from 105 ± 5% at 4.0 M HNO_3 to 69 ± 3% at 7.0 M HNO_3 for spiked blank soil and from 83 ± 5% at 4.0 M HNO_3 to 47 ± 17% at 7.0 M HNO_3 for SRM 2711. As a result, the recovery of inorganic mercury increased from 103 ± 1% at 4.0 M HNO_3 to 128 ± 5% at 7.0 M HNO_3 for spiked blank soil, and from 74 ± 8% at 4.0 M HNO_3 to 147 ± 19% at 7.0 M HNO_3 for SRM 2711. Therefore, 4.0 M HNO_3 was used as extraction solvent throughout the study.

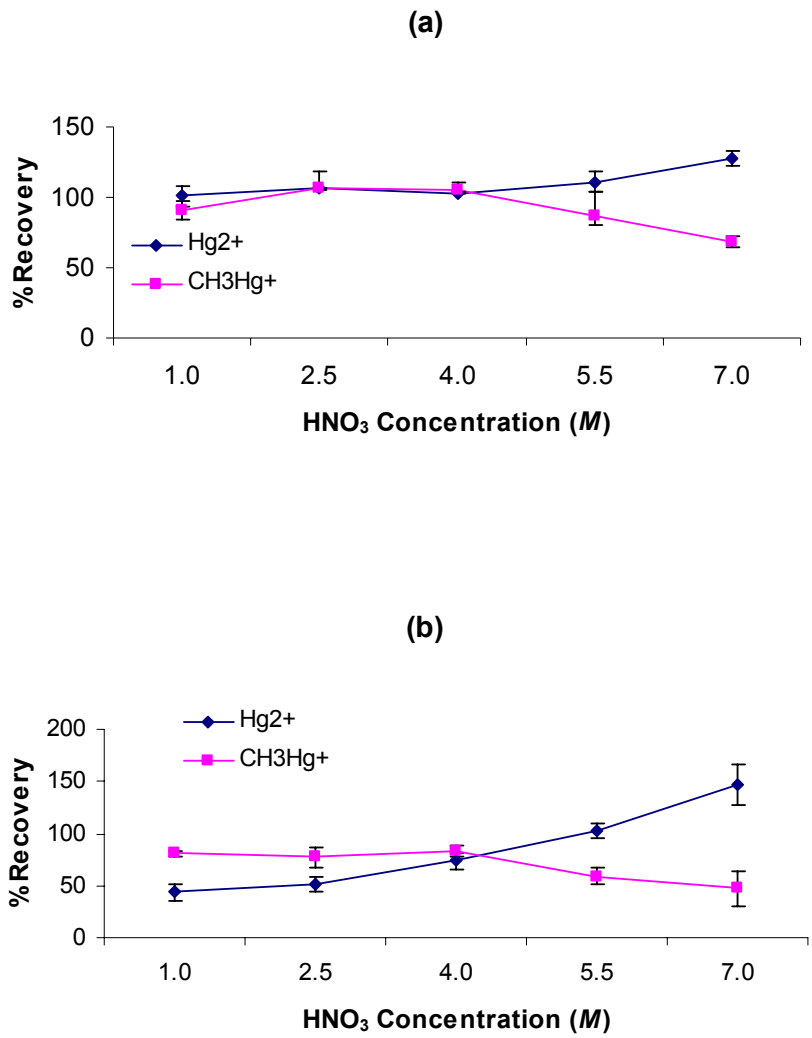


FIGURE 5-1. The effects of optimization of nitric acid concentration on the efficiency of extraction and stability of mercury species for (a) spiked blank soil and (b) SRM 2711 (spiked with methylmercury).

5.3.3 Optimization of Sample Weight

Effects of *sample weight* on the extraction efficiency and on the stability of mercury species were studied using the same blank soil and SRM 2711 spiked with methylmercury. Different amounts (0.25, 0.50, 0.75, 1.00 and 2.00 g) of blank soil and

SRM 2711 were weighed directly in the microwave vessel and were each spiked with a known amount of 100 μg/mL mixed mercury standard, and with a known amount of 100 μg/mL methylmercury. 10 mL of 4.0 M HNO_3 were added to each vessel and irradiated at 50 °C for 5 min. After filtration, the samples were analyzed using three different instruments (Table 5-III). From the total mercury results obtained from spiked blank soil, it is found that the recovery was nearly 100% for all the sample amounts studied: statistically, there was no significant difference between these results. But on the other hand, the recovery from SRM 2711 (spiked with methylmercury) was approximately 60% and also statistically indistinguishable at their 95% CL for all the sample amounts studied.

TABLE 5-III. Percent Recovery Results for Optimization of Sample Weight.

Sample	Sample Weight (g)	DMA-80 (%)	ICP-MS (%)	HPLC-ICP-MS (%)		
				Hg^{2+}	CH_3Hg^+	Average*
Blank Soil (Spiked)	0.25	108 ± 5	103 ± 1	102 ± 7	97 ± 3	100 ± 4
	0.50	97 ± 4	98 ± 1	100 ± 8	106 ± 5	103 ± 5
	0.75	92 ± 6	96 ± 1	91 ± 3	98 ± 1	95 ± 2
	1.00	90 ± 4	94 ± 1	92 ± 9	106 ± 7	99 ± 6
	2.00	94 ± 9	98 ± 1	100 ± 9	106 ± 4	103 ± 5
SRM 2711 (Spiked)	0.25	65 ± 4	61 ± 1	47 ± 3	74 ± 1	61 ± 2
	0.50	59 ± 4	57 ± 1	34 ± 4	85 ± 2	60 ± 2
	0.75	57 ± 3	60 ± 1	34 ± 1	92 ± 5	63 ± 3
	1.00	51 ± 2	49 ± 1	33 ± 2	73 ± 1	53± 1
	2.00	55 ± 2	57 ± 1	34 ± 2	87 ± 2	61 ± 1

Uncertainties are reported as 95% CL with n = 4. *Average = average of Hg^{2+} and CH_3Hg^+ data from HPLC-ICP-MS.

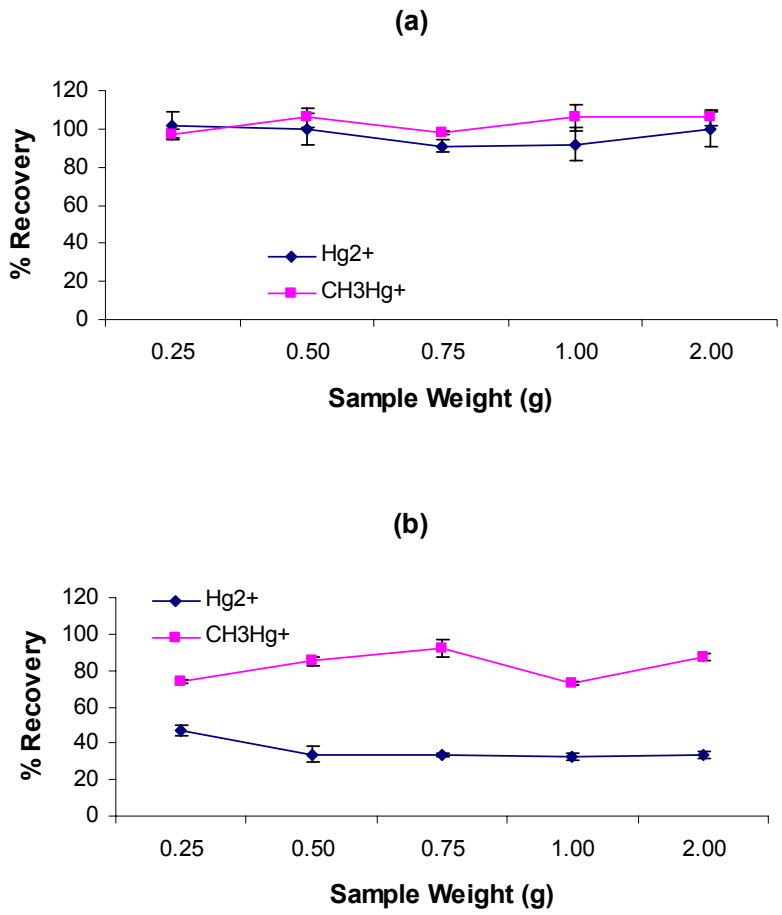


FIGURE 5-2. The effects of optimization of the sample weight on extraction efficiency for (a) spiked blank soil and (b) SRM 2711 (spiked with methylmercury).

The speciation data (Figure 5-2) indicates that the sample weight has no effect on the extraction efficiency at 50 °C. Almost 100% of inorganic mercury and methylmercury from spiked blank soil were recovered. But in SRM 2711, the recovery of inorganic mercury was poor (~ 35%). On the other hand, the recovery of spiked methylmercury from SRM 2711 was ~ 85% and was stable. The robustness of the extraction method is demonstrated in this study by optimizing the sample amount over

one order of magnitude. For the entire sample range tested, statistically identical recoveries were obtained from 0.25 g to a 2.0 g aliquot of sample. An intermediate 1.00 g sample size was used during rest of the evaluations.

5.3.4 Optimization of Irradiation Temperature

The effect of *irradiation temperature* on the extraction efficiency and stability of the mercury species was studied using spiked blank soils and SRM 2711 spiked with methylmercury at different temperatures (50, 60, 70, 80, 90, 100, 110, 120 and 130 °C). Each representative sample was weighed directly in a microwave vessel at 1.0 g concentration, along with 10 mL of 4.0 M HNO_3. Samples were irradiated in the microwave at different temperatures for 5 min. Samples were cooled to room temperature, filtered through 0.22 μm glass fiber filter and stored in the cold room until analyzed. The extracts were analyzed using three different instruments (Table 5-IV). It was found that the recoveries of the total mercury in spiked blank soil was nearly 100% for each of the temperatures studied, and are statistically indistinguishable at 95% CL. On the other hand, the recovery for total mercury in SRM 2711 increases from approximately 50% at 50 °C to 100% at 130 °C.

The speciation data are shown in Figure 5-3. It was found that the recovery results for both inorganic mercury and methylmercury in spiked blank soil are almost 100% and statistically indistinguishable at 95% CL throughout the temperature range studied here. However, the extraction efficiency of inorganic mercury in SRM 2711 increased from 33 ± 2% at 50 °C to 116 ± 10% at 130 °C. The recovery for methylmercury in SRM 2711 (spiked with methylmercury) also increased from 73 ± 1%

at 50 °C to 102 ± 7% at 100 °C, then decreased to 79 ± 11% at 130 °C due to the degradation of methylmercury to inorganic mercury. As the recovery for both inorganic mercury and methylmercury was approximately 100% at 100 °C, this temperature was used throughout the study.

TABLE 5-IV. Percent Recovery Results for Optimization of Irradiation Temperature.

Sample	Irradiation Temperature (°C)	DMA-80 (%)	ICP-MS (%)	HPLC-ICP-MS (%)		
				Hg^{2+}	CH_3Hg^+	Average*
Blank Soil (Spiked)	50	90 ± 4	94 ± 1	92 ± 9	106 ± 7	99 ± 6
	60	98 ± 2	99 ± 2	91 ± 10	98 ± 2	95 ± 5
	70	96 ± 1	101 ± 2	91 ± 9	102 ± 4	97 ± 5
	80	97 ± 1	95 ± 2	88 ± 6	99 ± 9	94 ± 5
	90	103 ± 5	97 ± 1	94 ± 8	105 ± 8	100 ± 6
	100	103 ± 6	97 ± 2	95 ± 2	101 ± 8	98 ± 4
	110	103 ± 2	101 ± 1	94 ± 4	99 ± 5	97 ± 3
	120	103 ± 6	100 ± 1	102 ± 10	98 ± 9	100 ± 7
	130	105 ± 2	97 ± 1	98 ± 10	96 ± 4	97 ± 5
SRM 2711 (Spiked)	50	51 ± 2	49 ± 1	33 ± 2	73 ± 1	53 ± 1
	60	57 ± 1	56 ± 1	31 ± 3	80 ± 8	56 ± 4
	70	64 ± 1	66 ± 2	48 ± 1	95 ± 6	72 ± 3
	80	71 ± 2	72 ± 1	69 ± 5	95 ± 7	82± 4
	90	82 ± 4	78 ± 2	76 ± 8	107 ± 9	92 ± 6
	100	93 ± 7	91 ± 2	98 ± 5	102 ± 7	100 ± 4
	110	94 ± 7	96 ± 1	109 ± 6	96 ± 7	103 ± 5
	120	92 ± 4	97 ± 1	112 ± 5	70 ± 7	91± 4
	130	97 ± 4	99 ± 1	116 ± 10	79 ± 11	98 ± 7

Uncertainties are reported as 95% CL with n = 4. *Average = average of Hg^{2+} and CH_3Hg^+ data from HPLC-ICP-MS.

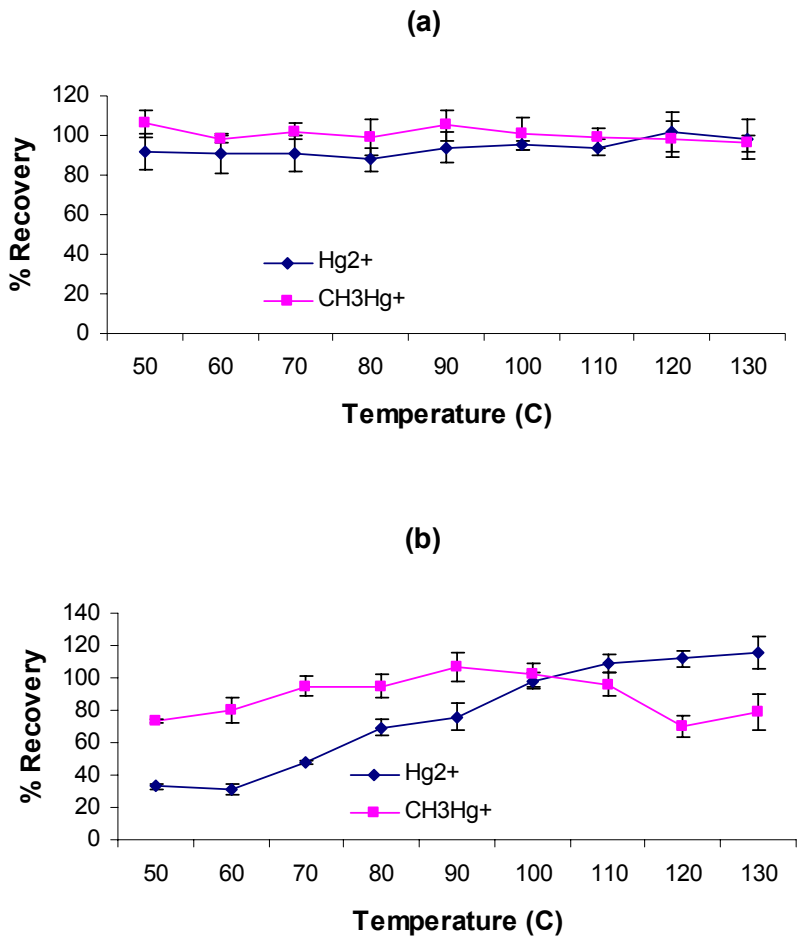


FIGURE 5-3. The effects of optimization of the irradiation temperature on extraction efficiency and stability of mercury species in (a) spiked blank soil and (b) SRM 2711 (spiked with methylmercury).

5.3.5 Optimization of Irradiation Time

The effect of *irradiation time* on the extraction efficiency and stability of mercury species was studied at different irradiation times (5, 10, 15, 20, 25 and 30 min.). Approximately 1.0 g sample of blank soil and SRM 2711 were weighed into microwave vessels and spiked with known amounts of 100 µg/mL mixed mercury standard and 100 µg/mL methylmercury standard. After addition of 10 mL of 4.0 M HNO_3 to each vessel,

the samples were irradiated at 100 °C for different amounts of time. The vessels were cooled to room temperature and extracts were filtered and stored in a cold room until analyzed. The extracts were again analyzed using three instruments. From the final total mercury results (Table 5-V), the recovery of mercury in both spiked blank soil and SRM 2711 (spiked with methylmercury) were nearly 100% and were statistically indistinguishable at 95% CL throughout the studied time periods. The speciation data for both spiked blank soil and SRM 2711 (spiked with methylmercury) are shown in Figure 5-4. From the speciation data, it was also found that both inorganic mercury and methylmercury were extracted 100% from the spiked blank soil with no distinguishable degradation of methylmercury during the studied time period. On the other hand, the recovery of both inorganic mercury and methylmercury in SRM 2711 (spiked with methylmercury) were nearly 100% and were stable up to 20 min, after which degradation of methylmercury occurred. As a result, the recovery of inorganic mercury increased and methylmercury recovery decreased, although at 95% CL, these changes in recovery were not distinguishable. In order to shorten the sample preparation time, it was decided to use 10 min as the optimum time for extraction.

The venting time used throughout this study was 3 min. The cooling rate of the vessels depends on the make, model and type of both the microwave and the vessels used. Therefore, the recommended venting or cooling time used may be more than 3 min.

5.3.6 Validation of the Developed and Optimized Method using Reference Soils and Standard Reference Materials (SRMs)

The microwave-assisted extraction method was validated by using two different sets of reference soil samples (Lot # 0611-01-02), prepared by Environmental Resource

Associates® for SAIC and United States Environmental Protection Agency (US EPA). The preparation of these reference soil samples is described elsewhere (*31*). In brief, two types of materials were prepared: one from 100% processed topsoil and labeled as Material-1, and a mixture of 75% processed topsoil and 25% Ottawa sand, labeled as Material-2. Both of these materials were then spiked with HgO (inorganic mercury), CH_3HgCl (organic mercury) and an equal mixture of HgO and CH_3HgCl (mixed mercury) in order to prepare a total of six samples.

TABLE 5-V. Percent Recovery Results for Optimization of Irradiation Time.

Sample	Irradiation Time (min.)	DMA-80 (%)	ICP-MS (%)	HPLC-ICP-MS (%)		
				Hg^{2+}	CH_3Hg^+	Average*
Blank Soil (Spiked)	5	103 ± 6	97 ± 2	95 ± 2	101 ± 8	98 ± 4
	10	107 ± 7	98 ± 3	91 ± 9	94 ± 8	93 ± 6
	15	106 ± 8	102 ± 1	92 ± 9	93 ± 9	93 ± 6
	20	105 ± 5	104 ± 2	94 ± 4	95 ± 11	95 ± 6
	25	104 ± 6	106 ± 2	91 ± 10	101 ± 5	96 ± 6
	30	107 ± 2	104 ± 2	91 ± 10	95 ± 3	93 ± 5
SRM 2711 (Spiked)	5	93 ± 7	91 ± 2	98 ± 5	102 ± 7	100 ± 4
	10	91 ± 5	99 ± 1	100 ± 9	97 ± 10	99 ± 7
	15	94 ± 5	97 ± 5	93 ± 10	94 ± 4	94 ± 5
	20	95 ± 4	102 ± 2	93 ± 8	93 ± 13	93 ± 8
	25	93 ± 5	100 ± 5	94 ± 10	90 ± 11	92 ± 7
	30	99 ± 5	100 ± 2	103 ± 9	88 ± 8	96 ± 6

Uncertainties are reported as 95% CL with n = 4. *Average = average of Hg^{2+} and CH_3Hg^+ data from HPLC-ICP-MS.

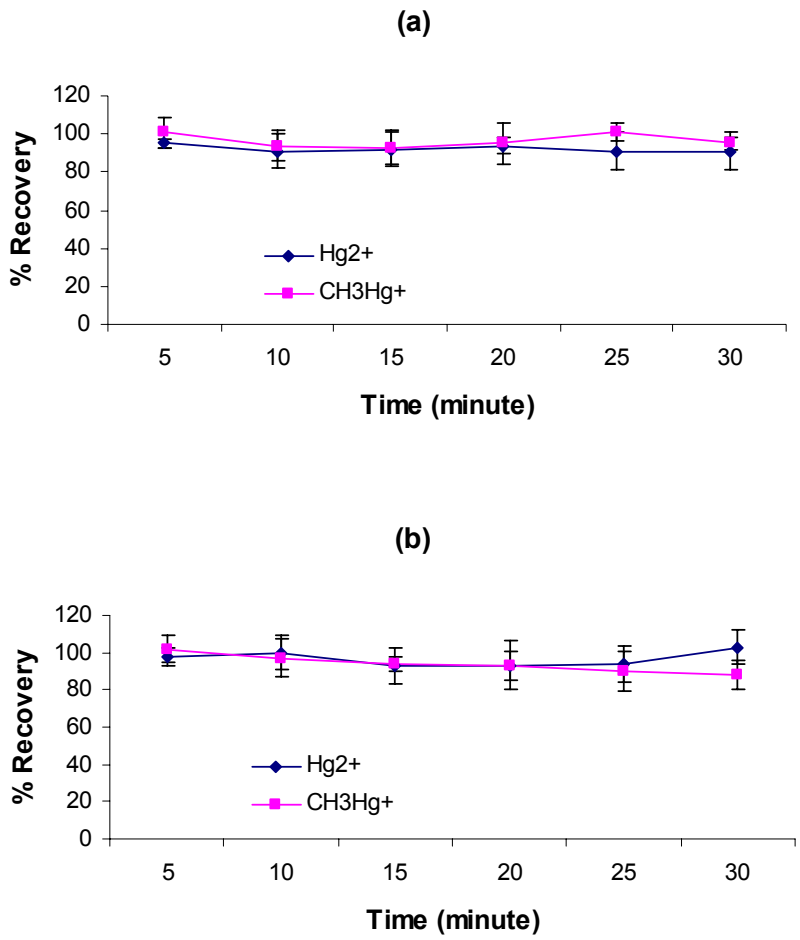


FIGURE 5-4. The effects of optimization of the irradiation time on extraction efficiency and stability of mercury species in (a) spiked blank soil and (b) SRM 2711 (spiked with methylmercury).

Due to the unavailability of a reference material containing both mercury species, SRM 1941a (Organics in Marine Sediment), SRM 2704 (Buffalo River Sediment) and SRM 2709 (San Joaquin Soil), each containing only inorganic mercury, were used for method validation. All of the reference soil samples and SRMs were analyzed directly with the DMA-80 using EPA Method 7473 protocol before extraction with microwave-assisted extraction method. Results are summarized in Table 5-VI. The results obtained

for different soil samples and SRMs from the direct mercury analyses, except for organic mercury in Material-1, are indistinguishable from their corresponding "made-to" or certified value at 95% CL.

TABLE 5-VI. Comparison of Different Analysis Methods for the Validation of the Microwave-Assisted Extraction Results. The Results are Expressed in μg/g at 95% CL, n = 3.

Sample	Certified / "made-to" value	Method 7473 (direct analysis)	HPLC-ICP-MS		
			Hg^{2+}	CH_3Hg^+	Average*
	(μg/g)	(μg/g)	(μg/g)	(μg/g)	(μg/g)
Material-1					
Inorganic Mercury	4.0	4.08 ± 0.16	4.26 ± 0.17	ND	4.26 ± 0.17
Organic Mercuy	4.0	3.58 ± 0.27	ND	3.81 ± 0.20	3.81 ± 0.20
Mixed Mercury	3.0 + 3.0	5.73 ± 0.58	3.02 ± 0.06	2.66 ± 0.07	5.68 ± 0.09
Material-2					
Inorganic Mercury	6.0	6.73 ± 1.04	6.06 ± 0.56	ND	6.06 ± 0.56
Organic Mercury	6.0	5.44 ± 0.62	ND	5.94 ± 0.52	5.94 ± 0.52
Standard Reference Materials					
SRM 1941a	0.5 ± 0.2	0.61 ± 0.02	0.67 ± 0.06	ND	0.67 ± 0.06
SRM 2704	1.44 ± 0.07	1.51 ± 0.05	1.40 ± .08	ND	1.40 ± 0.08
SRM 2709	1.40 ± 0.08	1.46 ± 0.03	1.28 ± 0.12	ND	1.28 ± 0.12

ND = not detectable. *Average = average of Hg^{2+} and CH_3Hg^+ data from HPLC-ICP-MS.

Approximately 1.0 g of each of the soil samples and SRMs was weighed in the microwave extraction vessels, and 10 mL of the 4.0 M HNO_3 and a magnetic stirrer bar was added to each vessel. The vessels were sealed and irradiated at 100 °C for 10 min. Vessels were cooled to room temperature (20-25 °C), and extracts were filtered and stored in the cold room until analyzed. Extracts were analyzed using three different

instruments usually within 2-3 days of extraction. As the results obtained from different instrumental analyses overlapped at 95% CL and were statistically indistinguishable, only the speciation results for different samples are summarized in Table 5-VI. It was found that the method is highly efficient in extracting different mercury species from the variety of matrices tested with approximately 100% recovery. With these optimized conditions it was observed that in the HPLC-ICP-MS analysis, that transformation of methylmercury to inorganic mercury, or vice versa, did not occur.

5.3.7 Application of EPA Method 6800 in the Validation of the Current Extraction Method under Study

EPA Method 6800 (Elemental and Speciated Isotope Dilution Mass Spectrometry, SIDMS) (*25*) was applied as a diagnostic tool to identify analytical biases in the developed microwave-assisted extraction (MAE) method. SIDMS was applied as an alternative detection method to identify the steps that might alter species distribution in the MAE method protocol. Interconversions that occur after spiking are traceable and can be quantitatively corrected by monitoring isotopes in each species (*32*). As SIDMS can measure the concentration of species at the time of spiking, one set of samples was spiked before extraction and another set of samples was spiked after extraction. In order to perform SIDMS analysis, the sample should be spiked with isotopically labeled species. Inorganic mercury labeled with isotopes is available commercially, but methylmercury labeled with isotopes is not available commercially. In this study, ^{199}HgO was used as labeled inorganic mercury and isotopically labeled methylmercury ($CH_3{}^{201}Hg^+$) was synthesized from ^{201}HgO using tetramethyltin. The synthesis and characterization of isotopically labeled methylmercury is described elsewhere (*27*).

TABLE 5-VII. The Deconvoluted Concentration and Transformation of Mercury Species in Material-1 using SIDMS Calculations.

	Deconvoluted Concentration		Interconversion	
	Hg^{2+} (μg/g)	CH_3Hg^+ (μg/g)	Hg^{2+} to CH_3Hg^+ (%)	CH_3Hg^+ to Hg^{2+} (%)
DSBE	3.05 ± 0.12	2.69 ± 0.10	1.3 ± 1.5	0.1 ± 1.4
DSAE	2.94 ± 0.07	2.62 ± 0.09	0.8 ± 1.5	0.7 ± 0.6

DSBE = double spiked before extraction.

DSAE = double spiked after extraction.

Uncertainties are expressed at 95% CL with n = 3.

The reference soil sample (Material-1) containing mixed mercury was used in this study for SIDMS analysis. Approximately 1.0 g portion of the soil sample was weighed into a microwave vessel (two sets of samples in triplicate). One set of the samples was double spiked with $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^+$ and equilibrated for 60 min. Ten mL of 4.0 M HNO_3 was added to each vessel and extracted using the MAE method. After filtering the extract, the second set of samples was then double spiked with the isotopically labeled species and stored in the cold room until analyzed with HPLC-ICP-MS. SIDMS calculations for species transformation corrections (*19*), were performed; the results are summarized in Table 5-VII. It was observed that the deconvoluted concentrations for each species obtained from both set of extractions overlap at the 95% CL and are statistically indistinguishable. Also, results for both species obtained from SIDMS calculations agree with the result obtained from method validation. Moreover, there is no statistically significant distinguishable interconversion using the developed MAE method. Correction of conversion was accomplished using EPA Method 6800 and

did not alter the accuracy of the analysis. EPA describes this diagnostic and quantitative method as being a legally definitive method for such active species. Each species transformation can be tracked and corrected through this procedure. In this study, it was used to monitor and correct for specific protocol steps and was found to provide the quality assurance that was necessary to evaluate the method under study.

5.4 Conclusions

A simple, fast and efficient closed vessel microwave-assisted extraction method for sample preparation and mercury speciation in soils and sediments has been developed in which, after extraction with 4.0 M HNO_3, inorganic and methylmercury concentrations were determined by DMA-80, ICP-MS and HPLC-ICP-MS techniques. The optimum conditions for microwave-assisted extraction of mercury species from soils and sediments were found to be 1.0g sample, 10 mL of 4.0 M HNO_3 and an irradiation time of 10 min at a temperature of 100 °C. The recoveries from the matrices analyzed were similar and quantitative. The proposed microwave-assisted extraction method offers the following advantages: 1) a notable reduction of solvent volume; 2) higher efficiency of extraction achievable under optimized conditions; 3) considerable time savings in the procedure of sample preparation; 4) no loss or interconversion of the target species; and, 5) the possibility of simultaneously extracting up to ten samples, resulting in increased sample output compared with conventional extraction techniques. Since the extracts are analyzed with HPLC-ICP-MS for speciation, there is no need for additional steps, such as clean-up or derivatization. Results obtained in the analyses of two types of specifically prepared reference soils and three standard reference materials (soils and

sediments) containing inorganic and methylmercury in an order of magnitude range verified the simplicity, efficiency, precision and accuracy of the proposed microwave-assisted extraction method for mercury speciation in soils and sediments. Moreover, the application of the EPA Method 6800 as a diagnostic tool significantly enhances the reliability of the proposed microwave-assisted extraction method.

5.5 References

(1) Mason, R. P.; Benoit, J. M. In: *Organometallic Compounds in the Environment*, Craig, P.J.; 2nd Ed.; John Wiley & Sons Ltd: England, **2003**, pp 57-99.

(2) Rodil, R.; Carro, A.M.; Lorenzo, R.A.; Abuin, M.; and Cela, R. *J. Chromatogr. A,* **2002**, *963*, 313-323.

(3) Ramalhosa, E.; Rio-Segade, S.; Pereira, E.; Vale, C.; Duarte, A. *J. Anal. At. Spectrom.* **2001**, *16*, 643-647.

(4) Westöö, G. *Acta. Chem. Scand*. **1967**, *20*, 1790-1800.

(5) Westöö, G. *Acta. Chem. Scand*. **1968**, *22*, 2277-2280.

(6) Hintelmann, H.; Wilken R.D. *Appl. Organomet. Chem*. **1993**, *7(3)*, 173-180.

(7) Quevauviller, P.; Donard, O.F.X.; Wasserman, J.C.; Martin, F.M.; Schneider, J. *Appl. Organomet. Chem*. **1992**, *6*, 221.

(8) Bloom, N.S. *Can. J. Fish. Aquat. Sci*. **1989**, *46*, 1131.

(9) Horvat, M.; Bloom, N.S.; Liang, L. *Anal. Chim. Acta* **1993**, *281(1)*, 135-152.

(10) Horvat, M.; Mandic, V.; Liang, L.; Bloom, N.S.; Padberg, S.; Lee, L.-H.; Hintelmann, H.; Benoit, J. *Appl. Organomet. Chem*. **1994**, *8*, 533.

(11) Horvat, M.; May, K.; Stoeppler, M.; Byrne, A.R. *Appl. Organomet. Chem*. **1988**, *2*, 515.

(12) Han, Y.; Kingston, H. M.; Boylan, H. M.; Rahman, G. M. M.; Shah, S.; Richter, R. C.; Link, D. D.; Bhandari, S. *Anal. Bioanal. Chem*. **2003**, *375(3)*, 428-436.

(13) Bloom, N. S.; Colman, J. A.; Barber, L. *Fresenius' J. Anal. Chem*. **1997**, *358(3)*, 371-377.

(14) Hintelmann, H.; Hempel, M.; Wilken, R. D. *Environ. Sci. Technol*. **1995**, *29*, 1845.

(15) Sanchez, J. E.; Sanz-Medel, A. *Talanta* **1998**, *47*, 509-524.

(16) Emteborg, H.; Bjorklund, E.; Odman, F.; Karlsson, L.; Mathiasson, L.; Frech, W.; Baxter, D. C. *Analyst* **1996**, *121*, 19.

(17) Hintelmann, H.; Falter, R.; Ilgen, G.; Evans, R. D. *Fresenius' J. Anal. Chem*. **1997**, *358*, 363-370.

(18) Hammerschmidt, C. R.; Fitzgerald, W. F. *Anal. Chem*. **2001**, *73*, 5930-5936.

(19) Rahman, G. M. M.; Kingston, H. M. S. *Anal. Chem*. **2004**, *76(13)*, 3548-3555.

(20) Vazquez, M. J.; Carro, A. M.; Lorenzo, R. A.; Cela, R. *Anal. Chem*. **1997**, *69*, 221-225.

(21) Vazquez, M. J.; Abuin, M.; Carro, A. M.; Lorenzo, R. A.; Cela, R. *Chemosphere* **1999**, *39(7)*, 1211-1224.

(22) Tseng, C. M.; Diego, A. D.; Martin, F. M.; Donard, O. F. X. *J. Anal. At. Spectrom*. **1997**, *12*, 629-635.

(23) Tseng, C. M.; Diego, A. D.; Martin, F. M.; Donard, O. F. X. *J. Anal. At. Spectrom*. **1997**, *12*, 743-750.

(24) Tseng, C. M.; Schmitt, V. O.; Diego, A. D.; Donard, O. F. X. *Am. Environ. Lab*. **1998**, *10*, 26-27.

(25) US EPA Method 6800. In *Solid Waste Manual-846, Update IV*, US Government Printing Office (GPO), Washington DC, **2000**.

(26) Rahman, G. M. M.; Kingston, H. M. S.; Bhandari, S. *Appl. Organomet. Chem*. **2003**, *17(12)*, 913-920.

(27) Kingston, H. M. S.; Walter, P. J.; Engelhart, W. G.; Parsons, P. J. In *Microwave Enhanced Chemistry: Fundamentals, Sample Preparation, and Applications*; Kingston, H. M. S.; Haswell, S. J., Eds.; American Chemical Society: Washington DC, **1997**; pp 697-745.

(28) US EPA Method 7473. In *Test Method for Evaluating Solid Waste, Physical/Chemical Methods SW 846, Update IVA*, US GPO: Washington DC, **2000**.

(29) Boylan, H. M.; Kingston, H. M. S. *Am. Lab*. **1998**, *30(19)*, 26.

(30) Wilken, R. D.; Hintelmann, H. *Water, Air, Soil Pollut*. **1991**, *56*, 427-437.

(31) Rahman, G. M. M.; Kingston, H. M. S.; Kern, J. C.; Yang, S. Y.; Hartwell, S. W.; Anderson, R. *Appl. Organomet. Chem*. **2004** (Submitted for publication).

(32) Dengwei, H.; Kingston, H. M.; Larget, B. In: *Comprehensive Analytical Chemistry*, Caruso, J. A.; Sutton, K. L.; Ackley, K. L.; Eds.; *Vol. 33*. Elemental Speciation: New Approaches for Trace Element Analysis. Elsevier: New York, **2000**, pp 277-313.

Chapter 6

Derivation of Generic Equation for SIDMS Analysis and Correction of 1, 2 and 3 Species Conversion Simultaneously

6.1 Introduction

The reactivity, toxicity and bioavailability of trace metals and organometallic compounds in soil, sediment, aquatic, effluent and flue gas are determined from the representative amount of the species present, rather than the total amount of the element. The determination of chemical species rather than the total elemental concentration is now considered to be a mature field of analytical chemistry. In addition, speciation analysis is more relevant to decision-making for environmental protection and remediation protocols. Accurate description of species distribution in any environmental sample helps cleanup programs to be more cost-effective.

The speciation analysis procedures are not that straightforward, unlike total elemental determinations. The primary requirement for speciation analysis is to preserve the speciation of the analyte in any given sample throughout the analytical procedure. The sample preparation is somewhat straightforward for aqueous samples, which needs either filtration or centrifugation. But it is more difficult for solid samples, which require at least one extraction step using either acidic, alkaline, or organic solvents, or a combination of those to bring the target analyte into solution. The nature of the extraction protocol employed may alter the speciation of the analyte in the sample during extraction, separation or analysis. Therefore, it is difficult to determine the exact true amount of the target species present in the sample before extraction. Such problems lead to biases and inaccuracies, which limit the application of these results in environmental

decision-making and severely reduce or eliminate their legal defensibility. Traditional methodologies do not provide defensible speciation results due to species transformations (*1-10*), and the method-induced errors can be as high as 50%.

In order to overcome the aforementioned problems, researchers are using conventional isotope dilution mass spectrometry (IDMS), a definitive analytical technique, for determining both total elemental and species specific concentrations (*3,11-20*). The IDMS can provide superior precision and accuracy over the conventional external calibration techniques, provided that the following prerequisites are met: (i) more than one, interference free, stable isotope is available for isotope ratio measurement; (ii) an isotopically enriched analogue of the analyte is available; (iii) complete equilibration between the spike and sample isotopes is achieved before or during extraction or measurement; (iv) the mass fraction concentration and isotopic abundances of the natural material and spike are well established; and (v) isotopes of the spike and sample are chemically stable (*21*). Any analyte formation or decomposition will give biased results. And, the IDMS measurement is unable either to make corrections for species transformations or to trace their source. Only the speciated isotope dilution mass spectrometry (SIDMS) (*22-27*), originally developed and validated for chromium speciation, can make the necessary corrections and trace the source of interconversion. Therefore, the purpose of this study is to extend the applicability of SIDMS technique to other environmental speciation systems containing two or three inter-related species.

The SIDMS equations for mercury speciation are based on understandings gleaned from chromium speciation equations and were utilized and validated (Chapter 4

and Chapter 5). Since for any target element it is very difficult to generate SIDMS equations and to construct corresponding Microsoft Excel worksheet for calculations, it is our objective to provide SIDMS generic equations for one, two and three species reflecting unidirectional and bidirectional transformations, and to construct generic Microsoft Excel worksheet applicable for any element in the periodic table, and to validate them using at least chromium and mercury speciation analyses data.

6.2 Bidirectional Transformation

6.2.1 Algorithms, Assumptions and Calculations

6.2.1.1 Two Species

Let's consider an environmental or biological sample containing two species of Z, and the species are K and L, with concentrations of C_x^K (μmol/g) and C_x^L (μmol/g), respectively. Weigh W_x gram of the sample, followed by the addition of W_s^K gram of ^{F}K spike (species K enriched with isotope "F") and W_s^L gram of ^{G}L spike (species L enriched with isotope "G") into the sample. After spiking, the sample contains $^{F}A_x C_x^K W_x + {}^{F}A_s^K C_s^K W_s^K$ μmol of ^{F}Z as K and $^{F}A_x C_x^L W_x + {}^{F}A_s^L C_s^L W_s^L$ μmol of ^{F}Z as L, where A represents the isotopic abundance.

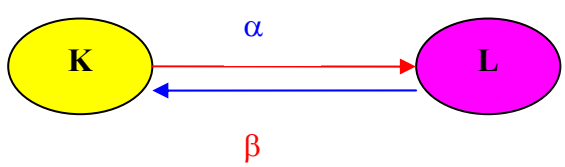


FIGURE 6-1 Schematic of bidirectional transformation for two species system. [α - fraction of species K converts to species L; β - fraction of species L converts to species K].

If these two species undergo bidirectional transformations after the spike isotopes equilibrate with the sample isotopes, the fraction of K that converts to L is α and the

fraction of L that converts to K is β (Figure 6-1). At any given time, the total amount of ^{F}Z in K form thus changes to

$\left({}^{F}A_x C_x^K W_x + {}^{F}A_s^K C_s^K W_s^K\right)(1-\alpha) + \left({}^{F}A_x C_x^L W_x + {}^{F}A_s^L C_s^L W_s^L\right)\beta$ after the interconversions between K and L.

Similarly, the total amount of ^{R}Z in K form is

$\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)(1-\alpha) + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\beta$.

Therefore, the expression for the isotope ratio of ^{F}Z to ^{R}Z in the K, $R_{F/R}^{K}$, can be constructed as Eq. 6.1. Following the similar procedure, we can construct equations Eq. 6.2 to Eq. 6.4.

$$R_{F/R}^{K} = \frac{\left({}^{F}A_x C_x^K W_x + {}^{F}A_s^K C_s^K W_s^K\right)(1-\alpha) + \left({}^{F}A_x C_x^L W_x + {}^{F}A_s^L C_s^L W_s^L\right)\beta}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)(1-\alpha) + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\beta} \qquad \text{Eq. 6.1}$$

$$R_{G/R}^{K} = \frac{\left({}^{G}A_x C_x^K W_x + {}^{G}A_s^K C_s^K W_s^K\right)(1-\alpha) + \left({}^{G}A_x C_x^L W_x + {}^{G}A_s^L C_s^L W_s^L\right)\beta}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)(1-\alpha) + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\beta} \qquad \text{Eq. 6.2}$$

$$R_{F/R}^{L} = \frac{\left({}^{F}A_x C_x^K W_x + {}^{F}A_s^K C_s^K W_s^K\right)\alpha + \left({}^{F}A_x C_x^L W_x + {}^{F}A_s^L C_s^L W_s^L\right)(1-\beta)}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)\alpha + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)(1-\beta)} \qquad \text{Eq. 6.3}$$

$$R_{G/R}^{L} = \frac{\left({}^{G}A_x C_x^K W_x + {}^{G}A_s^K C_s^K W_s^K\right)\alpha + \left({}^{G}A_x C_x^L W_x + {}^{G}A_s^L C_s^L W_s^L\right)(1-\beta)}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)\alpha + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)(1-\beta)} \qquad \text{Eq. 6.4}$$

where,

$R_{F/R}^{K}$ is the measured isotope ratio of ^{F}Z to ^{R}Z of K in the spiked sample

$R_{G/R}^{K}$ is the measured isotope ratio of ^{G}Z to ^{R}Z of K in the spiked sample

$R_{F/R}^{L}$ is the measured isotope ratio of ^{F}Z to ^{R}Z of L in the spiked sample

$R_{G/R}^{L}$ is the measured isotope ratio of ^{G}Z to ^{R}Z of L in the spiked sample

$^{F}A_x$ is the natural relative isotopic abundance of ^{F}Z in the sample

$^{R}A_x$ is the natural relative isotopic abundance of ^{R}Z in the sample

$^{G}A_x$ is the natural relative isotopic abundance of ^{G}Z in the sample

$^{F}A_s^K$ is the relative isotopic abundance of ^{F}Z in the ^{F}K spike

$^{R}A_s^K$ is the relative isotopic abundance of ^{R}Z in the ^{F}K spike

$^{G}A_s^K$ is the relative isotopic abundance of ^{G}Z in the ^{F}K spike

$^{F}A_s^L$ is the relative isotopic abundance of ^{F}Z in the ^{G}L spike

$^{R}A_s^L$ is the relative isotopic abundance of ^{R}Z in the ^{G}L spike

$^{G}A_s^L$ is the relative isotopic abundance of ^{G}Z in the ^{G}L spike

C_x^K is the concentration of *K* in the sample (μmol/g, unknown)

C_x^L is the concentration of *L* in the sample (μmol/g, unknown)

W_x is the weight of the sample (g)

C_s^K is the concentration of *K* in the ^{F}K spike (μmol/g)

W_s^K is the weight of the ^{F}K spike (g)

C_s^L is the concentration of *L* in the ^{G}L spike (μmol/g)

W_s^L is the weight of the ^{G}L spike (g)

α is the proportion of *K* converted to *L* after spiking (unknown)

β is the proportion of *L* converted to *K* after spiking (unknown)

From above equations, it is found that there are four unknowns in four equations. In order to make the expression simpler, assume that

$$C_x^K W_x = N_x^K, C_x^L W_x = N_x^L, C_S^K W_S = N_S^K, C_S^L W_S = N_S^L$$

At the beginning of the first iteration, we can assign N_X^L and α any values, for example, we assign both of them as 0. Now we need to determine the values for N_X^K and β. After careful derivations of Eqs 6.1 and 6.2, we can get the following equations:

$$\begin{cases} (1-\alpha)\left(R_{F/R}^K\,{}^R A_X - {}^F A_X\right)N_X^K + \left[R_{F/R}^K\left({}^R A_X N_X^L + {}^R A_S^L N_S^L\right) - \left({}^F A_X N_X^L + {}^F A_S^L N_S^L\right)\right]\beta \\ = \left(-R_{F/R}^K\,{}^R A_S^K + {}^F A_S^K\right)N_S^K(1-\alpha) \\ \\ (1-\alpha)\left(R_{G/R}^K\,{}^R A_X - {}^G A_X\right)N_X^K + \left[R_{G/R}^K\left({}^R A_X N_X^L + {}^R A_S^L N_S^L\right) - \left({}^G A_X N_X^L + {}^G A_S^L N_S^L\right)\right]\beta \\ = \left(-R_{G/R}^K\,{}^R A_S^K + {}^G A_S^K\right)N_S^K(1-\alpha) \end{cases}$$

We can rewrite the above equations as:

$$\begin{cases} \mathrm{A}_1 N_X^K + \mathrm{B}_1\beta = \mathrm{C}_1 \\ \\ \mathrm{A}_2 N_X^K + \mathrm{B}_2\beta = \mathrm{C}_2 \end{cases}$$

Where,

$$\begin{aligned} A_1 &= (1-\alpha)(R_{F/R}^K\,{}^R A_x - {}^F A_x) \\ B_1 &= [R_{F/R}^K({}^R A_x N_x^L + {}^R A_s^L N_s^L) - ({}^F A_x N_x^L + {}^F A_s^L N_s^L)] \\ C_1 &= (1-\alpha)N_s^K({}^F A_s^K - R_{F/R}^K\,{}^R A_s^K) \end{aligned}$$

and

$$\begin{aligned} A_2 &= (1-\alpha)(R_{G/R}^K\,{}^R A_x - {}^G A_x) \\ B_2 &= [R_{G/R}^K({}^R A_x N_x^L + {}^R A_s^L N_s^L) - ({}^G A_x N_x^L + {}^G A_s^L N_s^L)] \\ C_2 &= (1-\alpha)N_s^K({}^G A_s^K - R_{G/R}^K\,{}^R A_s^K) \end{aligned}$$

The solutions are

$$N_X^K = \frac{\begin{vmatrix} C_1 & B_1 \\ C_2 & B_2 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 \\ A_2 & B_2 \end{vmatrix}} \text{ and } \beta = \frac{\begin{vmatrix} A_1 & C_1 \\ A_2 & C_2 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 \\ A_2 & B_2 \end{vmatrix}}$$

or $N_x^K = \dfrac{(C_1B_2 - C_2B_1)}{(A_1B_2 - A_2B_1)}$ and $\beta = \dfrac{(A_1C_2 - A_2C_1)}{(A_1B_2 - A_2B_1)}$

Now we can use these two values (N_X^K and β) in Eqs 6.3 and 6.4 to solve for N_X^L and α .

The following two equations are obtained from rearrangement of Eqs 6.3 and 6.4.

$$\begin{cases} (1-\beta)\left(R_{F/R}^L\,{}^R A_X - {}^F A_X\right)N_X^L + \left[R_{F/R}^L\left({}^R A_X N_X^K + {}^R A_S^K N_S^K\right) - \left({}^F A_X N_X^K + {}^F A_S^K N_S^K\right)\right]\alpha \\ = \left(-R_{F/R}^L\,{}^R A_S^L + {}^F A_S^L\right)N_S^L(1-\beta) \\ \\ (1-\beta)\left(R_{G/R}^L\,{}^R A_X - {}^G A_X\right)N_X^L + \left[R_{G/R}^L\left({}^R A_X N_X^K + {}^R A_S^K N_S^K\right) - \left({}^G A_X N_X^K + {}^G A_S^K N_S^K\right)\right]\alpha \\ = \left(-R_{G/R}^L\,{}^R A_S^L + {}^G A_S^L\right)N_S^L(1-\beta) \end{cases}$$

We can rewrite them as:

$$\begin{cases} A_3 N_X^L + B_3\alpha = C_3 \\ \\ A_4 N_X^L + B_4\alpha = C_4 \end{cases}$$

Where,

$$A_3 = (1-\beta)(R_{F/R}^L\,{}^R A_x - {}^F A_x)$$
$$B_3 = [R_{F/R}^L({}^R A_x N_x^K + {}^R A_s^K N_s^K) - ({}^F A_x N_x^K + {}^F A_s^K N_s^K)]$$
$$C_3 = (1-\beta)N_s^L({}^F A_s^L - R_{F/R}^L\,{}^R A_s^L)$$

and

$$A_4 = (1-\beta)(R_{G/R}^L\,{}^R A_x - {}^G A_x)$$
$$B_4 = [R_{G/R}^L({}^R A_x N_x^K + {}^R A_s^K N_s^K) - ({}^G A_x N_x^K + {}^G A_s^K N_s^K)]$$
$$C_4 = (1-\beta)N_s^L({}^G A_s^L - R_{G/R}^L\,{}^R A_s^L)$$

again

$$N_X^L = \frac{\begin{vmatrix} C_3 & B_3 \\ C_4 & B_4 \end{vmatrix}}{\begin{vmatrix} A_3 & B_3 \\ A_4 & B_4 \end{vmatrix}} \quad \text{and } \alpha = \frac{\begin{vmatrix} A_3 & C_3 \\ A_4 & C_4 \end{vmatrix}}{\begin{vmatrix} A_3 & B_3 \\ A_4 & B_4 \end{vmatrix}}$$

$$\text{or } N_x^L = \frac{(C_3B_4 - C_4B_3)}{(A_3B_4 - A_4B_3)} \qquad \text{and } \alpha = \frac{(A_3C_4 - A_4C_3)}{(A_3B_4 - A_4B_3)}$$

Repeating the calculation, the variables N_X^K, N_X^L, α and β will converge to constant values, and these values are the solution of the equations.

Note: *Results should be discarded when ($\alpha + \beta$) > 80% because the interconversion will be too extensive and will cause inaccuracy and imprecision in the corrections. Samples should be re-spiked with isotopically enriched spikes and analyzed. The sample preservation conditions should be improved to retard conversion of the species.*

6.2.1.2 Three Species

Let's consider an environmental or biological sample containing three species of Z, and the species are *K, L*, and *M* with concentrations of C_x^K (μmol/g), C_x^L (μmol/g), and C_x^M (μmol/g), respectively. Weigh W_x gram of the sample, followed by the addition of W_s^K gram of ^{F}K spike (species *K* enriched with isotope "F"), W_s^L gram of ^{G}L spike (species *L* enriched with isotope "G"), and W_s^M gram of ^{H}M spike (species *M* enriched with isotope "H") into the sample. The total number of moles of each species present in the mixture at the end of the extraction step can be calculated taking into account the

original amount of the species present, their conversion, and the amount of species formed from the conversion of other species. For a polyisotopic element, the mass balance can be performed for each considered isotope. In this case, isotopes F, G, H, and R will be measured, where F, G and H are the reference isotopes in the spikes, and R is the reference isotope in the sample. If there is no conversion or transformation among the target species, then after spiking the sample should contain

$^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K$ μmol of ^{R}Z as *K*,

$^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L$ μmol of ^{R}Z as *L*, and

$^{R}A_x C_x^M W_x + {}^{R}A_s^M C_s^M W_s^M$ μmol of ^{R}Z as *M,*

where '*A*' represents the isotopic abundance. But if these three species undergo bidirectional transformations according to the following diagram after the spike isotopes equilibrate with the sample isotopes, then the total number of moles of each species will be a different number.

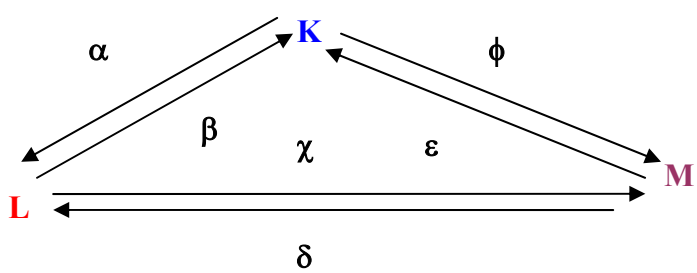


FIGURE 6-2. Bidirectional transformation for three species system. [α = the fraction of *K* converts to *L*; β = the fraction of *L* converts to *K*; χ = the fraction of *L* converts to *M*; δ = fraction of *M* converts to *L*; ε = fraction of *M* converts to *K*; and ϕ = fraction of *K* converts to *M*].

After interconversions among *K, L* and M, the total amount of ^{R}Z in *K* form thus becomes

$$\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)(1-\alpha-\phi) + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\beta + \left({}^{R}A_x C_x^M W_x + {}^{R}A_s^M C_s^M W_s^M\right)\varepsilon .$$

Similarly, the total amount of ^{R}Z in *L* form becomes

$$\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)\alpha + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)(1-\beta-\chi) + \left({}^{R}A_x C_x^M W_x + {}^{R}A_s^M C_s^M W_s^M\right)\delta$$

and the total amount of ^{R}Z in *M* form becomes

$$\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)\phi + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\chi + \left({}^{R}A_x C_x^M W_x + {}^{R}A_s^M C_s^M W_s^M\right)(1-\delta-\varepsilon) .$$

Equations for isotopes ^{F}Z, ^{G}Z and ^{H}Z can be constructed similar to the three equations for the ^{R}Z isotope. These twelve mass balance equations can be transformed into nine isotope ratio equations by dividing the equations obtained for ^{F}Z, ^{G}Z and ^{H}Z isotopes by the equations obtained for ^{R}Z isotope.

For species *K*:

$$R_{F/R}^K = \frac{\left({}^{F}A_x C_x^K W_x + {}^{F}A_s^K C_s^K W_s^K\right)(1-\alpha-\phi) + \left({}^{F}A_x C_x^L W_x + {}^{F}A_s^L C_s^L W_s^L\right)\beta + \left({}^{F}A_x C_x^M W_x + {}^{F}A_s^M C_s^M W_s^M\right)\varepsilon}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)(1-\alpha-\phi) + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\beta + \left({}^{R}A_x C_x^M W_x + {}^{R}A_s^M C_s^M W_s^M\right)\varepsilon}$$

$$R_{G/R}^K = \frac{\left({}^{G}A_x C_x^K W_x + {}^{G}A_s^K C_s^K W_s^K\right)(1-\alpha-\phi) + \left({}^{G}A_x C_x^L W_x + {}^{G}A_s^L C_s^L W_s^L\right)\beta + \left({}^{G}A_x C_x^M W_x + {}^{G}A_s^M C_s^M W_s^M\right)\varepsilon}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)(1-\alpha-\phi) + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\beta + \left({}^{R}A_x C_x^M W_x + {}^{R}A_s^M C_s^M W_s^M\right)\varepsilon}$$

$$R_{H/R}^K = \frac{\left({}^{H}A_x C_x^K W_x + {}^{H}A_s^K C_s^K W_s^K\right)(1-\alpha-\phi) + \left({}^{H}A_x C_x^L W_x + {}^{H}A_s^L C_s^L W_s^L\right)\beta + \left({}^{H}A_x C_x^M W_x + {}^{H}A_s^M C_s^M W_s^M\right)\varepsilon}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)(1-\alpha-\phi) + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\beta + \left({}^{R}A_x C_x^M W_x + {}^{R}A_s^M C_s^M W_s^M\right)\varepsilon}$$

For species *L*:

$$R_{F/R}^L = \frac{\left({}^{F}A_x C_x^K W_x + {}^{F}A_s^K C_s^K W_s^K\right)\alpha + \left({}^{F}A_x C_x^L W_x + {}^{F}A_s^L C_s^L W_s^L\right)(1-\beta-\chi) + \left({}^{F}A_x C_x^M W_x + {}^{F}A_s^M C_s^M W_s^M\right)\delta}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)\alpha + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)(1-\beta-\chi) + \left({}^{R}A_x C_x^M W_x + {}^{R}A_s^M C_s^M W_s^M\right)\delta}$$

$$R^{L}_{G/R} = \frac{\left({}^{G}A_x C^K_x W_x + {}^{G}A^K_s C^K_s W^K_s\right)\alpha + \left({}^{G}A_x C^L_x W_x + {}^{G}A^L_s C^L_s W^L_s\right)(1-\beta-\chi) + \left({}^{G}A_x C^M_x W_x + {}^{G}A^M_s C^M_s W^M_s\right)\delta}{\left({}^{R}A_x C^K_x W_x + {}^{R}A^K_s C^K_s W^K_s\right)\alpha + \left({}^{R}A_x C^L_x W_x + {}^{R}A^L_s C^L_s W^L_s\right)(1-\beta-\chi) + \left({}^{R}A_x C^M_x W_x + {}^{R}A^M_s C^M_s W^M_s\right)\delta}$$

$$R^{L}_{H/R} = \frac{\left({}^{H}A_x C^K_x W_x + {}^{H}A^K_s C^K_s W^K_s\right)\alpha + \left({}^{H}A_x C^L_x W_x + {}^{H}A^L_s C^L_s W^L_s\right)(1-\beta-\chi) + \left({}^{H}A_x C^M_x W_x + {}^{H}A^M_s C^M_s W^M_s\right)\delta}{\left({}^{R}A_x C^K_x W_x + {}^{R}A^K_s C^K_s W^K_s\right)\alpha + \left({}^{R}A_x C^L_x W_x + {}^{R}A^L_s C^L_s W^L_s\right)(1-\beta-\chi) + \left({}^{R}A_x C^M_x W_x + {}^{R}A^M_s C^M_s W^M_s\right)\delta}$$

For species *M*:

$$R^{M}_{F/R} = \frac{\left({}^{F}A_x C^K_x W_x + {}^{F}A^K_s C^K_s W^K_s\right)\phi + \left({}^{F}A_x C^L_x W_x + {}^{F}A^L_s C^L_s W^L_s\right)\chi + \left({}^{F}A_x C^M_x W_x + {}^{F}A^M_s C^M_s W^M_s\right)(1-\delta-\varepsilon)}{\left({}^{R}A_x C^K_x W_x + {}^{R}A^K_s C^K_s W^K_s\right)\phi + \left({}^{R}A_x C^L_x W_x + {}^{R}A^L_s C^L_s W^L_s\right)\chi + \left({}^{R}A_x C^M_x W_x + {}^{R}A^M_s C^M_s W^M_s\right)(1-\delta-\varepsilon)}$$

$$R^{M}_{G/R} = \frac{\left({}^{G}A_x C^K_x W_x + {}^{G}A^K_s C^K_s W^K_s\right)\phi + \left({}^{G}A_x C^L_x W_x + {}^{G}A^L_s C^L_s W^L_s\right)\chi + \left({}^{G}A_x C^M_x W_x + {}^{G}A^M_s C^M_s W^M_s\right)(1-\delta-\varepsilon)}{\left({}^{R}A_x C^K_x W_x + {}^{R}A^K_s C^K_s W^K_s\right)\phi + \left({}^{R}A_x C^L_x W_x + {}^{R}A^L_s C^L_s W^L_s\right)\chi + \left({}^{R}A_x C^M_x W_x + {}^{R}A^M_s C^M_s W^M_s\right)(1-\delta-\varepsilon)}$$

$$R^{M}_{H/R} = \frac{\left({}^{H}A_x C^K_x W_x + {}^{H}A^K_s C^K_s W^K_s\right)\phi + \left({}^{H}A_x C^L_x W_x + {}^{H}A^L_s C^L_s W^L_s\right)\chi + \left({}^{H}A_x C^M_x W_x + {}^{H}A^M_s C^M_s W^M_s\right)(1-\delta-\varepsilon)}{\left({}^{R}A_x C^K_x W_x + {}^{R}A^K_s C^K_s W^K_s\right)\phi + \left({}^{R}A_x C^L_x W_x + {}^{R}A^L_s C^L_s W^L_s\right)\chi + \left({}^{R}A_x C^M_x W_x + {}^{R}A^M_s C^M_s W^M_s\right)(1-\delta-\varepsilon)}$$

where,

$R^{K}_{F/R}$ is the measured isotope ratio of ^{F}Z to ^{R}Z of *K* in the spiked sample

$R^{K}_{G/R}$ is the measured isotope ratio of ^{G}Z to ^{R}Z of *K* in the spiked sample

$R^{K}_{H/R}$ is the measured isotope ratio of ^{H}Z to ^{R}Z of *K* in the spiked sample

$R^{L}_{F/R}$ is the measured isotope ratio of ^{F}Z to ^{R}Z of *L* in the spiked sample

$R^{L}_{G/R}$ is the measured isotope ratio of ^{G}Z to ^{R}Z of *L* in the spiked sample

$R^{L}_{H/R}$ is the measured isotope ratio of ^{H}Z to ^{R}Z of *L* in the spiked sample

$R^{M}_{F/R}$ is the measured isotope ratio of ^{F}Z to ^{R}Z of *M* in the spiked sample

$R^{M}_{G/R}$ is the measured isotope ratio of ^{G}Z to ^{R}Z of *M* in the spiked sample

$R^{M}_{H/R}$ is the measured isotope ratio of ^{H}Z to ^{R}Z of *M* in the spiked sample

${}^{R}A_x$ is the natural relative isotopic abundance of ^{R}Z in the sample

$^{F}A_x$ is the natural relative isotopic abundance of ^{F}Z in the sample

$^{G}A_x$ is the natural relative isotopic abundance of ^{G}Z in the sample

$^{H}A_x$ is the natural relative isotopic abundance of ^{H}Z in the sample

$^{R}A_s^K$ is the relative isotopic abundance of ^{R}Z in the ^{F}K spike

$^{F}A_s^K$ is the relative isotopic abundance of ^{F}Z in the ^{F}K spike

$^{G}A_s^K$ is the relative isotopic abundance of ^{G}Z in the ^{F}K spike

$^{H}A_s^K$ is the relative isotopic abundance of ^{H}Z in the ^{F}K spike

$^{R}A_s^L$ is the relative isotopic abundance of ^{R}Z in the ^{G}L spike

$^{F}A_s^L$ is the relative isotopic abundance of ^{F}Z in the ^{G}L spike

$^{G}A_s^L$ is the relative isotopic abundance of ^{G}Z in the ^{G}L spike

$^{H}A_s^L$ is the relative isotopic abundance of ^{H}Z in the ^{G}L spike

$^{R}A_s^M$ is the relative isotopic abundance of ^{R}Z in the ^{H}M spike

$^{F}A_s^M$ is the relative isotopic abundance of ^{F}Z in the ^{H}M spike

$^{G}A_s^M$ is the relative isotopic abundance of ^{G}Z in the ^{H}M spike

$^{H}A_s^M$ is the relative isotopic abundance of ^{H}Z in the ^{H}M spike

C_x^K is the concentration of *K* in the sample (μmol/g, unknown)

C_x^L is the concentration of *L* in the sample (μmol/g, unknown)

C_x^M is the concentration of *M* in the sample (μmol/g, unknown)

W_x is the weight of the sample (g)

C_s^K is the concentration of *K* in the ^{F}K spike (μmol/g)

W_s^K is the weight of the ^{F}K spike (g)

C_s^L is the concentration of *L* in the ^{G}L spike (μmol/g)

W_s^L is the weight of the ^{G}L spike (g)

C_s^M is the concentration of *M* in the ^{H}M spike (μmol/g)

W_s^M is the weight of the ^{H}M spike (g)

α is the proportion of *K* converted to *L* after spiking (unknown)

ϕ is the proportion of *K* converted to *M* after spiking (unknown)

β is the proportion of *L* converted to *K* after spiking (unknown)

χ is the proportion of *L* converted to *M* after spiking (unknown)

δ is the proportion of *M* converted to *L* after spiking (unknown)

ε is the proportion of *M* converted to *K* after spiking (unknown)

From above equations, it is observed that there are nine unknowns to solve using six simultaneous equations. These equations and their manipulation can be simplified using the following substitutions:

$$C_x^K W_x = N_x^K; C_x^L W_x = N_x^L; \mathrm{C}_\mathrm{x}^\mathrm{M} W_x = N_x^M; C_S^K W_S^K = N_S^K; C_S^L W_S^L = N_S^L; C_S^M W_S^M = N_S^M$$

At the beginning of the first iteration, we can assign N_X^L, N_X^M, α, ϕ, χ and δ any values, for example, we assign 0 for all of them. Now we need to know the values for N_X^K, β and ε.

After careful derivation of three equations for K species, we can get the following equations for species K:

$$\begin{cases} (1-\alpha-\phi)\left(R^K_{F/R}\,{}^R A_X - {}^F A_X\right)N^K_X + \beta\left[R^K_{F/R}\left({}^R A_X N^L_X + {}^R A^L_S N^L_S\right) - \left({}^F A_X N^L_X + {}^F A^L_S N^L_S\right)\right] \\ + \varepsilon\left[R^K_{F/R}\left({}^R A_X N^M_X + {}^R A^M_S N^M_S\right) - \left({}^F A_X N^M_X + {}^F A^M_S N^M_S\right)\right] = (1-\alpha-\phi)\left({}^F A^K_S - R^K_{F/R}\,{}^R A^K_S\right)N^K_S \\ \\ (1-\alpha-\phi)\left(R^K_{G/R}\,{}^R A_X - {}^G A_X\right)N^K_X + \beta\left[R^K_{G/R}\left({}^R A_X N^L_X + {}^R A^L_S N^L_S\right) - \left({}^G A_X N^L_X + {}^G A^L_S N^L_S\right)\right] \\ + \varepsilon\left[R^K_{G/R}\left({}^R A_X N^M_X + {}^R A^M_S N^M_S\right) - \left({}^G A_X N^M_X + {}^G A^M_S N^M_S\right)\right] = (1-\alpha-\phi)\left({}^G A^K_S - R^K_{G/R}\,{}^R A^K_S\right)N^K_S \\ \\ (1-\alpha-\phi)\left(R^K_{H/R}\,{}^R A_X - {}^H A_X\right)N^K_X + \beta\left[R^K_{H/R}\left({}^R A_X N^L_X + {}^R A^L_S N^L_S\right) - \left({}^H A_X N^L_X + {}^H A^L_S N^L_S\right)\right] \\ + \varepsilon\left[R^K_{H/R}\left({}^R A_X N^M_X + {}^R A^M_S N^M_S\right) - \left({}^H A_X N^M_X + {}^H A^M_S N^M_S\right)\right] = (1-\alpha-\phi)\left({}^H A^K_S - R^K_{H/R}\,{}^R A^K_S\right)N^K_S \end{cases}$$

We can rewrite these equations as:

$$\begin{cases} \mathrm{A}_1 N^K_X + \mathrm{B}_1\beta + C_1\varepsilon = \mathrm{D}_1 \\ \mathrm{A}_2 N^K_X + \mathrm{B}_2\beta + C_2\varepsilon = \mathrm{D}_2 \\ \mathrm{A}_3 N^K_X + \mathrm{B}_3\beta + C_3\varepsilon = \mathrm{D}_3 \end{cases}$$

Where,

$$\begin{aligned} A_1 &= (1-\alpha-\phi)(R^K_{F/R}\,{}^R A_x - {}^F A_x) \\ B_1 &= [R^K_{F/R}({}^R A_x N^L_x + {}^R A^L_s N^L_s) - ({}^F A_x N^L_x + {}^F A^L_s N^L_s)] \\ C_1 &= [R^K_{F/R}({}^R A_x N^M_x + {}^R A^M_s N^M_s) - ({}^F A_x N^M_x + {}^F A^M_s N^M_s)] \\ D_1 &= (1-\alpha-\phi)N^K_s({}^F A^K_s - R^K_{F/R}\,{}^R A^K_s) \end{aligned}$$

$$\begin{aligned} A_2 &= (1-\alpha-\phi)(R^K_{G/R}\,{}^R A_x - {}^G A_x) \\ B_2 &= [R^K_{G/R}({}^R A_x N^L_x + {}^R A^L_s N^L_s) - ({}^G A_x N^L_x + {}^G A^L_s N^L_s)] \\ C_2 &= [R^K_{G/R}({}^R A_x N^M_x + {}^R A^M_s N^M_s) - ({}^G A_x N^M_x + {}^G A^M_s N^M_s)] \\ D_2 &= (1-\alpha-\phi)N^K_s({}^G A^K_s - R^K_{G/R}\,{}^R A^K_s) \end{aligned}$$

$$A_3 = (1-\alpha-\phi)(R^K_{H/R}\,{}^R A_x - {}^H A_x)$$
$$B_3 = [R^K_{H/R}({}^R A_x N^L_x + {}^R A^L_s N^L_s) - ({}^H A_x N^L_x + {}^H A^L_s N^L_s)]$$
$$C_3 = [R^K_{H/R}({}^R A_x N^M_x + {}^R A^M_s N^M_s) - ({}^H A_x N^M_x + {}^H A^M_s N^M_s)]$$
$$D_3 = (1-\alpha-\phi)N^K_s({}^H A^K_s - R^K_{H/R}\,{}^R A^K_s)$$

The solutions are

$$N^K_X = \frac{\begin{vmatrix} D_1 & B_1 & C_1 \\ D_2 & B_2 & C_2 \\ D_3 & B_3 & C_3 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 & C_1 \\ A_2 & B_2 & C_2 \\ A_3 & B_3 & C_3 \end{vmatrix}} \;;\; \beta = \frac{\begin{vmatrix} A_1 & D_1 & C_1 \\ A_2 & D_2 & C_2 \\ A_3 & D_3 & C_3 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 & C_1 \\ A_2 & B_2 & C_2 \\ A_3 & B_3 & C_3 \end{vmatrix}} \text{ and } \varepsilon = \frac{\begin{vmatrix} A_1 & B_1 & D_1 \\ A_2 & B_2 & D_2 \\ A_3 & B_3 & D_3 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 & C_1 \\ A_2 & B_2 & C_2 \\ A_3 & B_3 & C_3 \end{vmatrix}}$$

or $N^K_x = \dfrac{(D_1B_2C_3 + B_1C_2D_3 + C_1B_3D_2) - (D_3B_2C_1 + D_2B_1C_3 + D_1C_2B_3)}{(A_1B_2C_3 + B_1C_2A_3 + C_1B_3A_2) - (A_3B_2C_1 + A_2B_1C_3 + A_1C_2B_3)}$;

$$\beta = \frac{(A_1D_2C_3 + D_1C_2A_3 + C_1D_3A_2) - (A_3D_2C_1 + A_2D_1C_3 + A_1C_2D_3)}{(A_1B_2C_3 + B_1C_2A_3 + C_1B_3A_2) - (A_3B_2C_1 + A_2B_1C_3 + A_1C_2B_3)} \text{ and}$$

$$\varepsilon = \frac{(A_1B_2D_3 + B_1D_2A_3 + D_1B_3A_2) - (A_3B_2D_1 + A_2B_1D_3 + A_1D_2B_3)}{(A_1B_2C_3 + B_1C_2A_3 + C_1B_3A_2) - (A_3B_2C_1 + A_2B_1C_3 + A_1C_2B_3)}$$

In the second iteration, we can use these three values for N^K_X , β and ε in equations for species L to solve for N^L_X , α and δ according to the following sets of equations.

$$
\begin{cases}
(1-\beta-\chi)\left(R^{L}_{F/R}\,{}^{R}A_{X}-{}^{F}A_{X}\right)N^{L}_{X}+\left[R^{L}_{F/R}\left({}^{R}A_{X}N^{K}_{X}+{}^{R}A^{K}_{S}N^{K}_{S}\right)-\left({}^{F}A_{X}N^{K}_{X}+{}^{F}A^{K}_{S}N^{K}_{S}\right)\right]\alpha \\
+[R^{L}_{F/R}\left({}^{R}A_{X}N^{M}_{X}+{}^{R}A^{M}_{S}N^{M}_{S}\right)-\left({}^{F}A_{X}N^{M}_{X}+{}^{F}A^{M}_{S}N^{M}_{S}\right)]\delta=\left({}^{F}A^{L}_{S}-R^{L}_{F/R}\,{}^{R}A^{L}_{S}\right)N^{L}_{S}(1-\beta-\chi) \\
\\
(1-\beta-\chi)\left(R^{L}_{G/R}\,{}^{R}A_{X}-{}^{G}A_{X}\right)N^{L}_{X}+\left[R^{L}_{G/R}\left({}^{R}A_{X}N^{K}_{X}+{}^{R}A^{K}_{S}N^{K}_{S}\right)-\left({}^{G}A_{X}N^{K}_{X}+{}^{G}A^{K}_{S}N^{K}_{S}\right)\right]\alpha \\
+[R^{L}_{G/R}\left({}^{R}A_{X}N^{M}_{X}+{}^{R}A^{M}_{S}N^{M}_{S}\right)-\left({}^{F}A_{X}N^{M}_{X}+{}^{F}A^{M}_{S}N^{M}_{S}\right)]\delta=\left({}^{G}A^{L}_{S}-R^{L}_{G/R}\,{}^{R}A^{L}_{S}\right)N^{L}_{S}(1-\beta-\chi) \\
\\
(1-\beta-\chi)\left(R^{L}_{H/R}\,{}^{R}A_{X}-{}^{H}A_{X}\right)N^{L}_{X}+\left[R^{L}_{H/R}\left({}^{R}A_{X}N^{K}_{X}+{}^{R}A^{K}_{S}N^{K}_{S}\right)-\left({}^{H}A_{X}N^{K}_{X}+{}^{H}A^{K}_{S}N^{K}_{S}\right)\right]\alpha \\
+[R^{L}_{H/R}\left({}^{R}A_{X}N^{M}_{X}+{}^{R}A^{M}_{S}N^{M}_{S}\right)-\left({}^{F}A_{X}N^{M}_{X}+{}^{F}A^{M}_{S}N^{M}_{S}\right)]\delta=\left({}^{H}A^{L}_{S}-R^{L}_{H/R}\,{}^{R}A^{L}_{S}\right)N^{L}_{S}(1-\beta-\chi)
\end{cases}
$$

We can rewrite them as:

$$
\begin{cases}
\mathrm{A}_4 N^{L}_{X}+\mathrm{B}_4\alpha+C_4\delta=\mathrm{D}_4 \\
\mathrm{A}_5 N^{L}_{X}+\mathrm{B}_5\alpha+C_5\delta=\mathrm{D}_5 \\
\mathrm{A}_6 N^{L}_{X}+\mathrm{B}_6\alpha+C_6\delta=\mathrm{D}_6
\end{cases}
$$

Where,

$$
\begin{aligned}
A_4 &= (1-\beta-\chi)(R^{L}_{F/R}\,{}^{R}A_x-{}^{F}A_x) \\
B_4 &= [R^{L}_{F/R}({}^{R}A_x N^{K}_{x}+{}^{R}A^{K}_{s}N^{K}_{s})-({}^{F}A_x N^{K}_{x}+{}^{F}A^{K}_{s}N^{K}_{s})] \\
C_4 &= [R^{L}_{F/R}({}^{R}A_x N^{M}_{x}+{}^{R}A^{M}_{s}N^{M}_{s})-({}^{F}A_x N^{M}_{x}+{}^{F}A^{M}_{s}N^{M}_{s})] \\
D_4 &= (1-\beta-\chi)N^{L}_{s}({}^{F}A^{L}_{s}-R^{L}_{F/R}\,{}^{R}A^{L}_{s})
\end{aligned}
$$

$$
\begin{aligned}
A_5 &= (1-\beta-\chi)(R^{L}_{G/R}\,{}^{R}A_x-{}^{G}A_x) \\
B_5 &= [R^{L}_{G/R}({}^{R}A_x N^{K}_{x}+{}^{R}A^{K}_{s}N^{K}_{s})-({}^{G}A_x N^{K}_{x}+{}^{G}A^{K}_{s}N^{K}_{s})] \\
C_5 &= [R^{L}_{G/R}({}^{R}A_x N^{M}_{x}+{}^{R}A^{M}_{s}N^{M}_{s})-({}^{G}A_x N^{M}_{x}+{}^{G}A^{M}_{s}N^{M}_{s})] \\
D_5 &= (1-\beta-\chi)N^{L}_{s}({}^{G}A^{L}_{s}-R^{L}_{G/R}\,{}^{R}A^{L}_{s})
\end{aligned}
$$

and

$$
\begin{aligned}
A_6 &= (1-\beta-\chi)(R^{L}_{H/R}\,{}^{R}A_x-{}^{H}A_x) \\
B_6 &= [R^{L}_{H/R}({}^{R}A_x N^{K}_{x}+{}^{R}A^{K}_{s}N^{K}_{s})-({}^{H}A_x N^{K}_{x}+{}^{H}A^{K}_{s}N^{K}_{s})] \\
C_6 &= [R^{L}_{H/R}({}^{R}A_x N^{M}_{x}+{}^{R}A^{M}_{s}N^{M}_{s})-({}^{H}A_x N^{M}_{x}+{}^{H}A^{M}_{s}N^{M}_{s})] \\
D_6 &= (1-\beta-\chi)N^{L}_{s}({}^{H}A^{L}_{s}-R^{L}_{H/R}\,{}^{R}A^{L}_{s})
\end{aligned}
$$

again the solutions are

$$N_X^L = \frac{\begin{vmatrix} D_4 & B_4 & C_4 \\ D_5 & B_5 & C_5 \\ D_6 & B_6 & C_6 \end{vmatrix}}{\begin{vmatrix} A_4 & B_4 & C_4 \\ A_5 & B_5 & C_5 \\ A_6 & B_6 & C_6 \end{vmatrix}} \; ; \; \alpha = \frac{\begin{vmatrix} A_4 & D_4 & C_4 \\ A_5 & D_5 & C_5 \\ A_6 & D_6 & C_6 \end{vmatrix}}{\begin{vmatrix} A_4 & B_4 & C_4 \\ A_5 & B_5 & C_5 \\ A_6 & B_6 & C_6 \end{vmatrix}} \text{ and } \delta = \frac{\begin{vmatrix} A_4 & B_4 & D_4 \\ A_5 & B_5 & D_5 \\ A_6 & B_6 & D_6 \end{vmatrix}}{\begin{vmatrix} A_4 & B_4 & C_4 \\ A_5 & B_5 & C_5 \\ A_6 & B_6 & C_6 \end{vmatrix}}$$

or $N_x^L = \dfrac{(D_4B_5C_6 + B_4C_5D_6 + C_4B_6D_5) - (D_6B_5C_4 + D_5B_4C_6 + D_4C_5B_6)}{(A_4B_5C_6 + B_4C_5A_6 + C_4B_6A_5) - (A_6B_5C_4 + A_5B_4C_6 + A_4C_5B_6)}$;

$$\alpha = \frac{(A_4D_5C_6 + D_4C_5A_6 + C_4D_6A_5) - (A_6D_5C_4 + A_5D_4C_6 + A_4C_5D_6)}{(A_4B_5C_6 + B_4C_5A_6 + C_4B_6A_5) - (A_6B_5C_4 + A_5B_4C_6 + A_4C_5B_6)} \text{ and}$$

$$\delta = \frac{(A_4B_5D_6 + B_4D_5A_6 + D_4B_6A_5) - (A_6B_5D_4 + A_5B_4D_6 + A_4D_5B_6)}{(A_4B_5C_6 + B_4C_5A_6 + C_4B_6A_5) - (A_6B_5C_4 + A_5B_4C_6 + A_4C_5B_6)}$$

Finally, in the third iteration, we can calculate the values for N_X^M, χ and ϕ from equations for species M. After careful derivation of these three equations, the following sets of equations are constructed.

$$\begin{cases} (1-\delta-\varepsilon)\left(R_{F/R}^M \, {}^R A_X - {}^F A_X\right) N_X^M + \left[R_{F/R}^M \left({}^R A_X N_X^K + {}^R A_S^K N_S^K\right) - \left({}^F A_X N_X^K + {}^F A_S^K N_S^K\right)\right]\phi \\ + [R_{F/R}^M \left({}^R A_X N_X^L + {}^R A_S^L N_S^L\right) - \left({}^F A_X N_X^L + {}^F A_S^L N_S^L\right)]\chi = \left({}^F A_S^M - R_{F/R}^M \, {}^R A_S^M\right) N_S^M (1-\delta-\varepsilon) \\ \\ (1-\delta-\varepsilon)\left(R_{G/R}^M \, {}^R A_X - {}^G A_X\right) N_X^M + \left[R_{G/R}^M \left({}^R A_X N_X^K + {}^R A_S^K N_S^K\right) - \left({}^G A_X N_X^K + {}^G A_S^K N_S^K\right)\right]\phi \\ + [R_{G/R}^M \left({}^R A_X N_X^L + {}^R A_S^L N_S^L\right) - \left({}^G A_X N_X^L + {}^G A_S^L N_S^L\right)]\chi = \left({}^G A_S^M - R_{G/R}^M \, {}^R A_S^M\right) N_S^M (1-\delta-\varepsilon) \\ \\ (1-\delta-\varepsilon)\left(R_{H/R}^M \, {}^R A_X - {}^H A_X\right) N_X^M + \left[R_{H/R}^M \left({}^R A_X N_X^K + {}^R A_S^K N_S^K\right) - \left({}^H A_X N_X^K + {}^H A_S^K N_S^K\right)\right]\phi \\ + [R_{H/R}^M \left({}^R A_X N_X^L + {}^R A_S^L N_S^L\right) - \left({}^H A_X N_X^L + {}^H A_S^L N_S^L\right)]\chi = \left({}^H A_S^M - R_{H/R}^M \, {}^R A_S^M\right) N_S^M (1-\delta-\varepsilon) \end{cases}$$

We can rewrite them as:

$$\begin{cases} \mathrm{A}_7 N_X^M + \mathrm{B}_7\phi + C_7\chi = \mathrm{D}_7 \\ \mathrm{A}_8 N_X^M + \mathrm{B}_8\phi + C_8\chi = \mathrm{D}_8 \\ \mathrm{A}_9 N_X^M + \mathrm{B}_9\phi + C_9\chi = \mathrm{D}_9 \end{cases}$$

Where,

$$A_7 = (1-\delta-\varepsilon)(R_{F/R}^M\,{}^R A_x - {}^F A_x)$$
$$B_7 = [R_{F/R}^M({}^R A_x N_x^K + {}^R A_s^K N_s^K) - ({}^F A_x N_x^K + {}^F A_s^K N_s^K)]$$
$$C_7 = [R_{F/R}^M({}^R A_x N_x^L + {}^R A_s^L N_s^L) - ({}^F A_x N_x^L + {}^F A_s^L N_s^L)]$$
$$D_7 = (1-\delta-\varepsilon)N_s^M({}^F A_s^M - R_{F/R}^M\,{}^R A_s^M)$$

$$A_8 = (1-\delta-\varepsilon)(R_{G/R}^M\,{}^R A_x - {}^G A_x)$$
$$B_8 = [R_{G/R}^M({}^R A_x N_x^K + {}^R A_s^K N_s^K) - ({}^G A_x N_x^K + {}^G A_s^K N_s^K)]$$
$$C_8 = [R_{G/R}^M({}^R A_x N_x^L + {}^R A_s^L N_s^L) - ({}^G A_x N_x^L + {}^G A_s^L N_s^L)]$$
$$D_8 = (1-\delta-\varepsilon)N_s^M({}^G A_s^M - R_{G/R}^M\,{}^R A_s^M)$$

and

$$A_9 = (1-\delta-\varepsilon)(R_{H/R}^M\,{}^R A_x - {}^H A_x)$$
$$B_9 = [R_{H/R}^M({}^R A_x N_x^K + {}^R A_s^K N_s^K) - ({}^H A_x N_x^K + {}^H A_s^K N_s^K)]$$
$$C_9 = [R_{H/R}^M({}^R A_x N_x^L + {}^R A_s^L N_s^L) - ({}^H A_x N_x^L + {}^H A_s^L N_s^L)]$$
$$D_9 = (1-\delta-\varepsilon)N_s^M({}^H A_s^M - R_{H/R}^M\,{}^R A_s^M)$$

and the solutions are

$$N_X^M = \frac{\begin{vmatrix} D_7 & B_7 & C_7 \\ D_8 & B_8 & C_8 \\ D_9 & B_9 & C_9 \end{vmatrix}}{\begin{vmatrix} A_7 & B_7 & C_7 \\ A_8 & B_8 & C_8 \\ A_9 & B_9 & C_9 \end{vmatrix}} \;;\; \phi = \frac{\begin{vmatrix} A_7 & D_7 & C_7 \\ A_8 & D_8 & C_8 \\ A_9 & D_9 & C_9 \end{vmatrix}}{\begin{vmatrix} A_7 & B_7 & C_7 \\ A_8 & B_8 & C_8 \\ A_9 & B_9 & C_9 \end{vmatrix}} \text{ and } \chi = \frac{\begin{vmatrix} A_7 & B_7 & D_7 \\ A_8 & B_8 & D_8 \\ A_9 & B_9 & D_9 \end{vmatrix}}{\begin{vmatrix} A_7 & B_7 & C_7 \\ A_8 & B_8 & C_8 \\ A_9 & B_9 & C_9 \end{vmatrix}}$$

or $N_x^M = \dfrac{(D_7B_8C_9 + B_7C_8D_9 + C_7B_9D_8) - (D_9B_8C_7 + D_8B_7C_9 + D_7C_8B_9)}{(A_7B_8C_9 + B_7C_8A_9 + C_7B_9A_8) - (A_9B_8C_7 + A_8B_7C_9 + A_7C_8B_9)}$;

$$\phi = \frac{(A_7D_8C_9 + D_7C_8A_9 + C_7D_9A_8) - (A_9D_8C_7 + A_8D_7C_9 + A_7C_8D_9)}{(A_7B_8C_9 + B_7C_8A_9 + C_7B_9A_8) - (A_9B_8C_7 + A_8B_7C_9 + A_7C_8B_9)} \text{ and}$$

$$\chi = \frac{(A_7B_8D_9 + B_7D_8A_9 + D_7B_9A_8) - (A_9B_8D_7 + A_8B_7D_9 + A_7D_8B_9)}{(A_7B_8C_9 + B_7C_8A_9 + C_7B_9A_8) - (A_9B_8C_7 + A_8B_7C_9 + A_7C_8B_9)}$$

Repeating the calculations, the variables $N_X^K, N_X^L, N_X^M, \alpha, \beta, \chi, \delta, \varepsilon$ and φ will converge to constant values, and these values are the solution of the equations.

Note: *Consider the species distribution in the spikes. If the prepared isotopic standards are 100% pure in the desired species, then they will not affect the total moles and isotopic abundances of each species present in the sample after triple spiking. But if isotopic standards are not 100% pure, then the total moles and isotopic abundances of each species will be changed after spiking. Therefore, the new total moles and new isotopic abundances should be calculated using the equations described in Sections 6.2.2 and 6.2.3.*

6.2.2 Calculation of Total Number of Moles and New Isotopic Abundances after Double Spiking

Let's consider W_x gram of the sample is double spiked by addition of W_s^K gram of ^{F}K spike [$^F C_{Spike}^K$, µg/g] and W_s^L gram of ^{G}L spike [$^G C_{Spike}^L$, µg/g]. Also consider that the ^{F}K spike contains $^K P_K$ % of *K* and $^L P_K$ % of *L*, where $^K P_K\% + {}^L P_K\% = 100\%$. On the other hand, consider that the ^{G}L spike contains $^K Q_L$ % of *K* and $^L Q_L$ % of *L*, where

${}^{L}Q_L\%+{}^{L}Q_L\% = 100\%$. Therefore, after double spiking the total moles of K (${}^{Total}Z^{K}_{Spike}$, μmol) and L (${}^{Total}Z^{L}_{Spike}$, μmol) contribution from spikes will be

$$ {}^{Total}Z^{K}_{Spike} = \frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{K}P_{K}\%)}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{K}Q_{L}\%)}{M^{L}_{s}} $$

and

$$ {}^{Total}Z^{L}_{Spike} = \frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{L}P_{K}\%)}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{L}Q_{L}\%)}{M^{L}_{s}} \text{ respectively.} $$

M^{K}_{S} and M^{L}_{S} are the average atomic mass for ^{F}K and ^{G}L spikes.

The new isotopic abundances for both spike standards can also be calculated using the following equations.

For ^{F}K spike:

$$ {}^{F}A^{K}_{S(New)} = \left[\frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{K}P_{K}\%)({}^{F}A^{K}_{S})}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{K}Q_{L}\%)({}^{F}A^{L}_{S})}{M^{L}_{s}} \right] \left[\frac{1}{{}^{Total}Z^{K}_{Spike}} \right] $$

$$ {}^{R}A^{K}_{S(New)} = \left[\frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{K}P_{K}\%)({}^{R}A^{K}_{S})}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{K}Q_{L}\%)({}^{R}A^{L}_{S})}{M^{L}_{s}} \right] \left[\frac{1}{{}^{Total}Z^{K}_{Spike}} \right] $$

$$ {}^{G}A^{K}_{S(New)} = \left[\frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{K}P_{K}\%)({}^{G}A^{K}_{S})}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{K}Q_{L}\%)({}^{G}A^{L}_{S})}{M^{L}_{s}} \right] \left[\frac{1}{{}^{Total}Z^{K}_{Spike}} \right] $$

For ^{G}L Spike:

$$ {}^{F}A^{L}_{S(New)} = \left[\frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{L}P_{K}\%)({}^{F}A^{K}_{S})}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{L}Q_{L}\%)({}^{F}A^{L}_{S})}{M^{L}_{s}} \right] \left[\frac{1}{{}^{Total}Z^{L}_{Spike}} \right] $$

$$ {}^{R}A^{L}_{S(New)} = \left[\frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{L}P_{K}\%)({}^{R}A^{K}_{S})}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{L}Q_{L}\%)({}^{R}A^{L}_{S})}{M^{L}_{s}} \right] \left[\frac{1}{{}^{Total}Z^{L}_{Spike}} \right] $$

$$ {}^{G}A^{L}_{S(New)} = \left[\frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{L}P_{K}\%)({}^{G}A^{K}_{S})}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{L}Q_{L}\%)({}^{G}A^{L}_{S})}{M^{L}_{s}} \right] \left[\frac{1}{{}^{Total}Z^{L}_{Spike}} \right] $$

6.2.3 Calculation of Total Number of Moles and New Isotopic Abundances after Triple Spiking

Let's consider W_x gram of the sample is triple spiked by the addition of W^{K}_{s} gram of ^{F}K spike [${}^{F}C^{K}_{Spike}$, µg/g], W^{L}_{s} gram of ^{G}L spike [${}^{G}C^{L}_{Spike}$, µg/g] and W^{M}_{s} gram of ^{H}M spike [${}^{H}C^{M}_{Spike}$, µg/g]. Also consider that the ^{F}K spike contains ${}^{K}P_{K}\%$ of *K*, ${}^{L}P_{K}\%$ of *L* and ${}^{M}P_{K}\%$ of *M*, where ${}^{K}P_{K}\%+{}^{L}P_{K}\%+{}^{M}P_{K}\% = 100\%$. Consider that the ^{G}L spike contains ${}^{K}Q_{L}\%$ of *K*, ${}^{L}Q_{L}\%$ of *L*, and ${}^{M}Q_{L}\%$ of *M*, where ${}^{K}Q_{L}\%+{}^{L}Q_{L}\%+{}^{M}Q_{L}\% = 100\%$. Also consider that the ^{H}M spike contains ${}^{K}R_{M}\%$ of *K*, ${}^{L}R_{M}\%$ of *L* and ${}^{M}R_{M}\%$ of *M*, where ${}^{K}R_{M}\%+{}^{L}R_{M}\%+{}^{M}R_{M}\% = 100\%$. Therefore, after triple spiking the total moles of *K* (${}^{Total}Z^{K}_{Spike}$, µmol), *L* (${}^{Total}Z^{L}_{Spike}$, µmol), and *M* (${}^{Total}Z^{M}_{Spike}$, µmol) contribution from spikes will be

$$ {}^{Total}Z^{K}_{Spike} = \frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{K}P_{K}\%)}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{K}Q_{L}\%)}{M^{L}_{s}} + \frac{({}^{H}C^{M}_{Spike})(W^{M}_{s})({}^{K}R_{M}\%)}{M^{M}_{s}} $$

$$ {}^{Total}Z^{L}_{Spike} = \frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{L}P_{K}\%)}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{L}Q_{L}\%)}{M^{L}_{s}} + \frac{({}^{H}C^{M}_{Spike})(W^{M}_{s})({}^{L}R_{M}\%)}{M^{M}_{s}} $$

$$ {}^{Total}Z^{M}_{Spike} = \frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{M}P_{K}\%)}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{M}Q_{L}\%)}{M^{L}_{s}} + \frac{({}^{H}C^{M}_{Spike})(W^{M}_{s})({}^{M}R_{M}\%)}{M^{M}_{s}} $$

respectively. M^{K}_{S}, M^{L}_{S} and M^{M}_{S} are the average atomic mass for ^{F}K, ^{G}L and ^{H}M spikes.

The new isotopic abundances for both spike standards can also be calculated using the following equations.

For ^{F}K spike:

$$^{F}A^{K}_{S(New)} = \left[\frac{(^{F}C^{K}_{Spike})(W^{K}_{s})(^{K}P_{K}\%)(^{F}A^{K}_{S})}{M^{K}_{s}} + \frac{(^{G}C^{L}_{Spike})(W^{L}_{s})(^{K}Q_{L}\%)(^{F}A^{L}_{S})}{M^{L}_{s}} + \frac{(^{H}C^{M}_{Spike})(W^{M}_{s})(^{K}R_{M}\%)(^{F}A^{M}_{S})}{M^{M}_{s}}\right]\left[\frac{1}{^{Total}Z^{K}_{Spike}}\right]$$

$$^{R}A^{K}_{S(New)} = \left[\frac{(^{F}C^{K}_{Spike})(W^{K}_{s})(^{K}P_{K}\%)(^{R}A^{K}_{S})}{M^{K}_{s}} + \frac{(^{G}C^{L}_{Spike})(W^{L}_{s})(^{K}Q_{L}\%)(^{R}A^{L}_{S})}{M^{L}_{s}} + \frac{(^{H}C^{M}_{Spike})(W^{M}_{s})(^{K}R_{M}\%)(^{R}A^{M}_{S})}{M^{M}_{s}}\right]\left[\frac{1}{^{Total}Z^{K}_{Spike}}\right]$$

$$^{G}A^{K}_{S(New)} = \left[\frac{(^{F}C^{K}_{Spike})(W^{K}_{s})(^{K}P_{K}\%)(^{G}A^{K}_{S})}{M^{K}_{s}} + \frac{(^{G}C^{L}_{Spike})(W^{L}_{s})(^{K}Q_{L}\%)(^{G}A^{L}_{S})}{M^{L}_{s}} + \frac{(^{H}C^{M}_{Spike})(W^{M}_{s})(^{K}R_{M}\%)(^{G}A^{M}_{S})}{M^{M}_{s}}\right]\left[\frac{1}{^{Total}Z^{K}_{Spike}}\right]$$

$$^{H}A^{K}_{S(New)} = \left[\frac{(^{F}C^{K}_{Spike})(W^{K}_{s})(^{K}P_{K}\%)(^{H}A^{K}_{S})}{M^{K}_{s}} + \frac{(^{G}C^{L}_{Spike})(W^{L}_{s})(^{K}Q_{L}\%)(^{H}A^{L}_{S})}{M^{L}_{s}} + \frac{(^{H}C^{M}_{Spike})(W^{M}_{s})(^{K}R_{M}\%)(^{H}A^{M}_{S})}{M^{M}_{s}}\right]\left[\frac{1}{^{Total}Z^{K}_{Spike}}\right]$$

For ^{G}L Spike:

$$^{F}A^{L}_{S(New)} = \left[\frac{(^{F}C^{K}_{Spike})(W^{K}_{s})(^{L}P_{K}\%)(^{F}A^{K}_{S})}{M^{K}_{s}} + \frac{(^{G}C^{L}_{Spike})(W^{L}_{s})(^{L}Q_{L}\%)(^{F}A^{L}_{S})}{M^{L}_{s}} + \frac{(^{H}C^{M}_{Spike})(W^{M}_{s})(^{L}R_{M}\%)(^{F}A^{M}_{S})}{M^{M}_{s}}\right]\left[\frac{1}{^{Total}Z^{L}_{Spike}}\right]$$

$$^{R}A^{L}_{S(New)} = \left[\frac{(^{F}C^{K}_{Spike})(W^{K}_{s})(^{L}P_{K}\%)(^{R}A^{K}_{S})}{M^{K}_{s}} + \frac{(^{G}C^{L}_{Spike})(W^{L}_{s})(^{L}Q_{L}\%)(^{R}A^{L}_{S})}{M^{L}_{s}} + \frac{(^{H}C^{M}_{Spike})(W^{M}_{s})(^{L}R_{M}\%)(^{R}A^{M}_{S})}{M^{M}_{s}}\right]\left[\frac{1}{^{Total}Z^{L}_{Spike}}\right]$$

$$^{G}A^{L}_{S(New)} = \left[\frac{(^{F}C^{K}_{Spike})(W^{K}_{s})(^{L}P_{K}\%)(^{G}A^{K}_{S})}{M^{K}_{s}} + \frac{(^{G}C^{L}_{Spike})(W^{L}_{s})(^{L}Q_{L}\%)(^{G}A^{L}_{S})}{M^{L}_{s}} + \frac{(^{H}C^{M}_{Spike})(W^{M}_{s})(^{L}R_{M}\%)(^{G}A^{M}_{S})}{M^{M}_{s}}\right]\left[\frac{1}{^{Total}Z^{L}_{Spike}}\right]$$

$$^{H}A^{L}_{S(New)} = \left[\frac{(^{F}C^{K}_{Spike})(W^{K}_{s})(^{L}P_{K}\%)(^{H}A^{K}_{S})}{M^{K}_{s}} + \frac{(^{G}C^{L}_{Spike})(W^{L}_{s})(^{L}Q_{L}\%)(^{H}A^{L}_{S})}{M^{L}_{s}} + \frac{(^{H}C^{M}_{Spike})(W^{M}_{s})(^{L}R_{M}\%)(^{H}A^{M}_{S})}{M^{M}_{s}}\right]\left[\frac{1}{^{Total}Z^{L}_{Spike}}\right]$$

For ^{H}M Spike:

$$^{F}A^{M}_{S(New)} = \left[\frac{(^{F}C^{K}_{Spike})(W^{K}_{s})(^{M}P_{K}\%)(^{F}A^{K}_{S})}{M^{K}_{s}} + \frac{(^{G}C^{L}_{Spike})(W^{L}_{s})(^{M}Q_{L}\%)(^{F}A^{L}_{S})}{M^{L}_{s}} + \frac{(^{H}C^{M}_{Spike})(W^{M}_{s})(^{M}R_{M}\%)(^{F}A^{M}_{S})}{M^{M}_{s}}\right]\left[\frac{1}{^{Total}Z^{M}_{Spike}}\right]$$

$$ {}^{R}A^{M}_{S(New)} = \left[\frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{M}P_{K}\%)({}^{R}A^{K}_{S})}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{M}Q_{L}\%)({}^{R}A^{L}_{S})}{M^{L}_{s}} + \frac{({}^{H}C^{M}_{Spike})(W^{M}_{s})({}^{M}R_{M}\%)({}^{R}A^{M}_{S})}{M^{M}_{s}} \right] \left[\frac{1}{{}^{Total}Z^{M}_{Spike}} \right] $$

$$ {}^{G}A^{M}_{S(New)} = \left[\frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{M}P_{K}\%)({}^{G}A^{K}_{S})}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{M}Q_{L}\%)({}^{G}A^{L}_{S})}{M^{L}_{s}} + \frac{({}^{H}C^{M}_{Spike})(W^{M}_{s})({}^{M}R_{M}\%)({}^{G}A^{M}_{S})}{M^{M}_{s}} \right] \left[\frac{1}{{}^{Total}Z^{M}_{Spike}} \right] $$

$$ {}^{H}A^{M}_{S(New)} = \left[\frac{({}^{F}C^{K}_{Spike})(W^{K}_{s})({}^{M}P_{K}\%)({}^{H}A^{K}_{S})}{M^{K}_{s}} + \frac{({}^{G}C^{L}_{Spike})(W^{L}_{s})({}^{M}Q_{L}\%)({}^{H}A^{L}_{S})}{M^{L}_{s}} + \frac{({}^{H}C^{M}_{Spike})(W^{M}_{s})({}^{M}R_{M}\%)({}^{H}A^{M}_{S})}{M^{M}_{s}} \right] \left[\frac{1}{{}^{Total}Z^{M}_{Spike}} \right] $$

6.2.4 Construction of Workable EXCEL Spreadsheets

The equations derived in Sections 6.2.1, 6.2.2 and 6.2.3 are programmed in Microsoft Excel spreadsheets and are available to assist with these solutions on CD-ROM and also on-line (*28*). Spreadsheets are constructed to be user friendly, such that one needs only to plug in information about the isotope abundances for both naturally abundant and isotopically enriched spike(s); measured isotope ratios; mass(es), concentration(s) and species composition(s) in the spike(s) added; and the mass of the sample originally spiked (before or after extraction). The original concentration(s) of the target species at the time of spiking along with the percent transformation(s) will be calculated, and tabulated in the same spreadsheet. Theses Excel spreadsheets will be provided to the US EPA to put those in their internet domain and to support the application of EPA Method 6800 by the interested scientific community.

6.2.5 Validation of SIDMS Equations

The SIDMS equations generated for two and three species in terms of bidirectional transformations were validated using chromium and mercury species data obtained from previous study.

6.2.5.1 Validation using Chromium as an example (Double Spiking)

In case of chromium speciation analysis, the data for road construction material (DOS-SC-10G) were used to calculate the Cr(VI) concentration and percent transformation using both the generic equations, and Dengwei Huo's equations (*29*). These two sets of calculations were applied to determine Cr(VI) concentration from the representative sample by spiking in solid (before extraction) and in alkaline extract (after extraction). Results are summarized and compared in Table 6-I. From the results, it is observed that both calculation techniques produce statistically indistinguishable results for both scenario (spiked before extraction and spiked after extraction) at their 95% confidence level (CL). The Cr(VI) concentration determined in DOS-SC-10G sample by spiking sample before extraction and by spiking extract after extraction was statistically indistinguishable at their 95% CL. The amount of Cr(III) converted to Cr(VI) was found to be much higher for results obtained from sample spiked before extraction than the sample spiked after extraction, which conclude that the extraction procedure is more prone to oxidize easily oxidizible Cr(III) species than extract storage or analysis steps.

The same data were used also to validate two additional assumptions and results were compared. Namely, the ^{53}Cr(VI) spike was assumed to contain 90% Cr(VI) and 10% Cr(III), the ^{50}Cr(III) spike to contain 95% Cr(III) and 5% Cr(VI). The final results based on these assumptions and calculations are shown in Table 6-II. From this set of data, it is also observed that both calculation techniques produce results that are indistinguishable at 95% CL. In this case the mean values for Cr(VI) concentration and percent Cr(III) converted to Cr(VI) were influenced by the new isotopic abundances but those values were statistically indistinguishable at their 95% CL.

TABLE 6-I. Chromium(VI) Found in DOS-SC-10G Sample.

Calculation Method	Cr (VI) Concentration (μg/g)	Cr(III) converted to Cr(VI) (%)
Spiked before extraction		
Generic Equations	221.6078 ± 23.7483	39.28 ± 1.55
Dengwei's Equations	221.5655 ± 23.7494	38.98 ± 1.54
Spiked after extraction		
Generic Equations	215.6603 ± 24.1049	9.61 ± 5.63
Dengwei's Equations	215.6602 ± 24.1049	9.61 ± 5.63

Uncertainties are at 95% CL, n = 3.

TABLE 6-II. Chromium(VI) Found in DOS-SC-10G Sample by Spiking with Impure Spikes.

Calculation Method	Cr (VI) Concentration (μg/g)	Cr(III) converted to Cr(VI) (%)
Spiked before extraction		
Generic Equations	199.4470 ± 21.3735	35.71 ± 1.65
Dengwei's Equations	199.4089 ± 21.3745	35.39 ± 1.63
Spiked after extraction		
Generic Equations	201.4821 ± 22.0643	3.17 ± 6.02
Dengwei's Equations	201.4807 ± 22.0643	3.17 ± 6.02

Uncertainties are at 95% CL, n = 3.

6.2.5.2 Validation using Mercury as another example (Double and Triple Spiking)

For validation of two species bidirectional transformation equations, data from a previous study for Material-1 extracted with EPA draft Method 3200 was used. The generic equation version of SIDMS calculations and the specific previous version (see Chapter 4), based on the guidelines of Dengwei's equations for chromium speciation, were applied for mercury species calculations. Results are compared in Table 6-III

(Case-1), and observed that there is no difference between these two sets of results at their 95% CL. It is also demonstrated that the percent transformation of inorganic mercury to methylmercury and vice versa for both calculations were negligible (negative).

TABLE 6-III. Mercury Speciation Results for Two Species Bidirectional Transformation.

	Hg^{2+} (μg/g)	CH_3Hg^+ (μg/g)	Hg^{2+} to CH_3Hg^+ (%)	CH_3Hg^+ to Hg^{2+} (%)
Case-1				
Generic Equation	2.709 ± 1.287	2.504 ± 0.881	- 4.64	- 0.34
Old Equation	2.709 ± 1.287	2.503 ± 0.881	- 4.64	- 0.34
Case-2				
Generic Equation	2.824 ± 1.231	2.389 ± 0.800	- 10.81	- 12.69
Old Equation	2.824 ± 1.232	2.388 ± 0.800	- 10.81	- 12.69

Uncertainties are at 95% CL, n = 3.

The same set of data was also used to validate the assumption that if the sample is spiked with materials that are not 100% pure in terms of species then the outcome should be different (Case-2). In this context, again it was assumed that the $^{199}Hg^{2+}$ spike contains 95% Hg^{2+} and 5% CH_3Hg^+; and the $CH_3{}^{201}Hg^+$ spike contains 90% CH_3Hg^+ and 10% Hg^{2+}. These results are also compared in Table 6-III. It is observed that both of these two calculation techniques (generic and old equations) produce statistically indistinguishable results at 95% CL, and percent transformation values are negligible.

The generic equations generated for three species bidirectional transformations were validated using only mercury as an example. In this case no real life data were

used. A synthetic sample containing 5.0 μg/g of inorganic mercury, 4.0 μg/g of methylmercury and 3.5 μg/g of ethylmercury was used. The concentration of the isotopically enriched inorganic mercury ($^{199}Hg^{2+}$) standard is 2.505 μg/g; isotopically enriched methylmercury ($CH_3{}^{201}Hg^{+}$) is 2.005 μg/g; and isotopically enriched ethylmercury ($C_2H_5{}^{200}Hg^{+}$) is 1.785 μg/g. It is assumed that a 1.525 g aliquot of the hypothetical sample was triple-spiked with three isotopically enriched mercury spike standards. The amount of each spike needed to be added to a sample entirely depends on the amount of sample taken, the concentration of each target species in the natural sample and the concentration of each isotopically enriched analogue species (the spike). In order to get better precision and accuracy in measurements, the synthetic sample was spiked with 0.4255 g of $^{199}Hg^{2+}$, 0.5055 g of $CH_3{}^{201}Hg^{+}$ and 0.2125 g of $C_2H_5{}^{200}Hg^{+}$. These spiking values were chosen to be with an optimal range given the known composition of the synthetic sample. The isotopic abundances of both the naturally abundant and the isotopically enriched mercury species used are shown in Table 6-IV.

Four different hypothetical scenarios were constructed and labeled as Case 1 to Case 4.

- Case-1: no transformation of the species take place;
- Case-2: 5% of inorganic mercury converts into both methylmercury and ethylmercury; 10% of methylmercury converts into both inorganic mercury and ethylmercury; and 5% of ethylmercury converts into both inorganic mercury and methylmercury;
- Case-3: 10% of both methylmercury and ethylmercury transformed into inorganic mercury

- Case-4: 5% of inorganic mercury converts into methylmercury and ethylmercury.

TABLE 6-IV. Isotopic Abundances of Different Mercury Species.

Isotopes	Natural Abundance (atom %)	$^{199}Hg^{2+}$ (atom %)	$CH_3{}^{201}Hg^{+}$ (atom %)	$C_2H_5{}^{200}Hg^{+}$ (atom %)	Atomic Mass
196	0.150	0.020	0.050	0.050	195.96581
198	9.970	1.630	0.080	0.080	197.96674
199	16.870	91.950	0.100	0.895	198.96825
200	23.100	4.920	0.450	96.410	199.96830
201	13.180	0.660	98.110	0.395	200.97028
202	29.860	0.730	1.180	2.090	201.97061
204	6.870	0.110	0.080	0.080	203.97347
Total	100.000	100.020	100.050	100.000	
Average atomic Mass	200.5991	199.0810	201.0736	200.0048	199.96907

The theoretical isotope ratios of 199/202, 200/202 and 201/202 for each of the target species were calculated based on all the information provided in previous paragraphs. The calculated isotope ratios are tabulated in Table 6-V and were used for final SIDMS calculations. The final results for each of the scenarios are listed in Table 6VI. From Table 6-VI, it is demonstrated that the results are 100% identical with those set up values. Therefore, it can be concluded that the Excel worksheet is error free and ready to use.

Note: *The unidirectional transformation equations for three species system are not constructed separately due the complexity and a number of possible diagrams (at least*

36, Section 8.2 Appendix A). To determine the initial concentration of three target species along with their transformation values for unidirectional conversions, the bidirectional equations may be used These sets of equations will produce the same results as do the unidirectional equations.

TABLE 6-V. Theoretical Isotope Ratios for Synthetic Samples.

	Inorganic Mercury			Methylmercury			Ethylmercury		
	199/202	***201/202***	***200/202***	***199/202***	***201/202***	***200/202***	***199/202***	***201/202***	***200/202***
Case 1	0.99528	0.44298	0.79408	0.56185	0.97961	0.77106	0.56429	0.44014	0.99876
Case 2	0.94615	0.48530	0.79934	0.59180	0.91675	0.78366	0.59235	0.49720	0.96132
Case 3	0.93878	0.48025	0.80496	0.56188	0.97967	0.77111	0.56429	0.44014	0.99876
Case 4	0.99528	0.44298	0.79408	0.58730	0.94819	0.77245	0.59298	0.44033	0.98514

TABLE 6-VI. Deconvoluted Concentration and Percent Transformation for Synthetic Sample.

Deconvoluted Concentration			% Transformation					
Hg^{2+} (μg/g)	CH_3Hg^+ (μg/g)	$C_2H_5Hg^+$ (μg/g)	Hg^{2+} to CH_3Hg^+	CH_3Hg^+ to Hg^{2+}	CH_3Hg^+ to $C_2H_5Hg^+$	$C_2H_5Hg^+$ to CH_3Hg^+	$C_2H_5Hg^+$ to Hg^{2+}	Hg^{2+} to $C_2H_5Hg^+$
5.000	4.000	3.500	0.00	0.00	0.00	0.00	0.00	0.00
5.000	3.998	3.500	5.01	10.00	10.00	5.02	5.00	5.00
5.000	3.998	3.500	0.01	10.00	0.00	0.02	10.00	0.00
5.000	3.998	3.500	5.01	0.00	0.00	0.02	0.00	5.00

6.3 Unidirectional Transformation

6.3.1 Algorithms, Assumptions and Calculations

Let's consider an aqueous sample containing two species of Z, and the species are *K* and *L*, with concentrations of C_x^K (μmol/g) and C_x^L (μmol/g), respectively. Weigh W_x gram

of the sample, followed by the addition of W_s^K gram of ^{F}K spike (species K enriched with isotope "F") and W_s^L gram of ^{G}L spike (species L enriched with isotope "G") into the sample. After spiking, the sample contains $^F A_x C_x^K W_x + {}^F A_s^K C_s^K W_s^K$ μmole of ^{F}Z as K and $^F A_x C_x^L W_x + {}^F A_s^L C_s^L W_s^L$ μmole of ^{F}Z as L, where A represents the isotopic abundance.

6.3.1.1 Conversion from K to L

When these two species undergo unidirectional transformations (***only from the species K to species L***) after the spike isotopes equilibrate with the sample isotopes, the fraction of K that converts to L will be α. Since no L converts to K, β will be zero. After conversion, the total amount of ^{F}Z in K form becomes $\left({}^F A_x C_x^K W_x + {}^F A_s^K C_s^K W_s^K\right)(1-\alpha)$. Similarly, the total amount of ^{R}Z in K form is $\left({}^R A_x C_x^K W_x + {}^R A_s^K C_s^K W_s^K\right)(1-\alpha)$. At the same time, the total amount of ^{F}Z in L form becomes $\left({}^F A_x C_x^K W_x + {}^F A_s^K C_s^K W_s^K\right)\alpha + \left({}^F A_x C_x^L W_x + {}^F A_s^L C_s^L W_s^L\right)$. And, the total amount of ^{R}Z in L form becomes $\left({}^R A_x C_x^K W_x + {}^R A_s^K C_s^K W_s^K\right)\alpha + \left({}^R A_x C_x^L W_x + {}^R A_s^L C_s^L W_s^L\right)$. Therefore, the isotope ratio of ^{F}Z to ^{R}Z in the K form, $R_{F/R}^K$, can be expressed as in Eq. 6.5. Following a similar procedure, we can construct equations Eq. 6.6 to Eq. 6.8.

$$R_{F/R}^K = \frac{\left({}^F A_x C_x^K W_x + {}^F A_s^K C_s^K W_s^K\right)}{\left({}^R A_x C_x^K W_x + {}^R A_s^K C_s^K W_s^K\right)} \qquad \text{Eq. 6.5}$$

$$R_{G/R}^K = \frac{\left({}^G A_x C_x^K W_x + {}^G A_s^K C_s^K W_s^K\right)}{\left({}^R A_x C_x^K W_x + {}^R A_s^K C_s^K W_s^K\right)} \qquad \text{Eq. 6.6}$$

$$R_{F/R}^L = \frac{\left({}^F A_x C_x^K W_x + {}^F A_s^K C_s^K W_s^K\right)\alpha + \left({}^F A_x C_x^L W_x + {}^F A_s^L C_s^L W_s^L\right)}{\left({}^R A_x C_x^K W_x + {}^R A_s^K C_s^K W_s^K\right)\alpha + \left({}^R A_x C_x^L W_x + {}^R A_s^L C_s^L W_s^L\right)} \qquad \text{Eq. 6.7}$$

$$R_{G/R}^{L} = \frac{\left({}^{G}A_x C_x^K W_x + {}^{G}A_s^K C_s^K W_s^K\right)\alpha + \left({}^{G}A_x C_x^L W_x + {}^{G}A_s^L C_s^L W_s^L\right)}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right)\alpha + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)} \qquad \text{Eq. 6.8}$$

These expressions can be simplified with the following substitutions

$$C_x^K W_x = N_x^K, C_x^L W_x = N_x^L, C_S^K W_S = N_S^K, C_S^L W_S = N_S^L$$

The value for N_X^K can be calculated from either Eq.6.5 or Eq. 6.6 as described below.

After careful rearrangement we get the following equations:

$$\begin{cases} \left(R_{F/R}^{K}\,{}^{R}A_X - {}^{F}A_X\right)N_X^K = \left(-R_{F/R}^{K}\,{}^{R}A_S^K + {}^{F}A_S^K\right)N_S^K \\ \left(R_{G/R}^{K}\,{}^{R}A_X - {}^{G}A_X\right)N_X^K = \left(-R_{G/R}^{K}\,{}^{R}A_S^K + {}^{G}A_S^K\right)N_S^K \end{cases}$$

We can rewrite the above equations as:

$$N_X^K = \frac{N_S^K\left({}^{F}A_S^K - R_{F/R}^{K}\,{}^{R}A_S^K\right)}{\left(R_{F/R}^{K}\,{}^{R}A_X - {}^{F}A_X\right)} \qquad \text{Eq. 6.9}$$

$$N_X^K = \frac{N_S^K\left({}^{G}A_S^K - R_{G/R}^{K}\,{}^{R}A_S^K\right)}{\left(R_{G/R}^{K}\,{}^{R}A_X - {}^{G}A_X\right)} \qquad \text{Eq.6.10}$$

Eq. 6.9 and Eq. 6.10 are the simple IDMS equations and the value for N_X^K can be determined using any one of those two equations.

Now we can substitute N_X^K value in the following equations to solve N_X^L and α

$$\begin{cases} \left(R_{F/R}^{L}\,{}^{R}A_X - {}^{F}A_X\right)N_X^L + \left[R_{F/R}^{L}\left({}^{R}A_X N_X^K + {}^{R}A_S^K N_S^K\right) - \left({}^{F}A_X N_X^K + {}^{F}A_S^K N_S^K\right)\right]\alpha \\ = \left(-R_{F/R}^{L}\,{}^{R}A_S^L + {}^{F}A_S^L\right)N_S^L \\ \left(R_{G/R}^{L}\,{}^{R}A_X - {}^{G}A_X\right)N_X^L + \left[R_{G/R}^{L}\left({}^{R}A_X N_X^K + {}^{R}A_S^K N_S^K\right) - \left({}^{G}A_X N_X^K + {}^{G}A_S^K N_S^K\right)\right]\alpha \\ = \left(-R_{G/R}^{L}\,{}^{R}A_S^L + {}^{G}A_S^L\right)N_S^L \end{cases}$$

We can rewrite them as:

$$\begin{cases} \mathrm{A}_3 N_X^L + \mathrm{B}_3 \alpha = \mathrm{C}_3 \\ \mathrm{A}_4 N_X^L + \mathrm{B}_4 \alpha = \mathrm{C}_4 \end{cases}$$

Where,

$$A_3 = (R_{F/R}^L \, {}^R A_x - {}^F A_x)$$
$$B_3 = [R_{F/R}^L ({}^R A_x N_x^K + {}^R A_s^K N_s^K) - ({}^F A_x N_x^K + {}^F A_s^K N_s^K)]$$
$$C_3 = N_s^L ({}^F A_s^L - R_{F/R}^L \, {}^R A_s^L)$$

and

$$A_4 = (R_{G/R}^L \, {}^R A_x - {}^G A_x)$$
$$B_4 = [R_{G/R}^L ({}^R A_x N_x^K + {}^R A_s^K N_s^K) - ({}^G A_x N_x^K + {}^G A_s^K N_s^K)]$$
$$C_4 = N_s^L ({}^G A_s^L - R_{G/R}^L \, {}^R A_s^L)$$

again

$$N_X^L = \frac{\begin{vmatrix} \mathrm{C}_3 & \mathrm{B}_3 \\ \mathrm{C}_4 & \mathrm{B}_4 \end{vmatrix}}{\begin{vmatrix} \mathrm{A}_3 & \mathrm{B}_3 \\ \mathrm{A}_4 & \mathrm{B}_4 \end{vmatrix}} \text{ and } \alpha = \frac{\begin{vmatrix} \mathrm{A}_3 & \mathrm{C}_3 \\ \mathrm{A}_4 & \mathrm{C}_4 \end{vmatrix}}{\begin{vmatrix} \mathrm{A}_3 & \mathrm{B}_3 \\ \mathrm{A}_4 & \mathrm{B}_4 \end{vmatrix}}$$

or $N_x^L = \dfrac{(C_3 B_4 - C_4 B_3)}{(A_3 B_4 - A_4 B_3)}$ and $\alpha = \dfrac{(A_3 C_4 - A_4 C_3)}{(A_3 B_4 - A_4 B_3)}$

Calculate the variables N_X^K, N_X^L and α using the expressions discussed above.

6.3.1.2 Conversion from L to K

If these two species undergo unidirectional transformations (***only from the species L to species K***) after the spike isotopes equilibrate with the sample isotopes, the fraction of *L* that converts to *K* will be β. Since no *K* converts to *L*, α is zero. Therefore, the total amount of ${}^{\mathrm{F}}\mathrm{Z}$ in *K* form becomes $\left({}^F A_x C_x^K W_x + {}^F A_s^K C_s^K W_s^K\right) + \left({}^F A_x C_x^L W_x + {}^F A_s^L C_s^L W_s^L\right)\beta$ after

the conversion from L to K. Similarly, the total amount of ^{R}Z in K form becomes $\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right) + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\beta$. At the same time, the total amount of ^{F}Z in L form becomes $\left({}^{F}A_x C_x^L W_x + {}^{F}A_s^L C_s^L W_s^L\right)(1-\beta)$, and, the total amount of ^{R}Z in L becomes $\left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)(1-\beta)$. Therefore, the expression of the isotope ratio of ^{F}Z to ^{R}Z in the K, $R_{F/R}^K$, can be constructed as Eq. 6.11. Following the similar procedure, we can construct equations Eq. 6.12 to Eq. 6.14.

$$R_{F/R}^K = \frac{\left({}^{F}A_x C_x^K W_x + {}^{F}A_s^K C_s^K W_s^K\right) + \left({}^{F}A_x C_x^L W_x + {}^{F}A_s^L C_s^L W_s^L\right)\beta}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right) + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\beta} \qquad \text{Eq. 6.11}$$

$$R_{G/R}^K = \frac{\left({}^{G}A_x C_x^K W_x + {}^{G}A_s^K C_s^K W_s^K\right) + \left({}^{G}A_x C_x^L W_x + {}^{G}A_s^L C_s^L W_s^L\right)\beta}{\left({}^{R}A_x C_x^K W_x + {}^{R}A_s^K C_s^K W_s^K\right) + \left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)\beta} \qquad \text{Eq. 6.12}$$

$$R_{F/R}^L = \frac{\left({}^{F}A_x C_x^L W_x + {}^{F}A_s^L C_s^L W_s^L\right)}{\left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)} \qquad \text{Eq. 6.13}$$

$$R_{G/R}^L = \frac{\left({}^{G}A_x C_x^L W_x + {}^{G}A_s^L C_s^L W_s^L\right)}{\left({}^{R}A_x C_x^L W_x + {}^{R}A_s^L C_s^L W_s^L\right)} \qquad \text{Eq. 6.14}$$

The following substitutions are used to simplify above equations

$$C_x^K W_x = N_x^K,\ C_x^L W_x = N_x^L,\ C_S^K W_S = N_S^K,\ C_S^L W_S = N_S^L$$

Now we can use Eq. 6.13 or Eq. 6.14 to solve the values for N_X^L. After careful rearrangements, Eqs. 6.13 and 6.14 can be written as follows:

$$\begin{cases} \left(R_{F/R}^L\, {}^{R}A_X - {}^{F}A_X\right)N_X^L = \left(-R_{F/R}^L\, {}^{R}A_S^L + {}^{F}A_S^L\right)N_S^L \\ \left(R_{G/R}^L\, {}^{R}A_X - {}^{G}A_X\right)N_X^L = \left(-R_{G/R}^L\, {}^{R}A_S^L + {}^{G}A_S^L\right)N_S^L \end{cases}$$

We can rewrite these equations as:

$$N_X^L = \frac{N_S^L({}^F A_S^L - R_{F/R}^L {}^R A_S^L)}{(R_{F/R}^L {}^R A_X - {}^F A_X)} \qquad \text{Eq. 6.15}$$

$$N_X^L = \frac{N_S^L({}^G A_S^L - R_{G/R}^L {}^R A_S^L)}{(R_{G/R}^L {}^R A_X - {}^G A_X)} \qquad \text{Eq. 6.16}$$

Eqs. 6.15 and 6.16 are the simplest form of the IDMS equations for species L and the value for N_X^L can be determined using any one of those two equations.

Now we can determine the values for N_X^K and β from Eqs. 6.11 or 6.12 by substituting value for N_X^L . After careful rearrangements of Eqs. 6.11 and 6.12, we can obtain the following equations:

$$\begin{cases} \left(R_{F/R}^K {}^R A_X - {}^F A_X\right) N_X^K + \left[R_{F/R}^K \left({}^R A_X N_X^L + {}^R A_S^L N_S^L\right) - \left({}^F A_X N_X^L + {}^F A_S^L N_S^L\right)\right]\beta \\ = \left(-R_{F/R}^K {}^R A_S^K + {}^F A_S^K\right) N_S^K \\ \\ \left(R_{G/R}^K {}^R A_X - {}^G A_X\right) N_X^K + \left[R_{G/R}^K \left({}^R A_X N_X^L + {}^R A_S^L N_S^L\right) - \left({}^G A_X N_X^L + {}^G A_S^L N_S^L\right)\right]\beta \\ = \left(-R_{G/R}^K {}^R A_S^K + {}^G A_S^K\right) N_S^K \end{cases}$$

We can rewrite these equations as:

$$\begin{cases} \mathrm{A}_1 N_X^K + \mathrm{B}_1 \beta = \mathrm{C}_1 \\ \\ \mathrm{A}_2 N_X^K + \mathrm{B}_2 \beta = \mathrm{C}_2 \end{cases}$$

Where,

$$A_1 = (R_{F/R}^K {}^R A_x - {}^F A_x)$$
$$B_1 = [R_{F/R}^K ({}^R A_x N_x^L + {}^R A_s^L N_s^L) - ({}^F A_x N_x^L + {}^F A_s^L N_s^L)]$$
$$C_1 = N_s^K ({}^F A_s^K - R_{F/R}^K {}^R A_s^K)$$

and

$$A_2 = (R^K_{G/R}\,{}^R A_x - {}^G A_x)$$
$$B_2 = [R^K_{G/R}({}^R A_x N^L_x + {}^R A^L_s N^L_s) - ({}^G A_x N^L_x + {}^G A^L_s N^L_s)]$$
$$C_2 = N^K_s({}^G A^K_s - R^K_{G/R}\,{}^R A^K_s)$$

The solutions are

$$N^K_X = \frac{\begin{vmatrix} C_1 & B_1 \\ C_2 & B_2 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 \\ A_2 & B_2 \end{vmatrix}} \text{ and } \beta = \frac{\begin{vmatrix} A_1 & C_1 \\ A_2 & C_2 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 \\ A_2 & B_2 \end{vmatrix}}$$

or $N^K_x = \dfrac{(C_1B_2 - C_2B_1)}{(A_1B_2 - A_2B_1)}$ and $\beta = \dfrac{(A_1C_2 - A_2C_1)}{(A_1B_2 - A_2B_1)}$

Calculate the variables N^K_X, N^L_X and β using the expressions discussed above.

Note: *In the unidirectional conversion, the new values for the total moles of* ${}^F K$ *and* ${}^G L$ *along with new isotopic abundances need to be calculated if the spikes are not in 100% pure form. The calculations are shown in Sections 6.2.2 and 6.2.3.*

6.3.2 Construction of Workable EXCEL® Spreadsheet

The equations derived in Sections 6.3.1.1 and 6.3.1.2 are programmed in Microsoft Excel spreadsheets and are available on CD-ROM and also on-line to assist with these solutions (*28*). The spreadsheet is user friendly and the user needs only to plug in all the required information as mentioned in Section 6.2.4. In this case, the user needs to define the mode of transformation, i.e., whether it is converting from first species to second species or vice versa.

***Note**: If the mode of species transformation is not well known and thus is no documentation to support an assumption, then the user should select the bidirectional transformation worksheet.*

6.3.3 Validation of SIDMS Equations

There was no real life data available to perform this type of validation study. Therefore, the isotope ratios, previously used for bidirectional transformation study, are applied in this study to validate the equations as well as the Excel worksheet.

6.3.3.1 Validation using Chromium

An acidic environment favors Cr(III), while a basic environment favors Cr(VI). When there is possibility of conversion of Cr(III) to Cr(VI) during alkaline extraction of Cr(VI) from samples, all the chromium present in the extract will be Cr(VI) form. Therefore, determination of Cr(III) concentration in sample is not possible by using SIDMS equations for unidirectional transformation, with double spiking before or after alkaline extraction. But the concentration of Cr(VI) and the percent of Cr(III) converted to Cr(VI) during extraction or analysis steps can be calculated (see Section 6.2.5.1).

For aqueous samples, the bidirectional transformation is possible with a higher tendency of conversion of Cr(VI) to Cr(III) during storage or analysis. In the pH range 1-2, only the Cr(VI) converts to Cr(III). If this pH range is set as analysis criterium then the original concentration of Cr(III) and Cr(VI) at the time of spiking and the amount of Cr(VI) converted to Cr(III) can be calculated by using the unidirectional SIDMS equations and the spreadsheet. The functionality of the two spreadsheets was tested using the very same isotope ratio used for bidirectional transformations and the results

are summarized in Table 6-VII. The reported percent conversion from Cr(VI) to Cr(III) is negative, because the isotope ratio used in this calculation is obtained from high pH system that stabilized/extracts Cr(VI).

TABLE 6-VII. Deconvoluted Cr(III) and Cr(VI) Concentrations and Percent Conversions Calculated using Unidirectional SIDMS Equations and Spreadsheet.

	Deconvoluted Concentration		% Transformation	
	Cr(III) (μg/g)	Cr(VI) (μg/g)	Cr(III) to Cr(VI)	Cr(VI) to Cr(III)
Case-1: Cr(III) to Cr(VI)	101.4 ± 41.8	219.4 ± 22.3	8 ± 5	----
Case-2: Cr(VI) to Cr(III)	156.7 ± 46.0	258.0 ± 13.3	----	- 21 ± 2

TABLE 6-VIII. Deconvoluted Hg^{2+} and CH_3Hg^+ Concentrations and Percent Conversions Calculated using Unidirectional SIDMS Equations and Spreadsheet.

	Deconvoluted Concentration		% Transformation	
	Hg^{2+} (μg/g)	CH_3Hg^+ (μg/g)	Hg^{2+} to CH_3Hg^+	CH_3Hg^+ to Hg^{2+}
Case-1: Hg^{2+} to CH_3Hg^+	3.335 ± 0.165	1.827 ± 0.289	8.89 ± 3.24	----
Case-2: CH_3Hg^+ to Hg^{2+}	1.790 ± 0.309	3.308 ± 0.617	----	46.58 ± 2.68

6.3.3.2 Validation using Mercury

In the literature, some scientists report artifact formation of methylmercury and some report artifact formation of inorganic mercury from organomercury species. In these situations the appropriate worksheet can be used to determine the concentration of both mercury species as well as the percent transformation. The isotope ratio data applied here for validation of the equations and worksheet functionality were obtained from

SONI-1 extraction for Material-1 (Chapter 4). The deconvoluted concentration and percent conversion results are summarized in Table 6-VIII.

6.4 Conclusions

The generic equations and workable Microsoft Excel worksheet for two and three species systems for both bidirectional and unidirectional transformations have been developed and validated using both real and hypothetical data. These equations and worksheet are applicable for determination of concentration of any species as well as the percent transformation that may occur after spiking.

6.5 References

(1) Falter, R.; Hintelmann, H.; Quevauviller, P. The workshop on "Sources of error in methylmercury determination during sample preparation, derivatization and detection", Wiesbaden, May 27-29, **1998**.

(2) Han, Y.; Kingston, H. M.; Boylan, H. M.; Rahman, G. M. M.; Shah, S.; Richter, R. C.; Link, D. D.; Bhandari, S. *Anal. Bioanal. Chem*. **2003**, *375*, 428-436.

(3) Hintelmann, H.; Falter, R.; Ilgen, G.; Evans, R. D. *Fresenius' J. Anal. Chem*. **1997**, *358(3)*, 363-370.

(4) Bloom, N. S.; Colman, J. A.; Barber, L. *Fresenius' J. Anal. Chem*. **1997**, *358(3)*, 371-377.

(5) Hintelmann, H. *Chemosphere* **1999**, *39(7)*, 1093-1105.

(6) Falter, R. *Chemosphere* **1999**, *39(7)*, 1051-1073.

(7) Falter, R. *Chemosphere* **1999**, *39(7)*, 1075-1091.

(8) Tseng, C. M.; De Diego, A.; Wasserman, J. C.; Amouroux, D.; Donard, O. F. X. *Chemosphere* **1999**, *39*, 1119-1136.

(9) Hammerschmidt, C. R.; Fitzgerland, W. F. *Anal. Chem*. **2001**, *73*, 5930-5936.

(10) Gaona, X.; Valiente, M. *Anal. Chim. Acta* **2003**, *480*, 219-230.

(11) Yang, L.; Colombini, V.; Maxwell, P.; Mester, Z.; Sturgeon, R. E. *J. Chromatogr. A* **2003**, *1011*, 135-142.

(12) Wilken, R. D.; Falter, R. *Appl. Orgnomet. Chem*. **1998**, *12*, 551-557.

(13) Snell, J. P.; Stewart, I. I.; Sturgeon, R. E.; Frech, W. *J. Anal. At. Spectrom*. **2000**, *15*, 1540-1545.

(14) Qvarnstrom, J.; Lambertsson, L.; Havarinasab, S.; Hultman, P.; Frech, W. *Anal. Chem*. **2003**, *75*, 4120-4124.

(15) Monperrus, M.; Rodriguez Martin-Doimeadios, R. C.; Scancar, J.; Amouroux, D.; Donard, O. F. X. *Anal. Chem*. **2003**, *75*, 4095-4102.

(16) Lambertsson, L.; Lundberg, E.; Nilsson, M.; Frech, W. *J. Anal. At. Spectrom*. **2001**, *16*, 1296-1301.

(17) Hintelmann, H.; Evans, R. D. *Fresenius' J. Anal. Chem*. **1997**, *358*, 378-385.

(18) Hintelmann, H.; Evans, R. D.; Villeneuve, J. Y. *J. Anal. At. Spectrom*. **1995**, *10*, 619-624.

(19) Rodriguez Martin-Doimeadios, R. C.; Monperrus, M.; Krupp, E.; Amouroux, D.; Donard, O. F. X. *Anal. Chem*. **2003**, *75*, 3202-3211.

(20) Rodriguez Martin-Doimeadios, R. C.; Krupp, E.; Amouroux, D.; Donard, O. F. X. *Anal. Chem*. **2002**, *74*, 2505-2512.

(21) Clough, R.; Belt, S.; Evans, E. H.; Fairman, B.; Catterick, T. *Appl. Spectrosc. Rev.* **2003**, *38(1)*, 101-132.

(22) Kingston, H. M. US Patent Number: 5,414,259; **1995**.

(23) Huo, D.; Kingston, H.M. *Anal. Chem.* **2000**, *72(20)*, 5047-5054.

(24) Huo, D.; Kingston, H. M. S.; Larget, B. In *Comprehensive Analytical Chemistry*, Caruso, J. A.; Sutton, K. L.; Ackley, K. L., Eds.; *Elemental Speciation: New Approaches for Trace Element Analysis*, Vol. 33; Elsevier: New York, **2000;** pp 277-313.

(25) Kingston, H. M.; Huo, D.; Lu, Y.; Chalk, S. *Spectrochim. Acta, Part B* **1998,** *53***,** 299-309.

(26) Huo, D.; Lu, Y.; Kingston, H. M. *Environ. Sci. Technol*. **1998,** *32***,** 3418.

(27) Kingston, H. M. S.; Huo, D. In *Solid Waste Manual – 846, Update IVA*, US Government Printing Office, Washington DC, **2000**.

(28) Kingston, H. M. S.; Huo, D.; Rahman, G. M. M.; Iyer, S.; Walter, P. SamplePrep Web, Available: http://www.sampleprep.duq.edu/.

(29) Huo, D. *Ph.D. Thesis*, Duquesne University, Pittsburgh, PA, **1999.**

Chapter 7

Application of Modified SIDMS Analysis for Cr(VI) in Selected Soil and Sludge Samples

7.1 Introduction

Chromium is a naturally occurring metal in the environment; it may exist in several oxidation states ranging from chromium(II) to chromium(VI). Chromium mainly occurs in the environment in hexavalent and trivalent oxidation states. The toxicity of a chromium species depends on its bioavailability, which is related in turn to its chemical forms (species) and concentrations. Trivalent chromium is more stable, relatively nontoxic and an essential nutrient in the human diet to maintain effective glucose, lipid and protein metabolism (*1*). On the other hand, hexavalent chromium can exist as chromium trioxide, dichromic acid and dichromate salts, which are all considered carcinogenic because they can easily diffuse through the cell membrane and oxidize biological molecules with toxic results (*2*).

Frequently, samples contain Cr(III) along with Cr(VI) and the concentration of Cr(III) is 10 to 1000 times greater than that of Cr(VI). Therefore, the preservation and stabilization of the oxidation states are essential for an accurate analysis. There are several factors that control the interconversion of Cr(III) and Cr(VI) species. These include the presence of oxidizing and reducing agents, the electrochemical potential of the oxidation and reduction reactions, UV light, presence of organic compounds and acid–base reactions. The standard reduction potential for the Cr(VI)/Cr(III) redox couple at high pH is negative [$CrO_4^{2-} + 4H_2O + 3e^- = Cr(OH)_2^+ + 6OH^-$ (E^0 = -0.13 V, pH = 14], which indicates that an alkaline medium favors the stabilization of Cr(VI). In acidic

media, the standard reduction potential for the Cr(VI)/Cr(III) redox couple is positive [$HCrO_4^- + 7H^+ + 3e^- = Cr^{3+} + 4H_2O$ (E^0 = +1.21 V, pH = 1], which favors Cr(III) stabilization. When pH < 10, Cr(VI) predominates as anionic species [$CrO_2^{2-}$and $HCrO_4^-$], whereas Cr(III) exists as cations [Cr^{3+}, $Cr(OH)^{2+}$, $Cr(OH)_2^+$] (*3*). In this case, pH is an important factor in determining the relative stabilities of Cr(III) and Cr(VI) in aqueous systems. Cr(III) is thermodynamically stable at low E_h and low pH, while high E_h and high pH is favorable for Cr(VI). Therefore, an alkaline medium favors the stabilization of Cr(VI). The distribution of Cr species upon pH and potential at 25 °C is shown in Figure 7-1.

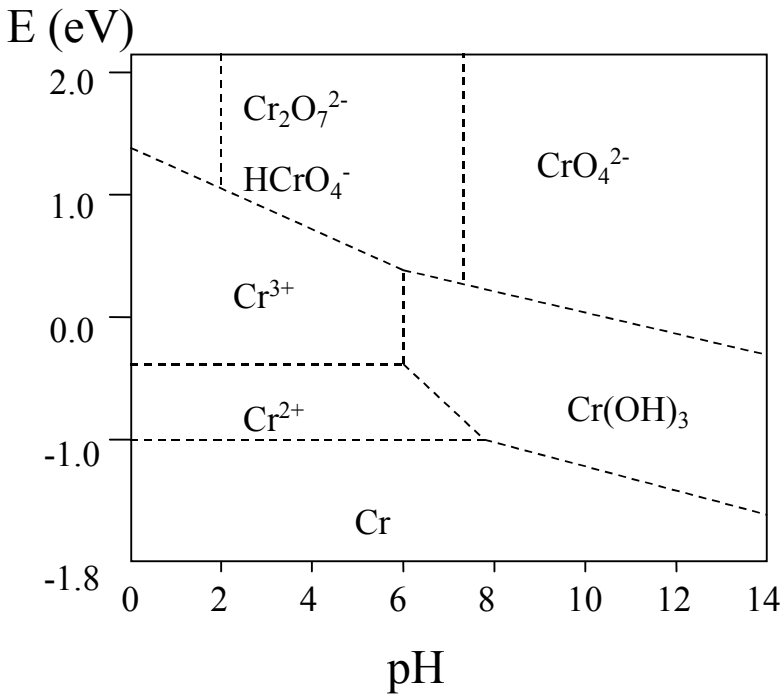


FIGURE 7-1 pH-E_h diagram of Cr Species (*4*).

The determination of Cr(VI) from solid samples requires two major steps: extraction and detection. During this study, all samples were extracted according to EPA Method 3060A (*Alkaline Digestion for Hexavalent Chromium*) (*5*). There are a number of methods available for the determination of chromium, e.g. atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS). These spectrometric methods determine total Cr, and a separation method prior to detection is necessary to provide any speciation information. A number of electrochemical and colorimetric techniques are also available to determine Cr(VI). However, none of these methods can correct for any interconversion or transformation of Cr(III) to Cr(VI) or Cr(VI) to Cr(III). Therefore, during this study, EPA Method 6800 (*Elemental and Speciated Isotope Dilution Mass Spectrometry, SIDMS*) was used as a determinative technique and correction tool (*6*). Any interconversions that occur after spiking are traceable and can be quantitatively corrected by monitoring isotopes in each species. Because SIDMS can measure species concentrations at the time of spiking, by spiking a sample both before and after its extraction, SIDMS can be used as diagnostic tool to identify a procedure that alters species distribution in a multiple step protocol. This method was developed specifically for active species that may undergo interconversion during their analysis, such as Cr(III) and Cr(VI). In some cases, and with further research, SIDMS can identify chemical interactions and conditions that alter species concentrations. SIDMS also can be used in several other sophisticated analyses where conversion is both required and deliberately or accidentally imposed on the analyte of interest. The method is based on the use of enriched separated isotope and has been

documented in the literature as a standard method. It has been extensively tested on soils, water and extracts in conjunction with specific analytical species preparation techniques such as EPA Method 3060A and EPA draft Method 3200. The fundamental theory of SIDMS in environmental systems is established and discussed in references (*7-12*).

The analysis of solid samples is not as straight forward as that of liquid samples. This topic has been discussed in several publications from the Kingston research group (*7,8,10,11,16,20*). The diversity of chemical forms and behaviors of Cr(III) subspecies causes difficulties evaluating the oxidation of Cr(III) because it is unlikely that the sample Cr(III) in soils will reach an equilibrium with the isotopically labeled spike ^{50}Cr(III) that is in aqueous form. Studies have shown that EPA Method 3060A effectively extracts Cr(VI) in strong alkaline solution at temperature of 90-95 °C. The total amount of Cr(VI) produced due to the method-induced oxidation of Cr(III) is limited in EPA Method 3060A and there is little Cr(VI) reduced to Cr(III) in hot alkaline extraction situation (*13-14*). However, it is also reported that the oxidation of Cr(III) is highly dependent on the chemical forms of Cr(III): Cr_2O_3 and aged precipitated $Cr(OH)_3$ are resistant to oxidation, while free Cr^{3+} and freshly precipitated $Cr(OH)_3$ are relatively easy to oxidize. Based on this information, samples are spiked with the ^{50}Cr(III) spike in Cr^{3+} aqueous form. Because the isotopically labeled Cr^{3+} is the most easily oxidizable Cr(III) subspecies and ^{50}Cr(III) is in relatively large quantity, ^{50}Cr(III) competes with the sample Cr(III) in oxidization. The addition of sufficient ^{50}Cr(III) spike, approximately equal to the total Cr amount in the sample, thus inhibits the transformation of the sample Cr(III) subspecies to Cr(VI) subspecies during extraction using EPA Method 3060A.

7.2 Experimental

7.2.1 Instrumentation

An Ethos-1600 microwave laboratory system (Milestone Monroe, CT, USA) equipped with temperature and pressure feedback control and magnetic stirring capability was used in the microwave digestion and extraction processes. Digestion and extractions were performed in closed TFM- and PFA-Teflon®-lined pressure vessels. The instrument was used in the class-1000 clean room. The samples were manipulated only in the clean room under class-1000 or class-100 conditions. Up to ten samples can be extracted simultaneously. The Teflon vessels have a capacity of 100 mL. A FAM–40 vacuum unit (Milestone, Sorisole (BG), Italy) was used to filter the digests and extracts. An Analytical *Plus* Electronic Balance (OHAUS, England), capable of weighing 0.00001g was used in this study to weigh samples and standards.

A ConstaMetric 4100Bio/MS polymeric inert pump (Thermo Separation, Riviera Beach, FL) and an ANX 4605 Cr anion-exchange column, PEEK 50 mm x 4.6 mm (CETAC Technologies, Omaha, NE) were used in this study to separate Cr(VI) and Cr(III). A six-port injection valve (Valco Vicci) was used between the pump and column. Because no special interface is required between the ANX 4605 Cr column and the ICP-MS, one outlet of the column is directly interfaced to the nebulizer of the ICP-MS with a piece of PFA tubing, and the other end is connected to a 50 μL TEFZEL™ sample loop (CETAC Technologies, Omaha, NE). Figure 7-2 shows a typical separation of Cr(III) and Cr(VI) using this system at a flow rate of 1.0 mL/min. An HP 4500 ICP-MS (Agilent Technologies, Palo Alto, CA, USA and Yokogawa Analytical Systems Inc., Tokyo, Japan) was used in this study. The sample delivery system consisted of

peristaltic pump and quartz spray chamber with concentric nebulizer and quartz torch. The instrument was fitted with platinum sampler and skimmer cones and optimized daily using 10 ppb tuning solution (Agilent Technologies, Palo Alto, CA, USA) containing Li, Y, Ce and Tl in 2% HNO_3. The operating conditions for the LC-ICP-MS set up are given in Table 7-I. Integration time per point was 0.5 s for both direct aspiration mode and Time Resolved Analysis (TRA) mode.

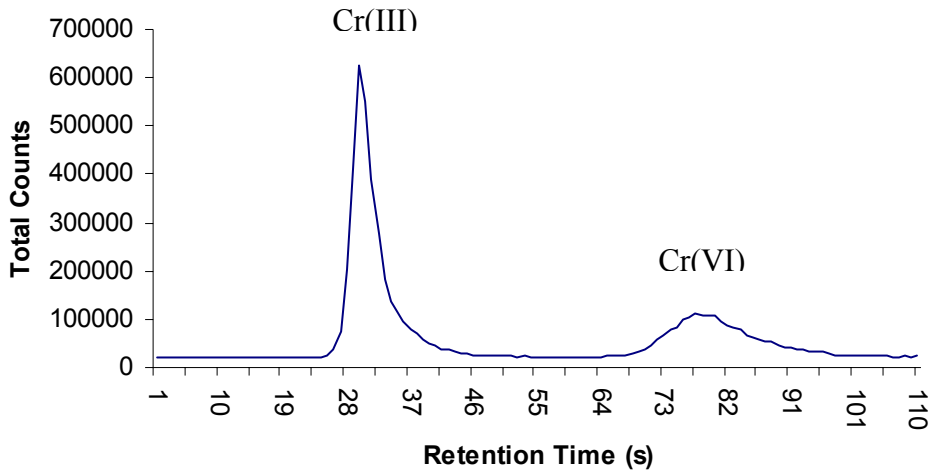


FIGURE 7-2. Typical chromatogram for separation of Cr(III) and Cr(VI). (*Flow rate*: 1.0 mL/min; *Eluent*: 0.06 *M* NO_3^-, pH = 3.0; *Column*: CETAC ANX 4605 Cr).

7.2.2 Reagents, Solutions and Samples

Double deionized (DDI) water (18 MΩ cm^{-1}), prepared from a Barnstead NANOpure Ultrapure Water System (Dubuque, IA, USA), was used in the preparation of all solutions throughout this study. Concentrated HNO_3 (69%) was purified by sub-boiling distillation of commercial HNO_3 (ACS plus, Fisher Scientific, Pittsburgh, PA, USA) using quartz still (Milestone, Sorisole (BG), Italy). Concentrated NH_3(aq)(15 M) was

prepared by bubbling high purity ammonia gas through deionized water. Concentrated HF(aq) (45-50%) (*Optima*, Fisher Scientific, Pittsburgh, PA, USA) was used. Extraction solution containing 2.5 M NaOH was prepared by dissolving 100.0 g of NaOH (98.0%) (Certified ACS, Fisher Scientific, Pittsburgh, PA, USA) in 1000 mL of DDI water, and approximately 0.742 g of Na_2CO_3 (99.6%, Fisher Scientific, Pittsburgh, PA, USA) was directly added to each extraction vessel to prepare 0.28 M Na_2CO_3 *in situ*.

TABLE 7-I. HPLC-ICP-MS Operating Conditions.

Plasma	
Plasma flow rate (L/min)	15.0
Auxiliary gas flow rate (L/min)	1.0
Radio frequency power (W)	1450
Sample cone	Platinum, 1.1 mm orifice
Skimmer cone	Platinum, 0.89 mm orifice
Measurement Parameters	
Analysis mode	Time resolved analysis (TRA)
Analysis isotopes	^{50}Cr, ^{52}Cr, and ^{53}Cr
Nebulizer gas flow rate (L/min)	0.93 – 1.00
Peristaltic pump rate (rpm)	0.25
Integration time per point (s)	0.5
Total analysis time (s)	180
Eluent flow rate (mL/min)	1.0

Five standard solutions were prepared, including $^{nat}Cr(III)$ standard, 1.0 mg/g in 1% HNO_3; $^{nat}Cr(VI)$ standard, 50 μg/g in 1% HNO_3; $^{50}Cr(III)$ spike, 1.0 mg/g in 1% HNO_3; $^{53}Cr(VI)$ spike, 150 μg/g in 1% NH_3(aq); and ^{srm}Cr (NIST SRM 979), 10 μg/g in 1% HNO_3. $^{nat}Cr(III)$ standard solution with natural isotopic abundance was prepared

from 99.999% Cr metal (Aldrich Chemical Co., Milwaukee, WI, USA) by dissolving it in hydrochloric acid; ^{nat}Cr(VI) standard solution with natural abundance was prepared from $K_2Cr_2O_7$ (NIST SRM 136e) in DDI water. The "nat" superscript stands for "natural isotopic abundance." Isotope-enriched materials were purchased from Isotech Inc. (Miamisburg, OH, USA). ^{50}Cr(III) spike solution was prepared from ^{50}Cr-enriched metal (Lot # 2691). ^{53}Cr(VI) spike solution was prepared from ^{53}Cr-enriched oxide (Lot # 2692). $^{iso\text{-}50}$Cr(III) spike, 1.0 mg/g in 1% HNO_3, was prepared by mixing ^{50}Cr(III) and ^{nat}Cr(III) in ratio of 1:6.89 by weight. The isotopic abundances of natural and isotope enriched chromium materials used in this study are reported in Table 7-II. The preparation procedure for each standard and characterization of standard solutions can be found in reference (*15*).

The eluent was prepared by using sub-boiling distilled HNO_3 and thulium standards. The final pH of the eluent was approximately 3.0 with 10 ppm thulium for optimum separation of Cr(III) and Cr(VI) on the column. The working solutions of ^{nat}Cr(VI) were prepared daily by weighing proper amount of stock ^{nat}Cr(VI) and diluting with DDI water to the desired mass. The working solutions of ^{nat}Cr(III) were prepared daily by weighing a proper amount of stock ^{nat}Cr(III) and diluting with 1% HNO_3 to the desired mass. ^{srm}Cr working solutions (0, 5, 10, 20, 30, 40, 50, 60, and 100 ppb) were prepared daily by measuring a proper amount of stock ^{srm}Cr and diluting with 1% HNO_3 to the desired volume. The calibration standards for determination of total Cr, V, Mn, Pb, and Ba at different concentrations were prepared by diluting with DDI water a high purity, NIST-traceable, multi-element standard, 10 μg/ml in 2% HNO_3, (High Purity Standards, Inc. #ICP-MS CS-M, Charleston, SC, USA).

TABLE 7-II: Isotope Abundances and Average Atomic Weight of Standards and Spikes.

Isotope	^{nat}Cr (%)	^{50}Cr(III) (%)	$^{iso-50}$Cr(III) (%)	^{53}Cr(VI) (%)
^{50}Cr	4.345	93.1	15.39	0.03
^{52}Cr	83.79	6.80	74.21	2.19
^{53}Cr	9.501	0.10	8.33	97.7
^{54}Cr	2.365	0.00	2.07	0.08
Average atomic weight	52.00	50.08	51.76	52.92

In the total of nine samples analyzed during this study, seven were obtained from *Environ* through *Environmental Standards Inc*. and two were environmental Standard Reference Materials, SRM 2704 (Buffalo River Sediment) and SRM 2711 (Montana Soil) (US Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD, USA). No certified speciated concentration of Cr(VI) was available for either SRM. The sample supplier provided information relating specific sample ID, sampling date and time, and sampling location. Refer to Table 7-III for details about the arrival, acceptance, storage, extraction and analysis.

Instrument grade liquid argon (Airgas Inc., Radnor, PA, USA) was used as nebulizer carrier gas for LC-ICP-MS. Polypropylene graduated tubes were used as extract and digest storage vials. Standards were prepared and stored in Teflon bottles. Polystyrene dilutions vials were used for preparation of all working sample solutions.

TABLE 7-III: Information Regarding Sample Processing.

Sample ID*	Sampling Date and Time	Location	Shipping Date and Time	Received Date[a,b]	Extraction Date	Analysis Date
LOV-SC-7F	01/02/03 @ 13:00	Pittsburg, CA	01/27/03 @ 16:00	01/28/03 @ 11:00	02/06/03	02/07/03
DOS-SC-7G	01/20/03 @ 11:40	Oakley, CA	01/27/03 @ 16:00	01/28/03 @ 11:00	02/06/03	02/07/03
DOS-SC-8G	01/20/03 @ 11:00	Oakley, CA	01/27/03 @ 16:00	01/28/03 @ 11:00	02/06/03	02/07/03
DOS-SC-9G	01/20/03 @ 10:20	Oakley, CA	01/27/03 @ 16:00	01/28/03 @ 11:00	02/06/03	02/07/03
DOS-SC-10G	01/20/03 @ 14:05	Oakley, CA	01/27/03 @ 16:00	01/28/03 @ 11:00	02/06/03	02/07/03
DOS-SC-11G	01/20/03 @ 13:40	Oakley, CA	01/27/03 @ 16:00	01/28/03 @ 11:00	02/08/03	02/09/03
DOS-SC-12G	01/20/03 @ 14:40	Oakley, CA	01/27/03 @ 16:00	01/28/03 @ 11:00	02/08/03	02/09/03

[a]Arrival Temperature = 1.0 °C; [b]Storage Temperature = 4.0 °C

*Samples analyzed in "as received" condition (wet).

7.2.3 Sample Preparation Procedures for Cr(VI)

7.2.3.1 Total Digestion Procedure: EPA Method 3052

All samples and SRMs (SRM 2704 and SRM 2711) were digested according to EPA Method 3052 (*Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices*). Approximately 0.5 g of representative aliquots were weighed into microwave vessels, and 9 mL of concentrated HNO_3 and 2 mL of concentrated HF were added to each vessel. Vessels were sealed and microwave irradiated at 180 ± 5 °C for 10 min. After digestion, the sample aliquots were filtered through filter paper and stored in cold room at 4 °C until analyzed.

7.2.3.2 Extraction: SIDMS Procedure

The sample preparation for Cr(VI) alkaline extraction follows the guideline of EPA Method 3060A (*5*). A closed vessel microwave system with temperature control and continuous auto stirring (Milestone, Monroe, CT, USA) was used as a heating device. The procedure is described as follows. Approximately 0.5 g of sample and 0.74 g of Na_2CO_3 were weighed into a microwave vessel. 5 mL of 2.5 M NaOH and a proper amount of $^{iso\text{-}50}Cr(III)$ and $^{53}Cr(VI)$ ("double-spiking") were added to the vessel. DDI water was added to the vessel so that the total volume of the liquid in each vessel was 25 mL. The amount of isotopic spike depends on the levels of Cr(VI) and Cr(III) in the sample. More $^{iso\text{-}50}Cr(III)$ gives better correction for oxidation. Three replicates of each sample and SRM were extracted during this study. Vessels were sealed and heated at 95 ± 2 °C in the microwave unit for 1 h with constant stirring. After extraction, the vessels were allowed to cool to room temperature and then the solutions were filtered using a 0.2 μm Millipore Glass Fiber Filter (Fisher Scientific, Pittsburgh, PA, USA). The filtered extracts were stored in cold room at 4 °C until analyzed. The filtrates were acidified to pH 5 to 6 with concentrated HNO_3 just before measurement.

7.2.3.3 Extraction: Method 3060A for Method 6800 and IDMS

A second aliquot of each sample and SRM was extracted again using EPA method 3060A and microwave energy as heat source. The extraction procedure is the same as described in paragraph *7.2.3.2, above,* except that (pre-extraction) double spiking with isotope-enriched standard was not employed. However, after extraction and filtration, a portion from each replicate was double-spiked (with known amount of isotope enriched

$^{iso\text{-}50}$Cr(III) and ^{53}Cr(VI) standard). These samples were then analyzed with HPLC-ICP-MS. The remaining portion was then divided into two more fractions. One of them was spiked only with ^{53}Cr(VI) in order to perform Isotope Dilution Mass Spectrometry (IDMS) measurements. The second fraction was analyzed for Cr(VI) with HPLC-ICP-MS.

7.2.3.4 Algorithms, Assumptions and Calculations

In EPA Method 6800, the algorithms for Cr(III) and Cr(VI) in aqueous sample have been demonstrated and are summarized below in Equations 7-1 to 7-4. Their derivation is based on these assumptions: spike isotopes and natural isotopes are equilibrated before species transformations; there are no selective losses of the species; and each isotopic spike has been converted completely to a single species (in this case, all Cr in ^{50}Cr(III) spike is in Cr(III) form; and all Cr in ^{53}Cr(VI) spike is in Cr(VI) form).

The following equations are solved simultaneously, using a Microsoft Excel spreadsheet. A programmed spreadsheet is available on-line (*16*) to assist with these computations.

$$R^{III}_{50/52} = \frac{\left({}^{50}A_x C^{III}_x W_x + {}^{50}A^{III}_s C^{III}_s W^{III}_s\right)(1-\alpha) + \left({}^{50}A_x C^{VI}_x W_x + {}^{50}A^{VI}_s C^{VI}_s W^{VI}_s\right)\beta}{\left({}^{52}A_x C^{III}_x W_x + {}^{52}A^{III}_s C^{III}_s W^{III}_s\right)(1-\alpha) + \left({}^{52}A_x C^{VI}_x W_x + {}^{52}A^{VI}_s C^{VI}_s W^{VI}_s\right)\beta} \qquad \text{Eqn. 7-1}$$

$$R^{III}_{53/52} = \frac{\left({}^{53}A_x C^{III}_x W_x + {}^{53}A^{III}_s C^{III}_s W^{III}_s\right)(1-\alpha) + \left({}^{53}A_x C^{VI}_x W_x + {}^{53}A^{VI}_s C^{VI}_s W^{VI}_s\right)\beta}{\left({}^{52}A_x C^{III}_x W_x + {}^{52}A^{III}_s C^{III}_s W^{III}_s\right)(1-\alpha) + \left({}^{52}A_x C^{VI}_x W_x + {}^{52}A^{VI}_s C^{VI}_s W^{VI}_s\right)\beta} \qquad \text{Eqn. 7-2}$$

$$R^{VI}_{50/52} = \frac{\left({}^{50}A_x C^{III}_x W_x + {}^{50}A^{III}_s C^{III}_s W^{III}_s\right)\alpha + \left({}^{50}A_x C^{VI}_x W_x + {}^{50}A^{VI}_s C^{VI}_s W^{VI}_s\right)(1-\beta)}{\left({}^{52}A_x C^{III}_x W_x + {}^{52}A^{III}_s C^{III}_s W^{III}_s\right)\alpha + \left({}^{52}A_x C^{VI}_x W_x + {}^{52}A^{VI}_s C^{VI}_s W^{VI}_s\right)(1-\beta)} \qquad \text{Eqn. 7-3}$$

$$R^{VI}_{53/52} = \frac{\left({}^{53}A_x C^{III}_x W_x + {}^{53}A^{III}_s C^{III}_s W^{III}_s\right)\alpha + \left({}^{53}A_x C^{VI}_x W_x + {}^{53}A^{VI}_s C^{VI}_s W^{VI}_s\right)(1-\beta)}{\left({}^{52}A_x C^{III}_x W_x + {}^{52}A^{III}_s C^{III}_s W^{III}_s\right)\alpha + \left({}^{52}A_x C^{VI}_x W_x + {}^{52}A^{VI}_s C^{VI}_s W^{VI}_s\right)(1-\beta)} \qquad \text{Eqn. 7-4}$$

where,

$R_{50/52}^{III}$ is the measured isotope ratio of ^{50}Cr to ^{52}Cr of Cr(III) in the spiked sample

$R_{53/52}^{III}$ is the measured isotope ratio of ^{53}Cr to ^{52}Cr of Cr(III) in the spiked sample

$R_{50/52}^{VI}$ is the measured isotope ratio of ^{50}Cr to ^{52}Cr of Cr(VI) in the spiked sample

$R_{53/52}^{VI}$ is the measured isotope ratio of ^{53}Cr to ^{52}Cr of Cr(VI) in the spiked sample

$^{50}A_x$ is the natural relative isotopic abundance of ^{50}Cr in the sample

$^{52}A_x$ is the natural relative isotopic abundance of ^{52}Cr in the sample

$^{53}A_x$ is the natural relative isotopic abundance of ^{53}Cr in the sample

$^{50}A_s^{III}$ is the relative isotopic abundance of ^{50}Cr in the ^{50}Cr(III) spike

$^{52}A_s^{III}$ is the relative isotopic abundance of ^{52}Cr in the ^{50}Cr(III) spike

$^{53}A_s^{III}$ is the relative isotopic abundance of ^{53}Cr in the ^{50}Cr(III) spike

$^{50}A_s^{VI}$ is the relative isotopic abundance of ^{50}Cr in the ^{53}Cr(VI) spike

$^{52}A_s^{VI}$ is the relative isotopic abundance of ^{52}Cr in the ^{53}Cr(VI) spike

$^{53}A_s^{VI}$ is the relative isotopic abundance of ^{53}Cr in the ^{53}Cr(VI) spike

C_x^{III} is the concentration of Cr(III) in the sample (μmol/g, unknown)

C_x^{VI} is the concentration of Cr(VI) in the sample (μmol/g, unknown)

W_x is the weight of the sample (g)

C_s^{III} is the concentration of Cr(III) in the ^{50}Cr(III) spike (μmol/g)

W_s^{III} is the weight of the ^{50}Cr(III) spike (g)

C_s^{VI} is the concentration of Cr(VI) in the ^{53}Cr(VI) spike (μmol/g)

W_s^{VI} is the weight of the ^{53}Cr(VI) spike (g)

α is the proportion of Cr(III) oxidized to Cr(VI) after spiking (unknown)

β is the proportion of Cr(VI) reduced to Cr(III) after spiking (unknown)

For Method 3060A extraction, several simplifying assumptions have been employed to aid the solution of the algorithms. These assumptions are based on the extreme stability afforded chromium species by the pH, as seen in the species chromatogram given in Figure 7-1. We have referred to these assumptions as one-way species degradations and they assist in analytical method development by reducing the bidirectionality of dynamic species to unidirectional degradation probabilities. Accordingly, we treat first β=0, because Cr(VI) is stable in alkaline solution and there is little Cr(VI) reduced to Cr(III) in the Method 3060A hot alkaline extraction. Second, because ^{50}Cr(III) spike is the dominant soluble Cr(III) species in pH 11 solutions and determines the isotopic ratio for soluble Cr(III) species in the final extract solution, we treat C_x^{III} = 0 due to its suppression by the isotopically labeled spike. Thereby, equations 7-1 through 7-4 are simplified to:

$$R_{50/52}^{VI} = \frac{\left({}^{50}A_s^{III} C_s^{III} W_s^{III}\right)\alpha + \left({}^{50}A_x C_x^{VI} W_x + {}^{50}A_s^{VI} C_s^{VI} W_s^{VI}\right)}{\left({}^{52}A_s^{III} C_s^{III} W_s^{III}\right)\alpha + \left({}^{52}A_x C_x^{VI} W_x + {}^{52}A_s^{VI} C_s^{VI} W_s^{VI}\right)} \qquad \text{Eqn. 7-5}$$

$$R_{53/52}^{VI} = \frac{\left({}^{53}A_s^{III} C_s^{III} W_s^{III}\right)\alpha + \left({}^{53}A_x C_x^{VI} W_x + {}^{53}A_s^{VI} C_s^{VI} W_s^{VI}\right)}{\left({}^{52}A_s^{III} C_s^{III} W_s^{III}\right)\alpha + \left({}^{52}A_x C_x^{VI} W_x + {}^{52}A_s^{VI} C_s^{VI} W_s^{VI}\right)} \qquad \text{Eqn. 7-6}$$

The four unknown factors in these two equations are the isotopic ratios of 50/52 and 53/52 for Cr(VI) species, C_x^{VI} and α in the final extract solution. , We can measure the isotopic ratios of 50/52 and 53/52 for Cr(VI) species, by using HPLC-ICP-MS. Although some of the Cr(VI) may transform to Cr(III) during the chromatographic separation and measurement, the isotopic ratios of Cr(VI) species are constant because no Cr(III) spike transforms to Cr(VI) in the acidic eluent. There remain only two unknown variables, C_x^{VI} and α. Equations 7-5 and 7-6 then become two equations in two unknowns and can be solved easily for the concentration of Cr (VI) in the samples, C_x^{VI}, and the fraction of Cr(III) transformed to Cr(VI), α. The algorithm solutions and assumptions are an extension of EPA Method 6800 specific to solid samples where equilibrium between the Cr(III) from the sample and the Cr(III) from isotopic spike usually is not achieved. This method has been published in a dissertation (*15*).

7.3 Results and Discussion

7.3.1 Total Elemental Analysis

The samples and the SRMs were digested according to EPA Method 3052 and were analyzed with ICP-MS in direct aspiration mode. The concentrations of total Cr, Mn, V, Ti, Pb, and Ba were determined from calibration curves produced by using multi-element standard. Results are summarized in Table 7-IV. The linear fit equations and corresponding correlation coefficients for Cr, V, Ti, Mn, Pb, and Ba are as follows (CPS vs. ppb): Y = 2217.6x – 1358.7 and R^2 = 0.9984 for Cr; Y = 20260x + 5633.6 and R^2 = 0.9987 for V; Y = 2030x + 566.08 and R^2 = 0.9987 for Ti; Y = 19346x + 3782.4 and R^2

= 0.9993 for Mn; Y = 5034x - 4133.6 and R^2 = 0.9940 for Pb; and Y = 2394x – 678.99 and R^2 = 0.9993 for Ba, respectively.

TABLE 7-IV: Summary for Total Digestion Analysis at 95% C.L.

Sample	Chromium (μg/g)	Vanadium (μg/g)	Titanium (μg/g)	Manganese (μg/g)	Lead (μg/g)	Barium (μg/g)
SRM 2704	133 ± 4	103 ± 4	4581 ± 59	551 ± 5	163 ± 5	417 ± 6
	(135 ± 5)*	(95 ± 4)	(4570 ± (4%))[a]	(555 ± (3%))[a]	(161 ± 17)	(414 ± (3%))[a]
SRM 2711	48 ± 1	83 ± 1	3048 ± 64	629 ± 10	1186 ± 19	736 ± 8
	(47)	(81.6 ± 2.9)	0.306 ± 0.023[b]	(638 ± 28)	(1162 ± 31)	(726 ± 38)
DOS-SC-7F	636 ± 9	1654 ± 39	5725 ± 94	8445 ± 200	103 ± 16	1263 ± 12
DOS-SC-7G	709 ± 19	1623 ± 50	4876 ± 106	6102 ± 695	70 ± 6	1495 ± 23
DOS-SC-8G	535 ± 10	1252 ± 27	5453 ± 118	10349 ± 135	55 ± 2	1758 ± 146
DOS-SC-9G	195 ± 13	1562 ± 83	7700 ± 507	3754 ± 256	53 ± 1	2268 ± 88
DOS-SC-10G	298 ± 7	1557 ± 29	9229 ± 322	3295 ± 60	50 ± 1	149 ± 7
DOS-SC-11G	723 ± 12	1726 ± 43	6971 ± 233	4368 ± 115	51 ± 1	2603 ± 125
DOS-SC-12G	692 ± 19	1817 ± 45	3803 ± 129	4656 ± 131	50 ± 3	2230 ± 193

[a] % error; [b]mass fraction (%)

*Values reported in parentheses are the certified values

n = 3 with 5 replicates of each digest

The purpose of total elemental analysis was to evaluate the sample matrix. From Table 7-IV, it is seen that all of the samples contain high amounts of Ti (3803 to 9229 μg/g) and V (1252 to 1817 μg/g). Therefore, there is a possibility of isobaric interference from Ti and V with ^{50}Cr. Since the samples contain 149 to 2603 μg/g of Ba and 50 to 103 μg/g Pb, there is possibility of producing highly water insoluble Cr(VI) compounds of Pb and Ba, specifically $PbCrO_4$ [K_{sp} = 2.8 x 10^{-13} at 25 °C] and $BaCrO_4$ [K_{sp} = 2.8 x 10^{-13} at 25 °C]. Therefore, necessary precautions should be taken during neutralization and analysis of Cr(VI), since $PbCrO_4$ precipitates out at lower pH. As an example,

analysis both with filtration and without filtration was acquired and the data are presented later in this study. The samples contain 3295 to 10349 μg/g of Mn. and very high Fe content (not quantified).

7.3.2 Cr(VI) Speciated Analysis

The SIDMS analysis depends on some fundamental operations: isotopic spike preparation and calibration, sample collection and sample spiking, sample species and spike species equilibration, sample extraction; species separation, isotope ratio measurements of each speciated component, and deconvolution of the species concentrations and species transformations.

For SIDMS analysis, samples were double spiked with known amounts of isotope enriched $^{iso\text{-}50}$Cr(III) and ^{53}Cr(VI) standards before and after extraction with EPA Method 3060A, using microwave energy as the heating source. The extracts were then filtered using 0.2 μm glass fiber filter and stored in cold room until analyzed. In these tests SIDMS equations were used as given, without any simplifying assumptions. Here transformations both of Cr(III) to Cr(VI) and of Cr(VI) to Cr(III) are evaluated and corrected for. These results are given in Table 7-V and 7-VI.

Sample and SRM aliquots were neutralized using concentrated HNO_3 just before the analysis by HPLC-ICP-MS to separate Cr(III) from Cr(VI). In order to do measurements of isotope ratios of each speciated component, the raw data obtained from the HPLC-ICP-MS needs to be processed. To date, no commercial software is available or found suitable for processing data acquired with the HPLC-ICP-MS system used in

this study. Experimental raw data were exported into Microsoft Excel for the appropriate processing.

TABLE 7-V. Cr(VI) Results in CA Samples: Extracted using EPA Method 3060A.

Sample	External Calibration	IDMS[a]	EPA Method 6800[b]	
	(μg/g)	(μg/g)	(μg/g)	Cr(III) converted to Cr(VI) (%)
SRM 2704	7 ± 2	12 ± 1	14 ± 2	10 ± 1
SRM 2711	7 ± 1	1.5 ± 0.1	12 ± 4	9 ± 4
DOS-SC-7F	342 ± 19	367 ± 25	342 ± 26	12 ± 3
DOS-SC-7G	440 ± 6	448 ± 17	409 ± 11	5 ± 2
DOS-SC-8G	245 ± 17	259 ± 12	242 ± 16	4 ± 1
DOS-SC-9G	164 ± 8	171 ± 7	184 ± 11	10 ± 2
DOS-SC-10G	199 ± 22	215 ± 17	214 ± 23	9 ± 2
DOS-SC-11G	277 ± 22	295 ± 15	319 ± 23	10 ± 2
DOS-SC-12G	291 ± 22	298 ± 18	328 ± 24	17 ± 4

Uncertainties are at 95% CL, n = 3

[a]Extracts were spiked with 53Cr(VI) spike after extraction

[b]Extracts were double spiked with iso-50Cr(III) and 53Cr(VI) after extraction

Following is an outline of the data acquisition and processing procedures.

General data acquisition:

- Set up and tune ICP-MS using direct aspiration mode;
- Perform experiments in order to determine deadtime (*17-18*);

- Connect the outlet of the chromatographic column to ICP-MS and stabilize the system. Inject sample through sample introduction loop and collect data in Time Resolved Analysis mode;
- Determine mass bias factors after every four hours (*19*);
- Export data as comma separated version (CSV) format for processing in Microsoft Excel.

TABLE 7-VI. Cr(VI) Results in CA Samples: Double Spiked Before Extraction with EPA Method 3060A.

Sample	EPA Method 6800 (SIDMS)			
	Cr(VI) before filtration	Cr(VI) after filtration	Cr(III) to Cr(VI) before filtration	Cr(III) to Cr(VI) after filtration
	(μg/g)	(μg/g)	(%)	(%)
SRM 2704	7 ± 3	8 ± 1	50 ± 2	45 ± 4
SRM 2711	NA	NA	NA	NA
DOS-SC-7F	308 ± 44	314 ± 28	56 ± 3	57 ± 2
DOS-SC-7G	341 ± 29	334 ± 36	46 ± 1	47 ± 1
DOS-SC-8G	206 ± 9	205 ± 16	48 ± 6	49 ± 7
DOS-SC-9G	141 ± 6	141 ± 14	33 ± 3	33 ± 2
DOS-SC-10G	223 ± 28	216 ± 17	41 ± 2	41 ± 1
DOS-SC-11G	289 ± 27	291 ± 22	19 ± 3	19 ± 3
DOS-SC-12G	278 ± 32	306 ± 29	20 ± 1	20 ± 1

Uncertainties are at 95% CL, n = 3

NA = not applicable

General data processing and measurement quality assurance:

- Calculate deadtime;

- Use the determined deadtime to correct the count rates; Integrate the counts for the background, the Cr(III) peak and the Cr(VI) peak by summing the deadtime-corrected count rates;
- Subtract background and calculate isotope ratio;
- Calculate mass bias factors for each isotope pair: $^{50}Cr/^{52}Cr$ and $^{53}Cr/^{52}Cr$; Correct mass biases in the sample isotope ratios.

An example for the magnitude of the deadtime correction on the isotope ratios is shown for conditions of pre-extraction spiking (Table 7-VII) and post-extraction spiking (Table 7-VIII). Note that the data in these tables represent one trial only and give total species mass, not total species concentration.

TABLE 7-VII: Method 6800 (SIDMS): Double Spiked Before Extraction (Sample LOV-SC-7F-1)

	Cr(III)		**Cr(VI)**	
	50/52	**53/52**	**50/52**	**53/52**
Isotope ratios without any correction	0.322854	0.321705	0.17381	0.193207
Deadtime corrected[a]	0.322797	0.32171	0.172985	0.193311
Mass bias corrected[b]	0.34335398	0.3178458	0.183965	0.190988
Deconvoluted result (μg) (Corrected)			183.95	
Deconvoluted result (μg) (w/o correction)			249.79	

[a]Determined deadtime for 50/52 = 9.880 ns; for 53/52 = 23.914 ns; and default deadtime = 22.5 ns

[b]Mass bias factor for 50/52 = 1.06368275; and for 53/52 = 0.987984

Isotope ratios (natural abundant): 50/52 = 0.05186; 53/52 = 0.11339

From Table 7-VII, it is observed that if the raw data were not corrected for deadtime and mass bias, then there was a chance to get ~ 35.79% more Cr(VI) for the sample LOV-SC-7F. This is an example of positive bias from the instrument.

TABLE 7-VIII: Method 6800 (SIDMS): Double Spiked After Extraction (Sample LOV-SC-7F-1)

	Cr(III)		Cr(VI)	
	50/52	**53/52**	**50/52**	**53/52**
Isotope ratios without any correction	0.193987	0.11967	0.122016	0.614282
Deadtime corrected[a]	0.220016	0.128283	0.124059	0.616274
Mass bias corrected[b]	0.20178303	0.1191569	0.113778	0.57243
Deconvoluted result (μg) (Corrected)			220.19	
Deconvoluted result (μg) (w/o correction)			173.17	

[a]Determined deadtime for 50/52 = 257.92 ns; for 53/52 = 133.043 ns; and default deadtime = 22.5 ns

[b]Mass bias factor for 50/52 = 0.91712771; and for 53/52 = 0.928856

Isotope ratios (natural abundant): 50/52 = 0.05186; 53/52 = 0.11339

From Table 7-VIII, it is observed that if the raw data were not corrected for deadtime and mass bias, then there was a chance to get ~ 21.34% less Cr(VI) for the sample LOV-SC-7F. This is an example of negative bias from the instrument.

Four quantification methods for the detection of Cr(VI) are used throughout this study. 1) *SIDMS:* in its most robust form, this method accounts simultaneously for both species transformations in the extraction procedure. SIDMS enables correction for the oxidation of Cr(III) and the reduction of Cr(VI) during extraction. Thus, the extraction/SIDMS procedures are capable of correcting for bi-directional species transformations that may occur in the determination of Cr(VI) in solid environmental

samples. 2) *EPA Method 6800:* this method was developed for the determination of species in aqueous solutions and soil extracts. It is applied after the extraction of species. Consequently, Method 6800 corrects for species transformations that might occur after, but not during, extraction; 3) *IDMS:* conventional isotope dilution mass spectrometry; and 4) *LC-ICP-MS:* traditional analysis and determination based on external calibration curve.

7.3.2.1 Isotope Dilution Mass Spectrometry (IDMS)

The other portion of the sample from EPA Method 3060A extract of each replicate (c.f., section 7.*2.3.3,* unspiked portion) was then divided into two sub-samples. One half was spiked only with ^{53}Cr(VI) in order to determine the Cr(VI) in different samples with IDMS. Sample aliquots were analyzed with ICP-MS in direct aspiration mode. The raw data were exported to Microsoft Excel for further processing. Data were corrected for deadtime and mass bias and the isotope ratio of ^{53}Cr/^{52}Cr in Cr(VI) species, ($R^{VI}_{53/52}$), was calculated for each sample. The final concentration of Cr(VI) was then determined from the following IDMS equations.

$$\begin{cases} C_x^{VI} = C_{std}^{VI} / M_x \\ C_s^{VI} = \dfrac{C_x^{VI} W_x}{W_s} \left(\dfrac{{}^{53}A_x - R^{VI}_{53/52}\,{}^{52}A_x}{R^{VI}_{53/52}\,{}^{52}A_s - {}^{53}A_s} \right) \\ C_{spike}^{VI} = C_s^{VI} M_s \end{cases} \qquad \text{Eqn. 7-7}$$

where, C_s^{VI} and C_x^{VI} are the concentrations of Cr(VI) in the isotope-enriched spike and natural standard in μmol/g, respectively. M_s and M_x are the average atomic weights of

the spike and the natural standard in g/mol, respectively. ${}^{53}A_s$ and ${}^{53}A_x$ are the atom fractions of ^{53}Cr for the spike and natural standard, respectively. ${}^{52}A_s$ and ${}^{52}A_x$ are the atom fractions of ^{52}Cr for the spike and natural standard, respectively. The final concentration of Cr(VI) in the representative samples is reported in Table 7-V (column 3). The samples contain Cr(VI) in the range of 171 to 448 μg/g. Table 7-V shows that the results from IDMS completely agree with those from Method 6800 (column 4) (double spiked after extraction) at 95% CL. All detection methods using post-extraction analysis (columns 2 – 4) in Table 7-V are in agreement and thus the difference between these determinations and those shown in columns 2-3 in Table 7-VI indicate the amount of Cr(III) converted to Cr(VI) during extraction. This demonstrates the need for the SIDMS result (columns 2-3, Table 7-VII) that corrects for additional Cr(VI) originating from Cr(III) in the sample matrix as artifact of the extraction process.

7.3.2.2 SIDMS Detection

As described earlier, samples and SRMs were double spiked with $^{\text{iso-50}}$Cr(III) and ^{53}Cr(VI) before extraction by Method 3060A. After extraction, samples were filtered and stored in cold room until analyzed. On the following day, samples were analyzed with HPLC-ICP-MS. Just before analysis, sample extracts were neutralized with concentrated HNO_3 to the pH range of 1 to 2. During instrumental analysis a quick reduction of a portion of Cr(VI) to Cr(III) was observed. The two chromatograms in Figure 7-3 were obtained by injecting the same extract 5 and 15 min after its acidification. The response at m/z 53 is shown to demonstrate the reduction of Cr(VI). Because the majority of ^{53}Cr is originally from ^{53}Cr(VI) spike in pure Cr(VI) form, the

observed ^{53}Cr as Cr(III) peak indicates the reduction of Cr(VI). In addition, since the alkaline extraction solution preserves Cr(VI), the reduction takes place after acidification.

Fresh extracts were then acidified with concentrated HNO_3 to pH 5 to 6, and analyzed with HPLC-ICP-MS. No reduction of Cr(VI) to Cr(III) was observed during a period of 15 min. The two chromatograms shown in Figure 7-4 were obtained by injecting the same extract at 1, 5 and 15 min after acidification. (Curves for 1 and 5 minutes are practically indistinguishable.) Therefore, throughout this study, all extracts were acidified to pH 5 to 6.

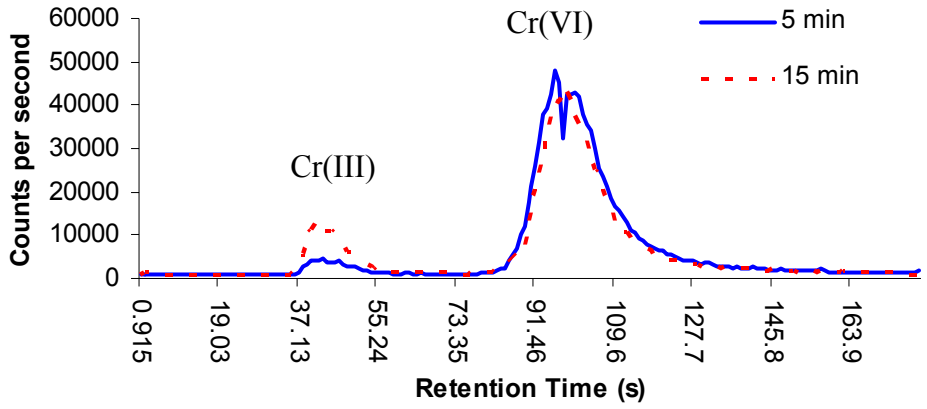


FIGURE 7-3. Reduction of Cr(VI) during measurement. Injection 5 and 15 min after acidification of extract to pH between 1 and 2 with concentrated HNO_3. Reduction of Cr(VI) is indicated by increase in the Cr(III) peak and decrease in the Cr(VI) peak.

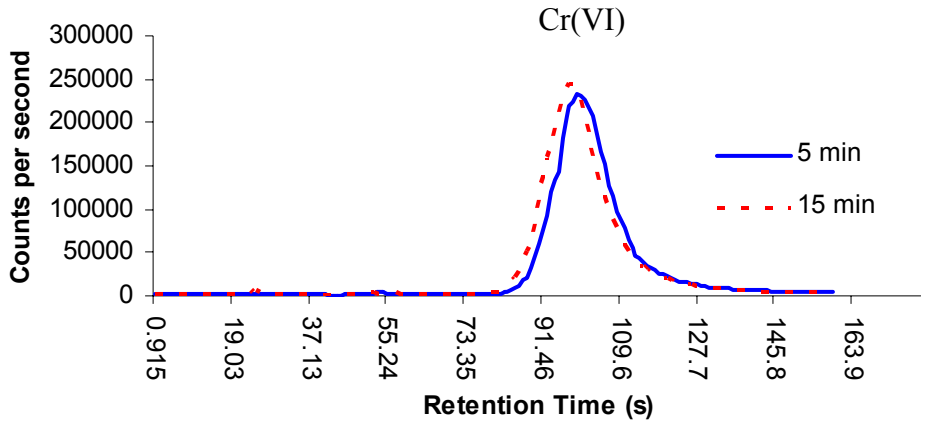


FIGURE 7-4: Cr(VI) measurements. Injection 5 and 15 min after acidification of extract to pH between 5 and 6. Peak for Cr(III) is not apparent.

After instrumental analysis, raw data were exported as CSV file to Microsoft Excel. Deadtime and mass bias corrected isotope ratios for 50/52 and 53/52 were calculated for Cr(VI) and Cr(III). As there is no Cr(III) present in the alkaline extract (pH 12), it was not detected in LC-ICP-MS measurements. Therefore, only the isotope ratios for Cr(VI) peak, $R^{VI}_{50/52}$ and $R^{VI}_{53/52}$ were considered and SIDMS calculations were performed to calculate the concentration of Cr(VI), where $R^{VI}_{50/52}$ and $R^{VI}_{53/52}$ are the isotope ratios of ^{50}Cr to ^{52}Cr and ^{53}Cr to ^{52}Cr in Cr(VI), respectively. The final concentration of Cr(VI) in the samples and the % transformation of Cr(III) to Cr(VI) during extraction are summarized in Table 7-VI.

The total sample matrix elemental analysis shows that the samples contain high amounts of Pb and Ba. Therefore, it was thought that the samples might contain Cr(VI) as insoluble $PbCrO_4$ and $BaCrO_4$. From literature it is found that the insoluble $BaCrO_4$

goes into solution as CrO_4^{2-}(aq) and Ba^{2+}(aq) during alkaline extraction, and then the Ba^{2+}(aq) reacts with available CO_3^{2-}(aq). The reaction can be expressed as:

$$BaCrO_4(s) + CO_3^{2-}(aq) \rightarrow BaCO_3(s) + CrO_4^{2-}(aq)$$

In the presence of high CO_3^{2-}(aq) concentration (0.28M), Ba^{2+}(aq) precipitates as $BaCO_3$, although the K_{sp} of $BaCO_3$ [5.1 x 10^{-9} at 25 °C] is higher than that of $BaCrO_4$ [K_{sp} = 1.2 x 10^{-10} at 25 °C]. The precipitation of $BaCO_3$ greatly decreases the concentration of the free Ba^{2+} (aq) ions in solution, driving the dissolution of $BaCrO_4$ and releasing CrO_4^{2-}(aq) as a free anion in solution. The precipitated $BaCO_3$ is removed from the extract during the filtration step. Therefore, when extracts are acidified from pH 12 to pH 5 to 6, CrO_4^{2-}(aq) cannot re-precipitate because nearly all Ba has been removed during filtration. The alkaline extraction thus takes advantage of the formation of $BaCO_3$ to release CrO_4^{2-}(aq) from $BaCrO_4$, and of subsequent filtration to remove Ba, preventing re-precipitation of $BaCrO_4$.

On the other hand, the dissolution of $PbCrO_4$ in the strong alkaline extraction solution does not require CO_3^{2-}(aq). The dissolution of $PbCrO_4$ involves the formation of $Pb(OH)_2$ [K_{sp} = 1.2 x 10^{-15} at 25 °C]. Although much of the Pb can be removed from the extract as $Pb(OH)_2$ during filtration, Pb still remains in the filtrate as complexes. Therefore, during acidification with HNO_3, there is a probability of re-precipitation of Pb as $PbCO_3$ or $PbCrO_4$. In order to determine if there is any loss of Cr(VI) during neutralization, the extracts were analyzed directly after neutralization and after filtration through 0.2 μm syringe filter.

Although no precipitation appeared during neutralization, replicates were filtered nonetheless just prior to HPLC-ICP-MS analysis. These results are summarized in Table

7-VI, and show that, within experimental error, the Cr(VI) values are the same before and after filtration. That is, lack of precipitation is confirmed and evidence of isotope equilibration is also indicated by these results. (Recall that after equilibration, the ratio has been established between the isotopes and this yields an accurate SIDMS determination (*20*).

Expected ranges for total Cr and Cr(VI) data were provided by Environmental Standards, Inc. and these estimates were used as the spiking targets for $^{iso\text{-}50}$Cr(III) and ^{53}Cr(VI) to optimize accuracy of the ratio measurements and to conserve the stable enriched isotope. Samples and SRMs were extracted in three replicates and each was analyzed three times to permit statistical evaluation of the samples (n = 3 x 3). Also shown in Table 7-V are the final concentrations of Cr(VI) found in the samples, compiled in μg/g.

Table 7-IV (column 2) shows that samples contain 195–723 μg/g of total Cr. From SIDMS analysis (spiked before extraction), samples are found to contain 141–341 μg/g of Cr(VI); in these samples 19 – 56% Cr(III) was converted to Cr(VI) during extraction process (Table 7-VI). The results obtained for the same extract aliquots after performing filtration (reported in column 3, Table 7-VI) completely agree with those obtained from direct analysis (without filtration). Therefore, it can be concluded that there is no loss of Cr(VI) or hindrance to isotope equilibration observed during acidification of the extracts. From this standpoint it was decided that during the rest of the study, no sample extracts needed to be filtered after acidification; all of the extracts were analyzed directly immediately after acidification.

According to Method 3060A, there is a chance of limited transformation of Cr(III) to Cr(VI) during extraction. The relatively large amounts of Cr(III) converted to Cr(VI) during the extraction procedure, reported in Table 7-VI (column 4), can be understood as follows. The extraction solvent used in this study contains higher concentration of NaOH (2.5 M) than that in Method 3060A (0.5 M). Method 3060A, regarded as the most appropriate extraction method for Cr(VI), uses a strong alkaline (pH > 12) solution in order to transfer all the soluble and insoluble salts of Cr(VI) from the sample matrix into solution and to prevent the reduction of Cr(VI) to Cr(III). However, under such high alkaline conditions most of the Cr(III), either from sample Cr(III) or from spike Cr(III), precipitates out as hydroxides, oxides and carbonates. While these precipitates are to be removed by filtration, it is reported in the literature (*12*) that freshly precipitated $Cr(OH)_3$ and Cr^{3+} present in the system have high possibility of oxidation to Cr(VI).

7.3.2.3 Method 6800 Detection

A second batch of samples and SRMs was extracted in triplicate with EPA Method 3060A and filtered. An aliquot of each replicate was double-spiked (with known amount of isotope enriched $^{iso\text{-}50}Cr(III)$ and $^{53}Cr(VI)$), and was analyzed with LC-ICP-MS. The goal of this study was to compare results obtained from SIDMS detection technique with those from EPA Method 6800 detection technique, and also to observe particularly whether chromium interconversion takes place during or after extraction.

As described in section *7.3.2.2*, raw data is exported from the instrument and used to make the necessary deadtime and mass bias corrections to the desired isotope

ratios. Table 7-V (column 4) shows that samples contain 184 – 409 μg/g of Cr(VI) under EPA Method 6800 protocol, and in this case 4%–17% of Cr(III) was converted to Cr(VI) (column 5). At 95% CL, these two sets of results (from SIDMS and Method 6800) overlap in most of the samples (four of seven). Results that do not overlap might be due to sample inhomogeneity, results obtained from different extractions, and conversion of some sample Cr(III) to Cr(VI) during extraction processes. Because change in isotope ratio is an extremely sensitive probe of change in species distribution, it is not analytically possible that conversions of isotopically enriched species would not be detected. That is, the isotope ratio is so sensitive to change in species abundance that significant change in the magnitude of either component of the ratio would be impossible to miss.

7.3.2.4 Method 3060A (External Calibration)

The remaining half of the sample aliquot from section *7.3.2.1* was analyzed by HPLC-ICP-MS for Cr(VI). Samples were acidified to pH 5 to 6 with concentrated HNO_3 immediately before analysis. During analysis with HPLC-ICP-MS, no peak was found in position of Cr(III) peak. There was only one peak present in position of Cr(VI). The purpose of these analyses was to evaluate the Method 3060A performance on these samples. Raw data was exported to Microsoft Excel and processed. In this case, data were not corrected for deadtime or mass bias. The final concentration of Cr(VI) in samples was calculated from the calibration curve by using ^{nat}Cr(VI) as calibration standard. The calibration curve for Cr(VI) is shown in Figure 7-5. The linear fit equation (CPS *vs.* ppb) and the corresponding R^2 value is as follows: Y = 1205.4x –

6333.9 and R^2 = 0.9979. All results for Cr(VI) from these measurements are summarized also in Table 7-V, column 2.

From Table 7-V (column 2), it is seen that samples contain Cr(VI) in the range of 164 to 440 μg/g. For four of the seven samples, Cr(VI) values from Method 3060A agree with those from SIDMS, Method 6800 and IDMS at 95% CL. The remaining three agree with Method 6800 and IDMS results. These results are obtained from the same batch of extractions that provide the IDMS data (column 3).

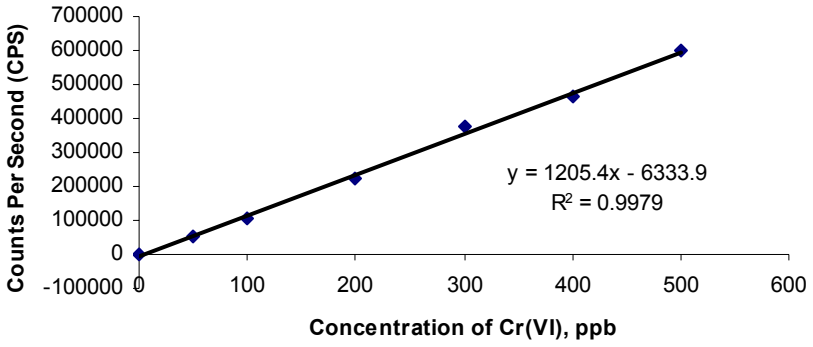


FIGURE 7-5: External calibration graph for determination of Cr(VI).

7.4 Conclusions

A suite of similar environmental solids samples has been examined for Cr content and speciation by several traditional and emerging diagnostic standard methods. Results with high precision and accuracy were obtained where methodology was appropriate to the analytical goal. Comparison of the method-specific results demonstrates several conclusions:

i) total Cr assay by external calibration (*EPA Method 3052*) is precise, accurate and appropriate for total chromium;

ii) the traditional method for species assay (*EPA Method 3060A*), while offering high precision, tends to over-estimate Cr(VI) concentration by as much as 35%;

iii) the over estimation by EPA Method 3060A may be due to an inherent inability of its methodology to acknowledge and control pertinent species chemistry;

iv) transformation of Cr(VI) occurs substantially during pre-extraction stages of the sample preparation and occurs only nominally during post-extraction activity;

v) Cr(VI) and Cr(III) transformations representing both depletion and amplification of each species are significant, are affected by sample preparation activities, and are quantified accurately and precisely by appropriate methodology (*EPA Method 6800 in practice as SIDMS enabled*);

vi) the difference between either column 2 or column 3 or column 4 of Table 7-V and column 2 or 3 of Table 7-VI is the amount of converted Cr(III) contributing to (or embedded in) the Cr(VI) results, as would be determined in these samples by any other method that is unable to diagnose and quantify species conversions. This difference is also the quantitative difference between methods, as gauged by the current samples. Column 2 or 3 of Table 7-VI, thus, represents the truest Cr(VI) assay for these samples with the converted Cr(III) removed.

7.5 References

(1) Li, Y.; Pradhan, N. K.; Foley, R.; Low, G. K. C. *Talanta* **2002**, *57*, 1143-1153.

(2) Wang, J.; Ashley, K.; Kennedy, E. R.; Neumeister, C. *Analyst* **1997**, *122*, 1307.

(3) Nieboer, E., Jusys, A. In: *Chromium in the Natural and Human Environments*, Nriagu, J. O.; Nieboer, E; Eds.; Wiley: New York, NY,**1988**, p 26.

(4) Weckhuysen, B. M.; Wachs, I. E.; Schoonheydt, R. A. *Chem. Rev*. **1996**, *96*, 3327 - 3349.

(5) EPA Method 3060A. In *Solid Waste Manual – 846,* US Government Printing Office (GPO): Washington DC, **1996**.

(6) Federal Register, May 8, **1998**, Vol.63, No. 89, pp. 25430-25438.

(7) Kingston, H. M. US Patent Number: 5,414,259; **1995**.

(8) Huo, D.; Kingston, H. M. *Anal. Chem.***2000**, *72(20)*, 5047-5054.

(9) Huo, D., Kingston, H. M. S.; Larget, B. In *Comprehensive Analytical Chemistry*, Caruso, J. A.; Sutton, K. L.; Ackley, K. L.; Eds.; *Elemental Speciation: New Approaches for Trace Element Analysis*, Vol. 33; Elsevier: New York, NY, **2000**, pp 277-313.

(10) Kingston, H. M.; Huo, D.; Lu, Y.; Chalk, S. *Spectrochim. Acta, Part B* **1998,** *53***,** 299-309.

(11) Huo, D.; Lu, Y.; Kingston, H. M. *Environ. Sci. Technol*. **1998,** *32***,** 3418.

(12) EPA Method 6800. In *Solid Waste Manual – 846, Update IVA*, US Government Printing Office (GPO): Washington DC, **2000**.

(13) Vitale, R. J.; Mussoline, G. R.; Petura, J. C.; James, B. R. *J. Environ. Qual*. **1994**, *23*, 1249-1256.

(14) James, B. R.; Petura, J. C.; Vitale, R. J.; Mussoline, G. R. *Environ. Sci. Technol*. **1995**, *29*, 2377-2381.

(15) Huo, D. *Ph.D. Thesis*, Duquesne University, Pittsburgh, PA, **1999**.

(16) Kingston, H. M.; Huo, D.; Rahman, G. M. M.; Iyer, S.; Walter, P. SamplePrep Web, Available: http://www.sampleprep.duq.edu/.

(17) Taylor, H. E. In *Inductively Coupled Plasma-Mass Spectrometry: Practices and Techniques*; Academic Press: San Diego, CA, USA, **2001**, p 48.

(18) VG Elemental. *VG PlasmaQuad System Manual*; VG Elemental Ltd: Winsford, England, **1989**.

(19) Jarvis, K.E.; Gray, A. L.; and Houk, R. S. In *Handbook of Inductively Coupled Plasma Mass Spectrometry*; Blackie Academic & Professional: London, UK, **1992**, p 315.

(20) Kingston, H. M.; International Patent publication ref. no. WO9939198A1, **1998**.

Appendices

Appendix-A: Publications

1. Rahman, G. M. M.; Isenhour, T. L.; Larget, B. Greenlaw, P. D. "Statistical Analysis of DOE EML QAP Data from 1982 to 1998". *J. Chem. Inf. Comput. Sci*., **2001**, 41, 1099-1105.
2. Han, Y.; Kingston, H. M.; Boylan, H. M.; Rahman, G. M. M.; Shah, S.; Richter, R. C.; Link, D. D.; Bhandari, S. "Speciation of mercury in soil and sediment by selective solvent and acid extraction". *Anal. Bioanal. Chem*. **2003**, 375, 428-436.
3. Rahman, G. M. M.; Kingston, H. M. S.; Bhandari, S. "Synthesis and Characterization of Isotopically Enriched Methylmercury ($CH_3{}^{201}Hg^+$)". *Appl. Organomet. Chem.* **2003**, 17(12), 913-920.
4. Rahman, G. M. M.; Kingston, H. M. S. "Application of Speciated Isotope Dilution Mass Spectrometry (SIDMS) to Evaluate Extraction Methods for Determining Mercury Speciation in Soils and Sediments." *Anal. Chem.* **2004,** 76(13), 3548-3555.
5. US EPA Draft Method 3200 (Mercury Species Separation by Selective Solvent Extraction and Acid Digestion). *Accepted* **2004**.
6. Rahman, G. M. M**.**; Kingston, H. M. S.; Kern, J. C.; Hartwell, S. W.; Anderson, R. F.; Yang, S. Y. "Inter-Laboratory Validation of EPA Method 3200 for Mercury Speciation Analysis using Prepared Soil Reference Materials." *Appl. Organomet. Chem.* **2004** (Reviewed).
7. Rahman, G. M. M.; Kingston, H. M. S. "Mercury Species Analysis Method Optimized Using Microwave Enhancements for Soils and Sediments." *J. Anal. At. Spectrom.* **2004** (Reviewed).
8. Rahman, G. M. M.; Kingston, H. M. S.; Towns, T. G.; Vitale, R.; Clay, K. "Analysis of hexavalent chromium in selected soil, sludge and sediment samples by modified speciated isotope dilution mass spectrometry." (Manuscript in Preparation).

Appendix-B: Poster Presentations

1. Rahman, G. M. Mizanur and Kingston, H. M. 'Skip'. "Development of closed vessel microwave-assisted extraction method for mercury speciation in soils and sediments". *Abstracts of Papers, 226th ACS National Meeting, New York, NY, United States*, **September 7-11, 2003 (2003), ANYL-102**.
2. Rahman, G. M. Mizanur**;** Kingston, H. M. 'Skip' and Bhandari, Sandeep. "Synthesis and use of isotope enriched methylmercury ($CH_3{}^{201}Hg^+$) in the characterization of mercury speciation methods". *Abstracts of Papers, 226th ACS National Meeting, New York, NY, United States*, **September 7-11, 2003 (2003), INOR-425**.

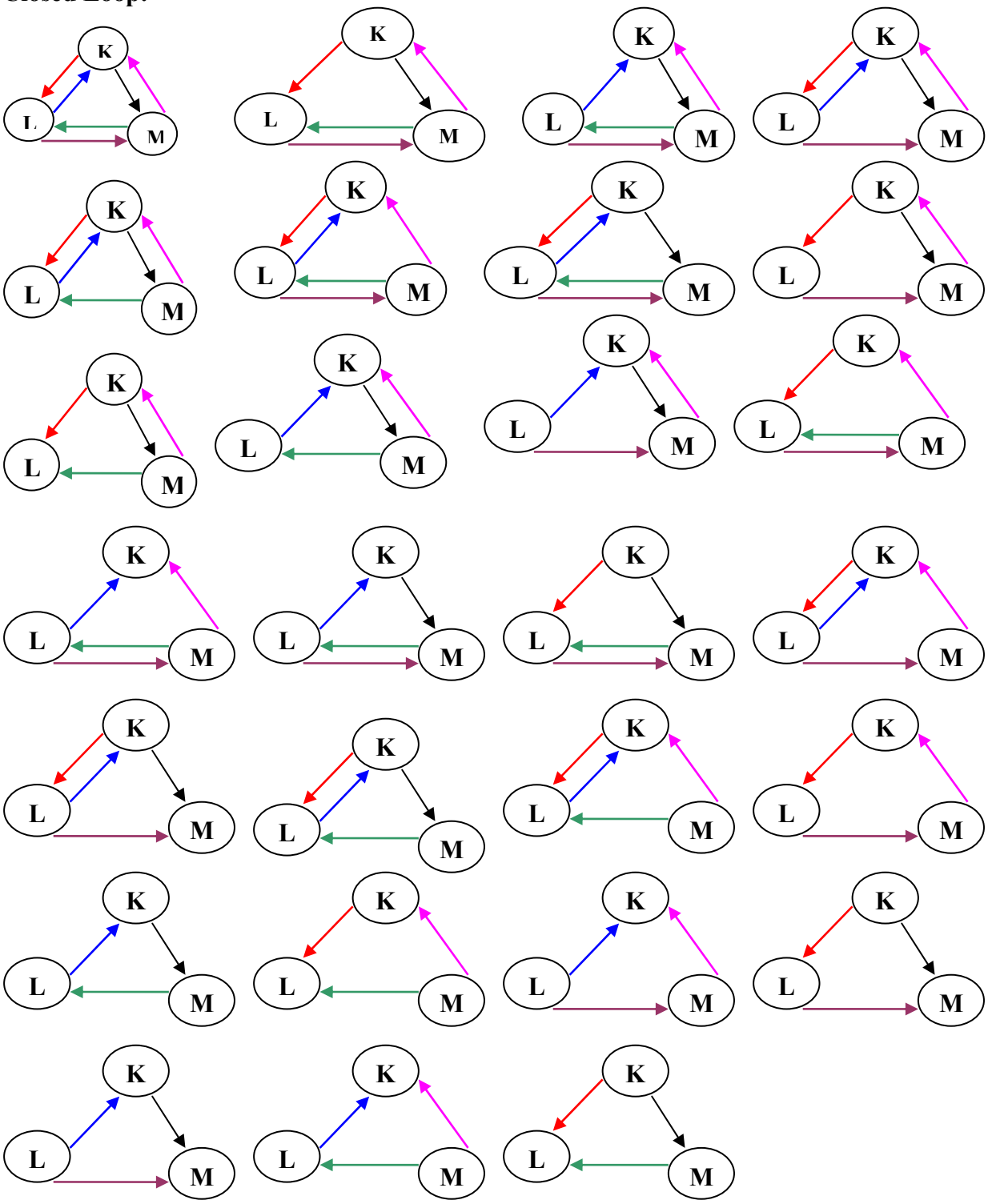
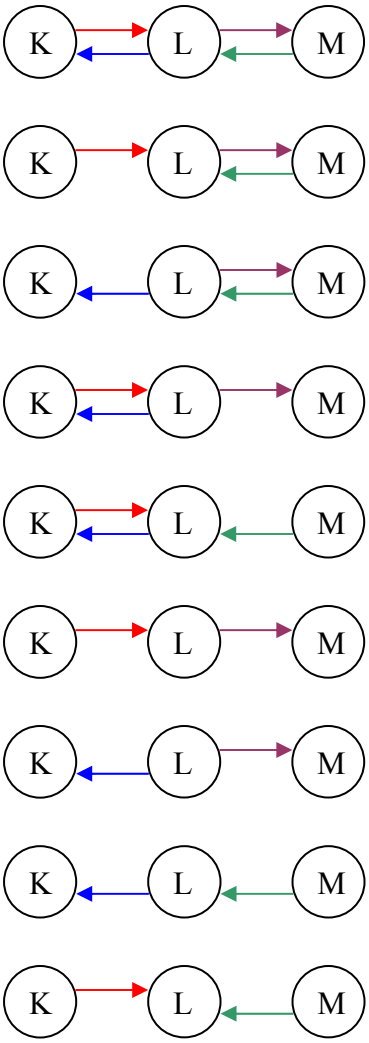
Appendix-C: Oral Presentations

1. Rahman, G. M. M**.**; Kingston, H. M. S.; Kern, J. C.; Hartwell, S. W.; Anderson, R. F.; Yang, S. Y. "Inter-Laboratory Validation of EPA Method 3200 for Mercury Speciation Analysis using Prepared Soil Reference Materials." NEMC 2004, The 20th Annual National Environmental Monitoring Conference, Washington, DC, **July 19-22, 2004**. Paper # 9.
2. Skip Kingston, Mizanur Rahman, John Kern, Matt Pamuku, Karin Rosen, Ye Han, Dingwei Huo, Theo Towns. "Elemental Speciation: An Environmental and Forensic Challenge and an Approach to the Analysis Uncertainty". *Enviro*Analysis-2004: The 5th Biennial Conference on Monitoring and Measurement of the Environment, Toronto, Canada, **May 17-21, 2004**.
3. G. M. Mizanur Rahman and H. M. Skip Kingston. "Mercury Speciation: An Inter-laboratory Validation of Proposed Method 3200 in Reference Soils." *PittCon 2004*, Chicago, IL, USA. **March 07-12, 2004**. Paper # 23900-200.
4. H. M. "Skip" Kingston; Mizanur Rahman; John Kern; Matt Pamuku; Karin Rosen; Ye Han; Dengwei Huo; and Theo Towns. "Enabling Speciated Isotope Dilution Mass Spectrometry for Difficult Species Sample Preparation and Analysis." 2004 Winter Conference on Plasma Spectrochemistry, Fort Lauderdale, FL, USA. **January 5 – 10, 2004**. Paper # Th 09.

5. Rahman, G. M. Mizanur; Kingston, H. M. 'Skip'. "Mercury Speciation, an Intra-Laboratory Evaluation of a Microwave-Assisted Extraction Method in Soils and Sediments". *PittCon 2003, Orlando, FL, USA*. **March 9 – 14, 2003**. Paper # 780.
6. Mizanur Rhaman; H. M. 'Skip' Kingston. "Efficiency of EPA Draft Method 3200 for the Determination of Inorganic and Methylmercury in Soils and Sediments". *FACSS 2002: The 29th Annual Meeting, Providence, RI, USA*. **October 13-17, 2002**. Paper # 269.
7. M. Rahman; Y. Han; H. M. Boylan and H. M. Kingston. "Determination of Methylmercury and Inorganic Mercury in Soils and Sediments by Draft Method 3200". *WTQA 2002 – 18th Annual Waste Testing & Quality Assurance Symposium, Arlington, VA, USA*. **August 11 – 15, 2002**. Paper # 66.
8. Mizanur Rahman; Ye Han; Helen Marie Boylan; Sejal M. Shah; H. M. 'Skip' Kingston. "Validation of EPA Draft Method 3200: Mercury Speciation by Selective Solvent Extraction and Acid Digestion". *PittCon 2002, New Orleans, LA, USA*. **March 17-22, 2002**. Paper # 1207.
9. M. Rahman; Y. Han; H. Boylan; D. Link; S. Shah, and H.M. Kingston. "Speciation of Mercury in Soil and Sediment by Selective Solvent and Acid Extraction, Draft Method 3200". WTQA *2001 – 17th Annual Waste Testing & Quality Assurance Symposium, Arlington, VA, USA*. **August 12 – 16, 2001**. Paper # 23.

Appendix-D: 36 Possible Transformations for a Three Species System

Closed Loop:



Open Loop: