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Accurate Quantitation of Hexavalent Chromium for Reference Standard Certification and Quality Control Testing using Speciated Isotope Dilution Mass Spectrometry

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ACCURATE QUANTITATION OF HEXAVALENT CHROMIUM FOR REFERENCE STANDARD CERTIFICATION AND QUALITY CONTROL TESTING USING SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY

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ABSTRACT

ACCURATE QUANTITATION OF HEXAVALENT CHROMIUM FOR REFERENCE STANDARD CERTIFICATION AND QUALITY CONTROL TESTING USING SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY

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The ability to perform accurate, repeatable, and defensible elemental and molecular speciated analysis is immensely significant for measurements that support human health, environmental science, and industry. This is especially true since trivalent chromium [Cr(III)] is necessary for proper nutrition, while hexavalent chromium [Cr(VI)] is extremely toxic, genotoxic, and carcinogenic. The dichotomous nature of chromium toxicity requires the use of an accurate analytical method that is capable of specific quantification of both Cr(III) and Cr(VI). Yet, the main challenges associated with speciated analysis are related to reactive species that are continuously transformed or converted to other species during sample processing. Due to this complexity, accurate determination of the concentrations and stabilities of the Cr(III) and Cr(VI) species

require a method that is capable of monitoring and correcting for interconversion, bias, and instrumental error.

Traditional quantitative methods, such as calibration curves, are unable to account for such species interconversion. However, Speciated Isotope Dilution Mass Spectrometry (SIDMS) chromium analysis by EPA Method 6800 and EPA Method 3060A includes the addition of known amounts of enriched 50-Cr(III) and 53-Cr(VI) isotope species to each sample containing naturally-occurring 52-Cr(III) and 52-Cr(VI) species, which ensures that oxidative/reductive interconversions are quantifiable. Differences between the known initial and final measured species concentrations for each isotope are determined to allow for mathematical correction of bidirectional species transformation by using isotope ratio calculations.

This dissertation demonstrates the certification of a new Sigma-Aldrich ambient-level hexavalent chromium standard reference material in soil matrix with SIDMS methodology. Using ion chromatography (IC) separation and inductively coupled plasma mass spectrometry (ICP-MS), the isotopic ratios were measured and used to calculate the initial concentrations of Cr(III) and Cr(VI) in the original unaltered sample. Challenges associated with the analytical method development are discussed along with details of the sample preparation, microwave-enhanced alkaline extraction, and quantitative data processing.

This dissertation also examines the concentrations and stability of Cr(VI) in a variety of dietary supplement samples by using a modified microwave-enhanced alkaline extraction protocol integrated with SIDMS, analysis by IC-ICPMS, and data processing according to EPA Method 6800. The results are presented along with discussion of the

Eh and pH phase diagram stability of Cr(VI) in dietary supplement samples. To ensure the quality and safety of chromium-containing dietary supplement products, manufacturers should be compelled to adopt routine analytical testing and controls for hexavalent chromium. The developed methods provide techniques for accurately measuring total chromium and hexavalent chromium concentrations in a robust variety of dietary supplement sample formulations.

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LIST OF ABBREVIATIONS

AI	Adequate Intake
AIT	Applied Isotope Technologies
CGMP	Current Good Manufacturing Practices
CI	Confidence Interval
COA	Certificate of Analysis
COPR	Chromium Ore Processing Residue
Cr(III)	Trivalent Chromium
Cr(VI)	Hexavalent Chromium
DSHEA	Dietary Supplement Health and Education Act of 1994
EDTA	Ethylenediaminetetraacetic Acid
Eh	Oxidation/Reduction Potential
EPA	United States Environmental Protection Agency
FDA	United States Food and Drug Administration
FD&C	Federal Food, Drug and Cosmetic Act
FSMA	Food Safety Modernization Act
HDPE	High-Density Polyethylene
HEPA	High-Efficiency Particulate Air
IARC	International Agency for Research on Cancer
IC	Ion Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IDMS	Isotope Dilution Mass Spectrometry
IUPAC	International Union for Pure and Applied Chemistry

KED	Kinetic Energy Discrimination
LOD	Limit of Detection
LOQ	Limit of Quantitation
MADL	Maximum Allowable Dose Level
MEC	Microwave-Enhanced Chemistry
NIH	United States National Institutes of Health
NIST	United States National Institute of Standards and Technology
ODS	United States Office of Dietary Supplements
ORNL	United States Oak Ridge National Laboratory
ORS^3	Octopole Reaction System
OSHA	United States Occupational Safety and Health Administration
PEEK	Polyether Ether Ketone
PP	Polypropylene
PTFE	Polytetrafluoroethylene
rIDMS	Reverse Isotope Dilution Mass Spectrometry
RSD	Relative Standard Deviation
SD	Standard Deviation
SIDMS	Molecular Speciated Isotope Dilution Mass Spectrometry
SRM	Standard Reference Material

CHAPTER ONE:

INTRODUCTION

The chemical characteristics and reactivity of an element are species-specific. Understanding analytical measurements at a speciated level is important for the accurate characterization and evaluation of complex chemical systems, such as those found in environmental science, geochemistry, toxicology, medicine, nutrition, forensics, and industry. The stability, mobility, and other physical properties of an element are directly related to the distribution of species. For living systems, the speciated form of an element or its compounds is often critical and directly affects whether it is nutritionally essential or highly toxic. Intricate physiological pathways required for maintaining life are regulated by coupled speciated systems. Yet, until recently, analytical methodologies only allowed for the determination of total, non-speciated characterization. New developments in analytical instrumentation and techniques now provide the opportunity for the identification and measurement of species in a particular system.

The International Union for Pure and Applied Chemistry (IUPAC) defines chemical species as chemical compounds that differ in isotopic composition, conformation, oxidation or electronic state, or in the nature of their complexed/covalently bound substituents [1]. For analytical chemistry, speciation analysis is defined as activities that identify and/or measure the quantities of individual chemical species in a sample [1]. Furthermore, speciation refers to the distribution of an element among the various chemical species in a system [1]. Such clarification is needed in order to avoid confusion and provide standardized terms for use within and outside the scientific community.

The distribution of an element's isotopic species varies in abundance and results from factors such as radioactive decay and physical separation. The formation of stable isotopes from the decay of radioactive elements is dependent on geological sources and time. For example, the age of organic material can be established by measuring carbon isotope ratios, which change as radioactive carbon-14 decays and is not replaced by atmospheric exchange. For elements without radioactive precursors, physical and biological processes may lead to separation and accumulation of isotopes by differences in chemical and inertial properties within the system. For example, the isotopic distribution of oxygen may be enriched as the element is partitioned between two phases in different bound forms. This effect, which is temperature-dependent, is used for long-term geological and climate studies [1]. Anthropogenic activities and industries are also capable of altering the distributions of isotopes in the environment. Additionally, kinetic isotope effects are observed in biological systems and may be used for physiological tracer studies.

Complexation of an element with different inorganic or organic compounds determines properties such as charge, solubility, mobility, and reactivity [1, 2]. The lability and reactivity of each species is determined by kinetics and thermodynamics. As an example, nickel oxides and sulfides are highly insoluble in water but may have bioavailability if the element is associated with biological ligands. Furthermore, species associated with organometallic compounds can bioaccumulate in fatty tissues and cross membrane barriers [1, 3]. Inorganic mercury (Hg^{2+}) is toxic to the kidney and corrosive to mucosal membranes, while methyl mercury (CH_3Hg^{+}) is capable of crossing the blood-brain barrier and causes central nervous system damage [1, 3]. Factors such as pH, concentrations, and stoichiometry mean that species cannot be separated from each other without changes in species distribution within the system. At the

macromolecular level, speciated analysis provides an opportunity to monitor the distribution of biomolecules in different states and local conformations. For example, most of the tripeptide glutathione is found in its reduced form under normal conditions in the human body. However, the oxidized form of glutathione (glutathione disulfide) is found during conditions that cause oxidative stress [4, 5]. Speciated analysis of glutathione therefore provides a clinical opportunity to measure oxidative stress and disease risk [4, 5].

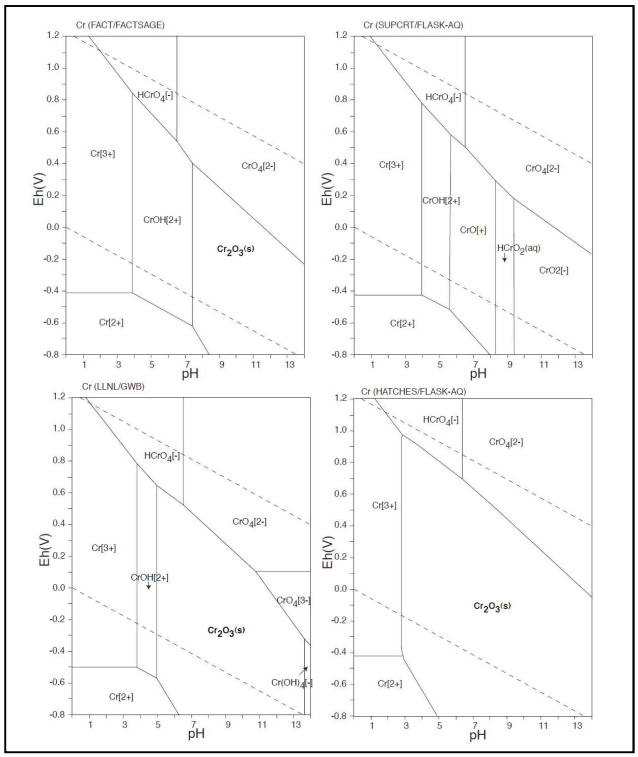
For inorganic elements, the oxidation state can have a significant role in determining the types of interactions that occur in environmental and biological systems. Different electronic and oxidation species have unique reactivity, solubility, stability, and physiological effects such as bioavailability and toxicity [6, 7]. For example, the iron (II) ion is soluble under physiological conditions and is capable of diffusing across membranes, while iron (III) does not readily enter cells and often participates in hydrolysis [1]. This difference has a profound effect on a large range of metabolic processes, including oxygen transport and enzyme activity. Also, trivalent chromium [Cr(III)] is an essential dietary mineral that provides proper sugar and lipid metabolism [1, 8-12]. Fresh foods and drinking water contain trivalent chromium, and dietary deficiency of Cr(III) is associated with diabetes, infertility, and cardiovascular disease [8-10]. However, hexavalent chromium [Cr(VI)] is highly toxic and is absorbed more readily than trivalent chromium by the lungs, gut, and skin [1, 8, 9]. Evidence suggests that Cr(VI) is carcinogenic, causes respiratory and dermal reactions, and damages the liver and kidneys [1, 8, 9, 13, 14]. While insoluble Cr(III) is the dominant natural species of chromium under most near-surface environmental conditions, Cr(VI) occurs naturally and is soluble in aqueous solutions, resulting in a highly mobile species in natural environments [6, 15, 16].

The ability to perform accurate, repeatable, and defensible speciated analysis is immensely significant for measurements that support human health, environmental science, and industry. This is especially true for chromium since the dichotomous nature of chromium toxicity requires the use of an accurate analytical method that is capable of specific quantification of both Cr(III) and Cr(VI). However, the main challenges and errors associated with speciated analysis are due to issues related to reactive species that are continuously transformed or converted to other species during sample processing prior to obtaining the necessary numerical measurements [17]. The presence of oxidizing and reducing agents, UV light, organic compounds, and changes in the pH and oxidation/reduction potential (Eh) of sample solutions may affect the interconversion of Cr(III) and Cr(VI) species [6, 17-20]. Cr(III) is thermodynamically stable in low Eh and low pH conditions, while high Eh and high pH favor the stability of Cr(VI). Specifically, the trivalent chromium species $Cr(H_2O)_6^{3+}(aq)$ is a moderately strong acid with a pK_a of ~4 and is successively deprotonated with increasing basic conditions, forming the sparingly soluble, neutral trihydroxochromium species [21]. In alkaline solutions, Cr(III) may show amphoteric behavior with the formation of soluble $Cr(OH)_4^-(aq)$ [22]. The dominant Cr(VI) species include $HCrO_4^-(aq)$ and $CrO_4^{2-}(aq)$. Other Cr(VI) species may form, however their formation requires Cr(VI) concentrations that are greater than those found in the natural environment [22]. Both $HCrO_4^-$ and CrO_4^{2-} can be reduced to Cr(III) by different reducing agents, such as Fe(II), phosphate, sulfide, and organic matter [12, 17]. Furthermore, Fe(II) hydroxide reduces Cr(VI) to Cr(III), which results in the formation of insoluble chromium and subsequent removal from solution [23].

Chromium species interconversions are best illustrated with Eh-pH diagrams, which indicate the most thermodynamically stable chromium species in a particular Eh-pH aqueous environment. However, an important caveat is that these diagrams are valid only for conditions

of chemical equilibrium and do not account for kinetic constraints, such as changes in chromium concentrations or when chromium is introduced into the system [21, 22]. Eh-pH diagrams describe diluted aqueous chromium solutions that are exposed to air and without complexing agents, other than water or OH^- [21]. Figure 1.1 is an example of four Eh-pH diagrams that compare thermodynamic databases as part of an open source project from the Research Center for Deep Geological Environments, Geological Survey of Japan [24]. These diagrams are useful for predicting the most probable, thermodynamically stable chromium species in the sample preparation in order to provide insight into the expected solution chemistry. In acidic media, the redox potential of the Cr(VI)/Cr(III) couple stabilizes the Cr(III) species. In alkaline conditions, the redox potential stabilizes the Cr(VI) species.

Figure 1.1: Chemical species of chromium as a function of pH and oxidation reduction potential (Eh). The four Eh-pH diagrams provide a comparison of thermodynamic databases as part of an open source project from the Research Center for Deep Geological Environments, Geological Survey of Japan. The diagrams are emended and from the Atlas of Eh-pH diagrams, National Institute of Advanced Industrial Science and Technology, Research Center for Deep Geological Environments, Geological Survey of Japan, Open File Report No. 419, pages 78-79, May 2005 [24].



Due to the complexity of potential species interconversions, accurate determination of the concentrations and stabilities of the Cr(III) and Cr(VI) species require a method that is capable of monitoring and correcting for interconversion, bias, and instrumental error. In general, most analytical laboratories have found that the accurate measurement of chromium species in environmental, biological, and industrial samples is difficult or not possible when using traditional analytical methods [8, 10, 17, 25-27]. Traditional analytical approaches attempt to produce static species, which is contradictory to the element's natural properties [17]. However, molecular speciated isotope dilution mass spectrometry (SIDMS), which is codified in EPA Method 6800, allows and mathematically corrects for species interconversions using additional degrees of freedom [26, 28]. This methodology has proven to be a powerful technique that allows for the accuracy, precision, and robustness needed to correct Cr(III)/Cr(VI) species interconversions [15, 17, 19, 29-31].

The use of isotopically-labelled species with SIDMS eliminates the need for external calibration curves and relies on direct mathematical determinations [25]. Traditional external calibration curves introduce bias from instrumental variables, uncertainty due to changes in the signal response with analyte concentration, and matrix influences due to the presence of shifting calibration data from the standards and actual samples [32]. Moreover, SIDMS is based on one of four International Union of Pure and Applied Chemistry's (IUPAC) definitive methods, which are methods that have exceptional scientific status and are capable of material certification. SIDMS provides measurements that are accurate and precise, enabling quantification of the concentration of each chromium species with interconversion correction.

For chromium SIDMS analysis by EPA Method 6800, known amounts of enriched 50-Cr(III) and 53-Cr(VI) isotope species are added to each sample containing naturally-occurring

52-Cr(III) and 52-Cr(VI) species, which ensures that oxidative/reductive interconversions are quantifiable. Differences between the known initial and final measured species concentrations for each isotope are determined to allow for mathematical correction of bidirectional species transformation by using isotope ratio calculations. Using ion chromatography (IC) separation and inductively coupled plasma mass spectrometry (ICP-MS), the 50/52-Cr(III), 53/52-Cr(III), 50/52-Cr(VI), and 53/52-Cr(VI) isotopic ratios are measured and used to calculate the initial concentrations of Cr(III) and Cr(VI) in the original unaltered sample. The use of SIDMS to mathematically determine the concentrations of both Cr(III) and Cr(VI), while providing bidirectional species transformation correction, is illustrated in the following equations:

$$R^{III}_{50/52} = \frac{\left({}^{50}A_X C^{III}_X W_X + {}^{50}A^{III}_S C^{III}_S W^{III}_S\right)(1 - \alpha) + \left({}^{50}A_X C^{VI}_X W_X + {}^{50}A^{VI}_S C^{VI}_S W^{VI}_S\right)(\beta)}{\left({}^{52}A_X C^{III}_X W_X + {}^{52}A^{III}_S C^{III}_S W^{III}_S\right)(1 - \alpha) + \left({}^{52}A_X C^{VI}_X W_X + {}^{52}A^{VI}_S C^{VI}_S W^{VI}_S\right)(\beta)}$$

$$R^{III}_{53/52} = \frac{\left({}^{53}A_X C^{III}_X W_X + {}^{53}A^{III}_S C^{III}_S W^{III}_S\right)(1 - \alpha) + \left({}^{53}A_X C^{VI}_X W_X + {}^{53}A^{VI}_S C^{VI}_S W^{VI}_S\right)(\beta)}{\left({}^{52}A_X C^{III}_X W_X + {}^{52}A^{III}_S C^{III}_S W^{III}_S\right)(1 - \alpha) + \left({}^{52}A_X C^{VI}_X W_X + {}^{52}A^{VI}_S C^{VI}_S W^{VI}_S\right)(\beta)}$$

$$R^{VI}_{50/52} = \frac{\left({}^{50}A_X C^{III}_X W_X + {}^{50}A^{III}_S C^{III}_S W^{III}_S\right)(\alpha) + \left({}^{50}A_X C^{VI}_X W_X + {}^{50}A^{VI}_S C^{VI}_S W^{VI}_S\right)(1 - \beta)}{\left({}^{52}A_X C^{III}_X W_X + {}^{52}A^{III}_S C^{III}_S W^{III}_S\right)(\alpha) + \left({}^{52}A_X C^{VI}_X W_X + {}^{52}A^{VI}_S C^{VI}_S W^{VI}_S\right)(1 - \beta)}$$

$$R^{VI}_{53/52} = \frac{\left({}^{53}A_X C^{III}_X W_X + {}^{53}A^{III}_S C^{III}_S W^{III}_S\right)(\alpha) + \left({}^{53}A_X C^{VI}_X W_X + {}^{53}A^{VI}_S C^{VI}_S W^{VI}_S\right)(1 - \beta)}{\left({}^{52}A_X C^{III}_X W_X + {}^{52}A^{III}_S C^{III}_S W^{III}_S\right)(\alpha) + \left({}^{52}A_X C^{VI}_X W_X + {}^{52}A^{VI}_S C^{VI}_S W^{VI}_S\right)(1 - \beta)}$$

Where,

$R^{III}_{50/52}$ = Measured isotope ratio of ^{50}Cr(III) to ^{52}Cr(III) in spiked sample

${}^{50}A_X$ = Atomic fraction of ^{50}Cr for sample

C^{III}_X (μmole/g) = Concentration of Cr(III) in the sample (unknown)

W_X (g) = Weight of the sample

${}^{50}A^{III}_S$ = Atomic fraction of ^{50}Cr in ^{50}Cr(III) spike

C^{III}_S (μmole/g) = Concentration of Cr(III) in ^{50}Cr(III) spike

W^{III}_s (g) = Weight of the in ^{50}Cr(III) spike

C^{VI}_X (μmole/g) = Concentration of Cr(VI) in the sample (unknown)

α = Percentage of Cr(III) oxidized to Cr(VI) after spiking (unknown)

β = Percentage of Cr(VI) reduced to Cr(III) after spiking (unknown).

Traditional methods for speciated analysis attempt to preserve the species during sample processing, species isolation/fractionation, and measurement [33]. These methods treat species interconversions as alternations to the original species concentrations. SIDMS is significantly different since the methodology does not prevent species interconversion, and instead measures the amount of the transformation and applies correction to deconvolute and determine the original species concentrations. For a two-species system, such as Cr(III) and Cr(VI) in an aqueous sample, the derivation is based on the following assumptions: (1) the isotopes of the sample and spiking standard solutions are in equilibrium before species interconversion; and (2) selective loss of a specific species does not occur [33].

EPA Method 6800 also describes the quantitative analytical technique of isotope dilution mass spectrometry (IDMS), which like SIDMS, utilizes the relationship between isotopes of a naturally-occurring sample and spiked isotopes of the standard solutions. Conventional IDMS, however, is not capable of determining and correcting for species interconversion. Instead, IDMS usually relies on the destruction of species to circumvent the need for complete equilibrium between the sample and spike standard [33]. Because of this, IDMS can be considered a particular form of SIDMS. However, the equations are less complex since they do not require additional terms for species interconversion monitoring and correction. The use of IDMS to mathematically determined the concentrations of total chromium content, without

species transformation correction, is illustrated in the following equations:

$$C_{Sample} = C_X M_X$$

$$C_S = \frac{C_{Spike}}{M_S}$$

$$C_X = \frac{C_S W_S}{W_X}\left(\frac{^{53}A_S - R_{53/52}\,^{52}A_S}{R_{53/52}\,^{52}A_X - ^{53}A_X}\right)$$

Where,

C_{Sample} (µg/g) = Concentration of the element in final sample solution

C_X (µmole/g) = Concentration of analyte in sample

M_X (g/mole) = Average atomic weight of sample

$^{53}A_X$ = Atomic fraction of ^{53}Cr for sample

$^{52}A_X$ = Atomic fraction of ^{52}Cr for sample

C_{Spike} (µg/g) = Concentration of isotopically-enriched spike

C_S (µmole/g) = Concentration of isotopically-enriched spike

M_S (g/mole) = Average atomic weight of isotopically-enriched spike

$^{53}A_S$ = Atomic fraction of ^{53}Cr for isotopically-enriched spike

$^{52}A_S$ = Atomic fraction of ^{52}Cr for isotopically-enriched spike

When isotope ^{50}Cr is used, ^{53}Cr is substituted with ^{50}Cr in the above equations.

The accurate determination of total chromium content can be achieved by using a single isotopically-enriched standard solution. This approach simplifies the sample preparation and mathematical manipulations needed for concentration determinations. The use of IDMS provides a method for total analysis that has been well evaluated for analytical merit, error

propagation, and detection [33]. It stands out as a method that provides results with unchallenged accuracy and precision in elemental analysis, and it corrects for matrix effects and (partial) analyte losses [34]. It is also considered a definitive method for trace element analyses, with its high metrological quality over traditional methods of standard additions, internal and external calibration [34].

Well characterized, pure, isotopically enriched standard solutions are required for analyte quantitation by IDMS and SIDMS methodology. Due to limited commercial availability, it is necessary to successfully synthesize the standard solutions and determine their concentrations, isotopic composition, molecular species composition, and purity. Chapter Two of this dissertation describes the synthesis and assessment of several isotopically enriched speciated chromium standard solutions and natural chromium standard solutions. The preparation procedures for speciated isotopically enriched chromium standards are not as straight-forward as preparing natural chromium standards. Since material that is enriched with a specific chromium isotope may only be commercially available in a few chemical forms, standard solution preparation requires dissolution of the material, chemical conversion of the chromium species to the intended form, stabilization of the intended chemical species, dilution to working standard concentrations, and assay value determinations.

The use of advanced analytical instrumentation with quantitation by EPA Method 6800 allows for accurate, precise, and repeatable measurements for a wide variety of analytes. ICP-MS instrumentation, for example, is capable of quickly and simultaneously detecting three-quarters of the periodic table at detection limits that are below parts-per-billion. These instruments require that the liquid phase samples are homogeneous at a molecular level. The use

of microwave-enhanced chemistry (MEC) supports the generation of the required homogeneous solutions and provides a method of fast, efficient, and reproducible sample preparation [2, 35].

When compared to other heating methods, MEC heats solutions more efficiently, reduces reaction timescales, and improves the level of reaction and process control [35]. Utilization of closed vessels for microwave sample digestion allows for higher reaction temperatures and system pressures, which increases reaction rates and decreases reaction times. The kinetic advantage of the higher temperatures achieved by MEC is described by the Arrhenius equation, which indicates that reaction rate exponentially increases with increasing temperature. Traditional heating methods such as flames, hotplates, mantles, and ovens transfer heat energy only to the parts of the solution in contact with the source. Heating is slow and limited to the solution's boiling point, pressure, colligative properties, and the properties of the solution container.

Yet, a solution directly absorbs microwave energy by dipole rotation and ionic conductance [2, 35]. Molecular dipoles align with the oscillations of the applied electric field and then randomize five billion times per second, which results in frictional heating [2, 35]. As ions interact with the polarity of the applied electric field, their accelerated flow meets resistance and generates heat in the solution [35]. These mechanisms allow for the solution to be heated much faster than convection and conduction, resulting in solutions that are superheated above their normal boiling points by as much as 5°C [35]. Furthermore, the closed vessels provide a microwave reflux action. This is characterized by the absence of ionic conductance heating in the gas phase, and removal of vapor phase molecules with condensation on the cooler surface of the vessel walls [35]. Microwave reflux action therefore maintains a lower than expected internal pressure within the vessel [35]. This methodology has been refined to include both

temperature and pressure feedback controls for the analytical microwave units, which improves sample preparation control, repeatability, and standardization [2, 35].

The overall advantages, control, and reproducibility of MEC makes it amenable for standardized sample preparation methods. EPA Method 3052, Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices, was developed around the use of MEC and rapidly produces sample digests suitable for inorganic elemental analysis by ICP-MS [36]. EPA Method 3052 provides the total decomposition of a sample and allows for the assessment of total elemental content. For example, total chromium analysis by EPA Method 3052 with quantitation by IDMS according to EPA Method 6800 provides a procedure that ensures complete equilibration of the endogenous chromium isotopes of the sample with those of the added isotopically enriched analytical chromium standard solutions.

Methods that were not specifically developed around the use of MEC, in many cases, can easily be adapted to utilize microwave assisted preparation techniques. Once such method is EPA Method 3060A, Alkaline Digestion for Hexavalent Chromium [37, 38]. This is important since many environmental laboratory certification programs require the use EPA Method 3060A for determination of Cr(VI) in soils. The method utilizes a hot alkaline digestion solution to quantitatively extract Cr(VI) from soluble, adsorbed, or precipitated forms of chromium compounds, while minimizing the interconversion of the chromium species [37, 38]. Alone, EPA Method 3060A is not capable of correcting for oxidation of Cr(III) and/or reduction of Cr(VI); however, the use of EPA Method 6800 provides for this correction. The accurate quantitation of speciated hexavalent chromium in the environment is especially important for monitoring industrial activities, such as mineral mining and processing.

Chapter Three of this dissertation describes the use of EPA Method 3052, EPA Method 3060A, and EPA Method 6800 to certify a new Sigma-Aldrich hexavalent chromium standard reference material in a soil matrix. The new low-level hexavalent chromium standard reference standard material will provide the scientific community with a standard material that supports quality assurance and quality control of the analytical methodology used for hexavalent chromium testing. New analysts and previously unexperienced laboratories have not had a material with well-characterized speciated chromium values to verify their mastery and proficiency in speciated analysis of hexavalent chromium. This new standard material enables validation within and between laboratories for hexavalent chromium data collection. The newly certified Sigma-Aldrich standard reference material will undoubtedly be used in the future to help mitigate the impact of mineral processing on the surrounding environment and assist in monitoring remediation of hexavalent chromium-containing waste materials produced during industrial activities.

Chapter Four discusses the determination of hexavalent chromium in a variety of dietary supplement formulations. As previously described, accurate quantitation of speciated chromium (trivalent and hexavalent chromium) is immensely significant not only for environmental measurements, but also for those that support human health and industry. Most multivitamin/multimineral vitamin formulations contain chromium. Although analysis of total chromium concentrations may be routinely and accurately made, the nature of chromium speciation requires the use of an accurate analytical method that is capable of specific quantification of both Cr(III) and Cr(VI) to provide information that may be used to improve human health and safety. Improved manufacturing practices and product quality control testing would help ensure that consumers are not exposed to unexpected concentrations of elemental

supplementation. Also, if there is inadequate quality control of hexavalent chromium in formulations marketed for prenatal support, both mother and child would be chronically exposed to a genotoxic and carcinogenic substance. The use of EPA Method 3052 with quantitation by IDMS according to EPA Method 6800 is examined to provide total chromium content of a variety of dietary supplement formulations. For quantitation of Cr(VI), microwave assisted sample digestion is performed in a 50 mM EDTA solution with EPA Method 6800 for Cr(VI) quantitation. Given the number of incorrectly and insufficiently labelled dietary supplements discovered during analysis, and the prevalence of hexavalent chromium in most of the multivitamin/multimineral vitamins, the routine use of these methods is recommended for quality assessment prior to the release of the finished products to the commercial marketplace.

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CHAPTER TWO:

SYNTHESIS AND CHARACTERIZATION OF ISOTOPICALLY ENRICHED CHROMIUM STANDARD SOLUTIONS

2.1 INTRODUCTION

According to the International Atomic Energy Agency, eighty of the first eighty-two elements in the periodic table have stable non-radioactive isotopes, with most elements having two or more isotopes. The distribution of stable isotopes was fixed during the galaxy's formation, which created constant isotopic ratios for almost all the elements found in terrestrial matter and the products made from natural ores, minerals, and raw materials. Although isotopes of the same element have unique masses that result from differences in neutron numbers, they also have nearly identical chemical characteristics and reactivity. Due to these properties, measuring and analyzing the distribution of isotopes is practical for wide variety of analytical applications, including nutrition research, environmental, medical, forensics, and agricultural studies [1]. These unique analytical measurements are achieved by adding a known quantity of an element that is enriched with a specific isotope during sample preparation. Once added to the sample, the natural, endogenous distribution of isotopes is artificially altered. The altered isotopic ratios are typically measured using a mass spectrometer to provide data for accurate quantitation of the original endogenous material.

This analytical approach is codified in United States EPA Method 6800, Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry [2]. Elemental isotope dilution mass spectrometry (IDMS) is a technique that is used for measuring total elemental concentrations in samples prepared using various dissolution, digestion, and/or extraction procedures. The sample

preparation should ensure complete decomposition and equilibrium between the endogenous and enriched isotopes. Additionally, when using an inductively coupled plasma mass spectrometer (ICP-MS) to measure the altered isotope distribution, the ionization process ensures that the endogenous and enriched isotopes are further "equilibrated," regardless of the initial molecular species of the sample and isotope standard. The accuracy of IDMS measurements is typically 0.5% to 0.001%, depending on the analytical equipment, techniques, and quality of the known data [1]. Unlike traditional calibration curve quantitation techniques, partial loss of analyte equilibrated with enriched spike does not impact the accuracy of IDMS measurements. Also, since the endogenous and enriched isotopes are at equilibrium in the sample preparation, physical and chemical interferences have less influences on IDMS measurements [1]. Using ICP-MS to measure the isotope ratios, high precision can also be achieved with relative standard deviation less than 0.5% [1, 2]. Furthermore, IDMS is considered a definitive method since it is capable of measuring and correcting biases that would greatly impact traditional methods of quantitation.

Analysis using IDMS works well for relatively stable species that do not interconvert or degrade during sample processing [1]. In IDMS sample preparations, all species are gathered into a single species and equilibration portion of the IDMS procedure. Some elements, ions, and molecules have a distribution of significant and chemically relevant species. However, interconversions between some reacting species is difficult to prevent and monitor. For example, although analysis of total chromium concentrations may be routinely and accurately made, the dichotomous toxicity of chromium requires the use of an accurate analytical method that is capable of specific quantification of both Cr(III) and Cr(VI). The presence of oxidizing and reducing agents, UV light, organic compounds, and changes in the pH and oxidation/reduction

potential (Eh) of sample solutions may affect the interconversion of Cr(III) and Cr(VI) species [3, 4]. Due to the potential for species interconversion, accurate determination of the concentrations and stabilities of the Cr(III) and Cr(VI) oxidation species therefore require a method that is capable of monitoring and correcting for interconversion, bias, and instrumental error. Traditional analytical approaches attempt to produce static species, which is contradictory to the element's natural properties [5]. However, molecular speciated isotope dilution mass spectrometry (SIDMS), which is codified in EPA Method 6800, allows and mathematically corrects for species interconversions using additional degrees of freedom [1, 2]. This is accomplished with enriched, isotopically-labelled Cr(III) and Cr(VI) spikes in the sample preparations. With IDMS, only one isotopically enriched standard solution is added to the sample. For chromium, the isotopically enriched standard is typically either 50-Cr(III) or 53-Cr(VI). While SIDMS retains the advantages of IDMS, it requires utilization of both enriched 50-Cr(III) and enriched 53-Cr(VI) isotope standards to provide a double spiked sample preparation. The addition of the enriched isotopic species adds to the naturally-occurring 52-Cr(III) and 52-Cr(VI) species of the endogenous sample material. This ensures that oxidative/reductive interconversions marked at the time of extraction, equilibrium, and species activity are quantifiable by measuring the final concentrations and oxidation states of the 50-Cr, 52-Cr, and 53-Cr isotopes. For example, ion chromatography (IC) may be used to separate the Cr(III) and Cr(VI) oxidative species into discretely eluting chromatographic peaks that are analyzed by ICP-MS during the real time elution from the chromatography column. The ICP-MS is used as an isotope detector to quantitate concentrations of the 50-Cr, 52-Cr, and 53-Cr isotopes in the eluting chromatographic peaks. The final 50/52-Cr(III), 53/52-Cr(III), 50/52-Cr(VI), and 53/52-Cr(VI) isotopic ratios are used to calculate the initial concentrations of Cr(III)

and Cr(VI) in the original unaltered sample, with correction for Cr(III) to Cr(VI) and Cr(VI) to Cr(III) conversions during extraction, equilibration, and analysis after the initial spiking of the natural samples.

The use of isotopically-labelled species with SIDMS eliminates the need for external calibration curves and relies on direct mathematical determinations [6]. Traditional external calibration curves introduce bias from instrumental variables, uncertainty due to changes in the signal response with analyte concentration, and matrix influences due to the presence of shifting calibration data from the standards and actual samples. The measurement of the isotope ratios in each sample is intrinsic and does not rely on the use of a previously established measurement. Moreover, SIDMS is based on one of four International Union of Pure and Applied Chemistry's (IUPAC) definitive methods, which are methods that have exceptional scientific status and are capable of material certification [7]. SIDMS provides measurements that are accurate and precise, enabling quantification of the concentration of each chromium species with interconversion correction.

Both IDMS and SIDMS use isotopically enriched standard solutions and require equilibration of the isotopically enriched species with the natural isotopic species of the sample for accurate analysis. The four naturally occurring isotopes of chromium are 50-Cr, 52-Cr, 53-Cr, and 54-Cr, which have natural abundances of 4.345%, 83.789%, 9.501% and 2.365%, respectively [4, 8, 9]. Enriched separated isotopes of various enrichment, purity, and molecular forms are generated by facilities such as the United States Oak Ridge National Laboratory (ORNL). Typically, the elemental enriched materials are available as oxides and metallic chromium, which require additional preparation before their use as isotopically enriched standards. To generate the isotopically enriched standard solutions that are required for

IDMS/SIDMS quantitation, the guidance provided in EPA Method 6800 was followed for the preparation of isotopically enriched standard solutions [2]. The new isotopically-enriched speciated chromium standards were synthesized and characterized to allow for further studies and assessment of chromium species in various research materials and projects.

2.2 MATERIALS AND METHODS

Two types of isotopically enriched speciated standard solutions were generated. The first was a trivalent chromium standard solution enriched in the 50-Cr isotope (50-Cr(III)) and the second was a hexavalent chromium standard enriched in the 53-Cr isotope (53-Cr(VI)). Two additional standard solutions were generated that provided Cr(III) and Cr(VI) solutions with natural chromium isotope distributions. For each standard solution, three different concentration levels were characterized.

2.2.1 REAGENTS AND MATERIALS

Potassium dichromate standard reference materials (SRM) 136e (99.984% ± 0.010%) and 136f (99.9954% ± 0.0044%) were purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland). The NIST COA documents for both NIST 136e and 136F are provided in Appendix 1. Chromium (III) nitrate nonahydrate (99.99% minimum, metal basis, Lot S08E051) was purchased from Alfa Aesar (Tewksbury, Massachusetts). Chromium metal isotopically enriched in 50-Cr (Batch 144980) was purchased from ORNL (Oak Ridge, Tennessee). Chromium oxide isotopically enriched in 53-Cr (Batch 177090) was purchased from ORNL (Oak Ridge, Tennessee). The ORNL COA documents for both 53-Cr and 50-Cr are provided in Appendix 1. Concentrated nitric acid (trace metal grade) and concentrated hydrochloric acid (trace metal grade) were purchased from Fisher Chemical (ThermoFisher Scientific, Waltham, Massachusetts). Concentrated perchloric acid 70% (Ultrex

II Ultrapure Reagent) and ammonium hydroxide 20% (Ultrex II Ultrapure Reagent) were purchased from J. T. Baker (VWR International, Radnor, Pennsylvania). Hydrogen peroxide 30-32% (Aristar Ultra) was purchased from VWR Chemicals BDH (VWR International, Radnor, Pennsylvania). Ethylenediaminetetraacetic acid, trisodium salt dihydrate (99%) was purchased from Acros Organics (ThermoFisher Scientific, Waltham, Massachusetts). Type I ultrapure water (18.2 MΩ-cm) was produced using a Barnstead EASYpure II RF/UV filtration system (ThermoFisher Scientific, Waltham, Massachusetts) and/or Evoqua Water Technologies PURELAB Flex filtration system (Pittsburgh, Pennsylvania). Polypropylene (PP) centrifuge tubes with high-density polyethylene (HDPE) lids were purchased from Fisher Scientific (ThermoFisher Scientific, Waltham, Massachusetts), VWR International (Radnor, Pennsylvania), and Globe Scientific Inc. (Mahwah, New Jersey).

2.2.2 INSTRUMENTATION

Analytical standards, reagents, and samples were prepared in a cleanroom laboratory environment that continuously filtered incoming air and recirculated cleaned laboratory air through a high-efficiency particulate air (HEPA) filtration system. Laminar flow benchtops and isolated hoods fitted with additional HEPA filtration systems isolated from the main laboratory were also utilized for preparation of standards and samples with trace-level analytes. A Mettler Toledo XS105 Excellence (Columbus, Ohio) analytical balance was utilized with 0.01 mg precision. Samples were prepared using a Milestone ETHOS UP microwave digestion system (Sorisole, Bergamo, Italy) equipped with a MAXI-44 easy TEMP high-throughput rotor and modified polytetrafluoroethylene (PTFE-TFM) vessels of 100-mL capacity. An Agilent Technologies 7700x inductively coupled plasma mass spectrometer (ICP-MS) (Santa Clara, California) was equipped with a micro-mist nebulizer, a quartz spray chamber, octopole reaction

system (ORS^3), and a quadrupole mass analyzer. The instrument was autotuned prior to analysis using an instrument tuning standard solution from Agilent Technologies and automated startup sequence. For direct sample introduction, spectrum mode of analysis (ICP-MS) was utilized with an ASX-520 autosampler (CETAC Automation, Omaha, Nebraska) that was contained within an anti-contamination enclosure. Time-resolved mode of analysis (IC-ICP-MS) was used for ion chromatography sample separations. A Metrohm 820 ion chromatography (IC) system (Herisau, Switzerland) was equipped with a Metrohm 858 Professional Sample Processor that was contained within an anti-contamination enclosure. The Metrohm ion chromatography system was metal free, with polyether ether ketone (PEEK) polymer material used for all connections, tubing, and column housing. The Metrohm 820 IC system was controlled using Metrohm IC Net 2.3, which was coupled to an independent Metrohm 850 Professional IC system running Metrohm MagicIC Net 3.1 to provide data communication and automation with the Agilent Technologies 7700x ICP-MS running MassHunter Workstation 4.2 software.

2.2.3 PREPARATION OF SPECIATED CHROMIUM STANDARDS

EPA Method 6800 is utilized for the determination of total elemental concentrations by IDMS and speciated elemental concentrations by SIDMS [2]. Also, the method provides guidance for the preparation of enriched speciated chromium standards [2]. To prepare the 53-Cr(VI) speciated standard solution, 71.6 mg of the 53-Cr oxide material supplied by ORNL was transferred into an acid-washed, 150-mL Pyrex-type glass beaker for dissolution. Under the cleanroom hood, approximately 8.0 g of concentrated perchloric acid was obtained in a polytetrafluoroethylene (PTFE) container. Using a small disposable plastic pipet, several aliquots of perchloric acid were used to rinse the vials that originally contained the oxide material. The rinses were transferred into the glass dissolution beaker and a final aliquot of

perchloric acid was used to rinse down the sides of the glass beaker. An acid-washed watch glass was used to cover the dissolution beaker and the solution was heated on a hotplate at 150°C. After approximately ninety minutes the hotplate temperature was increased to 185°C and maintained below the boiling point of perchloric acid (203°C). The dark green solution started to generate red crystals with additional heating. After a total heating time of four hours, approximately 2 mL of acid remained, and the beaker was removed from the hotplate. Approximately 10 mL of 18.2 MΩ-cm water was used to rinse the watch glass and sides of the dissolution beaker. The solution was dark yellow-orange without solids. Two 4.5 mL aliquots of ammonium hydroxide were used to adjust the solution to a final pH of approximately 10.5. Next, 300 mL of hydrogen peroxide as added to the light-yellow solution, which resulted in a dark yellow-brown color. The beaker was heated to 200°C for fifteen minutes to allow for oxidation of the chromium under alkaline conditions and removal of excess hydrogen peroxide from the solution. The beaker was removed from the hotplate and swirled, which resulted in the precipitation of the supersaturated solution. Approximately 10 mL of 18.2 MΩ-cm water was added to the beaker, and the beaker was stored overnight at ambient conditions in a PTFE enclosure. The solids dissolved into solution after it was heated to boiling for fifteen minutes. The resulting yellow solution was transferred into a PTFE storage container using several rinses from an additional 10 mL of 18.2 MΩ-cm water.

The 50-Cr(III) speciated standard solution was prepared by transferring 49.6 mg of the 50-Cr metal material supplied by ORNL into an acid-washed, 250-mL PTFE beaker for dissolution. Approximately 12.0 g of concentrated hydrochloric acid was transferred into the dissolution beaker. The solution was slowly heated on a hotplate until bubbles formed on the bottom of beaker, but the solution was not allowed to boil. The solution was heated until

approximately 2 mL of the solution remained. After cooling to ambient temperature, approximately 20 mL of 18.2 MΩ-cm water (1% nitric acid) was added to the beaker. The resulting blue green solution was transferred into a PTFE storage container.

Two separate 1000 µg/g natural Cr(VI) solutions were generated by dissolving NIST 136e and NIST 136f potassium dichromate in 18.2 MΩ-cm water (0.1% ammonia hydroxide). The resulting yellow solutions were individually transferred into PTFE storage containers and capped. The solutions were sonicated for 20 minutes to ensure complete solid dissolution. Attempts were made to generate natural Cr(III) solutions from NIST 136f through acidification with nitric acid and reduction with hydrogen peroxide. However, chromatographic analysis indicated incomplete species conversion. Instead, ultra-pure chromium (III) nitrate was obtained and dissolved in 18.2 MΩ-cm water (1% nitric acid) to generate a 1000 µg/g natural-Cr(III) solution. The resulting blue-green solution was transferred into a PTFE storage container and capped. The solution was sonicated for 20 minutes to ensure complete solid dissolution.

The four new standards [50-Cr(III), 53-Cr(VI), Nat-Cr(III), and Nat-Cr(VI)] were fully characterized before the solutions were diluted to targeted standard concentrations. Once characterized, preparations of approximately 100 µg/g and 10 µg/g were made by diluting each of the standard stock solutions. The diluted standard solutions were fully evaluated for species purity and chromium assay concentrations. The details of the characterization of the speciated chromium standards are described in Section 2.2.5. Briefly, reverse Isotope Dilution Mass Spectrometry (rIDMS) was used to determine the chromium assay content. The isotopic fractional distributions were determined by assessment of the solutions using ICP-MS. Each solution was examined by chromatography to ensure chromium species purity. Certificates of

Analysis (COA) were generated for the new standard solutions, which were included in reagent kits used for chromium analysis with additional research projects.

2.2.4 CHARACTERIZATION OF SPECIATED CHROMIUM STANDARDS

2.2.4.1 Total Chromium Analysis

EPA Method 3052, Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices, was used to prepare each batch of the natural and isotopically enriched speciated standard solutions for total chromium assessment [10]. EPA Method 6800, Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry, was used to quantitate the total elemental chromium concentrations of the digested samples [2]. Specifically, methodology for reverse IDMS (rIDMS) was followed according to EPA Method 6800, where the isotopically enriched speciated standard solutions are calibrated against a well-characterized assay material [2, 11]. For each isotopically enriched speciated chromium standard solution, eight independent rIDMS analyses were carried out with five injections per analysis. This methodology provides 40 data points for statistical workup (n = 40). The two separate solutions of NIST 136e and NIST 136f potassium dichromate in 18.2 MΩ-cm water (0.1% ammonia hydroxide) were used as the well-characterized assay material. Additionally, the isotope ratios of the unaltered (unspiked) standard solutions were measured by ICP-MS. To prepare each sample, an aliquot from an individual container of standard solution was transferred into a quartz weigh bottle. Using weigh by difference, 0.2500 g of the sample was quantitatively transferred directly into a microwave digestion vessel. Using weigh by difference, the sample was then spiked by quantitatively adding 0.2500 g of Nat-Cr(VI) [NIST 136e and NIST 136f solution] into the microwave digestion vessel. Using a transfer pipet, 9.0 mL of concentrated nitric acid and 1.0 mL of concentrated hydrochloric acid were added to the microwave vessel. A vented screw cap was

used to securely tighten the lid onto the microwave vessel. The microwave vessels were loaded into MAXI-44 easy TEMP high-throughput rotor, placed into the Milestone ETHOS UP microwave digestion system, and processed at 180°C for 9.5 minutes with a 5.5-minute ramp at 1800 watts. Once the samples cooled to ambient temperature, each microwave vessel was individually opened in a fume hood, and the digested sample was transferred into a labeled polypropylene 15-mL centrifuge tube and capped. The samples were held overnight at ambient temperature. The samples were centrifuged for 30 minutes at 3300 rpm. For each sample, 2.0 mL of the supernatant was transferred into a labelled polypropylene 50-mL centrifuge tube, brought to 20 mL with 18.2 MΩ-cm water, capped, and inverted ten times to mix. The diluted solutions were analyzed with ICP-MS using rIDMS according to EPA Method 6800.

2.2.4.2 Speciated Hexavalent Chromium Analysis

To determine the speciated chromium content of the chromium standard solutions, a hot alkaline digestion solution of 50 mM EDTA was selected for speciated chromium analysis. The high pH extraction solution supports extraction of Cr(VI) as a soluble chromate anion (CrO_4^{2-}) and formation of a $[Cr(III)EDTA]^-$ complex. The complexing of Cr(III) with EDTA prevents oxidation of Cr(III) compounds to Cr(VI) [12]. Furthermore, by chromatographically separating the Cr(III) peak as $Cr(EDTA)^-$ and the Cr(VI) peak as CrO_4^{2-}, the speciated purity of the chromium standard solutions were qualitatively verified. For each speciated standard, 0.2500 g of the solution was transferred into a microwave digestion vessel. The transferred solution was not spiked with additional standard solutions. Using a transfer pipet, 10 mL of 50 mM EDTA extraction solution was added to the microwave digestion vessel. A vented screw cap was used to securely tighten the lid onto the microwave vessel. The microwave vessels were loaded into MAXI-44 easy TEMP high-throughput rotor, placed

into the Milestone ETHOS UP microwave digestion system, and processed for ten minutes at 95°C with a 5-minute ramp at 1200 watts. Once the samples cooled to ambient temperature, each microwave vessel was individually opened in a fume hood, and the extracted sample was transferred into a labeled polypropylene 15-mL centrifuge tube and capped. The samples were held overnight at ambient temperature. The samples were centrifuged for 30 minutes at 3300 rpm. For each sample, the supernatant was completely transferred into an individually labeled polypropylene 50-mL centrifuge tube, brought to 35 mL with 18.2 MΩ-cm water, capped, and inverted ten times to mix. The diluted solutions were analyzed by IC-ICP-MS.

2.2.4.3 Instrument Methods

The samples for total chromium analysis were placed into an enclosed autosampler for direct sample introduction. The Agilent Technologies 7700x ICP-MS was set to spectrum mode of analysis (ICP-MS) and tuned with an automated startup sequence using an instrument tuning standard solution from Agilent Technologies. Table 2.1 provides tune settings that resulted from a typical autotune routine, which were used as the instrument parameters for total chromium analysis. For speciated chromium analysis, samples were placed into the enclosed autosampler for ion chromatography separation. The Metrohm 820 ion chromatography (IC) system was equipped with a set of Metrohm Metrosep A Supp 5 PEEK analytical and guard columns. An isocratic flow of a 2 mM EDTA solution at ambient temperature is used as the mobile phase for these columns and provides an anion exchange chromatographic separation mechanism. Table 2.2 provides details about the chromatographic system setup, including additional information about the column and mobile phase eluent.

Table 2.1: Agilent Technologies 7700x ICP-MS autotune settings for total chromium analysis by EPA Method 3052 and EPA Method 6800.

		Typical Autotune Parameters:	
RF power	1500 W		
RF matching	1.80 V	Extract 1	-125.0 V
Sampling depth	8.0 mm	Extract 2	-195.0 V
Carrier gas (Ar) flow	0.95 L min^{-1}	Omega bias	-85 V
Dilution gas (Ar) flow	0.15 L min^{-1}	Omega lens	4.4 V
ORS^3 gas (He) flow	5.0 mL min^{-1}	OctP bias	-18.0 V
Spray chamber temperature	2 °C	OctP RF	200 V
Data acquisition mode	Spectrum	Energy discrimination	4.0 V
Isotope monitored	^{50}Cr, ^{52}Cr, ^{53}Cr, ^{54}Cr		
Peak pattern	20 points/mass		
Replicates	5		
Sweeps/replicate	1000		
Integration time/mass	2 seconds		
Nebulizer pump	0.10 rps		
Sample uptake	60 seconds		
Stabilization	30 seconds		

The Agilent Technologies 7700x ICP-MS was set to time-resolved mode of analysis (IC-ICP-MS) and tuned with an automated startup sequence using an instrument tuning standard solution from Agilent Technologies. Table 2.3 provides tune settings that resulted from a typical autotune routine, which were used for the instrument parameters for speciated chromium analysis.

Table 2.2: Metrohm 820 Ion Chromatography Separation Center settings for speciated chromium analysis by 50 mM EDTA extraction and EPA Method 6800.

Column	Metrosep A Supp 5 PEEK column (Metrohm) containing polyvinyl alcohol with quaternary ammonium groups, 250 x 4.0 mm, 5 µm particle size, pH range 3 to 12; with Metrosep A Supp 5 guard column (5 x 4.0 mm, 5 µm particle size)
Mobile Phase	2 mmol L^{-1} EDTA in ultrapure water, pH 10 adjusted using ammonium hydroxide
Elution Mode	Isocratic
Flow Rate	0.8 mL min^{-1}
Column Temperature	Ambient
Injection Volume	100 µL

Table 2.3: Agilent Technologies 7700x ICP-MS autotune settings for speciated chromium analysis by 50 mM EDTA extraction and EPA Method 6800.

RF power	1500 W	*Typical Autotune Parameters:*	
RF matching	1.80 V	Extract 1	-125.0 V
Sampling depth	8.0 mm	Extract 2	-195.0 V
Carrier gas (Ar) flow	0.95 L min^{-1}	Omega bias	-85 V
Dilution gas (Ar) flow	0.15 L min^{-1}	Omega lens	4.4 V
ORS^3 gas (He) flow	5.0 mL min^{-1}	OctP bias	-18.0 V
Spray chamber temperature	2 °C	OctP RF	200 V
Data acquisition mode	Time resolved analysis	Energy discrimination	4.0 V
Isotope monitored	^{50}Cr, ^{52}Cr, ^{53}Cr, ^{54}Cr		
Integration time/mass	0.25 seconds		
Sampling period	1.006 sec		
Nebulizer pump	0.50 rps		

2.3 RESULTS AND DISCUSSION

2.3.1 TOTAL CHROMIUM ANALYSIS

The quantitation of total chromium in the standard solutions was performed according to EPA Method 3052 and EPA Method 6800 by ICP-MS (sample preparation outlined in section 2.2.5.1) using the instrument parameters provided in Table 2.1. Assessment of the method suitability was provided by the analysis of independent quality control sample preparations using NIST 136f, with suitability established by recoveries that ranged from approximately 90% - 110% of the theoretical concentrations. For each of the newly created standard solutions, an aliquot from each individual standard was subsampled four times and analyzed with five replicate measurements (n = 20). For the isotopically enriched standard solutions, carefully prepared assay standards of NIST 136e and NIST 136f were used for rIDMS quantitation of the total chromium content.

The results of the total chromium analysis of the 53-Cr(VI) standard solutions are summarized in Table 2.4 and Table 2.5, using NIST 136e and NIST 136f respectively. Table 2.6 provides the finalized assay values for the 53-Cr(VI) standards, which are an average of the NIST 136e and NIST 136f determinations. The tables provide total chromium (μg/g) with 95%

confidence intervals, 95% confidence intervals as percentages of the total assay values, standard deviations, and percent relative standard deviation. Three 53-Cr(VI) standard solutions were created and characterized. The finalized total chromium content of the standards were established as 822.2598 μg/g ± 7.3002 μg/g, 103.6183 μg/g ± 0.0947 μg/g, and 8.2519 μg/g ± 0.0326 μg/g (95% confidence intervals, n = 80). The 95% confidence intervals correspond to approximately 0.1% to 1.5% of the total chromium content. The percent relative standard deviations ranged from 0.2% to 3.1%, which indicate appropriate precision for standard solutions.

Similarly, the results of the total chromium analysis of the 50-Cr(III) standard solutions are summarized in Table 2.7 and Table 2.8, using NIST 136e and NIST 136f, respectively. Table 2.9 provides the finalized assay values for the 50-Cr(III) standards, which are the averages of the NIST 136e and NIST 136f determinations. The tables provide total chromium (μg/g) with 95% confidence intervals, 95% confidence intervals as percentages of the total assay values, standard deviations, and percent relative standard deviation. Three 50-Cr(III) standard solutions were created and characterized. The finalized total chromium content of the standards were established as 907.3397 μg/g ± 3.6921 μg/g, 99.9789 μg/g ± 0.1028 μg/g, and 8.0377 μg/g ± 0.0740 μg/g (95% confidence intervals, n = 80). The 95% confidence intervals correspond to approximately 0.1% to 3.7% of the total chromium content. The percent relative standard deviations ranged from 0.3% to 2.7%.

The isotope distributions of the standard solutions were determined by ICP-MS. During analysis, each of the standard solutions was examined without further spiking. Although the standard solutions required considerable preparation and processing, the final solutions are expected to retain the same distributions of the original materials acquired from ORNL. Any

measured deviations from the isotope ratios reported on the ORNL certificate of analysis (COA) reflect combined error from the sample preparation and instrumental analysis. For the 53-Cr(VI) standard solutions (Table 2.10), the percentage of 53-Cr isotope is expected to be 97.20%. Analysis provided 53-Cr isotope percentages that ranged from 97.40% to 97.90%. According to the ORNL COA, the percentage of 52-Cr is expected to be 2.65%. The measured 52-Cr percentages were found to be 1.95% to 2.38%. This indicates that no major changes to the isotope fractions occurred during solution preparation, such as contamination. Likewise, the measured isotopic ratios of the 50-Cr(III) standard solutions did not indicate difference from the isotope fractions certified by ORNL during sample preparation (Table 2.11). The measured 50-Cr isotope percentages range was 96.30% - 96.75%, which compares to the expected percentage of 96.05%. The measured 52-Cr was 2.94% to 3.31%, which closely compares to the ORNL COA value of 3.66%.

Table 2.4: Total chromium analysis using NIST 136e for speciated hexavalent chromium standard solutions isotopically enriched with 53-Cr(VI). For each concentration level, the standard solution was independently subsampled four times and analyzed with ICP-MS according to EPA Method 6800 using reverse isotope dilution mass spectrometry (rIDMS) with five replicate measurements for each sample (n = 20).

53-Cr(VI) Isotope Standard Solutions Calculated Concentrations by rIDMS with 136e (n = 20)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
821.6228	11.9276	1.4517%	25.4855	3.1018%
103.8427	0.1081	0.1041%	0.2309	0.2224%
8.2133	0.0218	0.2657%	0.0466	0.5678%

Table 2.5: Total chromium analysis using NIST 136f for speciated hexavalent chromium standard solutions isotopically enriched with 53-Cr(VI). For each concentration level, the standard solution was independently subsampled four times and analyzed with ICP-MS according to EPA Method 6800 using reverse isotope dilution mass spectrometry (rIDMS) with five replicate measurements for each sample (n = 20).

53-Cr(VI) Isotope Standard Solutions Calculated Concentrations by rIDMS with 136f (n = 20)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
822.8968	9.5816	1.1644%	20.4730	2.4879%
103.3940	0.0673	0.0651%	0.1438	0.1391%
8.2905	0.0592	0.7146%	0.1266	1.5268%

Table 2.6: Combined total chromium analysis using NIST 136e and NIST 136f for speciated hexavalent chromium standard solutions isotopically enriched with 53-Cr(VI). For each concentration level, the standard solution was independently subsampled eight times and analyzed with ICP-MS according to EPA Method 6800 using reverse isotope dilution mass spectrometry (rIDMS) with five replicate measurements for each sample (n = 40).

53-Cr(VI) Isotope Standard Solutions Calculated Concentrations by rIDMS Combined 136e and 136f Data Sets (n = 40)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
822.2598	7.3002	0.8878%	22.8263	2.7760%
103.6183	0.0947	0.0914%	0.2961	0.2858%
8.2519	0.0326	0.3951%	0.1019	1.2354%

Table 2.7: Total chromium analysis using NIST 136e for speciated trivalent chromium standard solutions isotopically enriched with 50-Cr(III). For each concentration level, the standard solution was independently subsampled four times and analyzed with ICP-MS according to EPA Method 6800 using reverse isotope dilution mass spectrometry (rIDMS) with five replicate measurements for each sample (n = 20).

50-Cr(III) Isotope Standard Solutions Calculated Concentrations by rIDMS with 136e (n = 20)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
913.3402	3.7525	0.4109%	8.0178	0.8779%
100.2084	0.1013	0.1011%	0.2165	0.2161%
8.1873	0.1429	1.7453%	0.2580	3.1517%

Table 2.8: Total chromium analysis using NIST 136f for speciated trivalent chromium standard solutions isotopically enriched with 50-Cr(III). For each concentration level, the standard solution was independently subsampled four times and analyzed with ICP-MS according to EPA Method 6800 using reverse isotope dilution mass spectrometry (rIDMS) with five replicate measurements for each sample (n = 20).

50-Cr(III) Isotope Standard Solutions Calculated Concentrations by rIDMS with 136f (n = 20)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
901.3392	5.4069	0.5999%	11.5529	1.2818%
99.7494	0.1091	0.1093%	0.2330	0.2336%
7.9255	0.0254	0.3204%	0.0543	0.6846%

Table 2.9: Combined total chromium analysis using NIST 136e and NIST 136f for speciated trivalent chromium standard solutions isotopically enriched with 50-Cr(III). For each concentration level, the standard solution was independently subsampled eight times and analyzed with ICP-MS according to EPA Method 6800 using reverse isotope dilution mass spectrometry (rIDMS) with five replicate measurements for each sample (n = 40).

50-Cr(III) Isotope Standard Solutions Calculated Concentrations by rIDMS Combined 136e and 136f Data Sets (n = 40)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
907.3397	3.6921	0.4069%	11.5443	1.2723%
99.9789	0.1028	0.1028%	0.3215	0.3215%
8.0377	0.0740	0.9201%	0.2153	2.6785%

Table 2.10: Measured isotopic composition for the speciated hexavalent chromium standard solutions enriched with 53-Cr(VI). For each concentration level, the standard solution was independently subsampled three times and analyzed by ICP-MS with four replicate measurements for each sample (n = 12). The isotopic composition provided on the ORNL Certificate of Analysis (COA) for the enriched chromium oxide is included for comparison.

53-Cr(VI) Isotope Standard Solutions **Measured Isotopic Composition in Atomic Percent (95% CI, n = 12)**				
µg/g	50-Cr (%)	52-Cr (%)	53-Cr (%)	54-Cr (%)
822.2598	0.04 ± 0.00110	1.97 ± 0.00734	97.87 ± 0.17885	0.11 ± 0.00082
103.6183	0.03 ± 0.00298	1.95 ± 0.00881	97.90 ± 0.31450	0.11 ± 0.00164
8.2519	0.06 ± 0.00250	2.38 ± 0.02553	97.40 ± 0.75654	0.16 ± 0.00392
ORNL COA	0.01 ± 0.00500	2.65 ± 0.02000	97.20 ± 0.02000	0.12 ± 0.00500

Table 2.11: Measured isotopic composition for the speciated trivalent chromium standard solutions enriched with 50-Cr(III). For each concentration level, the standard solution was independently subsampled three times and analyzed by ICP-MS with four replicate measurements for each sample (n = 12). The isotopic composition provided on the ORNL Certificate of Analysis (COA) for the enriched chromium metal is included for comparison.

50-Cr(III) Isotope Standard Solutions **Measured Isotopic Composition in Atomic Percent (95% CI, n = 12)**				
µg/g	50-Cr (%)	52-Cr (%)	53-Cr (%)	54-Cr (%)
907.3397	96.75 ± 0.34613	2.96 ± 0.00873	0.24 ± 0.00230	0.05 ± 0.00100
99.9789	96.75 ± 0.25762	2.94 ± 0.00966	0.25 ± 0.00760	0.05 ± 0.00064
8.0377	96.30 ± 0.15616	3.31 ± 0.01799	0.31 ± 0.00849	0.09 ± 0.00320
ORNL COA	96.05 ± 0.04000	3.66 ± 0.04000	0.24 ± 0.01000	0.05 ± 0.00500

For each of the newly created natural chromium standard solutions, an aliquot from each individual standard was subsampled four times and analyzed with five replicate measurements (n = 20). Isotopically enriched standard solutions of 50-Cr(III) and 53-Cr(VI) were used for IDMS quantitation of the total chromium content. The results of the total chromium analysis of the Nat-Cr(VI) standard solutions are summarized in Table 2.12 and Table 2.13 using 50-Cr(III) and 53-Cr(VI), respectively. Table 2.14 provides the finalized assay values for the Nat-Cr(VI) standards, which are an average of the 50-Cr(III) and 53-Cr(VI) determinations. The tables provide total chromium (µg/g) with 95% confidence intervals, 95% confidence intervals as percentages of the total assay values, standard deviations, and percent relative standard

deviation. Three Nat-Cr(VI) standard solutions were created and characterized. The finalized total chromium content of the standards were established as 1296.8849 μg/g ± 13.3890 μg/g, 101.6977 μg/g ± 0.3253 μg/g, and 8.7385 μg/g ± 0.0593 μg/g (95% confidence intervals, n = 80). The 95% confidence intervals correspond to approximately 0.7% to 1.0% of the total chromium content. The percent relative standard deviations ranged from 1.0% to 3.2%, which indicate appropriate precision for standard solutions.

Similarly, the results of the total chromium analysis of the Nat-Cr(III) standard solutions are summarized in Table 2.15 and Table 2.16 using 50-Cr(III) and 53-Cr(VI), respectively. Table 2.17 provides the finalized assay values for the Nat-Cr(III) standards, which are an average of the 50-Cr(III) and 53-Cr(VI) determinations. The tables provide total chromium (μg/g) with 95% confidence intervals, 95% confidence intervals as percentages of the total assay values, standard deviations, and percent relative standard deviation. Three Nat-Cr(III) standard solutions were created and characterized. The finalized total chromium content of the standards were established as 244.6492 μg/g ± 4.0711 μg/g, 126.0422 μg/g ± 0.3232 μg/g, and 7.9987 μg/g ± 0.0149 μg/g (95% confidence intervals, n = 80). The 95% confidence intervals correspond to approximately 0.2% to 1.7% of the total chromium content. The percent relative standard deviations ranged from 0.6% to 5.2%, which indicate appropriate precision for standard solutions.

Table 2.12: Total chromium analysis using 50-Cr(III) for speciated hexavalent chromium standard solutions with natural isotope distribution [Natural-Cr(VI)]. For each concentration level, the standard solution was independently subsampled four times and analyzed with ICP-MS according to EPA Method 6800 using isotope dilution mass spectrometry (IDMS) with five replicate measurements for each sample (n = 20).

Natural-Cr(VI) Standard Solutions Calculated Concentrations by IDMS with 50-Cr(III) (n = 20)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
1291.5404	27.3759	2.1196%	58.4937	4.5290%
100.8068	0.1146	0.1137%	0.2448	0.2429%
8.8044	0.1153	1.3092%	0.2463	2.7973%

Table 2.13: Total chromium analysis using 53-Cr(VI) for speciated hexavalent chromium standard solutions with natural isotope distribution [Natural-Cr(VI)]. For each concentration level, the standard solution was independently subsampled four times and analyzed with ICP-MS according to EPA Method 6800 using isotope dilution mass spectrometry (IDMS) with five replicate measurements for each sample (n = 20).

Natural-Cr(VI) Standard Solutions Calculated Concentrations by IDMS with 53-Cr(VI) (n = 20)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
1302.2295	5.0388	0.3869%	10.7663	0.8268%
102.5885	0.2936	0.2861%	0.6272	0.6114%
8.6726	0.0137	0.1580%	0.0293	0.3376%

Table 2.14: Total chromium analysis using 50-Cr(III) and 53-Cr(VI) for speciated hexavalent chromium standard solutions with natural isotope distribution [Natural-Cr(VI)]. For each concentration level, the standard solution was independently subsampled eight times and analyzed with ICP-MS according to EPA Method 6800 using isotope dilution mass spectrometry (IDMS) with five replicate measurements for each sample (n = 40).

Natural-Cr(VI) Standard Solutions Calculated Concentrations by IDMS Combined 50-Cr(III) and 53-Cr(VI) Data Sets (n = 40)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
1296.8849	13.3890	1.0324%	41.8647	3.2281%
101.6977	0.3253	0.3199%	1.0173	1.0003%
8.7385	0.0593	0.6790%	0.1855	2.1232%

Table 2.15: Total chromium analysis using 50-Cr(III) for speciated trivalent chromium standard solutions with natural isotope distribution [Natural-Cr(III)]. For each concentration level, the standard solution was independently subsampled four times and analyzed with ICP-MS according to EPA Method 6800 using isotope dilution mass spectrometry (IDMS) with five replicate measurements for each sample (n = 20).

Natural-Cr(III) Standard Solutions Calculated Concentrations by IDMS with 50-Cr(III) (n = 20)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
232.0999	0.3070	0.1323%	0.6559	0.2826%
126.0527	0.6465	0.5129%	1.3813	1.0958%
8.0285	0.0229	0.2850%	0.0489	0.6089%

Table 2.16: Total chromium analysis using 53-Cr(VI) for speciated trivalent chromium standard solutions with natural isotope distribution [Natural-Cr(III)]. For each concentration level, the standard solution was independently subsampled four times and analyzed with ICP-MS according to EPA Method 6800 using isotope dilution mass spectrometry (IDMS) with five replicate measurements for each sample (n = 20).

Natural-Cr(III) Standard Solutions Calculated Concentrations by IDMS with 53-Cr(VI) (n = 20)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
257.1985	0.3720	0.1446%	0.7948	0.3090%
126.0318	0.2028	0.1609%	0.4334	0.3439%
7.9689	0.0061	0.0764%	0.0130	0.1633%

Table 2.17: Total chromium analysis using 50-Cr(III) and 53-Cr(VI) for speciated trivalent chromium standard solutions with natural isotope distribution [Natural-Cr(III)]. For each concentration level, the standard solution was independently subsampled eight times and analyzed with ICP-MS according to EPA Method 6800 using isotope dilution mass spectrometry (IDMS) with five replicate measurements for each sample (n = 40).

Natural-Cr(III) Standard Solutions Calculated Concentrations by IDMS Combined 50-Cr(III) and 53-Cr(VI) Data Sets (n = 40)				
µg/g	95% CI (µg/g)	95% CI (%)	SD	%RSD
244.6492	4.0711	1.6641%	12.7295	5.2032%
126.0422	0.3232	0.2564%	1.0105	0.8017%
7.9987	0.0149	0.1857%	0.0464	0.5805%

2.3.2 SPECIATED CHROMIUM ANALYSIS

To determine the speciated chromium content of the chromium standard solutions, an alkaline digestion solution of 50 mM EDTA was selected for speciated chromium analysis. The prepared solutions were analyzed by IC-ICP-MS using the Metrosep A Supp 5 PEEK column.

The resulting chromatograms were used to verify the speciated purity of each of the standard solutions. For each chromatogram, the major isotopes of chromium (50-Cr, 52-Cr, 53-Cr, 54-Cr) are shown and reflect the targeted isotope enrichment. The retention time for Cr(III) was found to be approximately 2.5 minutes, and the retention time for Cr(VI) was found to be approximately 4.5 minutes. The small baseline fluctuation at approximately 1.25 minutes corresponds to an increase in system pressure from the sample injection. Figure 2.1 provides example chromatograms that were used for the assessment of the species purity of the chromium standard solutions. The chromatograms indicate that the solutions were synthesized with the desired purity since only one peak is evident for each of the solutions.

Figure 2.1: Example chromatograms for purity assessment of the speciated chromium standard solutions. A 50 mM EDTA alkaline extraction solution was used to prepare the samples, which were analyzed using IC-ICP-MS with a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column and 2 mM EDTA mobile phase. Examples of the resulting chromatograms are provided: (A) 53-Cr(VI) isotope standard; (B) 50-Cr(III) isotope standard; (C) Natural-Cr(VI) standard; and (D) Natural-Cr(III) standard. The retention time for Cr(III) was found to be approximately 2.5 minutes and the retention time for Cr(VI) was found to be approximately 4.5 minutes. The example chromatogram includes the ion count for each of the major isotopes of chromium.

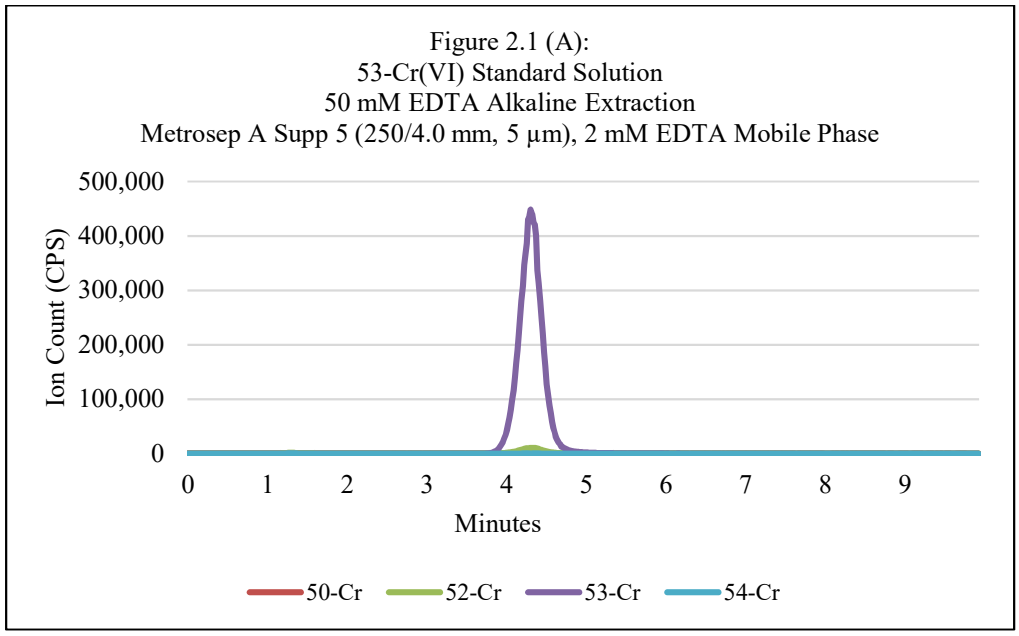


Figure 2.1 (B):
50-Cr(III) Standard Solution
50 mM EDTA Alkaline Extraction
Metrosep A Supp 5 (250/4.0 mm, 5 µm), 2 mM EDTA Mobile Phase

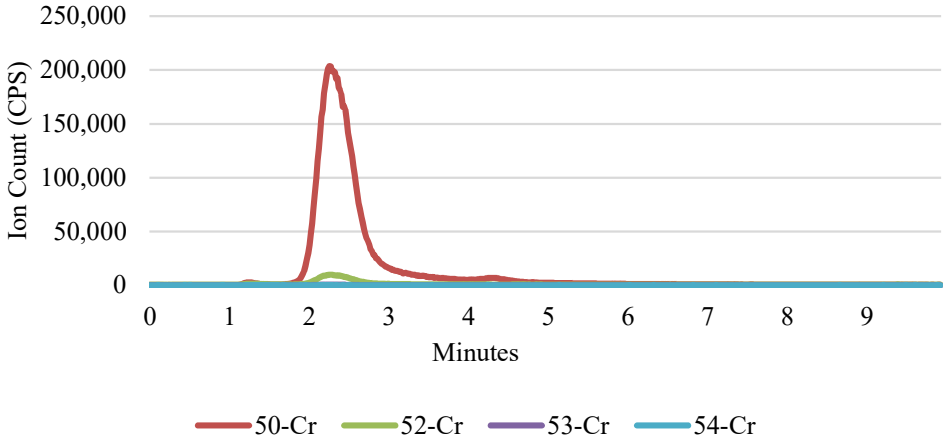
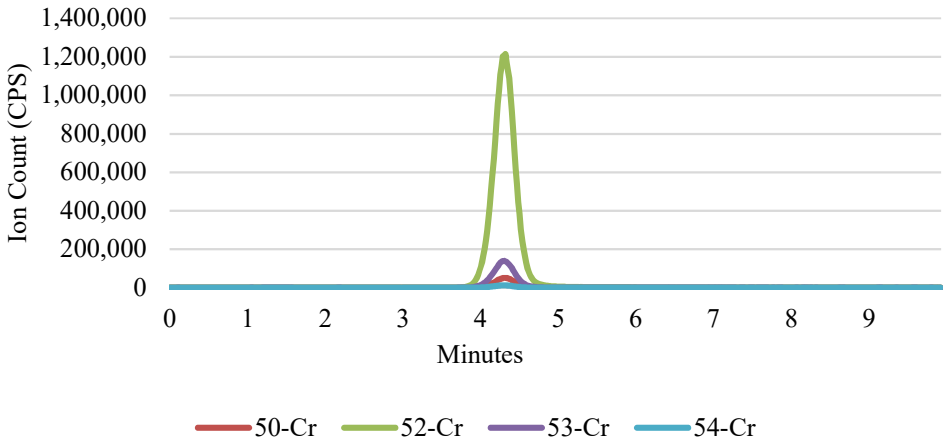
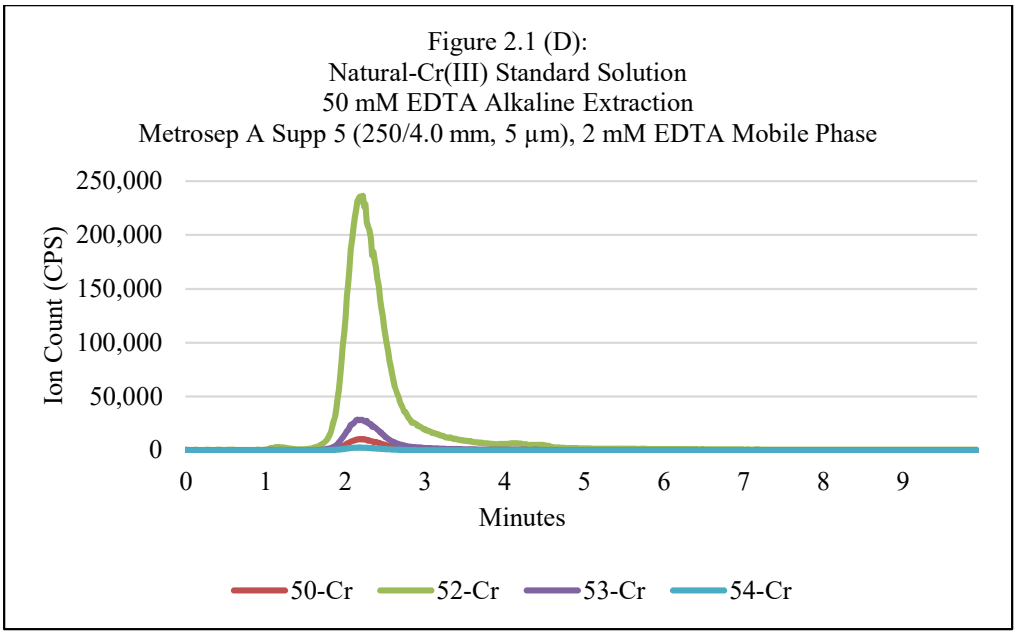


Figure 2.1 (C):
Natural-Cr(VI) Standard Solution
50 mM EDTA Alkaline Extraction
Metrosep A Supp 5 (250/4.0 mm, 5 µm), 2 mM EDTA Mobile Phase





2.4 CONCLUSIONS

These results indicate the successful synthesis and characterization of isotopically enriched speciated standard solutions. Assay values for the specific chromium species were assigned using rIDMS quantitation. Further analysis of the standards by ICP-MS indicate that the synthesized and diluted solutions maintained an isotopic distribution that matched the starting material obtained from ORNL. Additionally, speciated chromium analysis by IC-ICP-MS shows that each standard solution provides only one chromium species, which indicates standard solution purity. As such, these isotopically enriched standard solutions will be used for further IDMS/SIDMS quantitation using EPA Method 6800.

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CHAPTER THREE:

CERTIFICATION OF A NEW LOW-LEVEL HEXAVALENT CHROMIUM STANDARD REFERENCE MATERIAL IN A SOIL MATRIX

3.1 INTRODUCTION

Global demand for stainless steel is and will remain strong, which results in the yearly consumption of thousands of tons of chromium. Chromium is used in many metallic alloys, and it constitutes as much as 30% by weight in some stainless-steel fabrications. To produce these materials, the metallurgical industry extensively utilizes chromium, which enables iron-based alloys to be resistant to corrosion, oxidation, and wear [1]. Although stainless steel accounts for the majority of chromium consumption, chromium is also used in protective electroplate coatings, cast iron, and nonferrous alloys [2, 3]. Chromium is a gray, lustrous, hard, and brittle metal that is naturally found in the Earth's crust at an average concentration of 122 ppm (mg/kg) [3-5]. There are many types of chromium bearing ores, but the mineral chromite is the most commercially exploited ore used for chromium extraction [2-4]. The pure spinel mineral chromite is ideally composed of an oxide of iron and chromium ($Fe^{2+}Cr^{3+}{}_2O_4$) that contains 32% FeO and 68% Cr_2O_2, and it is often found in nature with other constituent impurities (Mg, Fe^{3+}, Al, Ti, and Mn) [2, 4, 5].

Low grade chromium containing ore is pulverized, mixed with lime and soda ash, and roasted to produce water soluble chromate and dichromate compounds, which are extensively utilized for leather tanning, pigments, catalysts, and wood preservatives [1, 3-7]. Higher grades of chromite ores that contain iron and 50-70% chromium are used for the production of ferrochrome (ferrochromium), which is a ferroalloy intermediate raw material for metallurgic

grade materials [4]. Approximately 75% of ferrochrome is used as an alloying agent for manufacturing stainless steel [1]. To produce ferrochrome, high grade ore is pulverized and melted in electric furnaces in the presence of reducing agents [4, 5].

Chromium ore is mined in over 20 countries, with 80% of the world's total mined in South Africa, Kazakhstan, India, and Turkey [2, 4, 5, 8, 9]. Of the 24.5 million tons of chromite mined worldwide in 2012, only 0.13% was mined in North America [2]. However, plans were announced in 2010 for a large-scale chromium mineral mining site in the James Bay Lowland of Northern Ontario, Canada [2, 9, 10]. Known as the Ring of Fire, it covers 5,000 square kilometers and contains massive amounts of chromite mineral deposits, which makes it the largest chromium containing mineral deposit discovery of the 21st century [2, 9, 10]. During the first 10 years of development, the site is estimated to have a potential of generating up to $9.4 billion in gross domestic product, $6.2 billion for Ontario's mining industry, and $2 billion in government revenue [9-11]. It is likely that development at the Ring of Fire will continue well into the future since it will strategically support uninterrupted stainless steel production in North America and is expected to meet North American needs for several centuries [2, 9].

The Ring of Fire is located in the Far North, which contains 40% of Ontario's Aboriginal population and 106 of Ontario's 133 First Nations [2, 10]. Although the local economies will benefit from the development of the mining industry and supporting infrastructure, the area forms part of the largest peatland in the world and is a naturally saturated environment [2, 9]. Careful environmental monitoring of the chromium ore excavation and processing is required to prevent contamination of the environment and reduce the risk of chromium exposure.

The toxicological disparity between Cr(III) and Cr(VI) is due to differences in the stability, mobility, and bioavailability of the chromium species in the environment [2, 12-14].

Insoluble trivalent chromium is the most dominant natural species of chromium under most near-surface environmental conditions [2, 12]. However, hexavalent chromium is known to occur naturally in at least 24 minerals [2, 15]. In surface or ground water, Cr(III) can be oxidized in the ambient environment when it is in a soluble form [2]. Cr(III) hydroxides are insoluble over a wide range of pH values and are not typically oxidized [16]. Yet, Cr(VI) can be reduced by various organic compounds, microorganisms, and inorganic species (aqueous Fe(III), magnetite, green rust, and zero valent iron) [2, 17]. When this occurs, it is possible for some organic compounds to form a soluble complex with Cr(III) [2, 4]. This facilitates the formation of Cr(VI) by natural oxidation when MnO_2 is present [2, 4, 17]. Since the vapor pressure of all chromium compounds is negligible, Cr(VI) in the ambient atmosphere is only associated with particle matter and atmospheric water [2]. Therefore, atmospheric chromium interconversion are typically water-phase reactions [2]. Since it is possible for Cr(VI) to exist naturally in the environment, assessment of the site's Cr(VI) background levels is required for future and continuous environmental monitoring. Also, once site development begins, Cr(III) may be converted to mobile Cr(VI) through mining and processing activities [2, 11].

However, current chromium reference materials that are required for analytical laboratory testing contain levels of hexavalent chromium that are not suitable for background environmental monitoring and are at least three orders of magnitude too high for background assessment. For example, National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2701 contains hexavalent chromium at a certified value of 551.2 mg/kg ± 34.5 mg/kg (*NIST Certificate of Analysis, 2018*). Since NIST SRM 2701 is made from chromium ore processing residue (COPR) and contains total chromium at a concentration of 42,600 mg/kg ± 1,200 mg/kg (*NIST Certificate of Analysis, 2018*), it is an unsuitable standard for ambient level

Cr(VI) measurements. Also, NIST SRM 2700 provides hexavalent chromium in contaminated soil, which is an active matrix (*NIST Certificate of Analysis, 2019*). It is necessary to perform hexavalent chromium quantitation using methodology that is capable of correcting for chromium species interconversion, or risk introduction of analytical bias and error during sample analysis [18]. Therefore, a new and different hexavalent chromium standard reference material in a soil matrix is needed to perform ambient level Cr(VI) background assessment measurements. It is also needed to monitor the impact of mineral processing on the surrounding environment and remediation of chromium-containing waste materials that may be produced during the operations. Sigma-Aldrich has produced, homogenized, and bottled a new ambient-level Cr(VI) standard reference material in a soil matrix, which will be certified by this study.

3.2 MATERIALS AND METHODS

3.2.1 SAMPLES

Three different batches of the candidate standard reference material were received from Sigma-Aldrich: LRAA7318, LRAA7319, and LRAA7320. For each batch of material, multiple, individually-labeled amber glass bottles in sealed air-tight packaging were available for testing. Once opened, the bottles were stored at ambient conditions in a cleanroom environment.

3.2.2 ANALYTICAL STANDARDS

Potassium dichromate standard reference materials (SRM) 136e and 136f were purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland). Isotopically enriched trivalent chromium standard solution in 0.5% nitric acid [50-Cr(III)], isotopically enriched hexavalent chromium standard solution in 0.1% ammonium hydroxide [53-Cr(VI)], natural trivalent chromium standard solution in 0.5% nitric acid [Nat-Cr(III)], and natural hexavalent chromium standard solution in 0.1% ammonium hydroxide [Nat-Cr(VI)] were

generated at Duquesne University for Applied Isotope Technologies (AIT) (Pittsburgh, Pennsylvania). Concentrations of the chromium standards solutions are provided in Table 3.1. Details of the generation and certification of the AIT standards at Duquesne University are previously described in a chapter two. Elemental vanadium in 2% nitric acid was purchased from High Purity Standards (North Charleston, South Carolina). Instrument tuning standard solutions were purchased from Agilent Technologies (Santa Clara, California).

Table 3.1: Concentrations of chromium standard solutions.

Standard Solution	Batch Number	Lot Number	Concentration (µg/g)
50-Cr(III)	144980-01-100	CR02192019B	95.9915 ± 0.0986
50-Cr(III)	144980-01-10	CR03152019C	7.7075 ± 0.0709
53-Cr(VI)	177090-01-100	CR03152019B	100.7669 ± 0.0947
53-Cr(VI)	177090-01-10	CR03152019C	8.0248 ± 0.0317
Nat-Cr(III)	S08E051-200	CR04252019A	244.6492 ± 4.0711
Nat-Cr(III)	S08E051-100	CR04252019B	126.0422 ± 0.3232
Nat-Cr(III)	S08E051-10	CR04252019C	7.9987 ± 0.0149
Nat-Cr(VI)	136F-01-1000	CR03282019A	1296.8849 ± 13.3890
Nat-Cr(VI)	136F-01-100	CR03282019B	101.6977 ± 0.3253
Nat-Cr(VI)	136F-01-10	CR03282019C	8.7385.0593

3.2.3 REAGENTS AND MATERIALS

Concentrated nitric acid (trace metal grade) and concentrated hydrochloric acid (trace metal grade) were purchased from Fisher Chemical (ThermoFisher Scientific, Waltham, Massachusetts). Hydrogen peroxide 30-32% (Aristar Ultra) was purchased from VWR Chemicals BDH (VWR International, Radnor, Pennsylvania). Sodium hydroxide pellets (99.998% metal basis) was purchased from Sigma-Aldrich (Saint Louis, Missouri). Sodium carbonate, anhydrous (99.95%) and ethylenediaminetetraacetic acid, trisodium salt dihydrate (99%) were purchased from Acros Organics (ThermoFisher Scientific, Waltham, Massachusetts). Potassium dihydrogen phosphate (KH_2PO_4, certified ACS) was purchased from

Fisher Chemical (ThermoFisher Scientific, Waltham, Massachusetts). Dipotassium hydrogen phosphate (K_2HPO_4, certified ACS) was purchased from VWR Chemicals BDH (VWR International, Radnor, Pennsylvania). Magnesium chloride (certified ACS) was purchased from J. T. Baker (VWR International, Radnor, Pennsylvania). Type I ultrapure water (18.2 MΩ-cm) was produced using a Barnstead EASYpure II RF/UV filtration system (ThermoFisher Scientific, Waltham, Massachusetts) and/or Evoqua Water Technologies PURELAB Flex filtration system (Pittsburgh, Pennsylvania). Polypropylene (PP) centrifuge tubes with high-density polyethylene (HDPE) lids were purchased from Fisher Scientific (ThermoFisher Scientific, Waltham, Massachusetts), VWR International (Radnor, Pennsylvania), and Globe Scientific Inc. (Mahwah, New Jersey).

3.2.4 INSTRUMENTATION

Analytical standards, reagents, and samples were prepared in a cleanroom laboratory environment that continuously recirculated laboratory air through a high-efficiency particulate air (HEPA) filtration system. Laminar flow benchtops and isolated hoods fitted with additional HEPA filtration systems were also utilized for preparation of standards and samples with trace-level analytes. A Mettler Toledo XS105 Excellence (Columbus, Ohio) analytical balance was utilized with 0.01 mg precision. Samples were prepared using a Milestone ETHOS UP microwave digestion system (Sorisole, Bergamo, Italy) equipped with a MAXI-44 easy TEMP high-throughput rotor and modified polytetrafluoroethylene (PTFE-TFM) vessels of 100-mL capacity. A Mettler Toledo (Columbus, Ohio) SevenCompact pH/Ion meter S220 equipped with an InLab Expert Pro-ISM PH probe (PN 30014096) and InLab Redox ORP probe (PN 51343200) was utilized to measure the sample pH, temperature, and Eh values. An Agilent Technologies 7700x inductively coupled plasma mass spectrometer (ICP-MS) (Santa Clara,

California) was equipped with a micro-mist nebulizer, a quartz spray chamber, octopole reaction system (ORS^3), and a quadrupole mass analyzer. The instrument was autotuned prior to analysis using an instrument tuning standard solution from Agilent Technologies and automated startup sequence. When needed, the resulting parameters of autotune were modified to allow for custom instrument tuning. For direct sample introduction, spectrum mode of analysis (ICP-MS) was utilized with an ASX-520 autosampler (CETAC Automation, Omaha, Nebraska) that was contained within an anti-contamination enclosure. Time-resolved mode of analysis (IC-ICP-MS) was used for ion chromatography sample separations. A Metrohm 820 ion chromatography (IC) system (Herisau, Switzerland) was equipped with a Metrohm 858 Professional Sample Processor that was contained within an anti-contamination enclosure. The Metrohm ion chromatography system was metal free, with polyether ether ketone (PEEK) polymer material used for all connections, tubing, and column housing. The Metrohm 820 IC system was controlled using Metrohm IC Net 2.3, which was coupled to an independent Metrohm 850 Professional IC system running Metrohm MagicIC Net 3.1 to provide data communication and automation with the Agilent Technologies 7700x ICP-MS running MassHunter Workstation 4.2 software.

3.2.5 SAMPLE PREPARATION

3.2.5.1 Total Chromium Analysis

In order to determine the total chromium content of each batch of the Sigma-Aldrich candidate reference standard material, sample decomposition was needed to ensure complete digestion of the sample matrix and solubility of the chromium analyte. EPA Method 3052, Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices, was used to rapidly produce sample digests that were suitable for analysis by ICP-MS [19]. EPA Method 6800, Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry, was used to

quantitate the total elemental chromium concentrations of the digested samples [20]. The use of EPA Method 3052 as a sample preparation procedure ensured that the endogenous chromium isotopes of the sample were in equilibrium with those of the added isotopically enriched analytical chromium standard solutions. The final isotope ratios of the spiked sample digests were measured by ICP-MS according to EPA Method 6800 using IDMS calculations.

To prepare each sample, an aliquot from an individual bottle of candidate standard reference material was transferred into a quartz weigh bottle. Using weigh by difference, 0.5000 g of the sample was quantitatively transferred directly into a microwave digestion vessel. Using weigh by difference, the sample was then spiked by quantitatively adding 0.1000 g of 50-Cr(III) [95.9915 μg/g] into the microwave digestion vessel. Using a transfer pipet, 9.0 mL of concentrated nitric acid, 1.0 mL of concentrated hydrochloric acid, and 1.0 mL of hydrogen peroxide (30%) were added to the microwave vessel. A vented screw cap was used to securely tighten the lid onto the microwave vessel. The samples were shaken to ensure that the solid sample material was dispersed into the reagents. Quality control samples were prepared using 0.1000 g of Nat-Cr(VI) [8.7385 μg/g], 0.1000 g of 50-Cr(III) [95.9915 μg/g], and the digestion reagents. Mass bias samples were prepared using 0.1000 g of Nat-Cr(III) [126.0422 μg/g], 0.1000 g of Nat-Cr(VI) [101.6977 μg/g], and the digestion reagents. To prepare an analytical blank, 0.1000 g of 50-Cr(III) [95.9915 μg/g] was transferred into a microwave digestion vessel. Once 9.0 mL of concentrated nitric acid, 1.0 mL of concentrated hydrochloric acid, and 1.0 mL of hydrogen peroxide (30%) were transferred into a tarred quartz weigh bottle and massed, the reagents were transferred into the microwave digestion vessel. The mass of the empty weigh bottle was then recorded. The microwave vessels were loaded into MAXI-44 easy TEMP high-throughput rotor, placed into the Milestone ETHOS UP microwave digestion system, and

processed at 180°C for 9.5 minutes with a 5.5-minute ramp at 1800 watts. Once the samples cooled to ambient temperature, each microwave vessel was individually opened in a fume hood, and the digested sample was transferred into a labeled polypropylene 15-mL centrifuge tube and capped. The samples were held overnight at ambient temperature. The samples were centrifuged for 30 minutes at 3300 rpm, or until the solid and liquid are well separated. For each sample, 2.0 mL of the supernatant was transferred into a labelled polypropylene 50-mL centrifuge tube, brought to 20 mL with 18.2 MΩ-cm water, capped, and inverted ten times to mix. The diluted solutions were analyzed by ICP-MS using EPA Method 6800.

3.2.5.2 Speciated Hexavalent Chromium Analysis

To determine the speciated hexavalent chromium content of each batch of the Sigma-Aldrich candidate reference standard material, it is necessary to extract Cr(VI) from the matrix material and account for any chromium species interconversion that may occur during sample processing. Without appropriate methodology, experimentally determined concentrations of Cr(III) and Cr(VI) may differ from the actual concentrations of the species in the indigenous sample since oxidation and reduction of chromium may be promoted by the laboratory reagents and measurement techniques. For determination of Cr(VI) in soils, laboratory certification programs require the use of EPA Method 3060A, Alkaline Digestion for Hexavalent Chromium [21, 22]. This method utilizes a hot alkaline digestion solution to quantitatively extract Cr(VI) from soluble, adsorbed or precipitated forms of chromium compounds, while minimizing the interconversion of the chromium species [21, 22]. The high pH extraction solution contains sodium hydroxide and sodium carbonate, which supports the extraction of Cr(VI) as a soluble chromate anion (CrO_4^{2-}) and precipitation of Cr(III) as a solid chromium hydroxide ($Cr(OH)_3$) [23]. The use of ethylenediaminetetraacetic acid (EDTA) as an additional extracting solution

allows EDTA complexing with Cr(III) and prevents oxidation of Cr(III) compounds to Cr(VI) [23]. Soluble forms of Cr(III) will complex as the anion $Cr(EDTA)^-$ [23]. Also, EDTA complexes with other metals that may be present in the sample matrix to form insoluble chromates [23]. Alone, EPA Method 3060A is not capable of correcting for oxidation of Cr(III) and/or reduction of Cr(VI), however the use of speciated isotope dilution mass spectrometry (SIDMS) provides for this correction. EPA Method 6800, Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry, was used to quantitate the speciated hexavalent chromium concentrations of the digested samples [20]. By chromatographically separating the Cr(III) peak as $Cr(EDTA)^-$ and the Cr(VI) peak as CrO_4^{2-}, the final isotope ratios of the spiked sample digests were measured by IC-ICP-MS. The concentration Cr(VI) in the indigenous sample was quantitated according to EPA Method 6800 using SIDMS calculations.

In addition to quantifying the concentrations of Cr(VI) in the EPA Method 3060A extracts for each of the samples, residues that remained after the extraction process were retained and tested for total chromium content according to EPA Method 3052. Microwave assisted acid digestion of the solid residues, which contained Cr(III) precipitates, allowed for quantitation of any remaining chromium with ICP-MS according to EPA Method 6800 using IDMS calculations. Using this approach, mass balance was achieved by summing the quantitative results from the speciated analysis (Cr(VI)) and the quantitative results from acid digestion of the extraction residues (Cr(III)). The summed chromium content of each sample was compared to the values obtained during a total chromium analysis previously determined by IDMS.

To prepare each sample, an aliquot from an individual bottle of candidate standard reference material was transferred into a quartz weigh bottle. Using weigh by difference, 0.5000 g of the sample was quantitatively transferred directly into a microwave digestion vessel. Using

weigh by difference, the sample was then spiked by quantitatively adding 0.1000 g of 50-Cr(III) [95.9915 μg/g] and 0.1000 g of 53-Cr(VI) [100.7669 μg/g] into the microwave digestion vessel. Using a transfer pipet, 12 mL of digestion solution (0.28 M Na_2CO_3 /0.5 M NaOH at pH 11.5 or greater) and 120 μL of phosphate buffer (0.5 M K_2HPO_4/0.5 M KH_2PO_4 at pH 7) was added to the microwave digestion vessel. Approximately 0.0240 g of Mg^{2+} (0.0960 g of anhydrous $MgCl_2$) was added to the sample. A vented screw cap was used to securely tighten the lid onto the microwave vessel. The samples were shaken to ensure that the solid sample material was dispersed into the reagents. Quality control samples were prepared using 0.1000 g of Nat-Cr(VI) [8.7385 μg/g], 0.1000 g of 50-Cr(III) [95.9915 μg/g], 0.1000 g of 53-Cr(VI) [100.7669 μg/g], and the digestion reagents. Mass bias samples were prepared using 0.1000 g of Nat-Cr(III) [126.0422 μg/g], 0.1000 g of Nat-Cr(VI) [101.6977 μg/g], and the digestion reagents. To prepare an analytical blank, 0.1000 g of 50-Cr(III) [95.9915 μg/g] and 0.1000 g of 53-Cr(VI) [100.7669 μg/g] were transferred into a microwave digestion vessel.

Once 12 mL of digestion solution, 120 μL of phosphate buffer, and 0.0240 g of Mg^{2+} were transferred into a tarred quartz weigh bottle and massed, the reagents were transferred into the microwave digestion vessel. The mass of the empty weigh bottle was then recorded. The microwave vessels were loaded into MAXI-44 easy TEMP high-throughput rotor, placed into the Milestone ETHOS UP microwave digestion system, and processed for one hour at 95°C with a 10-minute ramp at 1200 watts. Once the samples cooled to ambient temperature, each microwave vessel was individually opened in a fume hood, and the digested sample was transferred into a labeled polypropylene 15-mL centrifuge tube and capped. The samples were held overnight at ambient temperature. The emptied microwave digestion vessels were reserved for later use during mass balance analysis. The samples were centrifuged for 30 minutes at 3300 rpm, or until the

solid and liquid wee well separated. The supernatants were completely transferred into clean microwave digestion vessels. The solid residues in the emptied 15-mL centrifuge tubes were retained for further mass balance analysis. Using a transfer pipet, 10 mL of 50 mM EDTA was added into each microwave digestion vessel. Vented screw caps were used to securely tighten the lids onto the microwave vessels, which were loaded into MAXI-44 easy TEMP high-throughput rotor and placed into the Milestone ETHOS UP microwave digestion system. The samples were processed for 20 minutes at 95°C with a 10-minute ramp at 1200 watts with feedback temperature control. Once the samples cooled to ambient temperature, each microwave vessel was individually opened in a fume hood, and the sample extract was transferred into a labeled polypropylene 50-mL centrifuge tube and capped. For each sample, 2.0 mL of the supernatant was transferred into a labelled polypropylene 50-mL centrifuge tube, brought to 40 mL with 18.2 MΩ-cm water, capped, and inverted ten times to mix. The diluted solutions were analyzed by IC-ICP-MS using EPA Method 6800.

Mass balance analysis was performed by analyzing the EPA Method 3060A residues according to EPA Method 3052 and EPA Method 6800 using ICP-MS with IDMS calculations. For each 15 mL centrifuge tube containing sample residue, 4.5 mL of concentrated nitric acid was added into the tube and capped. In order to disperse the residue pellet in the nitric acid, the tube was vortexed for 10 seconds, inverted several times, and vortexed for an additional 10 seconds. The resulting sample was transferred into the corresponding, previously reserved, microwave digestion vessel. A second aliquot of 4.5 mL concentrated nitric acid was added into the tube, which was capped and inverted several times to allow for the transfer of any remaining residue into the microwave vessel. Using a transfer pipet, 1.0 mL of concentrated hydrochloric acid, and 1.0 mL of hydrogen peroxide (30%) were added to the microwave vessel. Vented

screw caps were used to securely tighten the lids onto the microwave vessels, which were loaded into MAXI-44 easy TEMP high-throughput rotor and placed into the Milestone ETHOS UP microwave digestion system. Once the samples were processed at 180°C for 9.5 minutes with a 5.5-minute ramp at 1800 watts, they were allowed to cool to ambient temperature. Each microwave vessel was individually opened in a fume hood, and the digested sample was transferred into a labeled polypropylene 15-mL centrifuge tube and capped. The samples were held overnight at ambient temperature. The samples were centrifuged for 30 minutes at 3300 rpm, or until the solid and liquid are well separated. For each sample, 2.0 mL of the supernatant was transferred into a labelled polypropylene 50-mL centrifuge tube, brought to 20 mL with 18.2 MΩ-cm water, capped, and inverted ten times to mix. The diluted solutions were analyzed by ICP-MS using EPA Method 6800.

3.2.6 INSTRUMENT METHODS

3.2.6.1 Initial Instrumentation Setup

The samples for total chromium analysis were placed into an enclosed autosampler for direct sample introduction. The Agilent Technologies 7700x ICP-MS was set to spectrum mode of analysis (ICP-MS) and tuned with an automated startup sequence using an instrument tuning standard solution from Agilent Technologies. Table 3.2 provides tune settings that resulted from a typical autotune routine, which were used as the initial instrument parameters for total chromium analysis. For speciated chromium analysis, samples were placed into the enclosed autosampler for ion chromatography separation. The Metrohm 820 ion chromatography (IC) system was equipped with a set of Metrohm Metrosep A Supp 5 PEEK analytical and guard columns. Additionally, comparison experiments were performed to examine the use of Metrohm Metrosep A Supp 17 PEEK analytical and guard columns. The Metrosep A Supp 5 column

contain polyvinyl alcohol with quaternary ammonium groups as the stationary phase. The Metrosep A Supp 17 column contain a polystyrene/divinylbenzene copolymer with quaternary ammonium groups as the stationary phase. An isocratic flow of a 2 mM EDTA solution at ambient temperature is used as the mobile phase for these columns and provides an anion exchange chromatographic separation mechanism. Table 3.3 provides details about the chromatographic system setup, including additional information about the column and mobile phase eluent. The Agilent Technologies 7700x ICP-MS was set to time-resolved mode of analysis (IC-ICP-MS) and tuned with an automated startup sequence using an instrument tuning standard solution from Agilent Technologies. Table 3.4 provides tune settings that resulted from a typical autotune routine, which were used for the initial instrument parameters for speciated chromium analysis. Additional method development experiments were performed to determine optimal instrument configuration and tune parameters, which are described in the next section.

Table 3.2: Agilent Technologies 7700x ICP-MS autotune settings for total chromium analysis by EPA Method 3052 and EPA Method 6800.

RF power	1500 W	*Typical Autotune Parameters:*	
RF matching	1.80 V	Extract 1	-125.0 V
Sampling depth	8.0 mm	Extract 2	-195.0 V
Carrier gas (Ar) flow	0.95 L min^{-1}	Omega bias	-85 V
Dilution gas (Ar) flow	0.15 L min^{-1}	Omega lens	4.4 V
ORS^3 gas (He) flow	5.0 mL min^{-1}	OctP bias	-18.0 V
Spray chamber temperature	2 °C	OctP RF	200 V
Data acquisition mode	Spectrum	Energy discrimination	4.0 V
Isotope monitored	^{50}Cr, ^{52}Cr, ^{53}Cr, ^{54}Cr		
Peak pattern	20 points/mass		
Replicates	5		
Sweeps/replicate	1000		
Integration time/mass	2 seconds		
Nebulizer pump	0.10 rps		
Sample uptake	60 seconds		
Stabilization	30 seconds		

Table 3.3: Metrohm 820 Ion Chromatography Separation Center settings for speciated chromium analysis by EPA Method 3060A and EPA Method 6800.

Column	Metrosep A Supp 5 PEEK column (Metrohm) containing polyvinyl alcohol with quaternary ammonium groups, 250 x 4.0 mm, 5 µm particle size, pH range 3 to 12; with Metrosep A Supp 5 guard column (5 x 4.0 mm, 5 µm particle size) Metrosep A Supp 17 PEEK column (Metrohm) containing polystyrene/ divinylbenzene copolymer with quaternary ammonium groups, 250 x 4.0 mm, 5 µm particle size, pH range 3 to 12; with Metrosep A Supp 17 guard column (5 x 4.0 mm, 5 µm particle size)
Mobile Phase	2 mmol L^{-1} EDTA in ultrapure water, pH 10 adjusted using ammonium hydroxide
Elution Mode	Isocratic
Flow Rate	0.8 mL min^{-1}
Column Temperature	Ambient
Injection Volume	100 µL

Table 3.4: Agilent Technologies 7700x ICP-MS autotune settings for speciated chromium analysis by EPA Method 3060A and EPA Method 6800.

		Typical Autotune Parameters:	
RF power	1500 W		
RF matching	1.80 V	Extract 1	-125.0 V
Sampling depth	8.0 mm	Extract 2	-195.0 V
Carrier gas (Ar) flow	0.95 L min^{-1}	Omega bias	-85 V
Dilution gas (Ar) flow	0.15 L min^{-1}	Omega lens	4.4 V
ORS^3 gas (He) flow	5.0 mL min^{-1}	OctP bias	-18.0 V
Spray chamber temperature	2 °C	OctP RF	200 V
Data acquisition mode	Time resolved analysis	Energy discrimination	4.0 V
Isotope monitored	^{50}Cr, ^{52}Cr, ^{53}Cr, ^{54}Cr		
Integration time/mass	0.25 seconds		
Sampling period	1.006 sec		
Nebulizer pump	0.50 rps		

3.2.6.2 Method Development

Analysis by ICP-MS is associated with interferences caused by atomic or molecular ions that have the same mass to charge ratio as the analyte [24]. In some cases, current software is capable of correcting for atomic isobaric interferences that occur when isotopes from two different elements have overlapping masses [24]. Yet, polyatomic interferences are ions that have the same mass as the analyte isotopes, but are generated by precursors from the sample matrix, reagents, plasma gases, and atmospheric gases [24]. Polyatomic interference from carbon, chlorine, nitrogen, and sulfur can interfere with the detection of the natural isotopes of

chromium, ^{50}Cr (4.3%), ^{52}Cr (83.8%), ^{53}Cr (9.6%), and ^{54}Cr (2.4%) [5, 24, 25]. For example, $^{40}Ar^{12}C^{+}$, $^{38}Ar^{14}N^{+}$, $^{36}Ar^{16}O^{+}$ and $^{36}Ar^{15}N^{1}H^{+}$ can interfere with the detection of the most abundant chromium isotope ($^{52}Cr^{+}$) [5, 24]. Furthermore, detection of $^{53}Cr^{+}$ can be disturbed by $^{36}Ar^{17}O^{+}$ or $^{40}Ar^{13}C^{+}$, and $^{50}Cr^{+}$ may have interferences from $^{36}Ar^{14}N^{+}$ [5, 24]. Several strategies have been proposed to reduce these inferences, including adding oxygen or air into the nebulizer gas, using cool plasma conditions, and increasing spectral resolution to distinguish chromium isotopes from those of the interference [25, 26]. However, for ICP-quadrupole MS, the use of a helium collision gas in an enclosed cell immediately before the quadrupole is one of the most popular methods for reducing polyatomic inferences [26]. Due to their larger size, the polyatomic species collide with the helium collision gas at a greater rate and loose more kinetic energy than the monatomic analyte ions of chromium [26, 27]. A voltage differential between the collision cell and the quadrupole mass analyzer provides kinetic energy discrimination (KED) of the interfering polyatomic ions [27]. Analyte ions are also affected by this process; however, it is to a lesser extent than the polyatomic ions, and the reduction of interferences results in a higher signal to noise ratio for the analyte [26]. For this work, helium was introduced as a collision gas into the octopole collision cell (third generation octopole reaction system, ORS^{3}) of the Agilent Technologies 7700x ICP-MS.

An experiment was performed to determine which helium collision cell gas flow rates provide optimal reduction of polyatomic interference for chromium analysis. A 2 mM EDTA solution was prepared for this experiment since the EDTA molecule provides a source of interfering carbon, nitrogen, and oxygen atoms. A similar solution is used as the mobile phase eluent for the chromatographic method, and a reduction of the resulting polyatomic inferences will also reduce baseline background counts and improve the signal to noise ratio for the

chromium analytes. No indigenous chromium was added to the EDTA solution. After setting the ICP-MS instrument tune parameters to those prescribed for total chromium analysis (Table 3.2), the collision cell gas flow rate was stepwise ramped from 0.0 mL/min to 10.0 mL/min for each subsequent direct sample injection of the EDTA solution. The total ion count per second was monitored for the m/z values of 50, 52, and 53. The experimental ion count corresponds to polyatomic interferences formed from the interaction of EDTA with the ICP plasma (e.g. $^{40}Ar^{12}C^{+}$). The results of this experiment are presented Figure 3.1, which indicate that the helium flow rate is optimized at 5.0 mL/min or higher since the interference ion counts for all chromium isotopes approach zero. However, it is important to note that an excessive flow rate of collision cell gas significantly impacts method analyte sensitivity during routine sample analysis.

To further examine the impact of the ICP-MS method tune parameters on analyte sensitivity and polyatomic interferences, an additional experiment was performed to determine optimal voltages for the ICP-MS lenses. A high purity vanadium standard was diluted with 1% ethanol (in 18.2 MΩ-cm water) to produce seven standard solutions ranging from 10 ppt to 1000 ppt. The mass of vanadium (m/z 51) was monitored as a surrogate analyte for chromium. The value of m/z 52 was monitored for generation of $^{40}Ar^{12}C^{+}$ and $^{36}Ar^{16}O^{+}$ polyatomic interferences from the dilute ethanol in each solution. The goal of this experiment was to reduce the amount of m/z 52 signal (carbon/oxygen polyatomic interferences) and to maximize the amount of m/z 51 signal (surrogate analyte). Thus, ICP tuning conditions that support the lowest ratio of polyatomic interferences and analyte ion counts (interference : analyte ratio) provide an optimized instrumentation method for chromium analysis. First, the standard solution at approximately 950 ppt was directly sampled after setting the ICP-MS instrument tune parameters to those prescribed for total chromium analysis in Table 3.5, which includes the use of an

increased collision cell gas flow rate. The ion count per second for m/z 51 and m/z 52 were monitoring in real-time using the Agilent MassHunter ICP software. The following tuning parameters were modulated to determine the impact of these adjustments on the m/z 51 and m/z 52 ion counts: carrier gas flow rate, gas switch configuration, dilution/makeup gas flow rate, extraction lens voltages, omega bias deflector voltage, omega collimator lens voltage, and octopole bias voltage. The adjustment of each of these tune parameters enhances or reduces the efficiency of ion transfer through the mass spectrometer. The signals were monitored until it was possible to reduce the m/z 52 signal and maximize the m/z 51 signal. The resulting optimized "chromium" tune mode is provided in Table 3.6 and is used for further chromium analysis and experiments. Next, each of the previously prepared vanadium standard solutions was analyzed using the initial and optimized ICP-MS tune modes (Table 3.5 and Table 3.6). The results of these experiments are illustrated in Figure 3.2, which indicate that the optimized ICP-MS tune mode reduces the ratio of polyatomic interferences by approximately half when compared to the initial tune parameters. As such, the optimized tune mode will help to ensure maximum analyte signal to noise ratio, even with high carbon matrix material or reagent solutions.

Table 3.5: Agilent Technologies 7700x ICP-MS initial settings for the tune parameters optimization experiment.

RF power	1500 W	*Tune Parameters:*	
RF matching	1.80 V	Extract 1	-125.0 V
Sampling depth	8.0 mm	Extract 2	-195.0 V
Carrier gas (Ar) flow	0.95 L min^{-1}	Omega bias	-85 V
Dilution gas (Ar) flow	0.15 L min^{-1}	Omega lens	4.4 V
ORS^3 gas (He) flow	6.0 mL min^{-1}	OctP bias	-18.0 V
Spray chamber temperature	2 °C	OctP RF	200 V
Data acquisition mode	Spectrum	Energy discrimination	4.0 V
Isotope monitored	^{50}Cr, ^{52}Cr, ^{53}Cr, ^{54}Cr		
Peak pattern	20 points/mass		
Replicates	5		
Sweeps/replicate	1000		
Integration time/mass	2 seconds		
Nebulizer pump	0.10 rps		
Sample uptake	60 seconds		
Stabilization	30 seconds		

Table 3.6: Agilent Technologies 7700x ICP-MS resulting optimized tune parameters for chromium analysis.

RF power	1500 W	*Optimized Parameters:*	
RF matching	1.80 V	Extract 1	-75.0 V
Sampling depth	8.0 mm	Extract 2	-125.0 V
Carrier gas (Ar) flow	0.82 L min^{-1}	Omega bias	-78 V
Makeup gas (Ar) flow	0.40 L min^{-1}	Omega lens	7.2 V
ORS^3 gas (He) flow	6.0 mL min^{-1}	OctP bias	-18.0 V
Spray chamber temperature	2 °C	OctP RF	200 V
Data acquisition mode	Spectrum or Time resolved	Energy discrimination	4.0 V
Isotope monitored	^{50}Cr, ^{52}Cr, ^{53}Cr, ^{54}Cr		
Peak pattern	20 points/mass		
Replicates	5		
Sweeps/replicate	1000		
Integration time/mass	2 seconds		
Nebulizer pump	0.10 rps/0.50 rps		
Sample uptake/Stabilization	60/30 seconds		

Figure 3.1: Reduction of polyatomic interferences with various collision cell gas flow rates. A 2 mM EDTA solution (without chromium) was directly injected as a sample for ICP-MS analysis. The ion count per second (CPS) were monitored for m/z values of 50, 52, and 53 as the helium collision cell flow rate was stepwise ramped for each subsequent sample injection. Interfering ion counts approach zero at helium flow rates greater than 5.0 mL/min for the m/z values corresponding to the major isotopes of chromium.

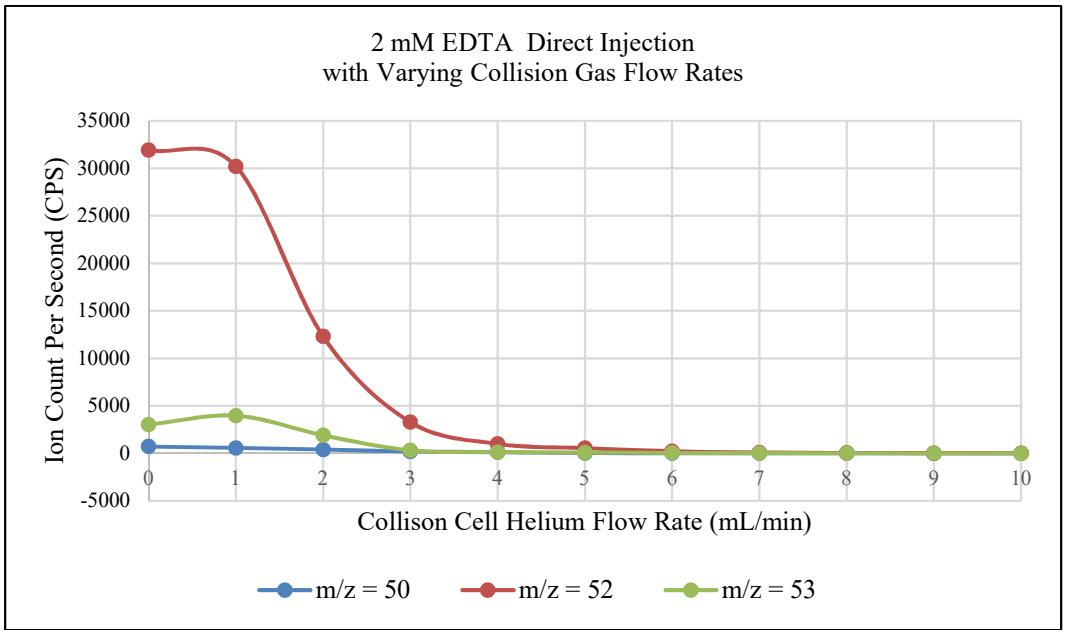
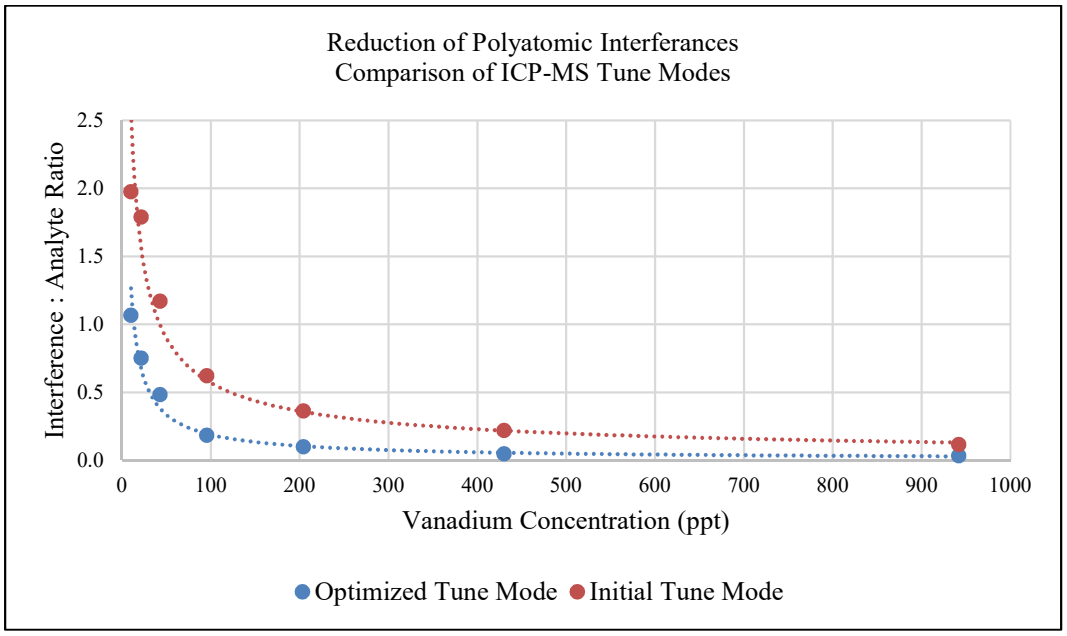


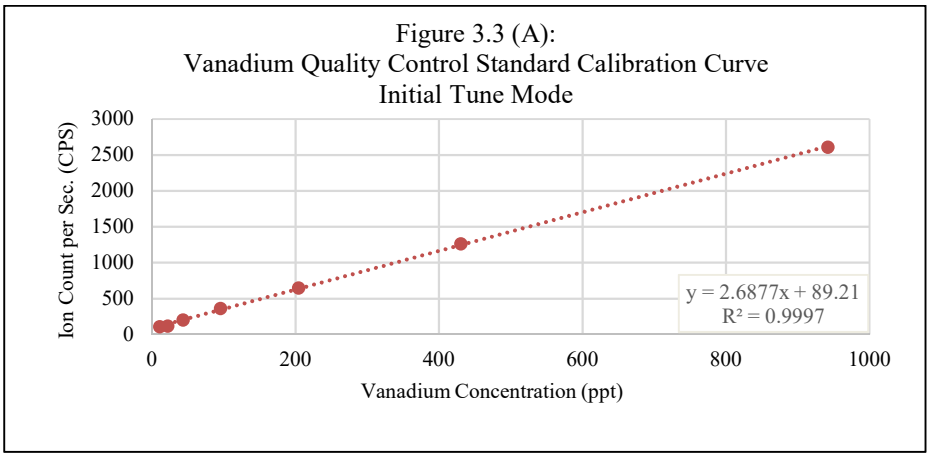
Figure 3.2: Reduction of polyatomic interferences with optimized ICP-MS tune mode parameters. High purity vanadium standards were prepared (1% ethanol in 18.2 MΩ-cm water) and directly injected as samples for ICP-MS analysis. The mass of vanadium (m/z 51) was monitored as a surrogate analyte for chromium. The value of m/z 52 was monitored for generation polyatomic interferences from the dilute ethanol in each solution. ICP tuning conditions that support the lowest ratio of polyatomic interferences and analyte ion counts (interference : analyte ratio) provide an optimized instrumentation method for chromium analysis.



To examine the impact of the optimized tune mode on analyte recovery and method sensitivity, quality control standard solutions were prepared in triplicate with a targeted vanadium concentration of approximately 150 ppt (1% ethanol in 18.2 MΩ-cm water). The seven previously prepared vanadium standards (10 ppt to 1000 ppt) and the quality control standards (150 ppt) were directly analyzed by using both the original and optimized ICP-MS tune modes. For each tune mode, a calibration curve was generated using the recoveries from the standard solutions (Figure 3.3A and Figure 3.3B). Both tune modes produced linear calibration curves with R^2 values of 0.9997. For each tune mode, the recoveries of the quality control standards where calculated from the resulting linear equations. Table 3.7 provides the calculated concentrations with 95% confidence intervals (n = 15) and the percent difference of the calculated concentrations from the theoretical concentrations. The optimized ICP-MS tune method provided approximately a 3% increase in accuracy when compared to the initial tune

method. Therefore, the optimized "chromium" tune mode (Table 3.6) was used for further chromium analysis and experiments.

Figure 3.3 (A and B): Standards solutions with vanadium concentrations ranging from 10 ppt to 1000 ppt were used to generated calibration curves using both the initial and optimized ICP-MS tune mode parameters. Both calibration curves indicate linearity with R^2 values of 0.9997. The resulting linear equations were used to calculate quality control standard recoveries.



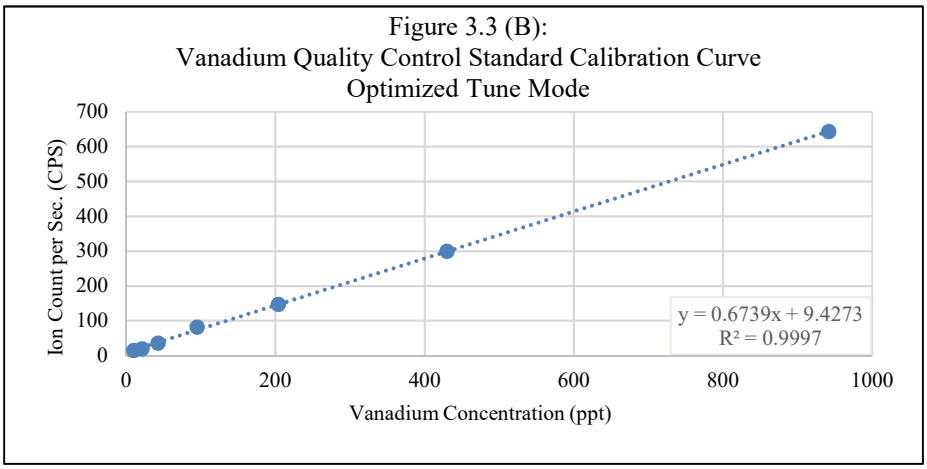


Table 3.7: Comparison of vanadium (m/z = 51) quality control standard recoveries using the initial and optimized ICP-MS tune mode parameters and calibration curve standards. The average concentrations (ppt) are reported with 95% confidence intervals (n = 15).

	Tune Parameters	
	Initial	**Optimized**
Average Recovery Concentration (ppt)	121.10 ± 4.70	125.40 ± 2.22
Average Percent Difference from Theoretical (%)	-18.58%	-15.63%

3.2.7 METHOD VALIDATION

Method validation was performed for the quantitation of total chromium by ICP-MS (sample preparation outlined in section 3.2.5.1) and speciated hexavalent chromium by IC-ICP-MS (sample preparation outlined in section 3.2.5.2) using the optimized instrument parameters provided in Table 3.3 and Table 3.6. For both methods, the following method validation parameters were evaluated: accuracy, precision, linearity, limit of detection (LOD), and limit of quantitation (LOQ). Method validation for speciated hexavalent chromium includes selectivity and specificity through analysis of the chromatographic peak separation.

3.2.7.1 Total Chromium Analysis

To perform method validation for total chromium analysis, NIST 136e Potassium Dichromate Standard Reference Material was used to prepare five standard solutions with total chromium theoretical concentrations at 3.6 µg/g, 15.3 µg/g, 74.0 µg/g, 297.6 µg/g, and 1389.4 µg/g in 18.2 MΩ-cm water (0.1% ammonium hydroxide). EPA Method 3052 (Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices) was utilized to prepare the standard solutions. EPA Method 6800 (Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry) was used to quantitate the total elemental chromium concentrations of the digested validation standard solutions according to IDMS calculations. The prepared standards were analyzed using optimized ICP-MS tune mode parameters.

Percent recoveries of the standard solutions support validation of the method accuracy and precision, which are provided in Table 3.8. The percent recovery of each standard solution is calculated using the following formula:

$$\text{Percent Recovery} = \frac{(\text{Experimental Concentration})}{(\text{Theoretical Concentration})} \times 100$$

The method validation standard recoveries range from 87.2% to 104.0% and indicate that the method has greater than ± 13% accuracy for this concentration range. The calculated percent difference in recoveries are shown in Figure 3.4, which were calculated according to the following equation:

$$\text{Percent Difference Recovery} = \frac{(\text{Experimental Concentration - Theoretical Concentration})}{(\text{Theoretical Concentration})} \times 100$$

The percent difference in recoveries provide an additional indicator of method accuracy and range from -3.8% to +11.7%. Method precision is evaluated using the resulting 95% CI (n = 12) values and percent relative standard deviation (%RSD) for the standard solutions. The method precision ranges from 0.297% to 0.962% relative standard deviation. Although traditional calibration curve quantitation is not utilized for EPA Method 6800 methodology, an assessment of method linearity was performed as part of the method validation. The linearity validation provides a statistical check of the homogeneity of the variances for a wide range of analyte concentration levels.

After generating a scatterplot that correlates the calculated experimentally determined concentration and theoretical concentration of each standard solution, a linear regression equation was generated for the data set with a reported R^2 value. Since the correlation coefficient was close to 1 (0.9999), it indicates that the method is linear throughout the validation concentration range. The results of the linearity method validation are provided in Figure 3.5. Limit of detection (LOD) is the lowest possible concentration that can be measured reliably. Typically, this value is statically calculated as the mean blank values plus three times the standard deviation of the blank samples [28-31]. Using the same approach, the limit of quantitation is statistically calculated as the mean blank values plus ten times the standard deviation of the blank samples [28-31]. The results of the statistical determination of both the

LOD and LOQ for this method are summarized in Table 3.9. The LOD was statistically determined to be 0.0017 µg/g and the LOQ was statistically determined to be 0.0031 µg/g. However, the LOQ was empirically measured during the accuracy method validation at 3.6 µg/g.

Table 3.8: Accuracy and precision method validation results for total chromium analysis. Five validation standard solutions were prepared using NIST Standard Reference Material 136e with total chromium theoretical concentrations ranging from approximately 3 µg/g to 1300 µg/g. EPA Method 3052 was utilized to prepare the standard solutions. EPA Method 6800 was used to quantitate the total elemental chromium concentrations of the digested validation standard solutions. The prepared standards were analyzed using optimized ICP-MS tune mode parameters. The percent recoveries of each standard solution are provided to support validation of the method accuracy. The resulting 95% CI (n = 12) and %RSD values for the standard solutions are provided to support validation of the method precision.

Accuracy and Precision Method Validation Total IDMS Chromium Analysis EPA Method 3052, Acid Digestion (n = 12, 95% CI)				
Theoretical Total Cr	IDMS Results			Percent Recovery
	Total Cr	95% CI	Percent RSD	
µg/g	*µg/g*	*µg/g*	*%*	*%*
1389.4	1210.8	4.532	0.513	87.2
297.56	273.82	0.561	0.297	92.0
74.018	67.431	0.198	0.420	91.1
15.280	15.886	0.056	0.581	104.0
3.582	3.696	0.022	0.962	103.2

Figure 3.4: Percent difference recovery method validation results for total chromium analysis. Five validation standard solutions were prepared using NIST Standard Reference Material 136e with total chromium theoretical concentrations ranging from 3 µg/g to 1300 µg/g. EPA Method 3052 was utilized to prepare the standard solutions. EPA Method 6800 was used to quantitate the total elemental chromium concentrations of the digested validation standard solutions. The prepared standards were analyzed using optimized ICP-MS tune mode parameters. The calculated percent difference in recoveries are shown, which indicate method accuracy.

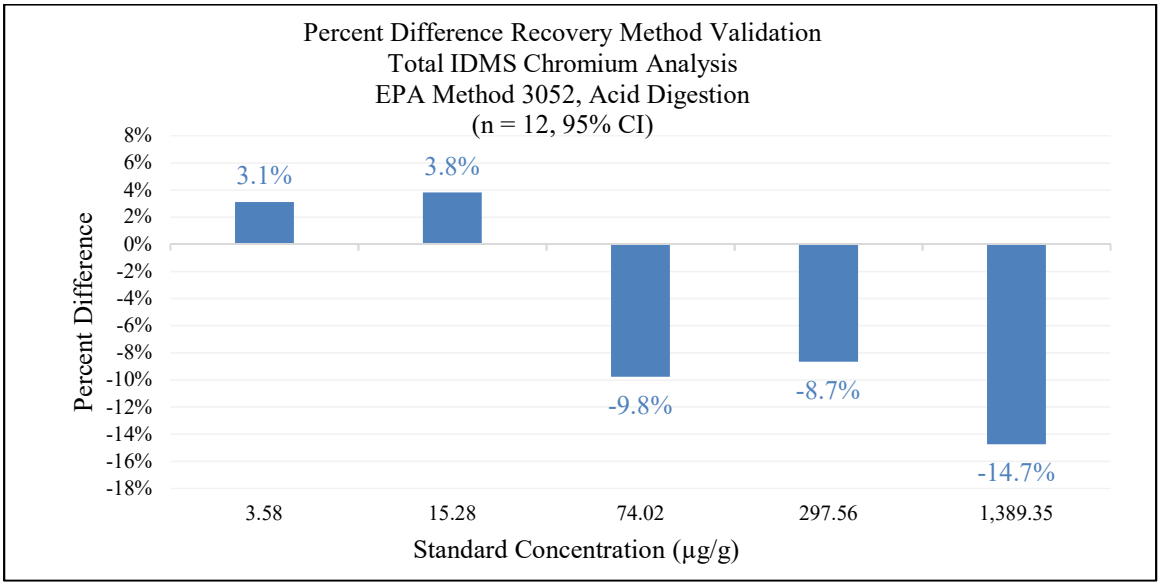


Figure 3.5: Linearity method validation results for total chromium analysis. Five validation standard solutions were prepared using NIST Standard Reference Material 136e with total chromium theoretical concentrations ranging from 3 µg/g to 1300 µg/g. EPA Method 3052 was utilized to prepare the standard solutions. EPA Method 6800 was used to quantitate the total elemental chromium concentrations of the digested validation standard solutions. The prepared standards were analyzed using optimized ICP-MS tune mode parameters. Linearity is shown with the R^2 value of 0.9999. The 95% CI (n = 12) error bars are not shown since they are not significant in this figure.

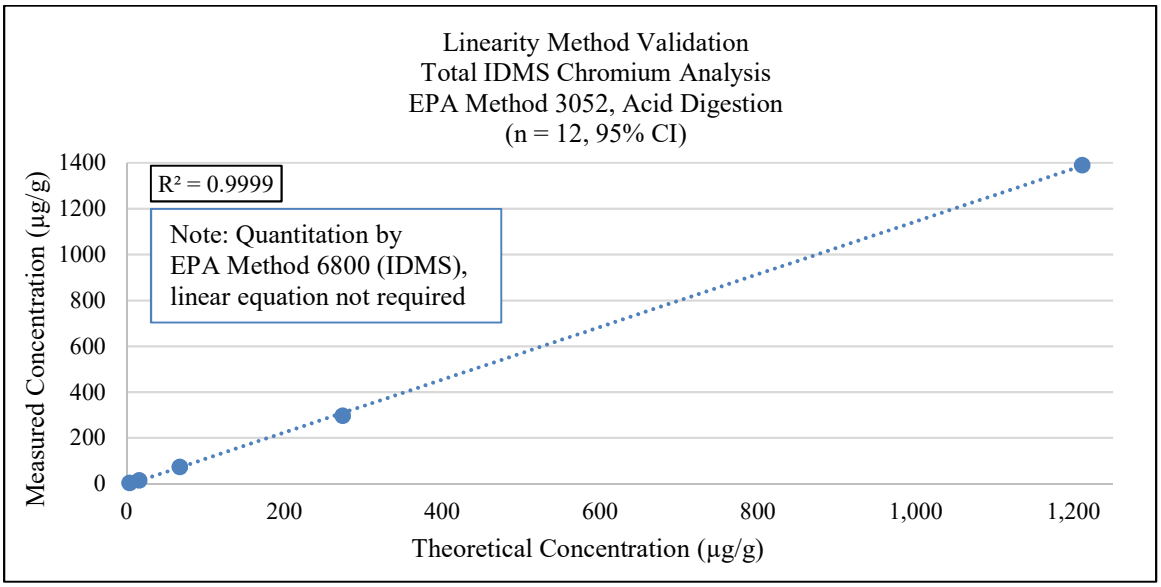


Table 3.9: Statistically determined limit of detection (LOD) and limit of quantitation (LOQ) method validation results for total chromium analysis. Blank solutions were prepared without chromium analyte and processed according to EPA Method 3052. EPA Method 6800 was used to quantitate the total elemental chromium concentrations of the digested blank solutions. The prepared solutions were analyzed using optimized ICP-MS tune mode parameters. The LOD and LOQ concentrations were statistically derived from the standard deviation (SD) of the blank mean (n = 15). The LOQ was empirically measured during the accuracy method validation at 3.6 µg/g.

Blank Determinations Total Chromium (n = 15)			Limit of Detection	Limit of Quantitation
Average	SD	95% CI	mean + 3(SD)	mean + 10(SD)
µg/g	µg/g	µg/g	µg/g	µg/g
0.0011	0.0002	0.0001	0.0017	0.0031

3.2.7.2 Speciated Hexavalent Chromium Analysis

To perform method validation for speciated chromium analysis, NIST 136e Potassium Dichromate Standard Reference Material was used to prepare six standard solutions with hexavalent chromium theoretical concentrations at 0.3113 µg/g, 0.6219 µg/g, 1.2415 µg/g, 2.4799 µg/g, 4.9409 µg/g, and 10.133 µg/g in 18.2 MΩ-cm water (0.1% ammonium hydroxide). To prepare the standard solutions, EPA Method 3060A was utilized to digest the standard

solutions and followed by extraction with a 50 mM EDTA. EPA Method 6800 (Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry) was used to quantitate the speciated hexavalent chromium concentrations of the digested validation standard solutions according to SIDMS calculations. In order to validate the method for selectivity and specificity, the separation of Cr(III) and Cr(VI) was examined by sampling standard solutions that contained Nat-Cr(III) at 244.6492 µg/g and Nat-Cr(VI) at 8.7386 µg/g. The Nat-Cr(III) concentration was much higher than the Nat-Cr(VI) concentration since formation of an insoluble Cr(III) complex is favored with the EPA Method 3060A digestion. The resulting sample was not spiked with isotope standards; however, it was processed according to EPA Method 3060A with a 50 mM EDTA extraction. The prepared standards and specificity sample were analyzed using optimized IC-ICP-MS tune mode parameters and a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase.

The selectivity and specificity of the method for Cr(III) and Cr(VI) was validated using a natural chromium solution. An example chromatogram is provided in Figure 3.6, which indicates the complete separation of the $[Cr(III)EDTA]^-$ and $[Cr(VI)O_4]^{2-}$ species. The three major isotopes of chromium (50-Cr, 52-Cr, 53-Cr) are shown and correspond to the expected isotopic distribution of a natural chromium sample. The retention time for Cr(III) was found to be approximately 3.25 minutes, and the retention time for Cr(VI) was found to be approximately 4.30 minutes. The small baseline fluctuation at approximately 1.15 minutes corresponds to an increase in system pressure from the sample injection. In Table 3.10, the percent recoveries of the standard solutions support validation of the method accuracy and precision. The percent recovery of each standard solution is calculated using the following formula:

$$\text{Percent Recovery} = \frac{(\text{Experimental Concentration})}{(\text{Theoretical Concentration})} \times 100$$

The method validation standard recoveries range from 89.3% to 104.3% and indicate that the method has greater than ± 11% accuracy for this concentration range. The calculated percent difference in recoveries are shown in Figure 3.7, which were calculated according to the following equation:

$$\text{Percent Difference Recovery} = \frac{(\text{Experimental Concentration - Theoretical Concentration})}{(\text{Theoretical Concentration})} \times 100$$

The percent difference in recoveries provide an additional indicator of method accuracy and range from -10.7% to +2.6%. Method precision is evaluated using the resulting 95% CI (n = 12) values and percent relative standard deviation (%RSD) for the standard solutions. The method precision ranges from 0.974% to 8.604% relative standard deviation. Although traditional calibration curve quantitation is not utilized for EPA Method 6800 methodology, an assessment of method linearity was performed as part of the method validation. After generating a scatterplot that correlates the calculated experimentally determined concentration and theoretical concentration of each standard solution, a linear regression equation was generated for the data set with a reported R^2 value. Since the correlation coefficient was close to 1 (0.9999), it indicates that the method is linear throughout the validation concentration range. The results of the linearity method validation are provided in Figure 3.8. Limit of detection (LOD) is the lowest possible concentration that can be measured reliably. The results of the statistical determination of both the LOD and LOQ for this method are summarized in Table 3.11. The LOD was statistically determined to be 0.0122 µg/g, and the LOQ was statistically determined to be 0.0262 µg/g. However, the LOQ was empirically measured during the accuracy method validation at 0.3113 µg/g.

Figure 3.6: Method validation results for selectivity and specificity of the speciated hexavalent chromium analysis. A validation standard solution was prepared using solutions that contained Nat-Cr(III) at 244.6492 µg/g and Nat-Cr(VI) at 8.7386 µg/g. EPA Method 3060A was utilized to digest the standard solutions, followed by extraction with a 50 mM EDTA solution. The prepared standards were analyzed using optimized IC-ICP-MS tune mode parameters and a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase. The resulting chromatogram indicates complete separation of the $[Cr(III)EDTA]^-$ and $[Cr(VI)O_4]^{2-}$ species.

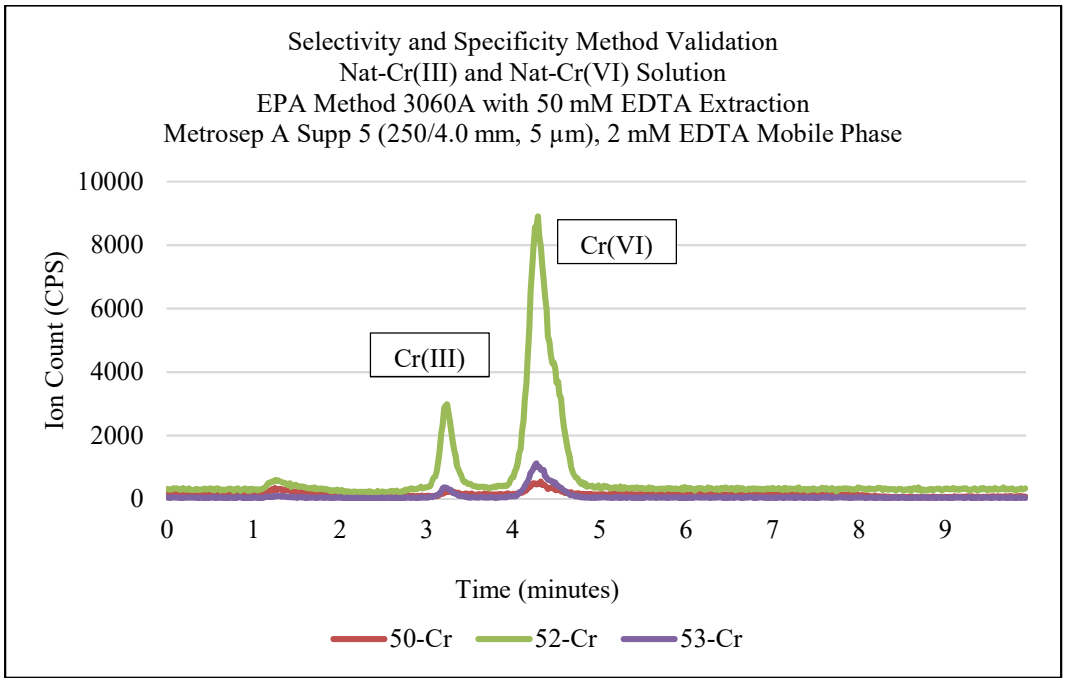


Table 3.10: Accuracy and precision method validation results for speciated hexavalent chromium analysis. Six validation standard solutions were prepared using NIST Standard Reference Material 136e in 0.1% ammonium hydroxide with hexavalent chromium theoretical concentrations ranging from approximately 0.3 µg/g to 10 µg/g. EPA Method 3060A was utilized to digest the standard solutions, followed by extraction with a 50 mM EDTA solution. EPA Method 6800 was used to quantitate the speciated hexavalent chromium concentrations of the digested validation standard solutions. The prepared standards were analyzed using optimized IC-ICP-MS tune mode parameters. The percent recoveries of each standard solution are provided to support validation of the method accuracy. The resulting 95% CI (n = 12) and %RSD values for the standard solutions are provided to support validation of the method precision.

Accuracy and Precision Method Validation Speciated SIDMS Hexavalent Chromium Analysis EPA Method 3060A with 50 mM EDTA, Alkaline Extraction (n = 12, 95% CI)				
Theoretical Cr(VI)	SIDMS Results			Percent Recovery
	Speciated Cr	95% CI	Percent RSD	
µg/g	*µg/g*	*µg/g*	*%*	*%*
10.133	10.395	0.091	1.581	102.6
4.9409	5.1512	0.028	0.974	104.3
2.4799	2.5010	0.027	1.949	100.8
1.2415	1.2409	0.059	8.604	100.0
0.6219	0.6012	0.021	6.330	96.7
0.3113	0.2782	0.016	8.101	89.3

Figure 3.7: Percent difference recovery method validation results for speciated hexavalent chromium analysis. Six validation standard solutions were prepared using NIST Standard Reference Material 136e in 0.1% ammonium hydroxide with hexavalent chromium theoretical concentrations ranging from approximately 0.3 µg/g to 10 µg/g. EPA Method 3060A was utilized to digest the standard solutions, followed by extraction with a 50 mM EDTA solution. EPA Method 6800 was used to quantitate the speciated hexavalent chromium concentrations of the digested validation standard solutions. The prepared standards were analyzed using optimized IC-ICP-MS tune mode parameters. The calculated percent difference in recoveries are shown, which indicate method accuracy.

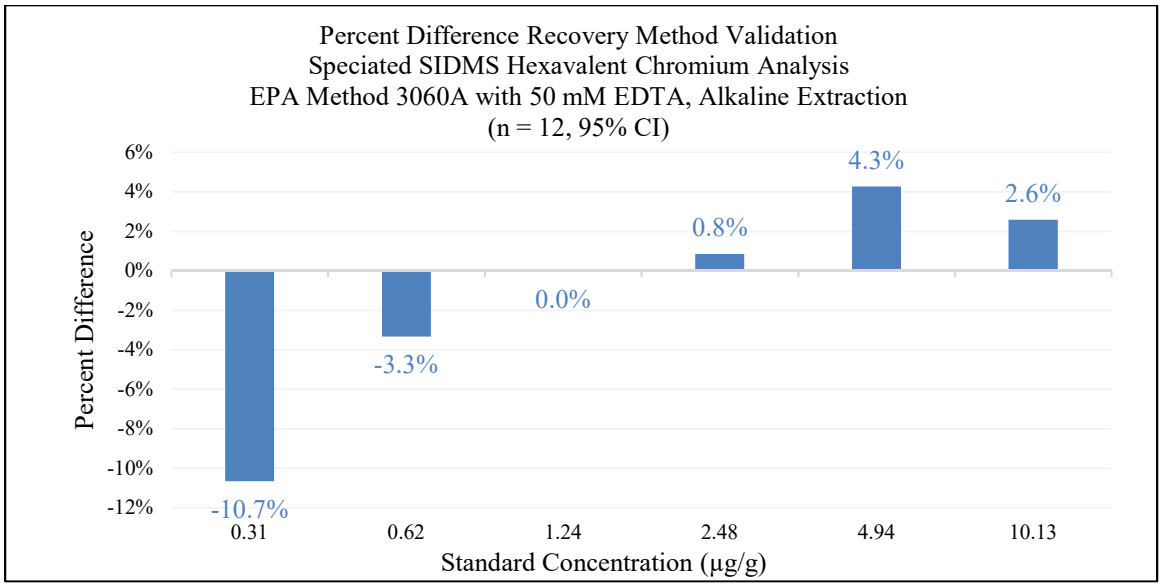


Figure 3.8: Linearly method validation results for speciated hexavalent chromium analysis. Six validation standard solutions were prepared using NIST Standard Reference Material 136e in 0.1% ammonium hydroxide with hexavalent chromium theoretical concentrations ranging from approximately 0.3 µg/g to 10 µg/g. EPA Method 3060A was utilized to digest the standard solutions, followed by extraction with a 50 mM EDTA solution. EPA Method 6800 was used to quantitate the speciated hexavalent chromium concentrations of the digested validation standard solutions. The standards were analyzed using optimized IC-ICP-MS tune mode parameters. Linearity is shown with the R^2 value of 0.9999. The 95% CI (n = 12) error bars are not shown since they are not significant in this figure.

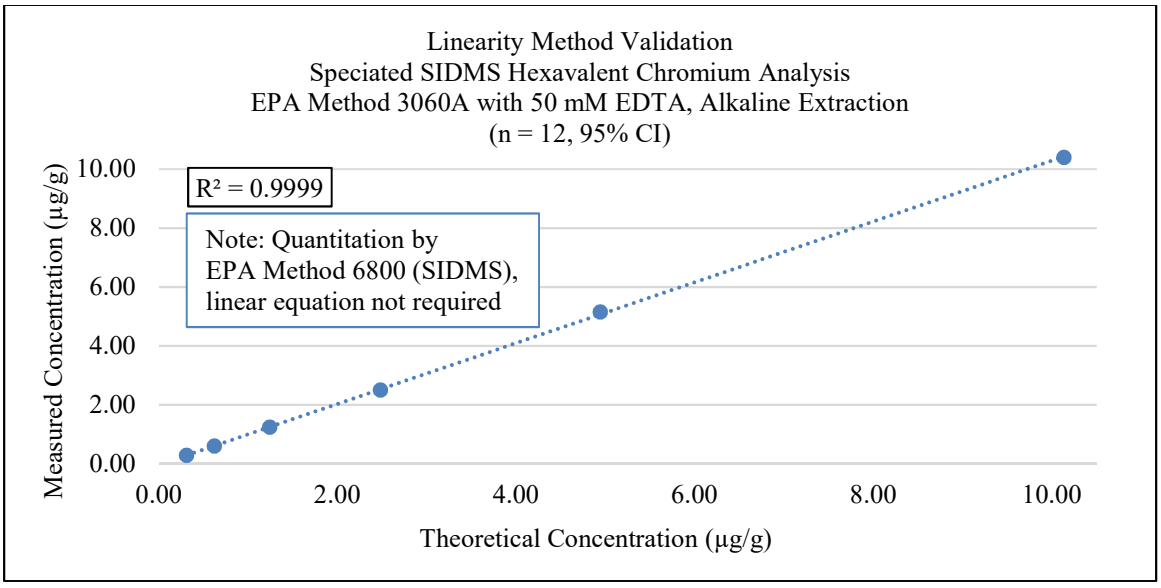


Table 3.11: Statistically determined limit of detection (LOD) and limit of quantitation (LOQ) method validation results for speciated hexavalent chromium analysis. Blank solutions were prepared without chromium analyte and processed according to EPA Method 3060A with 50 mM EDTA extraction. EPA Method 6800 was used to quantitate the speciated chromium concentrations of the digested blank solutions. The prepared solutions were analyzed using optimized IC-ICP-MS tune mode parameters. The LOD and LOQ concentrations were statistically derived from the standard deviation (SD) of the blank mean (n = 15). The LOQ was empirically measured during the accuracy method validation at 0.3113 µg/g.

Blank Determinations Hexavalent Chromium (n = 15)			Limit of Detection	Limit of Quantitation
Average	SD	95% CI	mean + 3(SD)	mean + 10(SD)
µg/g	µg/g	µg/g	µg/g	µg/g
0.0062	0.0020	0.0011	0.0122	0.0262

3.3 Results and Discussion

Method validation experiments performed with chromium standard solutions prepared from NIST SRM 136e indicate that the optimized methods developed for total chromium and speciated hexavalent chromium analysis are accurate and precise. Also, the validation indicates chromium quantitation by EPA Method 6800 (IDMS and SIDMS) provides a linear fit when the resulting calculated concentrations are compared to the corresponding theoretical concentrations of the validation standard solutions. The validated limit of quantitation provides confidence that the lowest concentrations of chromium are quantitated with accuracy. Method validation work for the speciated chromium analytical method shows specificity and selectivity for both the Cr(III) and Cr(VI) species. As such, the methods were determined to be suitable to use for certification of the new Sigma-Aldrich low-level hexavalent chromium standard reference material. Multiple individually-labeled bottles were subsampled for batches LRAA7318, LRAA7319, and LRAA7320, and tested for total chromium and hexavalent chromium content.

Each total chromium and speciated hexavalent chromium analysis included assessment of system suitability and quality control standards. A mass bias standard solution was prepared for each analysis using both Nat-Cr(III) [126.0422 µg/g] and Nat-Cr(VI) [101.6977 µg/g], and was

analyzed at the beginning and end of each sample set in replicate injections. The data was used to determine and mathematically correct method and/or instrument bias that resulted in a deviation from the theoretical isotope fraction distribution of natural chromium. This mathematical correction was applied to the data before performing EPA Method 6800 concentration calculations. Also, multiple replicate preparations of the reagent blank were analyzed for each sample set and the resulting calculated chromium concentrations subtracted from the determined sample concentrations. The analytical blank concentrations were routinely found to be less than 10 ppb and below the empirically validated limit of quantitation. Finally, replicate quality control standard solutions were prepared using the natural chromium primary standards outlined in Table 3.1. The quality control standards were assessed for each sample set, with acceptable and valid sample set recoveries within ±15% of the theoretical values. Data was collected using Agilent Technologies MassHunter Workstation software and exported to Microsoft Excel for further processing and statistical workup.

3.3.1 TOTAL CHROMIUM ANALYSIS

The quantitation of total chromium in the three batches of the Sigma-Aldrich low-level hexavalent chromium standard reference material (LRAA7318, LRAA7319, LRAA7320) was performed according to EPA Method 3052 and EPA Method 6800 by ICP-MS (sample preparation outlined in section 3.2.5.1) using the optimized instrument parameters provided in Table 3.6. For each batch of material, four independent bottles of material (B1, B2, B3, and B4) were obtained. An aliquot from each individual bottle of candidate standard reference material was transferred into a quartz weigh bottle, which was then subsampled four times and analyzed with five replicate measurements (n = 20). The results of the total chromium analysis are summarized in Table 3.12 (LRAA7318), Table 3.13 (LRAA7319), and Table 3.14 (LRAA7320).

The tables provide the mean chromium concentrations (μg/g) with their corresponding standard deviations (SD), percent relative standard deviations (%RSD, SD as a percent of mean), 95% confidence interval (95% CI), and number of replicate measurements (n).

LRAA7318 was found to have an average total chromium concentration of 28.0586 ± 0.4314 μg/g with a %RSD value of less than 10%. The mean values of the individual bottles of LRAA7318 range from 27.1500 μg/g to 29.2348 μg/g with %RSD values of less than 10%. A graphical representation of the data is provided in Figure 3.9, which indicates the average total chromium concentrations with 95% CI error bars. The blue shaded region of the chart provides the 95% CI range of the average batch measurements.

Similarly, LRAA7319 was found to have an average total chromium concentration of 21.8959 ± 0.3465 μg/g with a %RSD value of less than 10%. The mean values of the individual bottles of LRAA7319 range from 20.8533 μg/g to 22.4954 μg/g with %RSD values of less than 10%. A graphical representation of the data is provided in Figure 3.10, which indicates the average total chromium concentrations with 95% CI error bars. The blue shaded region of the chart provides the 95% CI range of the average batch measurements.

Finally, LRAA7320 was found to have an average total chromium concentration of 21.4216 ± 0.2428 μg/g with a %RSD value of less than 10%. The mean values of the individual bottles of LRAA7320 range from 20.8440 μg/g to 21.6640 μg/g with %RSD values of less than 10%. A graphical representation of the data is provided in Figure 3.11, which indicates the average total chromium concentrations with 95% CI error bars. The blue shaded region of the chart provides the 95% CI range of the average batch measurements.

Intra-batch homogeneity of total chromium concentration was demonstrated by the overlap of the majority of the individual bottle 95% CI concentration ranges with the

corresponding overall mean 95% CI concentration ranges. Notably, the 95% CI for LRAA7318 Bottle 2 and LRAA7319 Bottle 4 did not overlap with the overall mean 95% CI for the respective batch. Further statistical analysis was performed by a two sample t-test assuming equal variances to generate two-tail p-values, with comparison to an alpha value of 0.05 (95% CI). When the chromium replicate concentrations of LRAA7318 Bottle 2 were compared to all other LRAA7318 total chromium replicate values, a p-value of 0.00134 was found, which is less than the alpha value of 0.05. This indicates that Bottle 2 is statistically different than LRAA7318 Bottles 1, 3, and 4. Likewise, when the chromium replicate concentrations of LRAA7319 Bottle 4 were compared to all other LRAA7319 total chromium replicate values, a p-value of 0.00036 was found, which is less than the alpha value of 0.05. This indicates that Bottle 4 is statistically different than LRAA7319 Bottles 1, 2, and 3. To examine inter-batch homogeneity, a statistical two sample t-test assuming equal variances was used to generate two-tail p-values for comparison of the batch bottle means, with comparison to an alpha value of 0.05 (95% CI). The mean for LRAA7318 was statistically different than the mean for LRAA7319 ($p = 4.73 \times 10^{-5}$) and LRAA7320 ($p = 1.29 \times 10^{-5}$). However, the means for LRAA7319 and LRAA7320 were not statistically different ($p = 0.29$).

Table 3.12: Total chromium analysis for candidate standard reference material LRAA7318. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with ICP-MS according to EPA Method 6800 (IDMS) with five replicate measurements for each sample (n = 20).

Total Chromium Analysis					
Sample Name	Average µg/g	SD µg/g	Percent RSD %	95% CI µg/g	Number of Samples (n)
LRAA7318-B1	27.1500	0.4378	1.6127	0.2049	20
LRAA7318-B2	29.2348	1.4579	4.9869	0.6823	20
LRAA7318-B3	27.4575	2.4371	8.8757	1.1406	20
LRAA7318-B4	28.3923	2.1314	7.5069	0.9975	20
Average	28.0586	1.9383	6.9082	0.4314	80

Figure 3.9: Total chromium analysis for candidate standard reference material LRAA7318. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with ICP-MS according to EPA Method 6800 (IDMS) with five replicate measurements for each sample (n = 20). The blue shaded region of the chart provides the 95% CI range of the average measurements.

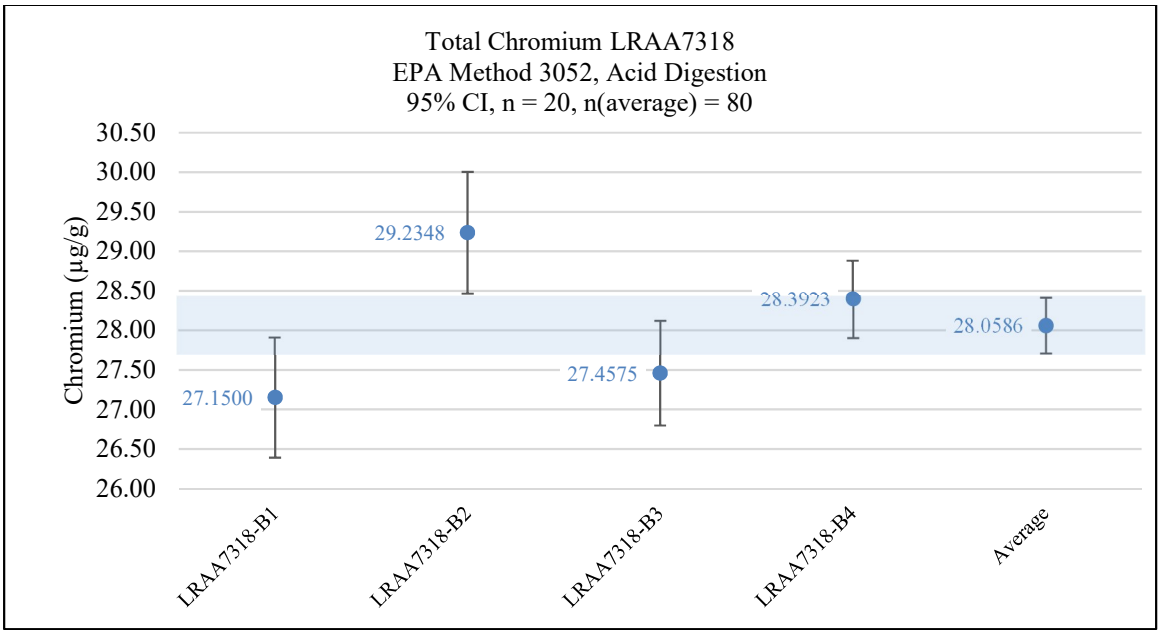


Table 3.13: Total chromium analysis for candidate standard reference material LRAA7319. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with ICP-MS according to EPA Method 6800 (IDMS) with five replicate measurements for each sample (n = 20).

Total Chromium Analysis					
Sample Name	Average µg/g	SD µg/g	Percent RSD %	95% CI µg/g	Number of Samples (n)
LRAA7319-B1	22.2144	1.6256	7.3178	0.7608	20
LRAA7319-B2	22.0204	1.6441	7.4664	0.7695	20
LRAA7319-B3	22.4954	1.4122	6.2777	0.6609	20
LRAA7319-B4	20.8533	1.0449	5.0107	0.4890	20
Average	21.8959	1.5568	7.1102	0.3465	80

Figure 3.10: Total chromium analysis for candidate standard reference material LRAA7319. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with ICP-MS according to EPA Method 6800 (IDMS) with five replicate measurements for each sample (n = 20). The blue shaded region of the chart provides the 95% CI range of the average measurements.

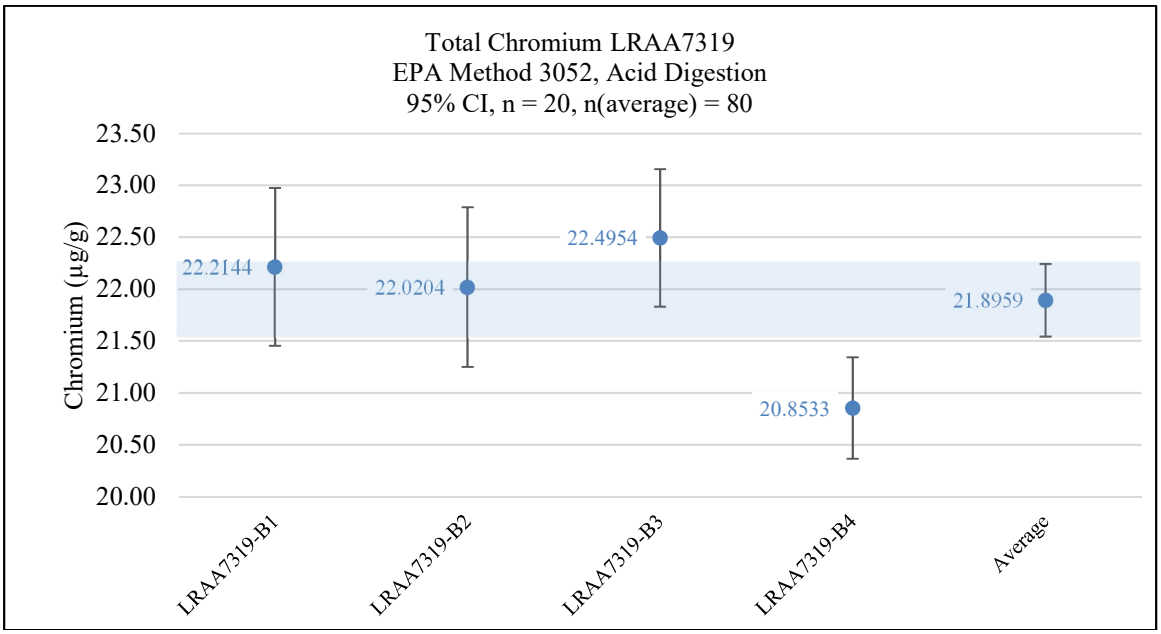
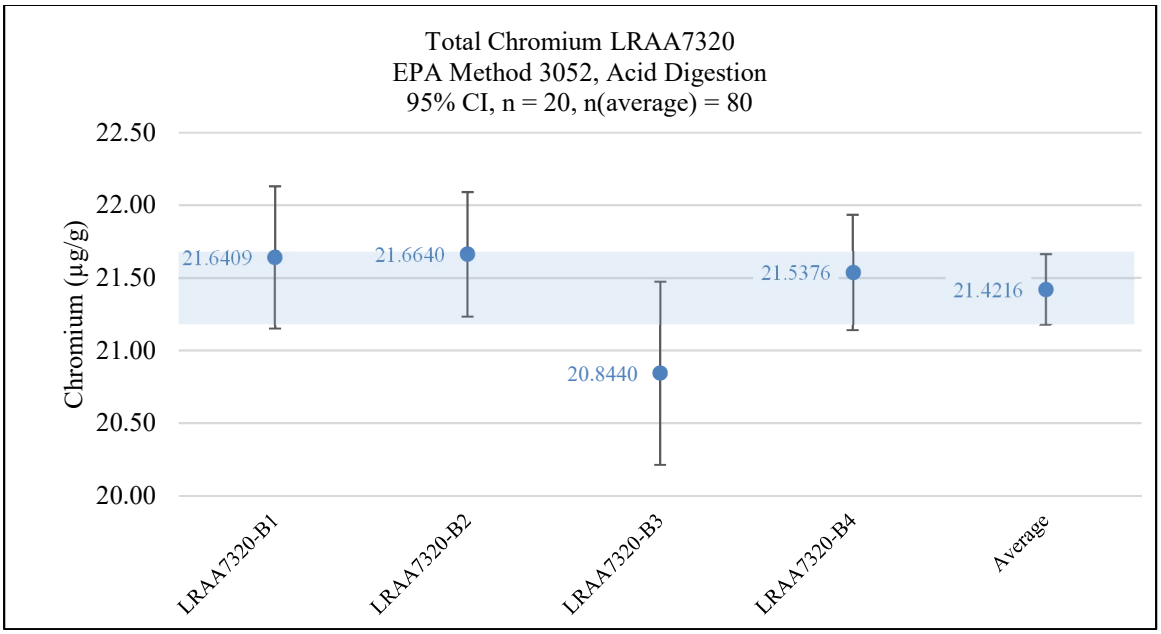


Table 3.14: Total chromium analysis for candidate standard reference material LRAA7320. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with ICP-MS according to EPA Method 6800 (IDMS) with five replicate measurements for each sample (n = 20).

Total Chromium Analysis					
Sample Name	Average µg/g	SD µg/g	Percent RSD %	95% CI µg/g	Number of Samples (n)
LRAA7320-B1	21.6409	1.0474	4.8399	0.4902	20
LRAA7320-B2	21.6640	0.9129	4.2139	0.4273	20
LRAA7320-B3	20.8440	1.3501	6.4771	0.6319	20
LRAA7320-B4	21.5376	0.8486	3.9403	0.3972	20
Average	21.4216	1.0912	5.0941	0.2428	80

Figure 3.11: Total chromium analysis for candidate standard reference material LRAA7320. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with ICP-MS according to EPA Method 6800 (IDMS) with five replicate measurements for each sample (n = 20). The blue shaded region of the chart provides the 95% CI range of the average measurements.



3.3.2 SPECIATED HEXAVALENT CHROMIUM ANALYSIS

The quantitation of speciated hexavalent chromium in the three batches of the Sigma-Aldrich low-level hexavalent chromium standard reference material (LRAA7318, LRAA7319, LRAA7320) was performed according to EPA Method 3060A with 50 mM EDTA and EPA Method 6800 by IC-ICP-MS (sample preparation outlined in section 3.2.5.2) using the optimized instrument parameters provided in Table 3.3 and Table 3.6. Two ion chromatography columns were compared: (1) Metrosep A Supp 5 PEEK column (Metrohm) containing polyvinyl alcohol with quaternary ammonium groups, 250 x 4.0 mm, 5 μm particle size, and pH range 3 to 12, with Metrosep A Supp 5 guard column (5 x 4.0 mm, 5 μm particle size); and (2) Metrosep A Supp 17 PEEK column (Metrohm) containing polystyrene/ divinylbenzene copolymer with quaternary ammonium groups, 250 x 4.0 mm, 5 μm particle size, and pH range 3 to 12, with Metrosep A Supp 17 guard column (5 x 4.0 mm, 5 μm particle size).

For each batch of material, four independent bottles of material (B1, B2, B3, and B4) were obtained. An aliquot from each individual bottle of candidate standard reference material was transferred into a quartz weigh bottle, which was then subsampled four times and analyzed with five replicate measurements (n = 20). The results of the speciated hexavalent chromium analysis are summarized in Table 3.15 and Table 3.16 (LRAA7318), Table 3.17 and Table 3.18 (LRAA7319), and Table 3.19 and Table 3.20 (LRAA7320). The tables provide the mean hexavalent chromium concentrations (μg/g) with their corresponding standard deviations (SD), percent relative standard deviations (%RSD, SD as a percent of mean), 95% confidence interval (95% CI), and number of replicate measurements (n).

Using the Metrosep A Supp 5 column, LRAA7318 was found to have an average hexavalent chromium concentration of 3.9009 ± 0.1373 μg/g with a %RSD value of less than

20%. The mean values of the individual bottles of LRAA7318 range from 3.5775 µg/g to 4.0386 µg/g with %RSD values of less than approximately 20%. A graphical representation of the data is provided in Figure 3.12, which indicates the average hexavalent chromium concentrations with 95% CI error bars. The blue shaded region of the chart provides the 95% CI range of the average batch measurements using the Metrosep A Supp 5 column. Similarly, using the Metrosep A Supp 17 column, LRAA7318 was found to have an average hexavalent chromium concentration of 3.7926 ± 0.1233 µg/g with a %RSD value of less than 20%. The mean values of the individual bottles of LRAA7318 range from 3.7284 µg/g to 3.9560 µg/g with %RSD values of less than 20%. A graphical representation of the data is provided in Figure 3.12, which indicates the average hexavalent chromium concentrations with 95% CI error bars. The orange shaded region of the chart provides the 95% CI range of the average batch measurements using the Metrosep A Supp 17 column.

Using the Metrosep A Supp 5 column, LRAA7319 was found to have an average hexavalent chromium concentration of 0.5059 ± 0.0198 µg/g with a %RSD value of less than 20%. The mean values of the individual bottles of LRAA7319 range from 0.4770 µg/g to 0.5369 µg/g with %RSD values of less than approximately 20%. A graphical representation of the data is provided in Figure 3.13, which indicates the average hexavalent chromium concentrations with 95% CI error bars. The blue shaded region of the chart provides the 95% CI range of the average batch measurements using the Metrosep A Supp 5 column. Similarly, using the Metrosep A Supp 17 column, LRAA7319 was found to have an average hexavalent chromium concentration of 0.5013 ± 0.0182 µg/g with a %RSD value of less than 20%. The mean values of the individual bottles of LRAA7319 range from 0.4728 µg/g to 0.5369 µg/g with %RSD values of approximately less than 20%. A graphical representation of the data is provided in Figure 3.13,

which indicates the average hexavalent chromium concentrations with 95% CI error bars. The orange shaded region of the chart provides the 95% CI range of the average batch measurements using the Metrosep A Supp 17 column.

Using the Metrosep A Supp 5 column, LRAA7320 was found to have an average hexavalent chromium concentration of 0.4981 ± 0.0201 µg/g with a %RSD value of less than 20%. The mean values of the individual bottles of LRAA7320 range from 0.4573 µg/g to 0.5532 µg/g with %RSD values of less than approximately 20%. A graphical representation of the data is provided in Figure 3.14, which indicates the average hexavalent chromium concentrations with 95% CI error bars. The blue shaded region of the chart provides the 95% CI range of the average batch measurements using the Metrosep A Supp 5 column. Similarly, using the Metrosep A Supp 17 column, LRAA7320 was found to have an average hexavalent chromium concentration of 0.5197 ± 0.0168 µg/g with a %RSD value of less than 20%. The mean values of the individual bottles of LRAA7320 range from 0.4899 µg/g to 0.5552 µg/g with %RSD values of less than 20%. A graphical representation of the data is provided in Figure 3.14, which indicates the average hexavalent chromium concentrations with 95% CI error bars. The orange shaded region of the chart provides the 95% CI range of the average batch measurements using the Metrosep A Supp 17 column.

Intra-batch homogeneity of speciated hexavalent chromium concentration was demonstrated by the overlap of the individual bottle 95% CI concentration ranges with the corresponding overall mean 95% CI concentration ranges. To examine intra-batch comparison between the Metrosep A Supp 5 and Metrosep A Supp 17 columns, a statistical two sample t-test assuming equal variances was used to generate two-tail p-values for comparison of the batch bottle means, by comparison to an alpha value of 0.05 (95% CI). The means generated using

each column were not statistically different for LRAA7318 ($p = 0.40$), LRAA7319 ($p = 0.87$), and LRAA7320 ($p = 0.44$). Therefore, the data generated from both columns are statistically comparable. To examine inter-batch homogeneity, a statistical two sample t-test assuming equal variances was used to generate two-tail p-values for comparison of the batch bottle means, with comparison to an alpha value of 0.05 (95% CI). For the Metrosep A Supp 5 column, the mean for LRAA7318 was statistically different than the mean for LRAA7319 ($p = 7.60 \times 10^{-8}$) and LRAA7320 ($p = 7.96 \times 10^{-8}$). However, the means for LRAA7319 and LRAA7320 were not statistically different ($p = 0.76$). Likewise, for the Metrosep A Supp 17 column, the mean for LRAA7318 was statistically different than the mean for LRAA7319 ($p = 1.94 \times 10^{-9}$) and LRAA7320 ($p = 1.95 \times 10^{-9}$). However, the means for LRAA7319 and LRAA7320 were not statistically different ($p = 0.48$).

Example chromatograms are provided in Figure 3.15 for speciated hexavalent chromium analysis according to EPA Method 3060A with 50 mM EDTA by IC-ICP-MS using the Metrosep A Supp 5 PEEK column. The three major isotopes of chromium (50-Cr, 52-Cr, 53-Cr) are shown and reflect the addition of the isotopically enriched standard solutions to each sample. The retention time for Cr(III) was found to be approximately 2.97 minutes, and the retention time for Cr(VI) was found to be approximately 3.97 minutes. The small baseline fluctuation at approximately 1.22 minutes corresponds to an increase in system pressure from the sample injection. Figure 3.16 provides example chromatograms for speciated hexavalent chromium analysis according to EPA Method 3060A with 50 mM EDTA by IC-ICP-MS using the Metrosep A Supp 17 column. The retention time for Cr(III) was approximately 2.83 minutes, Cr(VI) at approximately 5.32 minutes, and a system peak at approximately 1.11 minutes. Using this method of analysis, the resulting sample chromatograms routinely lacked a Cr(III) peak that

provided ion counts for the three major isotopes of chromium above a 10:1 signal to noise ratio. This is due to the fact that EPA Method 3060A with 50 mM EDTA alkaline digestion supports the extraction of Cr(VI) as a soluble chromate anion (CrO_4^{2-}) and precipitation of Cr(III) as a solid chromium hydroxide ($Cr(OH)_3$) [23]. As a result, peak area integration was not routinely performed for the Cr(III) peak. Quantitation of hexavalent chromium in LRAA7318, LRAA7319, and LRAA7320 by EPA Method 6800 was performed using IDMS calculations. Additional analysis to account for the differences between total and speciated chromium concentrations is examined in Section 3.3.3 (Mass Balance Analysis).

To further understand the types of chromium species that are expected to be formed during the EPA Method 3060A with 50 mM EDTA alkaline digestion, an additional experiment was performed using four samples from one bottle of each batch (LRAA7318, LRAA7319, and LRAA7320). These samples and four solution blanks were processed according to the speciated hexavalent chromium protocol. After filtering the samples, a Mettler Toledo SevenCompact pH/Ion meter S220 equipped with an InLab Expert Pro-ISM pH probe and InLab Redox ORP probe was utilized to measure the sample pH, temperature, and Eh values. These values are compared to Eh-pH diagram references found in literature to predict the most probable, thermodynamically stable chromium species in the sample preparations in order to provide insight into the expected solution chemistry. The results from this experiment are provided in Figure 3.17 and indicate that the formation of the soluble $[Cr(VI)O_4]^{2-}$ ionic species is expected during sample preparation.

Table 3.15: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7318 using a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column and 2 mM EDTA mobile phase. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with IC-ICP-MS according to EPA Method 6800 (SIDMS) with five replicate measurements for each sample (n = 20).

Speciated Hexavalent Chromium Metrosep A Supp 5 Column with 2 mM EDTA Mobile Phase					
Sample Name	Average	SD	Percent RSD	95% CI	Number of Samples (n)
	µg/g	µg/g	%	µg/g	
LRAA7318-B1	3.9800	0.8624	21.6690	0.4036	20
LRAA7318-B2	3.5775	0.6091	17.0272	0.2851	20
LRAA7318-B3	4.0143	0.3297	8.2124	0.1543	20
LRAA7318-B4	4.0386	0.4243	10.5050	0.2110	20
Average	3.9009	0.6131	15.7173	0.1373	80

Table 3.16: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7318 using a Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography column and 2 mM EDTA mobile phase. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with IC-ICP-MS according to EPA Method 6800 (SIDMS) with five replicate measurements for each sample (n = 20).

Speciated Hexavalent Chromium Metrosep A Supp 17 Column with 2 mM EDTA Mobile Phase					
Sample Name	Average	SD	Percent RSD	95% CI	Number of Samples (n)
	µg/g	µg/g	%	µg/g	
LRAA7318-B1	3.9560	0.6179	15.6186	0.2892	20
LRAA7318-B2	3.7511	0.7020	18.7138	0.3383	20
LRAA7318-B3	3.7295	0.5027	13.4782	0.2353	20
LRAA7318-B4	3.7284	0.2764	7.4122	0.1374	20
Average	3.7926	0.5468	14.4181	0.1233	80

Figure 3.12: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7318 using a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column (blue data points) and Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography column (orange data points) with 2 mM EDTA mobile phase. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with IC-ICP-MS according to EPA Method 6800 (SIDMS) with five replicate measurements for each sample (n = 20). The shaded regions of the chart (blue and orange) provide the 95% CI range of the average measurements.

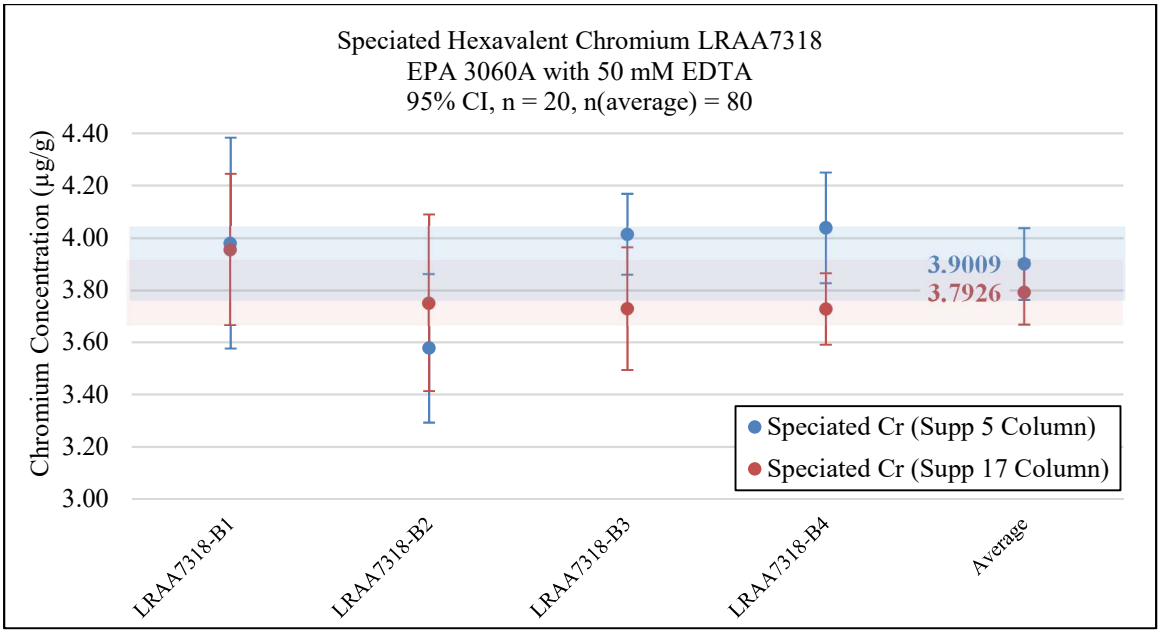


Table 3.17: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7319 using a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column and 2 mM EDTA mobile phase. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with IC-ICP-MS according to EPA Method 6800 (SIDMS) with five replicate measurements for each sample (n = 20).

Speciated Hexavalent Chromium Metrosep A Supp 5 Column with 2 mM EDTA Mobile Phase					
Sample Name	Average	SD	Percent RSD	95% CI	Number of Samples (n)
	µg/g	µg/g	%	µg/g	
LRAA7319-B1	0.4918	0.1044	21.2322	0.0489	20
LRAA7319-B2	0.5181	0.0976	18.8345	0.0457	20
LRAA7319-B3	0.4770	0.0671	14.0758	0.0314	20
LRAA7319-B4	0.5369	0.0767	14.2800	0.0359	20
Average	0.5059	0.0892	17.6261	0.0198	80

Table 3.18: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7319 using a Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography column and 2 mM EDTA mobile phase. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with IC-ICP-MS according to EPA Method 6800 (SIDMS) with five replicate measurements for each sample (n = 20).

Speciated Hexavalent Chromium Metrosep A Supp 17 Column with 2 mM EDTA Mobile Phase					
Sample Name	Average	SD	Percent RSD	95% CI	Number of Samples (n)
	µg/g	µg/g	%	µg/g	
LRAA7319-B1	0.4728	0.1023	21.6377	0.0479	20
LRAA7319-B2	0.4751	0.0627	13.2020	0.0294	20
LRAA7319-B3	0.5243	0.0486	9.2613	0.0241	20
LRAA7319-B4	0.5369	0.0783	14.5789	0.0377	20
Average	0.5013	0.0801	15.9789	0.0182	80

Figure 3.13: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7319 using a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column (blue data points) and Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography column (orange data points) with 2 mM EDTA mobile phase. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with IC-ICP-MS according to EPA Method 6800 (SIDMS) with five replicate measurements for each sample (n = 20). The shaded regions of the chart (blue and orange) provide the 95% CI range of the average measurements.

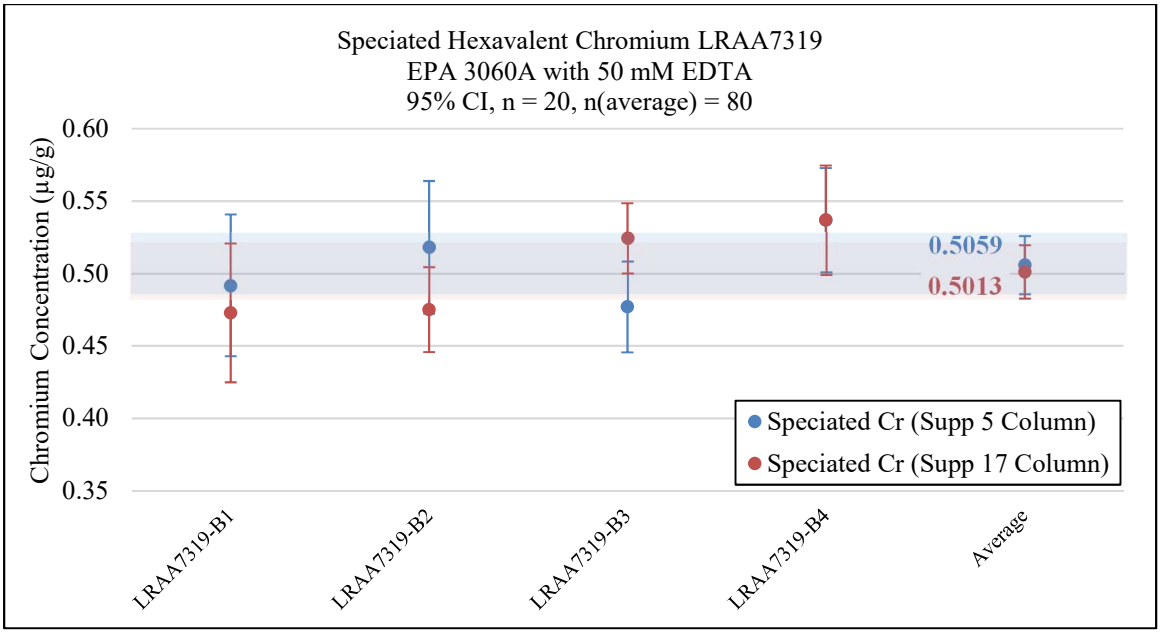


Table 3.19: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7320 using a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column and 2 mM EDTA mobile phase. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with IC-ICP-MS according to EPA Method 6800 (SIDMS) with five replicate measurements for each sample (n = 20).

Speciated Hexavalent Chromium Metrosep A Supp 5 Column with 2 mM EDTA Mobile Phase					
Sample Name	Average	SD	Percent RSD	95% CI	Number of Samples (n)
	µg/g	µg/g	%	µg/g	
LRAA7320-B1	0.4573	0.1081	23.6399	0.0506	20
LRAA7320-B2	0.5037	0.0633	12.5691	0.0296	20
LRAA7320-B3	0.4783	0.0914	19.1139	0.0428	20
LRAA7320-B4	0.5532	0.0657	11.8695	0.0307	20
Average	0.4981	0.0901	18.0907	0.0201	80

Table 3.20: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7320 using a Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography column and 2 mM EDTA mobile phase. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with IC-ICP-MS according to EPA Method 6800 (SIDMS) with five replicate measurements for each sample (n = 20).

Speciated Hexavalent Chromium Metrosep A Supp 17 Column with 2 mM EDTA Mobile Phase					
Sample Name	Average	SD	Percent RSD	95% CI	Number of Samples (n)
	µg/g	µg/g	%	µg/g	
LRAA7320-B1	0.5552	0.1000	18.0111	0.0468	20
LRAA7320-B2	0.4967	0.0434	8.7458	0.0203	20
LRAA7320-B3	0.4899	0.0772	15.7672	0.0361	20
LRAA7320-B4	0.5369	0.0524	9.7571	0.0245	20
Average	0.5197	0.0755	14.5379	0.0168	80

Figure 3.14: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7320 using a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column (blue data points) and Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography column (orange data points) with 2 mM EDTA mobile phase. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with IC-ICP-MS according to EPA Method 6800 (SIDMS) with five replicate measurements for each sample (n = 20). The shaded regions of the chart (blue and orange) provide the 95% CI range of the average measurements.

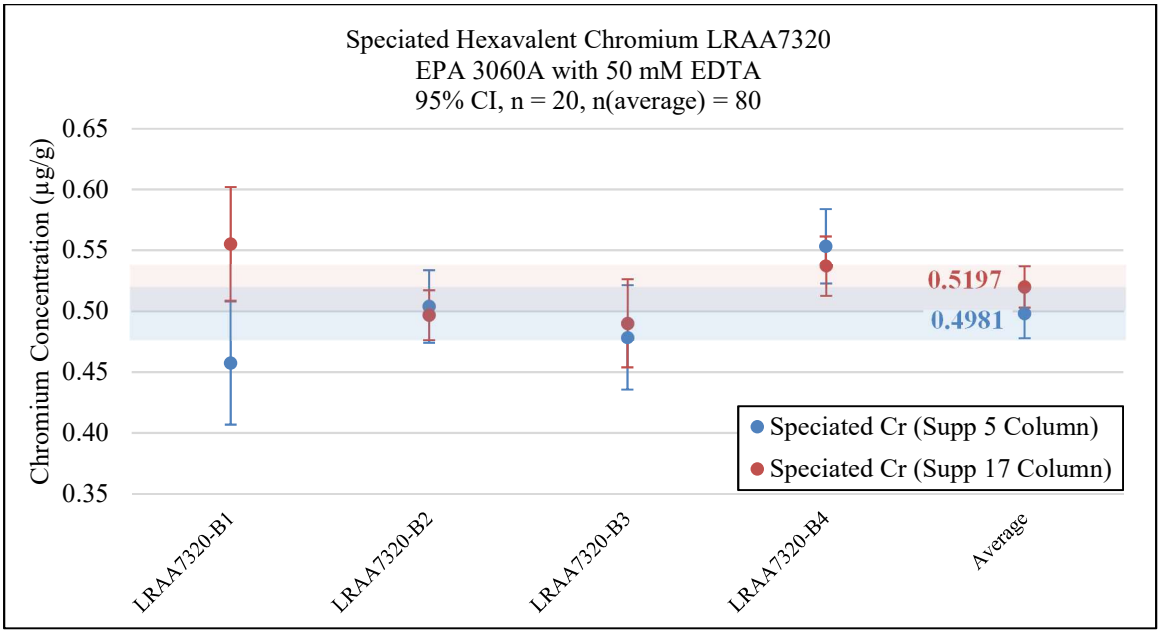
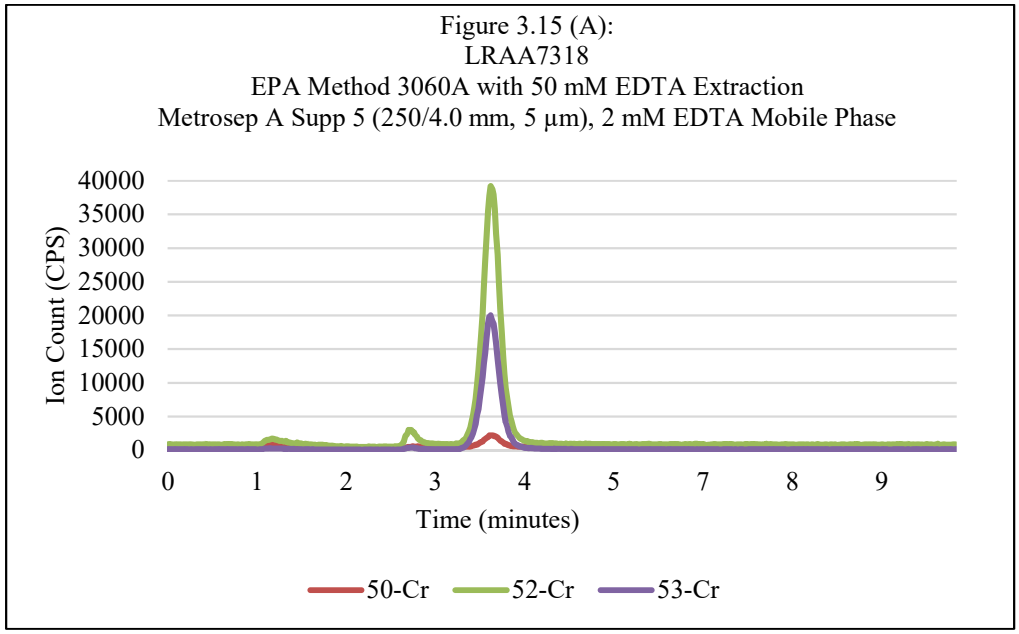
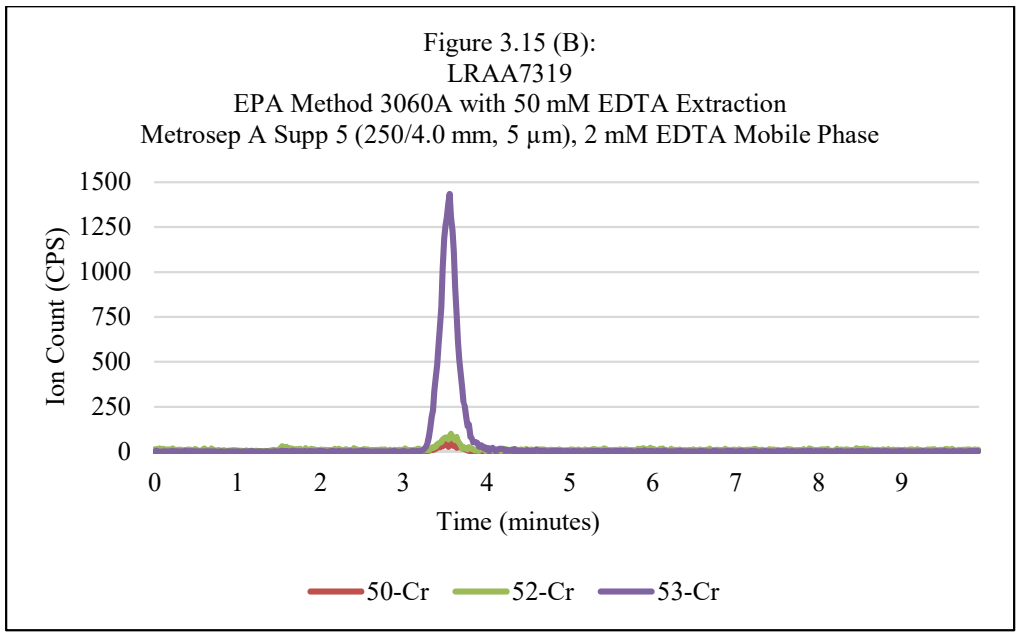


Figure 3.15: Example chromatograms for speciated hexavalent chromium analysis. EPA Method 3060A was utilized to digest the samples, followed by extraction with a 50 mM EDTA solution. The prepared, spiked samples were analyzed using optimized IC-ICP-MS tune mode parameters and a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase. Examples of the resulting chromatograms are provided for batches LRAA7318 (Figure 3.15 A), LRAA7319 (Figure 3.15 B), and LRAA7320 (Figure 3.15 C). The retention time for Cr(III) was found to be approximately 2.97 minutes and the retention time for Cr(VI) was found to be approximately 3.97 minutes. The small baseline fluctuation at approximately 1.22 minutes corresponds to an increase in system pressure from the sample injection. The example chromatogram includes the ion count for each of the major isotopes of chromium.





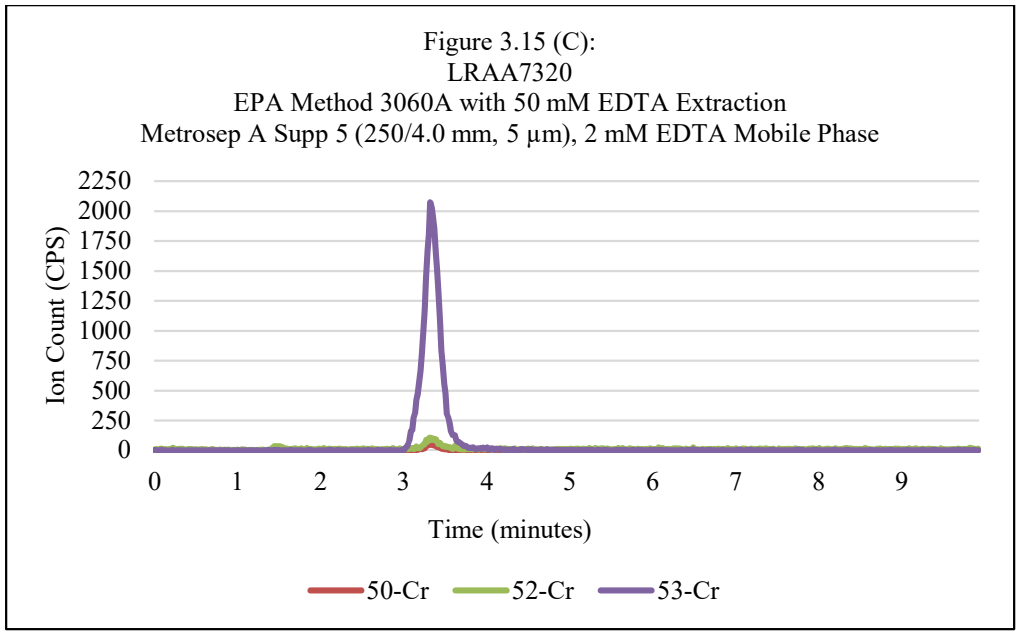


Figure 3.16: Example chromatograms for speciated hexavalent chromium analysis. EPA Method 3060A was utilized to digest the samples, followed by extraction with a 50 mM EDTA solution. The prepared samples were analyzed using optimized IC-ICP-MS tune mode parameters and a Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase. Example for the resulting chromatograms are provided for batches LRAA7318 (Figure 3.16 A), LRAA7319 (Figure 3.16 B), and LRAA7320 (Figure 3.16 C). The retention time for Cr(III) was found to be approximately 2.83 minutes and the retention time for Cr(VI) was found to be approximately 5.32 minutes. The small baseline fluctuation at approximately 1.11 minutes corresponds to an increase in system pressure from the sample injection. The example chromatogram includes the ion count for each of the major isotopes of chromium.

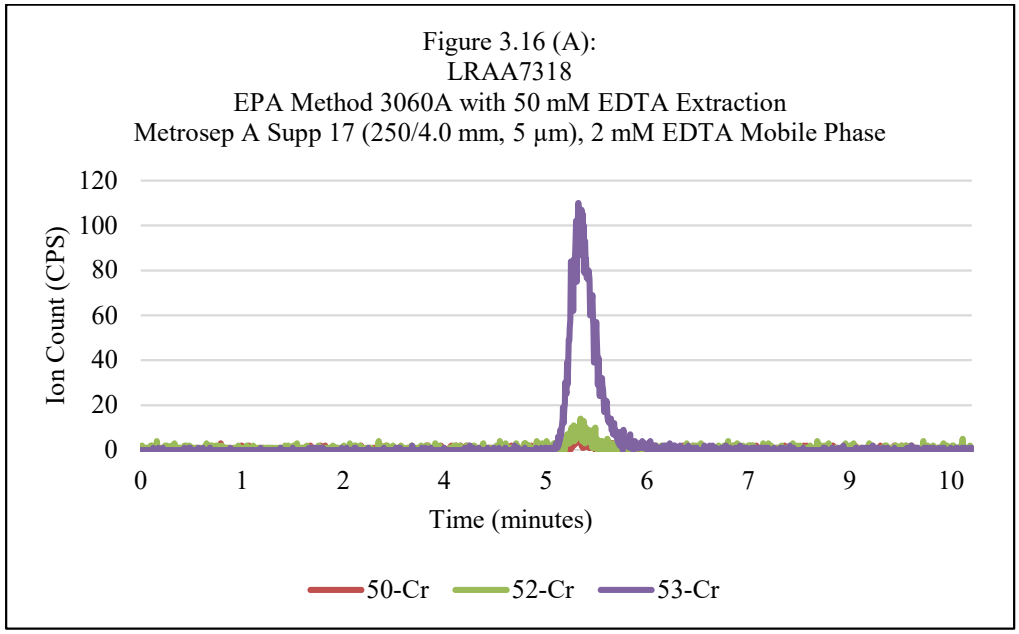


Figure 3.16 (B):
LRAA7319
EPA Method 3060A with 50 mM EDTA Extraction
Metrosep A Supp 17 (250/4.0 mm, 5 µm), 2 mM EDTA Mobile Phase

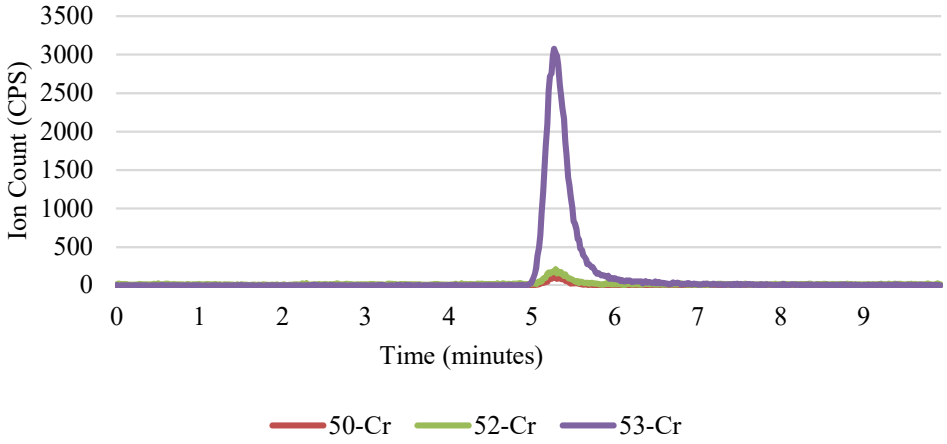


Figure 3.16 (C):
LRAA7320
EPA Method 3060A with 50 mM EDTA Extraction
Metrosep A Supp 17 (250/4.0 mm, 5 µm), 2 mM EDTA Mobile Phase

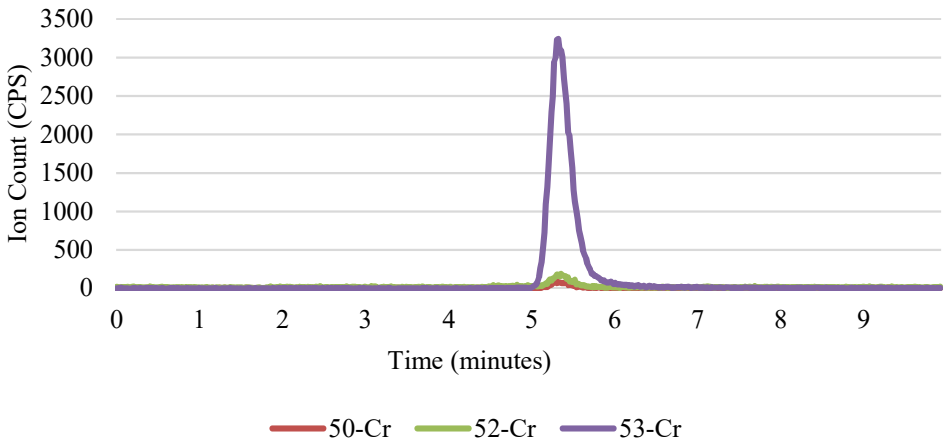
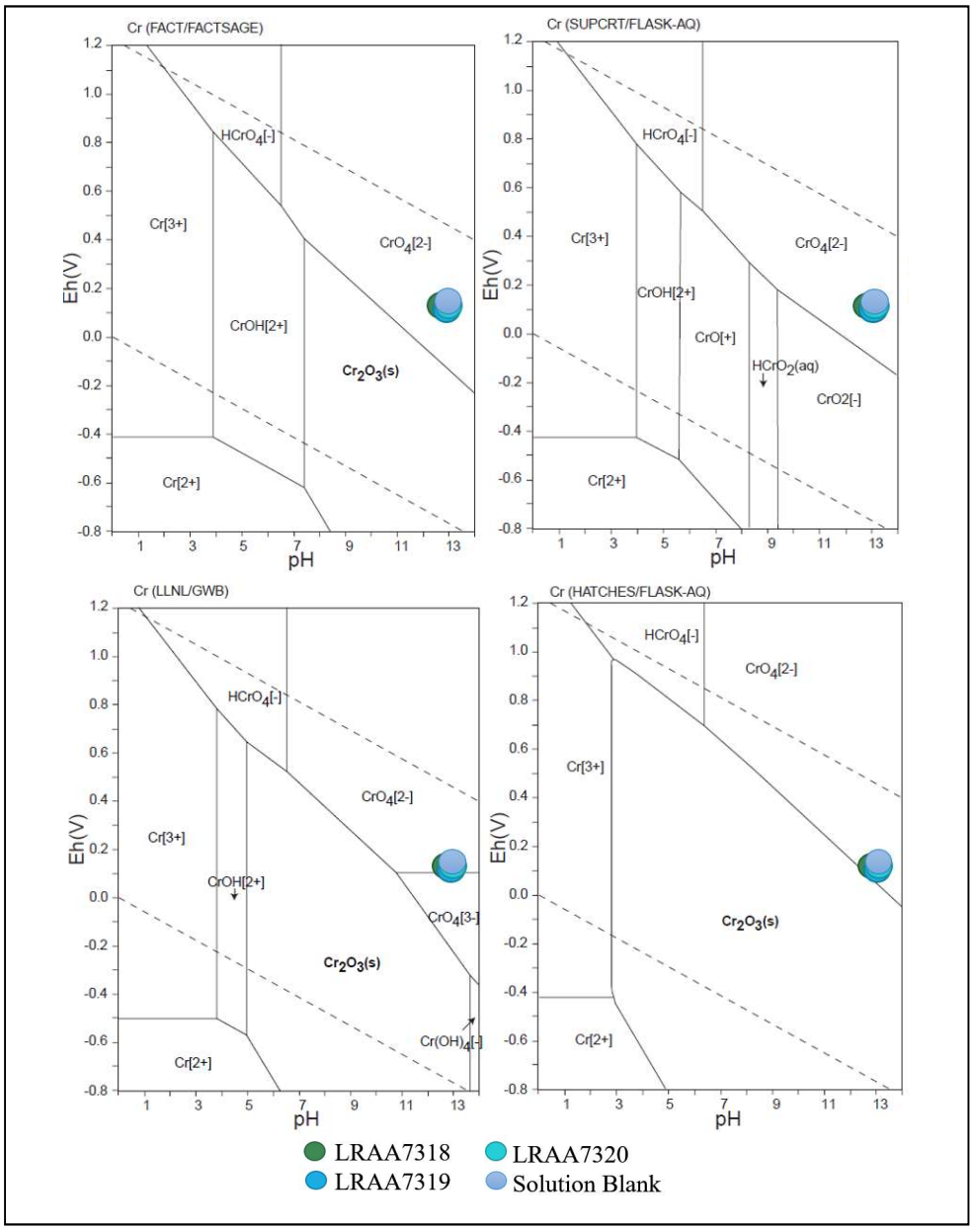


Figure 3.17: Evaluation of sample pH and Eh values using Mettler Toledo pH and Redox ORP probes. EPA Method 3060A with 50 mM EDTA was used to prepare four samples of LRAA7318, LRAA7319, and LRAA7320 with four solution blanks for speciated hexavalent chromium analysis. After filtering the samples, pH and oxidation/reduction potential values (Eh) were compared and superimposed onto Eh-pH diagram references found in literature to predict the most probable, thermodynamically stable chromium species in the sample. The results indicate that the formation of soluble $[Cr(VI)O_4]^{2-}$ ionic species is expected during sample preparation. The four Eh-pH diagrams provide a comparison of thermodynamic databases as part of an open source project from the Research Center for Deep Geological Environments, Geological Survey of Japan. The diagrams are emended and from the Atlas of Eh-pH diagrams, National Institute of Advanced Industrial Science and Technology, Research Center for Deep Geological Environments, Geological Survey of Japan, Open File Report No. 419, pages 78-79, May 2005 [32].



3.3.3 MASS BALANCE ANALYSIS

Results from the total chromium analysis of the Sigma-Aldrich candidate reference standard material indicate chromium concentrations of 28.0586 ± 0.4314 µg/g for batch LRAA7318, 21.8959 ± 0.3465 µg/g for batch LRAA7319, and 21.4216 ± 0.2428 µg/g for LRAA7320. Yet, the concentrations for speciated hexavalent chromium were found to be approximately 3.8 µg/g for LRAA7318 and approximately 0.50 µg/g for LRAA7319 and LRAA7320. To account for these differences, it is important to consider that the stability of the chromium species is influenced by several factors, including the pH and oxidation/reduction potential (Eh) of the extraction solution, which makes it difficult to simultaneously extract both Cr(III) and Cr(VI) during speciated analysis. The alkaline digestion solution used in the hexavalent chromium analysis supports extraction of Cr(VI) as a soluble chromate anion (CrO_4^{2-}) and precipitation of Cr(III) as a solid chromium hydroxide ($Cr(OH)_3$). Therefore, in order to have a comprehensive chromium speciation analysis, it is important to account for the concentrations of the insoluble Cr(III) hydroxide residues that precipitate out of solution during the EPA 3060A extraction. To further validate the analytical results, mass balance was examined by comparing the total elemental chromium content to the sum of measured concentrations of the chromium species [Cr(III) + Cr(VI)]. This strategy was implemented by reserving the insoluble EPA Method 3060A residues as speciated trivalent chromium samples. The residue samples were then acid digested according to EPA Method 3052, with trivalent chromium species quantification by EPA Method 6800 using ICP-MS with IDMS calculations. Section 3.2.5.2 describes the mass balance sample preparation. While the 53-Cr(VI) isotopically enriched standard was utilized for quantification of the hexavalent chromium species by IDMS calculations, the 50-Cr(III) isotopically enriched standard was utilized for quantification of the

trivalent chromium species by IDMS calculations. The samples were double spiked with both isotopically enriched standards during the initial speciated sample preparation, and no additional standards were added to the insoluble residues during acid digestion by EPA Method 3052. Also, although it is possible for soluble Cr(III) to be derivatized with EDTA during the speciated chromium analysis and directly determined using SIDMS calculations, no recovery of the $[Cr(III)EDTA]^-$ complex above LOQ was found with IC-ICP-MS analysis.

The results for the mass balance analysis for candidate standard reference material batch LRAA7318 are presented in Table 3.21 and illustrated in Figure 3.18. The previously reported total chromium content concentrations for each of the four independent bottles of sample material (B1, B2, B3, and B4) are summarized in the table. The total chromium content is compared to the sum of speciated chromium analysis [Cr(III) + Cr(VI)]. The Cr(III) concentrations were found by acid digesting (EPA Method 3052) sample preparation residues from the hexavalent chromium analysis (EPA Method 3060A). The Cr(VI) concentrations were previously found by speciated hexavalent chromium analysis (EPA Method 3060A with 50 mM EDTA) using Metrosep A Supp 5 (250/4.0 mm, 5 µm) and Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography columns with 2 mM EDTA mobile phase. The sum of speciated chromium analysis with the Metrosep A Supp 5 column [Cr(III) + Cr(VI)] range from 33.3276 µg/g to 36.8740 µg/g. This compares to the average total chromium analysis concentration of 28.0586 µg/g. The difference between the total chromium and speciated chromium analysis [Cr(III) + Cr(VI)] range from +21.3455% to +29.8734%. The sum of speciated chromium analysis with the Metrosep A Supp 17 column [Cr(III) + Cr(VI)] range from 33.0428 µg/g to 36.5638 µg/g. This compares to the average total chromium analysis concentration of 28.0586 µg/g. The difference between the total chromium and speciated chromium analysis [Cr(III) +

Cr(VI)] range from +20.3416% to +28.7809%. Due to an apparent bias, the 95% confidence intervals for the total chromium concentrations and summed speciated chromium concentrations do not directly overlap. However, the average speciated chromium results agree with the average total chromium with a difference of less than 25%.

The results for the mass balance analysis for candidate standard reference material batch LRAA7319 are presented in Table 3.22 and illustrated in Figure 3.19. The previously reported total chromium content concentrations for each of the four independent bottles of sample material (B1, B2, B3, and B4) are summarized in the table. The total chromium content is compared to the sum of speciated chromium analysis [Cr(III) + Cr(VI)]. The sum of speciated chromium analysis with the Metrosep A Supp 5 column [Cr(III) + Cr(VI)] range from 22.2285 µg/g to 25.6132 µg/g. This compares to the average total chromium analysis concentration of 21.8959 µg/g. The difference between the total chromium and speciated chromium analysis [Cr(III) + Cr(VI)] range from +0.9452% to +14.8678%. The sum of speciated chromium analysis with the Metrosep A Supp 17 column [Cr(III) + Cr(VI)] range from 24.3906 µg/g to 26.0867 µg/g. This compares to the average total chromium analysis concentration of 21.8959 µg/g. The difference between the total chromium and speciated chromium analysis [Cr(III) + Cr(VI)] range from +9.1728% to +20.2872%. Due to an apparent bias, the 95% confidence intervals for the total chromium concentrations and summed speciated chromium concentrations do not directly overlap. However, the average speciated chromium results agree with the average total chromium with a difference of less than 15%.

The results for the mass balance analysis for candidate standard reference material batch LRAA7320 are presented in Table 3.23 and illustrated in Figure 3.20. The previously reported total chromium content concentrations for each of the four independent bottles of sample

material (B1, B2, B3, and B4) are summarized in the table. The total chromium content is compared to the sum of speciated chromium analysis [Cr(III) + Cr(VI)]. The sum of speciated chromium analysis with the Metrosep A Supp 5 column [Cr(III) + Cr(VI)] range from 23.5545 μg/g to 27.8952 μg/g. This compares to the average total chromium analysis concentration of 21.4216 μg/g. The difference between the total chromium and speciated chromium analysis [Cr(III) + Cr(VI)] range from +8.7261% to +28.8006%. The sum of speciated chromium analysis with the Metrosep A Supp 17 column [Cr(III) + Cr(VI)] range from 23.8656 μg/g to 25.0896 μg/g. This compares to the average total chromium analysis concentration of 21.4216 μg/g. The difference between the total chromium and speciated chromium analysis [Cr(III) + Cr(VI)] range from +10.1624% to +20.3687%. Due to an apparent bias, the 95% confidence intervals for the total chromium concentrations and summed speciated chromium concentrations do not directly overlap. However, the average speciated chromium results agree with the average total chromium with a difference of less than approximately 20%.

Table 3.21: Mass balance analysis for candidate standard reference material LRAA7318. The total chromium content according to EPA Method 3052 is compared to the sum of speciated chromium analysis [Cr(3) + Cr(6)]. The Cr(3) concentrations were found by acid digesting (EPA Method 3052) sample preparation residues from the hexavalent chromium analysis (EPA Method 3060A). The Cr(6) concentrations were found by speciated hexavalent chromium analysis (EPA Method 3060A with 50 mM EDTA) using Metrosep A Supp 5 (250/4.0 mm, 5 µm) and Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography columns with 2 mM EDTA mobile phase. The results of the hexavalent chromium analysis were reported in Tables 3.15 and 3.16. Four independent bottles (B1, B2, B3, and B4) were each subsampled and analyzed with five replicate measurements for each sample (n = 20). The difference between the total chromium and speciated chromium analysis [Cr(3) + Cr(6)] are provided.

	EPA 3052	**Metrosep Supp 5 Column 3060A Residues Mass Balance**			**Metrosep Supp 17 Column 3060A Residues Mass Balance**		
Sample Name	*Total Cr*	*Cr(3) + Cr(6)*	*Difference*	*Difference*	*Cr(3) + Cr(6)*	*Difference*	*Difference*
	µg/g	*µg/g*	*µg/g*	*%*	*µg/g*	*µg/g*	*%*
LRAA7318-B1	27.1500	33.5450	6.3950	23.5544	33.5210	6.3710	23.4660
LRAA7318-B2	29.2348	35.6067	6.3719	21.7955	35.7803	6.5455	22.3894
LRAA7318-B3	27.4575	33.3276	5.8701	21.3788	33.0428	5.5853	20.3416
LRAA7318-B4	28.3923	36.8740	8.4817	29.8734	36.5638	8.1715	28.7809
Average	28.0586	34.8366	6.7780	24.1565	34.7283	6.6697	23.7705

Figure 3.18: Mass balance analysis for candidate standard reference material LRAA7318. The figure compares the following: (1) total chromium content according to EPA Method 3052; (2) Cr(3) concentrations by acid digestion (EPA Method 3052) of the sample preparation residues from the hexavalent chromium analysis (EPA Method 3060A with 50 mM EDTA); and (3) sum of speciated chromium analysis [Cr(3) + Cr(6)] as mass balance values for both the Metrosep A Supp 5 and Metrosep A Supp 17 columns. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with five replicate measurements for each sample (n = 20). The Metrosep A Supp 5 and Metrosep A Supp 17 preparations shared the same stock solution and have the same EPA Method 3060A residues.

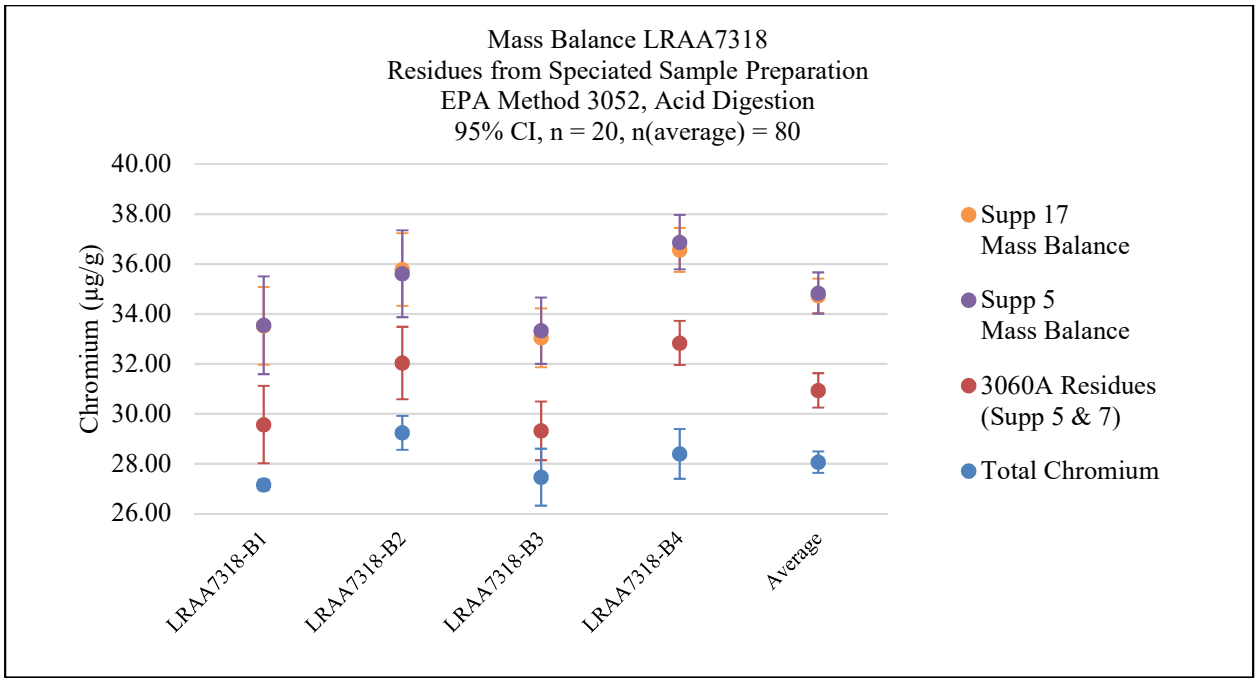


Table 3.22: Mass balance analysis for candidate standard reference material LRAA7319. The total chromium content according to EPA Method 3052 is compared to the sum of speciated chromium analysis [Cr(3) + Cr(6)]. The Cr(3) concentrations were found by acid digesting (EPA Method 3052) sample preparation residues from the hexavalent chromium analysis (EPA Method 3060A). The Cr(6) concentrations were found by speciated hexavalent chromium analysis (EPA Method 3060A with 50 mM EDTA) using Metrosep A Supp 5 (250/4.0 mm, 5 µm) and Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography columns with 2 mM EDTA mobile phase. The results of the hexavalent chromium analysis were reported in Tables 3.15 and 3.16. Four independent bottles (B1, B2, B3, and B4) were each subsampled and analyzed with five replicate measurements for each sample (n = 20). The difference between the total chromium and speciated chromium analysis [Cr(3) + Cr(6)] are provided.

	EPA 3052	**Metrosep Supp 5 Column 3060A Residues Mass Balance**			**Metrosep Supp 17 Column 3060A Residues Mass Balance**		
Sample Name	*Total Cr*	*Cr(3) + Cr(6)*	*Difference*	*Difference*	*Cr(3) + Cr(6)*	*Difference*	*Difference*
	µg/g	*µg/g*	*µg/g*	*%*	*µg/g*	*µg/g*	*%*
LRAA7319-B1	22.2144	25.4161	3.2017	14.4128	26.0867	3.8722	17.4312
LRAA7319-B2	22.0204	22.2285	0.2081	0.9452	24.3906	2.3702	10.7634
LRAA7319-B3	22.4954	25.6132	3.1178	13.8595	24.5589	2.0635	9.1728
LRAA7319-B4	20.8533	23.9537	3.1004	14.8678	25.0838	4.2305	20.2872
Average	21.8959	24.3028	2.4070	10.9928	25.0290	3.1331	14.3092

Figure 3.19: Mass balance analysis for candidate standard reference material LRAA7319. The figure compares the following: (1) total chromium content according to EPA Method 3052; (2) Cr(3) concentrations by acid digestion (EPA Method 3052) of the sample preparation residues from the hexavalent chromium analysis (EPA Method 3060A with 50 mM EDTA); and (3) sum of speciated chromium analysis [Cr(3) + Cr(6)] as mass balance values for both the Metrosep A Supp 5 and Metrosep A Supp 17 columns. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with five replicate measurements for each sample (n = 20).

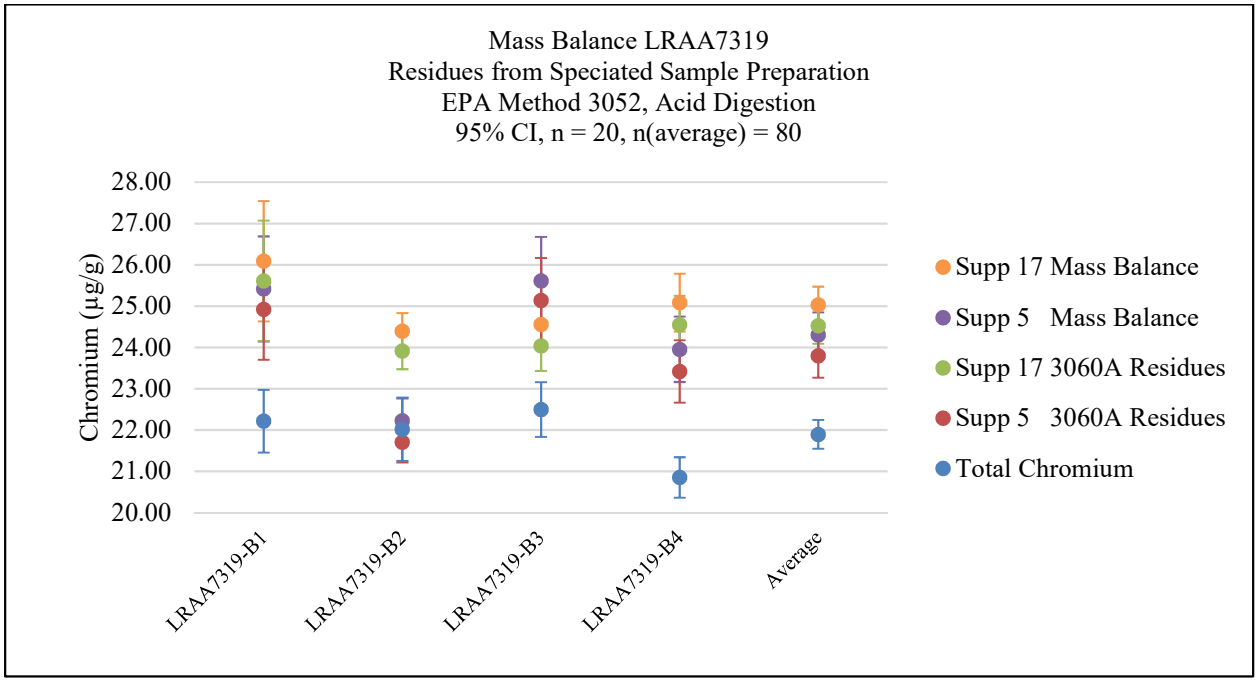
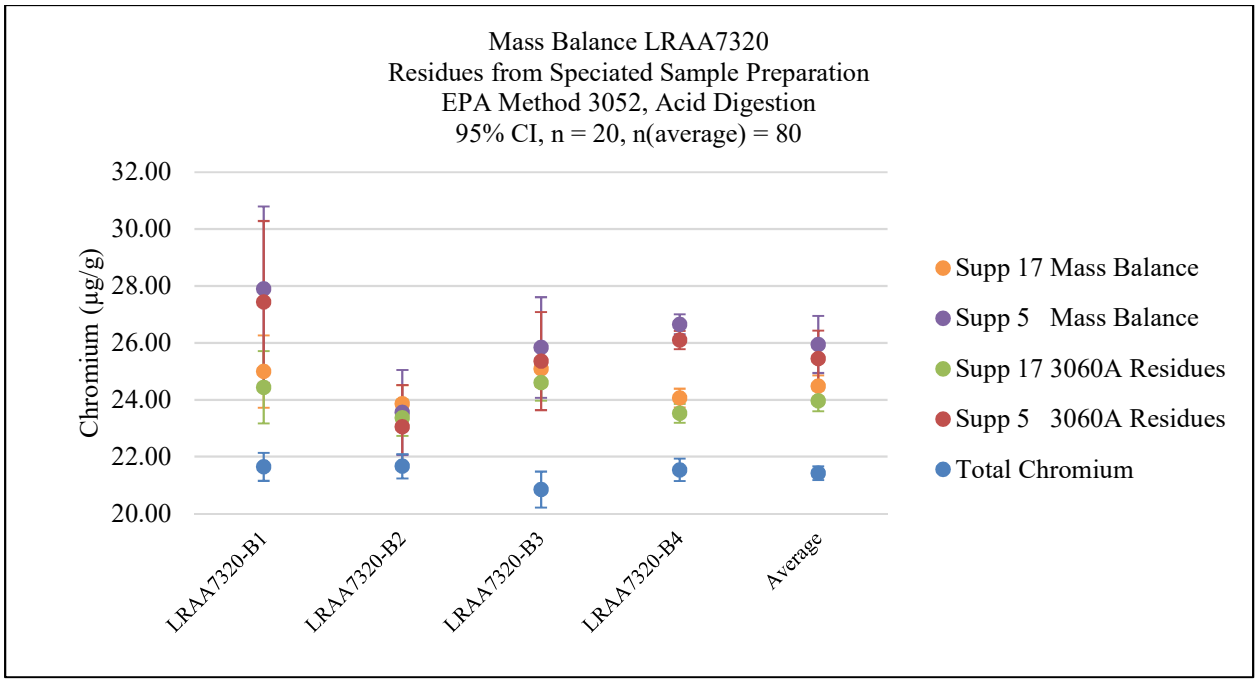


Table 3.23: Mass balance analysis for candidate standard reference material LRAA7320. The total chromium content according to EPA Method 3052 is compared to the sum of speciated chromium analysis [Cr(3) + Cr(6)]. The Cr(3) concentrations were found by acid digesting (EPA Method 3052) sample preparation residues from the hexavalent chromium analysis (EPA Method 3060A). The Cr(6) concentrations were found by speciated hexavalent chromium analysis (EPA Method 3060A with 50 mM EDTA) using Metrosep A Supp 5 (250/4.0 mm, 5 µm) and Metrosep A Supp 17 (250/4.0 mm, 5 µm) ion chromatography columns with 2 mM EDTA mobile phase. The results of the hexavalent chromium analysis were reported in Tables 3.15 and 3.16. Four independent bottles (B1, B2, B3, and B4) were each subsampled and analyzed with five replicate measurements for each sample (n = 20). The difference between the total chromium and speciated chromium analysis [Cr(3) + Cr(6)] are provided.

	EPA 3052	**Metrosep Supp 5 Column 3060A Residues Mass Balance**			**Metrosep Supp 17 Column 3060A Residues Mass Balance**		
Sample Name	*Total Cr*	*Cr(3) + Cr(6)*	*Difference*	*Difference*	*Cr(3) + Cr(6)*	*Difference*	*Difference*
	µg/g	*µg/g*	*µg/g*	*%*	*µg/g*	*µg/g*	*%*
LRAA7320-B1	21.6409	27.8952	6.2543	28.9006	24.9921	3.3512	15.4857
LRAA7320-B2	21.6640	23.5545	1.8904	8.7261	23.8656	2.2016	10.1624
LRAA7320-B3	20.8440	25.8373	4.9933	23.9557	25.0896	4.2456	20.3687
LRAA7320-B4	21.5376	26.6542	5.1167	23.7569	24.0594	2.5218	11.7088
Average	21.4216	25.9443	4.5227	21.1130	24.4840	3.0624	14.2957

Figure 3.20: Mass balance analysis for candidate standard reference material LRAA7320. The figure compares the following: (1) total chromium content according to EPA Method 3052; (2) Cr(3) concentrations by acid digestion (EPA Method 3052) of the sample preparation residues from the hexavalent chromium analysis (EPA Method 3060A with 50 mM EDTA); and (3) sum of speciated chromium analysis [Cr(3) + Cr(6)] as mass balance values for both the Metrosep A Supp 5 and Metrosep A Supp 17 columns. Four independent bottles (B1, B2, B3, and B4) were each subsampled four times and analyzed with five replicate measurements for each sample (n = 20).



3.3.4 COMPARISON OF ISOTOPE STANDARD CONCENTRATIONS

In order to evaluate potential bias and method error, and additional speciated hexavalent chromium analysis for candidate standard reference material LRAA7318, LRAA7319, and LRAA7320 was performed using low-concentration isotope standards (50-Cr(III) at 7.7075 µg/g and 53-Cr(VI) at 8.0248 µg/g, outlined in Table 3.1) and Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase. The samples were prepared using EPA Method 3060A with 50 mM EDTA and EPA Method 6800 by IC-ICP-MS (sample preparation outlined in section 3.2.5.2) using the optimized instrument parameters provided in Table 3.3 and Table 3.6.

For each batch of material, one bottle of material (B3) was obtained. An aliquot from each individual bottle of candidate standard reference material was transferred into a quartz weigh bottle, which was then subsampled four times and analyzed with five replicate measurements (n = 20). The results of the speciated hexavalent chromium analysis are summarized in Table 3.24 for LRAA7318, LRAA7319, and LRAA7320. Speciated hexavalent chromium analysis results for candidate standard reference material LRAA7318, LRAA7319, and LRAA7320 using the original concentration isotope standards (50-Cr(III) at 95.9915 µg/g and 53-Cr(VI) at 100.7669 µg/g, outlined in Table 3.1) and Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase are summarized in Table 3.25. The results were previously reported in Table 3.15, Table 3.17, and Table 3.19. The tables provide the mean hexavalent chromium concentrations (µg/g) with their corresponding standard deviations (SD), percent relative standard deviations (%RSD, SD as a percent of mean), 95% confidence interval (95% CI), and number of replicate measurements (n).

Using the low-concentration standards, LRAA7318 (B3) was found to have an average hexavalent chromium concentration of 3.7793 ± 0.1538 µg/g with a %RSD value of approximately 20%. The resulting speciated hexavalent chromium concentration is comparable to the previously reported average hexavalent chromium concentration for LRAA7318 (3.9009 ± 0.1373 µg/g) since there is overlap of the 95% CI concentration ranges. Similarly, using the low-concentration standards, LRAA7319 (B3) was found to have an average hexavalent chromium concentration of 0.5351 ± 0.0191 µg/g with a %RSD value of less than 15%. The resulting speciated hexavalent chromium concentration is comparable to the previously reported average hexavalent chromium concentration for LRAA7319 (0.5059 ± 0.0198 µg/g) since there is overlap of the 95% CI concentration ranges. Finally, using the low-concentration standards, LRAA7320 (B3) was found to have an average hexavalent chromium concentration of 0.4441 ± 0.0066 µg/g with a %RSD value of less than 20%. The resulting speciated hexavalent chromium concentration is comparable to the previously reported average hexavalent chromium concentration for LRAA7320 (0.4981 ± 0.0201 µg/g) since the difference between the values are approximately 10%. However, there is not overlap of the 95% CI concentration ranges.

Mass balance analysis for candidate standard reference material LRAA7318, LRAA7319, and LRAA7320 using low-concentration isotope standards (50-Cr(III) at 7.7075 µg/g and 53-Cr(VI) at 8.0248 µg/g) was also performed. The results for the mass balance analysis Table 3.26 and illustrated in Figure 3.21. The previously reported total chromium content concentrations are also summarized in the table. The total chromium content is compared to the sum of speciated chromium analysis [Cr(III) + Cr(VI)]. The difference between the total chromium and speciated chromium analysis [Cr(III) + Cr(VI)] was +17.7404% for LRAA7318, +2.7338% for LRAA7319, and -1.9888% for LRAA7320. As shown in Figure 3.21, the 95% confidence

interval for the total chromium concentration and summed speciated chromium concentration for LRAA7318 do not directly overlap. However, the 95% confidence intervals for the total chromium concentrations and summed speciated chromium concentrations directly overlap for both LRAA7319 and LRAA7320. This experiment indicates that the use of 50-Cr(III) and 53-Cr(VI) enriched isotope standard solutions with concentrations of approximately 10 µg/g and 100 µg/g are comparable when determining hexavalent chromium concentrations. The use of the 10 µg/g isotope standard solutions provided improved results for mass balance determinations.

Table 3.24: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7318, LRAA7319, and LRAA7320 using low-concentration isotope standards (50-Cr(III) at 7.7075 µg/g and 53-Cr(VI) at 8.0248 µg/g) and Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase. For each batch of material, bottle three (B3) was subsampled four times and analyzed with IC-ICP-MS according to EPA Method 6800 (SIDMS) with five replicate measurements for each sample (n = 20).

Speciated Hexavalent Chromium Low-Concentration Isotope Standards Metrosep A Supp 5 Column with 2 mM EDTA Mobile Phase								
Sample Name	**Average**	**95% CI**	**95% CI Range**			**SD**	**Percent RSD**	**Number of Samples (n)**
	µg/g	**µg/g**	**µg/g**		**µg/g**	**µg/g**	**%**	
LRAA7318-B3	3.7793	0.1538	3.6255	to	3.9331	0.2777	23.6399	20
LRAA7319-B3	0.5351	0.0191	0.5159	to	0.5542	0.0409	12.5691	20
LRAA7320-B3	0.4441	0.0066	0.4375	to	0.4508	0.0141	19.1139	20

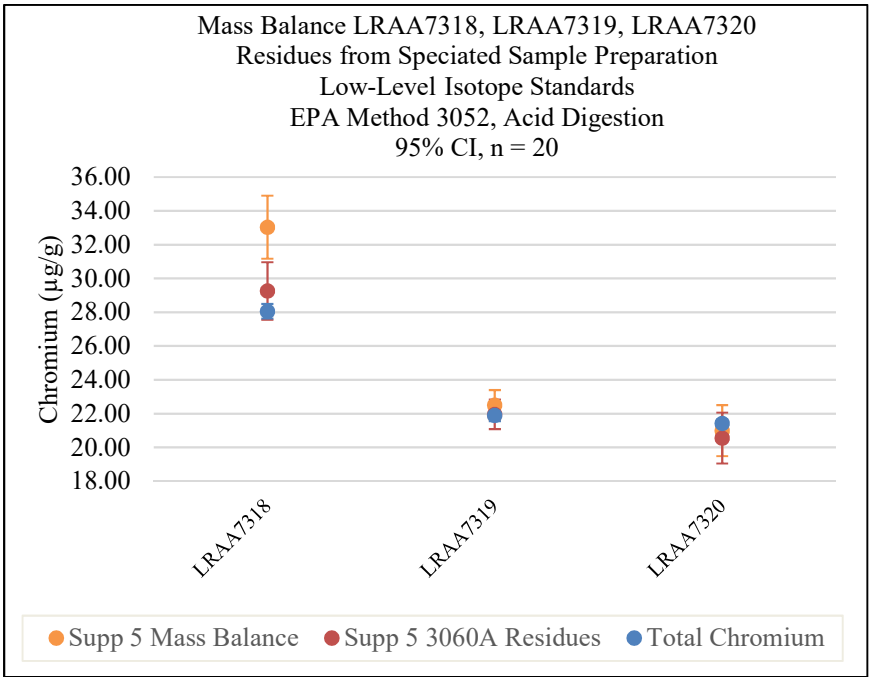
Table 3.25: Speciated hexavalent chromium analysis for candidate standard reference material LRAA7318, LRAA7319, and LRAA7320 using high-concentration isotope standards (50-Cr(III) at 95.9915 µg/g and 53-Cr(VI) at 100.7669 µg/g) and Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase. The previously reported averages (Table 3.15, 3.17, and 3.19) are summarized (n = 80).

Speciated Hexavalent Chromium High-Concentration Isotope Standards Metrosep A Supp 5 Column with 2 mM EDTA Mobile Phase								
Sample Name	**Average**	**95% CI**	**95% CI Range**			**SD**	**Percent RSD**	**Number of Samples (n)**
	µg/g	**µg/g**	**µg/g**		**µg/g**	**µg/g**	**%**	
LRAA7318-AVG	3.9009	0.1373	3.7636	to	4.0382	0.6131	15.7173	80
LRAA7319-AVG	0.5059	0.0198	0.4861	to	0.5257	0.0892	17.6261	80
LRAA7320-AVG	0.4981	0.0201	0.4780	to	0.5182	0.0901	18.0907	80

Table 3.26: Mass balance analysis for candidate standard reference material LRAA7318, LRAA7319, and LRAA7320 using low-concentration isotope standards (50-Cr(III) at 7.7075 µg/g and 53-Cr(VI) at 8.0248 µg/g). The total chromium content according to EPA Method 3052 is compared to the sum of speciated chromium analysis [Cr(3) + Cr(6)]. The Cr(3) concentrations were found by acid digesting (EPA Method 3052) sample preparation residues from the hexavalent chromium analysis (EPA Method 3060A). The Cr(6) concentrations were found by speciated hexavalent chromium analysis (EPA Method 3060A with 50 mM EDTA) using Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography columns with 2 mM EDTA mobile phase. The results of the hexavalent chromium analysis were reported in Table 3.25. Bottle three (B3) was subsampled four times and analyzed with five replicate measurements for each sample (n = 20). The difference between the total chromium and speciated chromium analysis [Cr(3) + Cr(6)] are provided.

	EPA 3052	**Metrosep Supp 5 Column 3060A Residues Mass Balance**		
Sample Name	*Total Cr*	*Cr(3) + Cr(6)*	*Difference*	*Difference*
	µg/g	*µg/g*	*µg/g*	*%*
LRAA7318	28.0586	33.0363	4.9777	17.7404
LRAA7319	21.8959	22.4945	0.5986	2.7338
LRAA7320	21.4216	20.9956	-0.4260	-1.9888

Figure 3.21: Mass balance analysis for candidate standard reference material LRAA7318, LRAA7319, and LRAA7320 using low-concentration standards for sample preparations (50-Cr(III) at 7.7075 µg/g and 53-Cr(VI) at 8.0248 µg/g). The figure compares the following: (1) total chromium content according to EPA Method 3052; (2) Cr(3) concentrations by acid digestion (EPA Method 3052) of the sample preparation residues from the hexavalent chromium analysis (EPA Method 3060A with 50 mM EDTA); and (3) sum of speciated chromium analysis [Cr(3) + Cr(6)] as mass balance values for the Metrosep A Supp 5 column. Bottle three (B3) was subsampled four times and analyzed with five replicate measurements for each sample (n = 20).



3.4 CONCLUSION

The total chromium and speciated hexavalent chromium content of a new Sigma-Aldrich hexavalent chromium standard reference material in a soil matrix was effectively quantitated using EPA Method 6800. Accuracy, precision, linearity, specificity and selectivity, limit of quantitation, and limit of detection of the sample preparation and analytical methods were fully validated. EPA Method 3052 was used for acid digestion of the sample, and EPA Method 6800 with IDMS was used to quantitate the total chromium content of the material. Furthermore, EPA Method 3060A with 50 mM EDTA was used for speciated chromium sample preparation, while EPA Method 6800 with IDMS/SIDMS was used to quantitate hexavalent chromium in the material. Also, mass balance analysis was performed to compare the total chromium content to the sum of the speciated chromium analysis [Cr(III) + Cr(VI)]. For the mass balance assay, EPA Method 3052 was used for acid digestion of the speciated chromium extraction residues, and EPA Method 6800 with IDMS was used for chromium quantification.

Using this methodology, LRAA7318 was determined to have an average total chromium concentration of 28.0586 ± 0.4314 µg/g with a %RSD value of less than 10%. The average hexavalent chromium concentrations of 3.9009 ± 0.1373 µg/g and 3.7926 ± 0.1233 µg/g were determined with %RSD values of less than 20% using the Metrosep A Supp 5 and Metrosep A Supp 17 columns, respectively. LRAA7319 was found to have an average total chromium concentration of 21.8959 ± 0.3465 µg/g with a %RSD value of less than 10%. The average hexavalent chromium concentrations of 0.5059 ± 0.0198 µg/g and 0.5013 ± 0.0182 µg/g were determined with %RSD values of less than 20% using the Metrosep A Supp 5 and Metrosep A Supp 17 columns, respectively. LRAA7320 was found to have an average total chromium concentration of 21.4216 ± 0.2428 with a %RSD value of less than 10%. The average

hexavalent chromium concentrations of 0.4981 ± 0.0201 µg/g and 0.5197 ± 0.0168 µg/g were determined with %RSD values of less than 20% using the Metrosep A Supp 5 and Metrosep A Supp 17 columns, respectively.

The speciated hexavalent chromium determinations using the Metrosep A Supp 5 and Metrosep A Supp 17 ion chromatography columns were compared, and the data statistically supports column equivalency for hexavalent chromium quantitation. Also, the results from the mass balance assay determinations indicate that the sums of speciated chromium content [Cr(III) + Cr(VI)] were within 11% to 24% of the total chromium content. Finally, experiments were performed that indicate 50-Cr(III) and 53-Cr(VI) enriched isotope standard solutions with concentrations of approximately 10 µg/g and 100 µg/g produce comparable results when utilized to prepare samples for determination of hexavalent chromium concentrations.

Development and certification of this new Sigma-Aldrich hexavalent chromium standard reference material in a soil matrix will provide the scientific community with a standard material that supports quality assurance and quality control of the analytical methodology used for hexavalent chromium testing. Considering the expected growth in chromium ore excavation and processing, this new standard will be a valuable addition to the analytical materials used for performing ambient level Cr(VI) background assessment measurements. This new standard will undoubtedly be used in the future to help mitigate the impact of mineral processing on the surrounding environment and assist in monitoring remediation of hexavalent chromium-containing waste materials produced during industrial activities. Previously, a low-level hexavalent chromium soil standard has not been available for method and operator validation of EPA Method 6800. New analysts and previously unexperienced laboratories have not had a material with a well-characterized speciated value to verify their certification of mastery and

proficiency in speciated analysis of hexavalent chromium. This material enables validation within and between laboratories for hexavalent chromium data collection.

3.5 REFERENCES

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CHAPTER FOUR:

DETERMINATION OF HEXAVALENT CHROMIUM

IN A ROBUST VARIETY OF DIETARY SUPPLEMENT FORMULATIONS

4.1 INTRODUCTION

Chromium is found in nature predominately as trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)). At low does, Cr(III) is an essential dietary mineral that provides proper carbohydrate, lipid, and protein metabolism [1-3]. Also, biologically active Cr(III) facilitates the interaction of insulin with cellular receptor sites and improves glucose adsorption [1, 2]. Trivalent chromium is relatively non-toxic, and is considered to be important for human nutrition [2]. The Institute of Medicine panel on micronutrients at the United States National Academy of Sciences concluded that an adequate intake (AI) of chromium is 35 μg/day and 25 μg/day for young men and women, respectively [4]. Trivalent chromium can be obtained in microgram quantities by consuming fruits, vegetables, grains, and meats [2]. Although fresh foods and drinking water contain chromium, human intake is often considered inadequate and deficiency of chromium is associated with diabetes, infertility, and cardiovascular disease [1-3]. For this reason, chromium is often provided in dietary supplement formulations, which are marketed as multivitamin/multimineral nutritional supplements, prenatal support supplements, and weight loss products. Chromium picolinate is commonly used by supplement manufactures since this form of the element, which contains one chromium atom chelated with three molecules of picolinic acid, has been shown to have improved absorption and intracellular uptake [2].

However, hexavalent chromium is highly toxic and is absorbed more readily than Cr(III) by the lungs, gut, and skin [1, 5]. Evidence suggests that Cr(VI) is carcinogenic, causes

respiratory and dermal reactions, and damages the liver and kidneys [6]. The risks associated with Cr(VI) to human health are recognized by national and international organizations, including the United States Environmental Protection Agency (EPA), Occupational Safety and Health Administration (OSHA), International Agency for Research on Cancer (IARC), National Institutes of Health - National Toxicology Program (NTP), and the European Union (EU) [3, 7]. The EPA is investigating the need to revise chromium drinking water regulations and California's Proposition 65 (Safe Drinking Water and Toxic Enforcement Act of 1986) includes Cr(VI) on a list of chemicals that cause cancer, birth defects, or reproductive problems [6, 8]. The inhalation carcinogenicity of Cr(VI) is well established, yet evidence of the carcinogenicity potential of Cr(VI) by oral ingestion has been slow to develop [5]. In 2008, the National Institutes of Health released a technical report detailing toxicological and carcinogenesis results from a two-year study involving chronic exposure to a soluble form of Cr(VI) [5]. The study showed that similar doses of Cr(VI) and Cr(III) resulted in significantly higher concentrations of Cr(VI) in tissues, indicating that Cr(VI) is well absorbed and distributed [7]. It was once thought that low-pH gastric reduction of Cr(VI) to the less permeable/bioavailable Cr(III) occurs efficiently in the stomach, and oral exposure to Cr(VI) would not result in toxicity or carcinogenicity [7]. However, the study concluded that even low, environmentally relevant doses of Cr(VI) escape reduction in the stomach [5, 7]. Also, the study provided clear evidence that Cr(VI) exposure by oral route is carcinogenic in the gastrointestinal tract [5, 7].

Dietary supplements that are formulated with chromium compounds such as chromium chelates, chromium picolinate, chromium nicotinate, and chromium chloride are intended to be safe. However trivalent chromium readily oxidizes to hexavalent chromium, which is highly toxic, carcinogenic, genotoxic, and an internationally regulated species. Because of this

dichotomy, it is important to ensure that the initial chromium raw material is not adulterated with Cr(VI). Also, dietary supplements contain a complex mixture of additional ingredients, such as various vitamins, minerals, ions, organic material, and coatings, which may lead to conversion of Cr(III) to Cr(VI). The final speciated form of chromium found in the finished product is kinetically dependent on processes that impact temperature, pH, and oxidizing/reducing potential of the supplement formulation. Therefore, the control of manufacturing processes, design of supplement formulations, and routine Cr(VI) analytical testing are imperative for maintaining the production of safe, chromium-containing finished products.

Dietary supplement safety is regulated by the United States Food and Drug Administration (FDA) under the Dietary Supplement Health and Education Act of 1994 (DSHEA), which amended the Federal Food, Drug and Cosmetic Act (FD&C Act) and significantly enhanced the framework for dietary supplement regulations [9]. Under United States law, dietary supplements are defined as food, with DSHEA further defining dietary supplements as products that supplement the diet and contain one or more of the following ingredients: vitamin, mineral, herb or botanical, amino acid, substance for supplementing dietary intake, metabolite, or concentrate/extract [9, 10]. DSHEA provided procedures for addressing product safety, regulations for labelling and health claims, and guidance for establishing Current Good Manufacturing Practices (CGMP). Also, the Office of Dietary Supplements (ODS) was established within the National Institutes of Health (NIH) [9]. In 2011, the Food Safety Modernization Act (FSMA) further amended the FD&C Act to provide the FDA with new enforcement tools and authority for mandatory recalls [9] Today, there is rapid growth of the multi-billion dollar dietary supplement industry, with evidence of increased risk from unsafe and adulterated products [9, 10]. To ensure the quality and safety of chromium-containing dietary

supplement products, manufactures should be compelled to adopt routine analytical testing and controls for hexavalent chromium.

Although analysis of total chromium concentrations may be routinely and accurately made, the nature of chromium speciation requires the use of an accurate analytical method that is capable of specific quantification of both Cr(III) and Cr(VI) to provide information that may be used to improve human health and safety. Analytical laboratories have found that the accurate measurement of chromium species in environmental, biological, dietary, and industrial samples is difficult when using traditional analytical methods. This is mainly due to the interconversion between the different chromium species during sample processing and instrumental analysis. Most analytical methods used for the determination of hexavalent chromium use alkaline extraction solutions. However, the alkaline solution may oxidize Cr(III), while the reverse transformation may occur during neutralization and acidification of the extraction. This is due to the correlation of the Cr(III)/Cr(VI) species distribution with the sample oxidation/reduction potential (Eh) and pH values. Trivalent chromium is thermodynamically stable under low Eh and low pH, while high Eh and high pH favor the existence of Cr(VI) [11, 12]. Also, sample matrix components, such as iron (II) and manganese (III, IV) hydroxides/oxides, play a role in the interconversion between Cr(III) and Cr(VI) [12]. The oxidation of Cr(III) is dependent on the chemical forms: Cr_2O_3 and aged $Cr(OH)_3$ are resistant to oxidation; Cr^{3+} and freshly precipitated $Cr(OH)_3$ are relatively easy to oxidize [13].

Analysis of speciated chromium in dietary supplement samples with traditional methods provide erroneous results since the batch formulations contain ingredients that promote chromium oxidation and reduction. Accurate determination of the concentrations and stabilities of the Cr(III) and Cr(VI) species therefore require a method that is capable of correcting for interconversion,

bias, and instrumental error. Molecular speciated isotope dilution mass spectrometry (SIDMS) is outlined in EPA Method 6800, and involves a novel technique that includes enriched, isotopically-labelled Cr(III) and Cr(VI) spikes in the sample preparations to correct for the Cr(III)/Cr(VI) interconversion during sample extraction and instrumental analysis [14]. The correction of species interconversion is accomplished by measuring changes in the Cr(III)/Cr(VI) isotope ratios to provide mathematical corrections to the calculations [14]. The addition of standards containing 50-Cr(III) and 53-Cr(VI) isotope species to a sample, which contains the more abundant, naturally-occurring 52-Cr(III) and 52-Cr(VI) species, ensures that any oxidative/reductive interconversions are quantifiable by measuring the final concentrations and oxidation states of the 50-Cr, 52-Cr, and 53-Cr isotopes. For example, ion chromatography (IC) may be used to separate the Cr(III) and Cr(VI) oxidative species into discretely eluting chromatographic peaks that are then analyzed by inductively coupled plasma mass spectroscopy (ICP-MS).

The ICP-MS detector is used to quantitate concentrations of the 50-Cr, 52-Cr, and 53-Cr isotopes in the eluting chromatographic peaks. The final 50/52-Cr(III), 53/52-Cr(III), 50/52-Cr(VI), and 53/52-Cr(VI) isotopic ratios are used to calculate the initial concentrations of Cr(III) and Cr(VI) in the original unaltered sample. The use of isotopically-labelled species with SIDMS eliminates the need for external calibration measurements. Traditional external calibration measurements introduce bias, shift, and uncertainty due to changes in the signal response with analyte concentration, unequal distribution of calibration levels, presence of outlier calibration data points, matrix bias, and instrumentation drift [15]. The calculation of the isotope ratios in each sample is intrinsic and does not rely on the use of a previously established measurement. Therefore, SIDMS provides measurements that are accurate and precise at trace

concentration levels and is a powerful technique that allows correction for Cr(III)/Cr(VI) species interconversions.

Traditional methods of speciation analysis use selective isolation and/or derivatization methods that stabilize and target a single chromium species [2, 16]. For example, EPA Method 7196A (Hexavalent Chromium by Colorimetry) involves the reaction of diphenylcarbazide with Cr(VI) to form a complex that is detected using UV-Vis spectrophotometry [17, 18]. However, these types of methods often have multiple interference, exhibit reduced sensitivity, and have low repeatability and legal defensibility since they do not measure species interconversion [2]. Analytical techniques that use on-line hyphenated techniques are capable of direct analysis of specific chromium species and do not rely on previously-separated fractions [2]. Reverse phase chromatography and ion chromatography are the most widely used separation methods [16]. Methods utilizing chromatography to provide separation of discrete chromium species can be coupled to mass spectrometry for element-specific detection with high selectivity and sensitivity. These methods are most often coupled to a highly sensitive, element-specific detector such as ICP-MS [2, 3, 6, 16]. ICP-MS instruments equipped with collision cell technology reduce interferences from polyatomic species, and newer triple quadrupole (QQQ) ICP instruments provide improved reduction of polyatomic interferences with even higher selectivity and specificity [2]. Furthermore, the use on-line hyphenated techniques with EPA Method 6800 allows for quantitation of specific chromium species with accuracy and precision.

Several methods have been developed that allow for extraction of chromium for dietary supplement samples. One approach uses EPA Method 3060A (Alkaline Digestion for Hexavalent Chromium), which was developed to address the deficiencies found with EPA Method 7196A and accounts for the possible Cr(III) oxidation [6, 19, 20]. This method utilizes a

hot alkaline digestion solution to quantitatively extract Cr(VI) from soluble, adsorbed, or precipitated forms of chromium compounds, while minimizing the interconversion of the chromium species [19, 20]. For samples that contain high concentrations of Cr(III), magnesium (Mg^{2+}) is added to suppress oxidation of Cr(III) to Cr(VI), and the majority of Cr(III) is precipitated out of solution as Cr(III) hydroxides. The addition of magnesium is optional when an analytical method is used that corrects for possible method induced chromium species interconversion. More recently, the use of additional complexing agents such as ethylenediaminetetraacetic acid (EDTA), ethylenediamine-N,N'-disuccinic acid (EDDS), 2,6-pyridinedicarboxylic acid (PDCA), or diethylenetriaminepentaacetic acid (DTPA) have shown improved extraction and stabilization of the chromium compounds [3, 21]. The use of EDTA extraction has been used to provide complexation with Cr(III) and allow chromatographic separation of both Cr(III) and Cr(VI) species [3, 21]. This approach supports the formation of a $[Cr(III)EDTA]^-$ complex, prevents oxidation of Cr(III) to Cr(VI) in solution, and extracts Cr(VI) as a soluble anionic species [3, 21].

A method was successfully developed that combines Direct Isotope Dilution Mass Spectrometry (D-IDMS), EPA Method 6800, microwave sample extraction, and ion chromatography-inductively coupled plasma mass spectrometry (IC-ICP-MS) to quantitatively determine the amount of hexavalent chromium in a range of dietary supplement sample formulations. The study determined if levels of Cr(VI) exceed the maximum allowable dose level (MADL) of 8.2 µg per day regulatory limit established by California Proposition 65 [22].

4.2 MATERIALS AND METHODS

4.2.1 SAMPLES

Twenty commercially-available multimineral/multivitamin dietary supplement formulations were obtained by the research laboratory from a variety of sources, such as retail pharmacies, department stores, grocery stores, and online marketplace ordering. The products were marketed for general supplementation, prenatal support, or men/women-specific nutritional supplementation. The dietary supplement products were solid dose tablets, solid dose caplets, and flavored gummies. In some cases, multiple lot numbers of the same supplement formulation were sampled. Products from multiple states were obtained, which for some dietary supplements, provided samples that had both different and identical lot numbers for testing. Assessment of NIST multivitamin/multielement tablets standard reference material (NIST SRM 3280) was used as a reference material during testing. To achieve homogeneity, fifteen (15) separate specimens from the bottle of each product were milled to a particle size of 300 μm using a Retsch knife mill equipped with titanium blades and a polycarbonate/polypropylene sample chamber. The samples were stored in closed polypropylene tubes and kept in a desiccator, until subsampled for analyses. Gummy supplements were left in the original sealed bottle and opened four days prior to analysis.

4.2.2 ANALYTICAL STANDARDS

Multivitamin/multielement tablets standard reference material (SRM) 3280 was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland). Potassium dichromate standard reference materials (SRM) 136e and 136f were purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland). Isotopically enriched trivalent chromium standard solution in 0.5% nitric acid [50-

Cr(III)], isotopically enriched hexavalent chromium standard solution in 0.1% ammonium hydroxide [53-Cr(VI)], natural trivalent chromium standard solution in 0.5% nitric acid [Nat-Cr(III)], natural hexavalent chromium standard solution in 0.1% ammonium hydroxide [Nat-Cr(VI)] were provided by Applied Isotope Technologies (AIT) (Pittsburgh, Pennsylvania). Concentrations of the chromium standards solutions are provided in Table 4.1. Instrument tuning standard solutions were purchased from Agilent Technologies (Santa Clara, California).

Table 4.1: Concentrations of chromium standard solutions.

Standard Solution	Batch Number	Lot Number	Concentration (µg/g)
50-Cr(III)	030129-04-A	CR10172008A	726.5679 ± 19.6097
50-Cr(III)	030129-04-A	CR04272010A	726.5679 ± 19.6097
53-Cr(VI)	ISO040803-06-B	CR608262004A	95.60 ± 2.99
53-Cr(VI)	SH1332	CR10172009A	10.1049 ± 0.3195
Nat-Cr(III)	139002	CR05182010A	9.7431
Nat-Cr(VI)	139002	R05182010A	9.1140

4.2.3 REAGENTS AND MATERIALS

Concentrated nitric acid (Aristar Plus, trace metal grade) and concentrated hydrochloric acid (Aristar Ultra, trace metal grade) were purchased from VWR Chemicals BDH (VWR International, Radnor, Pennsylvania). Hydrogen peroxide 30-32% (Aristar Ultra) was purchased from VWR Chemicals BDH (VWR International, Radnor, Pennsylvania). Ethylenediamine-tetraacetic acid (EDTA), trisodium salt dihydrate (99%) was purchased from Acros Organics (ThermoFisher Scientific, Waltham, Massachusetts). Type I ultrapure water (18.2 MΩ-cm) was produced using a Barnstead EASYpure II RF/UV filtration system (ThermoFisher Scientific, Waltham, Massachusetts) and/or Evoqua Water Technologies PURELAB Flex filtration system (Pittsburgh, Pennsylvania). Polypropylene (PP) centrifuge tubes with high-density polyethylene (HDPE) lids were purchased from Fisher Scientific (ThermoFisher Scientific, Waltham,

Massachusetts), VWR International (Radnor, Pennsylvania), and Globe Scientific Inc. (Mahwah, New Jersey).

4.2.4 INSTRUMENTATION

Analytical standards, reagents, and samples were prepared in a cleanroom laboratory environment that continuously recirculated laboratory air through a high-efficiency particulate air (HEPA) filtration system. Laminar flow benchtops and isolated hoods fitted with additional HEPA filtration systems were also utilized for preparation of standards and samples with trace-level analytes. Retsch Knife Mill Grindomix GM 200 (Haan, Germany) with titanium blades and a polycarbonate/polypropylene sample chamber was utilized for grinding and homogenizing dietary supplement samples to a final sample fineness of less than 300 µm. A Mettler Toledo XS105 Excellence (Columbus, Ohio) analytical balance was utilized with 0.01 mg precision. Samples were prepared using a Milestone ETHOS UP microwave digestion system (Sorisole, Bergamo, Italy) equipped with a MAXI-44 easy TEMP high-throughput rotor and modified polytetrafluoroethylene (PTFE-TFM) vessels of 100-mL capacity. A Mettler Toledo SevenCompact pH/Ion meter S220 equipped with an InLab Expert Pro-ISM PH probe (PN 30014096) and InLab Redox ORP probe (PN 51343200) was utilized to measure the sample pH, temperature, and Eh values. An Agilent Technologies 7700x inductively coupled plasma mass spectrometer (ICP-MS) (Santa Clara, California) was equipped with a micro-mist nebulizer, a quartz spray chamber, octopole reaction system (ORS^3), and a quadrupole mass analyzer. The instrument was autotuned prior to analysis using an instrument tuning standard solution from Agilent Technologies and automated startup sequence. For direct sample introduction, spectrum mode of analysis (ICP-MS) was utilized with an ASX-520 autosampler (CETAC Automation, Omaha, Nebraska) that was contained within an anti-contamination enclosure. Time-resolved

mode of analysis (IC-ICP-MS) was used for ion chromatography sample separations. A Metrohm 820 ion chromatography (IC) system (Herisau, Switzerland) was equipped with a Metrohm 858 Professional Sample Processor that was contained within an anti-contamination enclosure. The Metrohm ion chromatography system was metal free, with polyether ether ketone (PEEK) polymer material used for all connections, tubing, and column housing. The Metrohm 820 IC system was controlled using Metrohm IC Net 2.3, which was coupled to an independent Metrohm 850 Professional IC system running Metrohm MagicIC Net 3.1 to provide data communication and automation with the Agilent Technologies 7700x ICP-MS running MassHunter Workstation 4.2 software.

4.2.5 SAMPLE PREPARATION

4.2.5.1 Total Chromium Analysis

In order to determine the total chromium content of each batch of dietary supplement samples, sample decomposition was needed to ensure complete digestion of the sample matrix and solubility of the chromium analyte. EPA Method 3052, Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices, was used to rapidly produce sample digests that were suitable for analysis by ICP-MS [23]. EPA Method 6800, Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry, was used to quantitate the total elemental chromium concentrations of the digested samples [14]. The use of EPA Method 3052 as a sample preparation procedure ensured that the endogenous chromium isotopes of the sample were in equilibrium with those of the added isotopically enriched analytical chromium standard solutions. The final isotope ratios of the spiked sample digests were measured by ICP-MS according to EPA Method 6800 using IDMS calculations.

To prepare samples of dietary supplement tablets, fifteen (15) separate tablets were selected from the product bottle and weighed to determine the average mass per tablet. The tablets were milled at medium speed for one minute in reverse direction and two minutes in forward direction using a Retsch knife mill equipped with titanium blades and a polycarbonate/polypropylene sample chamber. The homogenized tablet material was transferred into an individually-labelled 50-mL polypropylene centrifuge tube, capped, and stored in a desiccator cabinet until subsampled. For samples of dietary supplement capsules, fifteen (15) separate capsules from the product bottle were selected and emptied into a tarred individually-labelled 50-mL polypropylene centrifuge tube and weighed to determine the average mass for the contents of one capsule. The centrifuge tube was capped and stored in a desiccator cabinet until subsampled. The gummy supplements were prepared by using a ceramic knife to subdivide the sample into the required aliquot at the time of sample preparation. Using weigh by difference, 0.5000 g of the sample was quantitatively transferred directly into a microwave digestion vessel. Using weigh by difference, the sample was then spiked by quantitatively adding 0.0500 g of 50-Cr(III) [726.5679 μg/g] into the microwave digestion vessel. Using a transfer pipet, 9.0 mL of concentrated nitric acid, 1.0 mL of concentrated hydrochloric acid, and 1.0 mL of hydrogen peroxide (30%) were added to the microwave vessel. A vented screw cap was used to securely tighten the lid onto the microwave vessel. The samples were shaken to ensure that the solid sample material was dispersed into the reagents. Mass bias samples were prepared using 0.1000 g of Nat-Cr(III) [9.7431 μg/g] and the digestion reagents. To prepare an analytical blank, 0.0500 g of 50-Cr(III) [726.5679 μg/g] was transferred into a microwave digestion vessel. Once 9.0 mL of concentrated nitric acid, 1.0 mL of concentrated hydrochloric acid, and 1.0 mL of hydrogen peroxide (30%) were transferred into a tarred quartz weigh bottle and massed, the reagents were

transferred into the microwave digestion vessel. The mass of the empty weigh bottle was then recorded. The microwave vessels were loaded into MAXI-44 easy TEMP high-throughput rotor, placed into the Milestone ETHOS UP microwave digestion system, and processed at 180°C for 9.5 minutes with a 5.5-minute ramp at 1800 watts.

Once the samples cooled to ambient temperature, each microwave vessel was individually opened in a fume hood, and the digested sample was transferred into a labeled polypropylene 15-mL centrifuge tube and capped. The samples were held overnight at ambient temperature. The samples were centrifuged for 30 minutes at 3300 rpm. For each sample, 1.0 mL of the supernatant was transferred into a labelled polypropylene 50-mL centrifuge tube, brought to 20 mL with 18.2 MΩ-cm water, capped, and inverted ten times to mix. The diluted solutions were analyzed by ICP-MS using EPA Method 6800.

4.2.5.2 Speciated Hexavalent Chromium Analysis

To determine the speciated hexavalent chromium content of each batch of the dietary supplement samples, it is necessary to extract Cr(VI) from the matrix material and account for any chromium species interconversion that may occur during sample processing. Without appropriate methodology, experimentally determined concentrations of Cr(III) and Cr(VI) may differ from than the actual concentrations of the species in the indigenous sample since oxidation and reduction of chromium may be promoted by the laboratory reagents and measurement techniques. A method that uses a hot alkaline digestion solution of 50 mM EDTA to quantitatively extract Cr(VI) from the sample material was selected for speciated chromium analysis. The high pH extraction solution supports the extraction of Cr(VI) as a soluble chromate anion (CrO_4^{2-}) and formation of a $[Cr(III)EDTA]^-$ complex. The complexing of Cr(III) with EDTA prevents oxidation of Cr(III) compounds to Cr(VI) [24]. Also, EDTA complexes with

other metals that may be present in the dietary supplement sample matrix to form insoluble complexes [24]. EPA Method 6800, Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry, was used to quantitate the speciated hexavalent chromium concentrations of the digested samples [14]. By chromatographically separating the Cr(III) peak as $Cr(EDTA)^-$ and the Cr(VI) peak as CrO_4^{2-}, the final isotope ratios of the spiked sample digests were measured by IC-ICP-MS. The concentration Cr(VI) in the indigenous sample was quantitated according to EPA Method 6800 using IDMS calculations.

Using weigh by difference, 0.2500 g of the sample was quantitatively transferred directly into a microwave digestion vessel. Using weigh by difference, the sample was then spiked by quantitatively adding 0.0150 g of 50-Cr(III) [726.5679 μg/g] and 0.0600 g of 53-Cr(VI) [95.60 μg/g] into the microwave digestion vessel. Using a transfer pipet, 10 mL of 50 mM EDTA extraction solution was added to the microwave digestion vessel. A vented screw cap was used to securely tighten the lid onto the microwave vessel. The samples were shaken to ensure that the solid sample material was dispersed into the reagent. Mass bias samples were prepared using 0.0600 g of Nat-Cr(III) [9.7431 μg/g], 0.0600 g of Nat-Cr(VI) [9.1140 μg/g], and the extraction reagent. To prepare an analytical blank, 0.0150 g of 50-Cr(III) [726.5679 μg/g] and 0.0600 g of 53-Cr(VI) [95.60 μg/g] were transferred into a microwave digestion vessel. Once 10 mL of extraction solution was transferred into a tarred quartz weigh bottle and massed, the reagent was transferred into the microwave digestion vessel. The mass of the empty weigh bottle was then recorded. The microwave vessels were loaded into MAXI-44 easy TEMP high-throughput rotor, placed into the Milestone ETHOS UP microwave digestion system, and processed for ten minutes at 95°C with a 5-minute ramp at 1200 watts. Once the samples cooled to ambient temperature, each microwave vessel was individually opened in a fume hood, and the extracted

sample was transferred into a labeled polypropylene 15-mL centrifuge tube and capped. The samples were held overnight at ambient temperature. The samples were centrifuged for 30 minutes at 3300 rpm, or until the solid and liquid are well separated. For each sample, the supernatant was completely transferred into an individually labeled polypropylene 50-mL centrifuge tube, brought to 35 mL with 18.2 MΩ-cm water, capped, and inverted ten times to mix. The diluted solutions were analyzed by IC-ICP-MS using EPA Method 6800.

4.2.6 INSTRUMENT METHODS

The samples for total chromium analysis were placed into an enclosed autosampler for direct sample introduction. The Agilent Technologies 7700x ICP-MS was set to spectrum mode of analysis (ICP-MS) and tuned with an automated startup sequence using an instrument tuning standard solution from Agilent Technologies. Table 4.2 provides tune settings that resulted from a typical autotune routine, which were used as the instrument parameters for total chromium analysis. For speciated chromium analysis, samples were placed into the enclosed autosampler for ion chromatography separation. The Metrohm 820 ion chromatography (IC) system was equipped with a set of Metrohm Metrosep A Supp 5 PEEK analytical and guard columns. An isocratic flow of a 2 mM EDTA solution at ambient temperature is used as the mobile phase for these columns and provides an anion exchange chromatographic separation mechanism. Table 4.3 provides details about the chromatographic system setup, including additional information about the column and mobile phase eluent. The Agilent Technologies 7700x ICP-MS was set to time-resolved mode of analysis (IC-ICP-MS) and tuned with an automated startup sequence using an instrument tuning standard solution from Agilent Technologies. Table 4.4 provides tune settings that resulted from a typical autotune routine, which were used for the instrument parameters for speciated chromium analysis.

Table 4.2: Agilent Technologies 7700x ICP-MS autotune settings for total chromium analysis by EPA Method 3052 and EPA Method 6800.

		Typical Autotune Parameters:	
RF power	1500 W		
RF matching	1.80 V	Extract 1	-125.0 V
Sampling depth	8.0 mm	Extract 2	-195.0 V
Carrier gas (Ar) flow	0.95 L min^{-1}	Omega bias	-85 V
Dilution gas (Ar) flow	0.15 L min^{-1}	Omega lens	4.4 V
ORS^3 gas (He) flow	5.0 mL min^{-1}	OctP bias	-18.0 V
Spray chamber temperature	2 °C	OctP RF	200 V
Data acquisition mode	Spectrum	Energy discrimination	4.0 V
Isotope monitored	^{50}Cr, ^{52}Cr, ^{53}Cr, ^{54}Cr		
Peak pattern	20 points/mass		
Replicates	5		
Sweeps/replicate	1000		
Integration time/mass	2 seconds		
Nebulizer pump	0.10 rps		
Sample uptake	60 seconds		
Stabilization	30 seconds		

Table 4.3: Metrohm 820 Ion Chromatography Separation Center settings for speciated chromium analysis by 50 mM EDTA extraction and EPA Method 6800.

Column	Metrosep A Supp 5 PEEK column (Metrohm) containing polyvinyl alcohol with quaternary ammonium groups, 250 x 4.0 mm, 5 µm particle size, pH range 3 to 12; with Metrosep A Supp 5 guard column (5 x 4.0 mm, 5 µm particle size)
Mobile Phase	2 mmol L^{-1} EDTA in ultrapure water, pH 10 adjusted using ammonium hydroxide
Elution Mode	Isocratic
Flow Rate	0.8 mL min^{-1}
Column Temperature	Ambient
Injection Volume	100 µL

Table 4.4: Agilent Technologies 7700x ICP-MS autotune settings for speciated chromium analysis by 50 mM EDTA extraction and EPA Method 6800.

		Typical Autotune Parameters:	
RF power	1500 W		
RF matching	1.80 V	Extract 1	-125.0 V
Sampling depth	8.0 mm	Extract 2	-195.0 V
Carrier gas (Ar) flow	0.95 L min^{-1}	Omega bias	-85 V
Dilution gas (Ar) flow	0.15 L min^{-1}	Omega lens	4.4 V
ORS^3 gas (He) flow	5.0 mL min^{-1}	OctP bias	-18.0 V
Spray chamber temperature	2 °C	OctP RF	200 V
Data acquisition mode	Time resolved analysis	Energy discrimination	4.0 V
Isotope monitored	^{50}Cr, ^{52}Cr, ^{53}Cr, ^{54}Cr		
Integration time/mass	0.25 seconds		
Sampling period	1.006 sec		
Nebulizer pump	0.50 rps		

Analysis by ICP-MS is associated with interferences caused by atomic or molecular ions that have the same mass to charge ratio as the analyte [25]. In some cases, current software is capable of correcting for atomic isobaric interferences that occur when isotopes from two different elements have overlapping masses [25]. Yet, polyatomic interferences are ions that have the same mass as the analyte isotopes, but are generated by precursors from the sample matrix, reagents, plasma gases, and atmospheric gases [25]. However for ICP-quadrupole MS, the use of a helium collision gas in an enclosed cell immediately before the quadrupole is one of the most popular methods for reducing polyatomic inferences [26]. An experiment was performed to determine which helium collision cell gas flow rates provide optimal reduction of polyatomic interference for chromium analysis. A 2 mM EDTA solution was prepared for this experiment since the EDTA molecule provides a source of interfering carbon, nitrogen, and oxygen atoms. The results of this experiment are presented in Chapter 3 - Figure 3.1, which indicate that the helium flow rate is optimized at 5.0 mL/min or higher since the interference ion count for all chromium isotopes approach zero.

4.2.7 METHOD VALIDATION

Method validation was performed for the quantitation of total chromium by ICP-MS (sample preparation outlined in section 4.2.5.1) and speciated hexavalent chromium by IC-ICP-MS (sample preparation outlined in section 4.2.5.2), using the instrument parameters provided in Table 4.2, Table 4.3 and Table 4.4. For both methods, the following method validation parameters were evaluated: accuracy, precision, linearity, limit of detection (LOD), and limit of quantitation (LOQ). Method validation for speciated hexavalent chromium includes selectivity and specificity through analysis of the chromatographic peak separation and resolution.

4.2.7.1 Total Chromium Analysis

To perform method validation for total chromium analysis, NIST 136e Potassium Dichromate Standard Reference Material was used to prepare five standard solutions with total chromium theoretical concentrations at 3.6 µg/g, 15.3 µg/g, 74.0 µg/g, 297.6 µg/g, and 1389.4 µg/g in 18.2 MΩ-cm water (0.1% ammonium hydroxide). EPA Method 3052 (Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices) was utilized to prepare the standard solutions. EPA Method 6800 (Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry) was used to quantitate the total elemental chromium concentrations of the digested validation standard solutions according to IDMS calculations.

The results of the method validation experiments for total chromium analysis are outlined in Chapter 3 – Section 3.2.7.1. In summary, the method validation standard recoveries ranged from 87.2% to 104.0%, which indicates greater than ± 13% accuracy for this concentration range. The method precision ranged from 0.297% to 0.962% relative standard deviation. The method was determined to be linear throughout the validation concentration range since the correlation coefficient was close to 1 (0.9999). The LOD was statistically determined to be 0.0017 µg/g and the LOQ was statistically determined to be 0.0031 µg/g. However, the LOQ was empirically measured during the accuracy method validation at 3.6 µg/g.

4.2.7.2 Speciated Hexavalent Chromium Analysis

To perform method validation for speciated chromium analysis, NIST 136e Potassium Dichromate Standard Reference Material was used to prepare six standard solutions with hexavalent chromium theoretical concentrations at 0.9092 µg/g, 3.6965 µg/g, 15.886 µg/g, 67.431 µg/g, 273.82 µg/g and 1210.8 µg/g in 18.2 MΩ-cm water (0.1% ammonium hydroxide). To prepare the standard solutions, a 50 mM EDTA alkaline solution was utilized to extract the

standard solutions. EPA Method 6800 (Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry) was used to quantitate the speciated hexavalent chromium concentrations of the digested validation standard solutions according to SIDMS calculations. In order to validate the method for selectivity and specificity, the separation of Cr(III) and Cr(VI) was examined by sampling standard solutions that contained Nat-Cr(III) at 9.7431 µg/g and Nat-Cr(VI) at 9.1140 µg/g. The resulting sample was not spiked with isotope standards; however, it was processed with a 50 mM EDTA extraction solution. The prepared standards and specificity sample were analyzed using IC-ICP-MS and a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase.

The selectivity and specificity of the method for Cr(III) and Cr(VI) was validated using a natural chromium solution. An example chromatogram is provided in Figure 4.1, which indicates the complete separation of the $[Cr(III)EDTA]^-$ and $[Cr(VI)O_4]^{2-}$ species. The three major isotopes of chromium (50-Cr, 52-Cr, 53-Cr) are shown and correspond to the expected isotopic distribution of a natural chromium sample. The retention time for Cr(III) was found to be approximately 1.5 minutes and the retention time for Cr(VI) was found to be approximately 4.3 minutes. In Table 4.5, the percent recoveries of the standard solutions support validation of the method accuracy and precision. The percent recovery of each standard solution is calculated using the following formula:

$$\text{Percent Recovery} = \frac{(\text{Experimental Concentration})}{(\text{Theoretical Concentration})} \times 100$$

The method validation standard recoveries range from 90.8% to 112.1% and indicate that the method has greater than ± 12% accuracy for this concentration range. The calculated percent difference in recoveries are shown in Figure 4.2, which were calculated according to the following equation:

$$\text{Percent Difference Recovery} = \frac{(\text{Experimental Concentration - Theoretical Concentration})}{(\text{Theoretical Concentration})} \times 100$$

The percent difference in recoveries provide an additional indicator of method accuracy and range from -9.2% to +12.1%. Method precision is evaluated using the resulting 95% CI (n = 12) values and percent relative standard deviation (%RSD) for the standard solutions. The method precision ranges from 0.434% to 4.094% relative standard deviation. Although traditional calibration curve quantitation is not utilized for EPA Method 6800 methodology, an assessment of method linearity was performed as part of the method validation.

After generating a scatterplot that correlates the calculated experimentally determined concentration and theoretical concentration of each standard solution, a linear regression equation was generated for the data set with a reported R^2 value. Since the correlation coefficient was close to 1 (0.9998), it indicates that the method is linear throughout the validation concentration range. The results of the linearity method validation are provided in Figure 4.3. Limit of detection (LOD) is the lowest possible concentration that can be measured reliably. The results of the statistical determination of both the LOD and LOQ for this method are summarized in Table 4.6. The LOD was statistically determined to be 0.0029 µg/g and the LOQ was statistically determined to be 0.0046 µg/g. However, the LOQ was empirically measured during the accuracy method validation at 0.9092 µg/g.

Figure 4.1: Method validation results for selectivity and specificity of the speciated Cr(VI) analysis. A validation standard solution was prepared using solutions that contained Nat-Cr(III) at 9.7431 µg/g and Nat-Cr(VI) at 9.1140 µg/g. A 50 mM EDTA alkaline extraction solution was used to extract the standard solutions. The prepared solutions were analyzed using IC-ICP-MS and a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase. The resulting chromatogram indicates complete separation of the $[Cr(III)EDTA]^-$ and $[Cr(VI)O_4]^{2-}$ species.

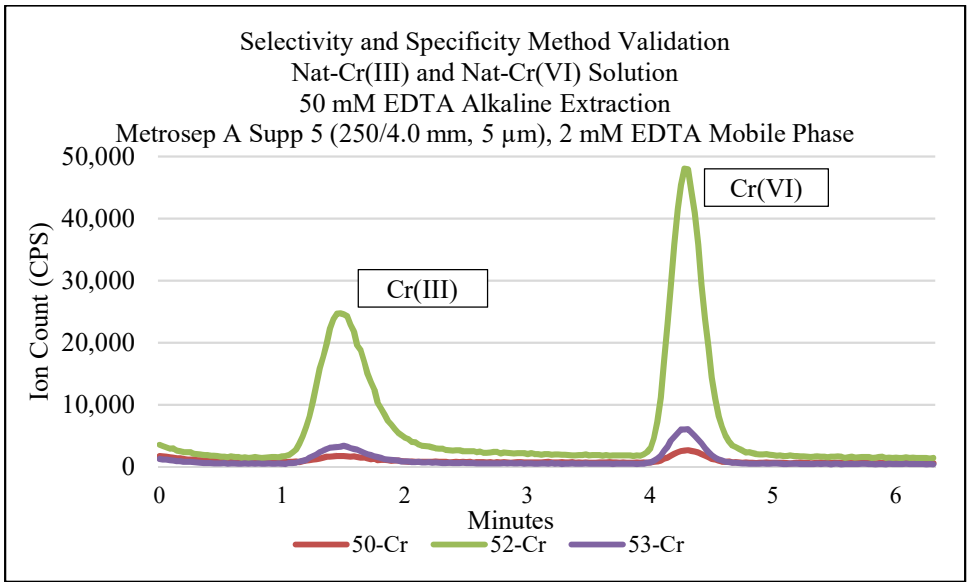


Table 4.5: Accuracy and precision method validation results for speciated Cr(VI) analysis. Six validation standard solutions were prepared using NIST Standard Reference Material 136e in 0.1% ammonium hydroxide with hexavalent chromium theoretical concentrations ranging from approximately 1.0 µg/g to 1200 µg/g. A 50 mM EDTA alkaline extraction solution was used to extract the standard solutions. EPA Method 6800 was used to quantitate the speciated Cr(VI) concentrations of the extracted validation standard solutions. The prepared standards were analyzed using IC-ICP-MS. The percent recoveries of each standard solution are provided to support validation of the method accuracy. The resulting 95% CI (n = 12) and %RSD values for the standard solutions are provided to support validation of the method precision.

Accuracy and Precision Method Validation Speciated SIDMS Hexavalent Chromium Analysis 50 mM EDTA Alkaline Extraction (n = 12, 95% CI)				
Theoretical Cr(VI)	SIDMS Results			Percent Recovery
	Speciated Cr	95% CI	Percent RSD	
µg/g	*µg/g*	*µg/g*	*%*	*%*
1210.8	1266.9	14.16	1.759	104.6
273.82	304.43	1.200	0.621	111.2
67.431	75.563	0.420	0.875	112.1
15.886	14.864	0.041	0.434	93.6
3.6965	3.3578	0.020	0.954	90.8
0.9092	1.0164	0.028	4.094	111.8

Figure 4.2: Percent difference recovery method validation results for speciated Cr(VI) analysis. Six validation standard solutions were prepared using NIST Standard Reference Material 136e in 0.1% ammonium hydroxide with hexavalent chromium theoretical concentrations ranging from approximately 1.0 µg/g to 1200 µg/g. A 50 mM EDTA alkaline extraction solution was used to extract the standard solutions. EPA Method 6800 was used to quantitate the speciated Cr(VI) concentrations of the digested validation standard solutions. The prepared standards were analyzed using IC-ICP-MS. The calculated percent difference in recoveries are shown, which indicate method accuracy.

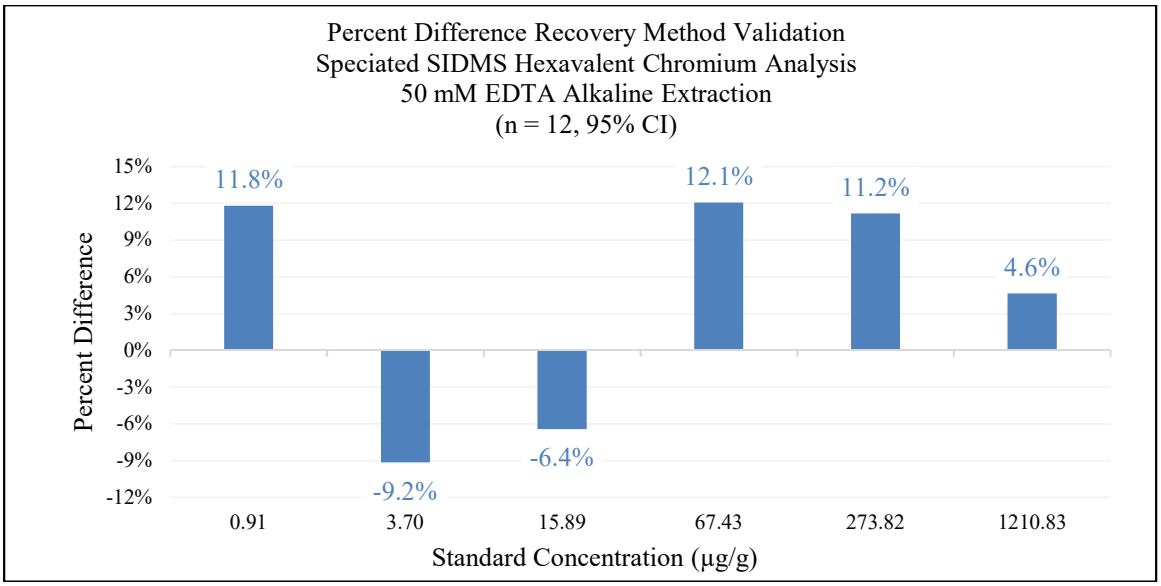


Figure 4.3: Linearly method validation for speciated Cr(VI) analysis. Six validation standards were prepared using NIST Standard Reference Material 136e in 0.1% ammonium hydroxide with Cr(VI) theoretical concentrations ranging from approximately 1.0 µg/g to 1200 µg/g. A 50 mM EDTA alkaline extraction solution was used to extract the standard solutions. EPA Method 6800 was used to quantitate the speciated Cr(VI) concentrations of the digested validation standard solutions. The standards were analyzed using IC-ICP-MS. Linearity is shown with the R^2 value of 0.9998. The 95% CI (n = 12) error bars are not shown since they are not significant in this figure.

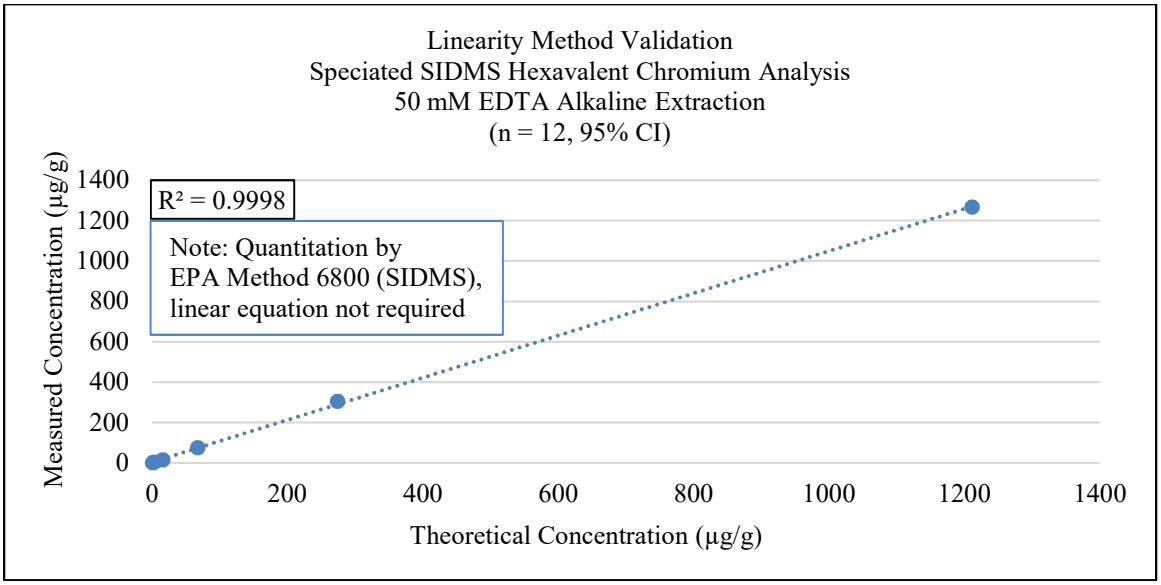


Table 4.6: Statistically determined limit of detection (LOD) and limit of quantitation (LOQ) method validation results for speciated Cr(VI) analysis. Blank solutions were prepared without chromium analyte and processed with a 50 mM EDTA alkaline extraction solution. EPA Method 6800 was used to quantitate the speciated chromium concentrations of the digested blank solutions. The prepared solutions were analyzed using IC-ICP-MS. The LOD and LOQ concentrations were statistically derived from the standard deviation (SD) of the blank mean (n = 12). The LOQ was empirically measured during the accuracy method validation at 0.9092 µg/g.

Blank Determinations Hexavalent Chromium (n = 12)			Limit of Detection	Limit of Quantitation
Average	SD	95% CI	mean + 3(SD)	mean + 10(SD)
µg/g	µg/g	µg/g	µg/g	µg/g
0.0022	0.0002	0.0002	0.0029	0.0046

4.3 RESULTS AND DISCUSSION

Method validation experiments performed with chromium standard solutions prepared from NIST SRM 136e indicate that the optimized methods developed for total chromium and speciated hexavalent chromium analysis are accurate and precise. Also, the validation indicates chromium quantitation by EPA Method 6800 (IDMS and SIDMS) provides a linear fit when the resulting calculated concentrations are compared to the corresponding theoretical concentrations of the validation standard solutions. The validated limit of quantitation provides confidence that the lowest concentrations of chromium are quantitated with accuracy. Method validation work for the speciated chromium analytical method shows specificity and selectivity for both Cr(III) and Cr(VI) species. As such, the methods were determined to be suitable to use for quantitation of total chromium and speciated hexavalent chromium concentrations in dietary supplement samples. Multiple chromium-containing dietary supplement brands, each with unique formulations, were tested for total chromium and hexavalent chromium content.

A mass bias standard solution was prepared for each analysis using both Nat-Cr(III) [9.7431 µg/g] and Nat-Cr(VI) [9.1140 µg/g] and was analyzed at the beginning and end of each sample set in replicate injections. The data was used to determine and mathematically correct method and/or instrument bias that resulted in a deviation from the theoretical isotope fraction

distribution of natural chromium. This mathematical correction was applied to the data before performing EPA Method 6800 concentration calculations. Also, multiple replicate preparations of the reagent blank were analyzed for each sample set and the resulting calculated chromium concentrations subtracted from the determined sample concentrations. The analytical blank concentrations were routinely found to be less than 10 ppb and below the empirically validated limit of quantitation. Data was collected using Agilent Technologies MassHunter Workstation software and exported to Microsoft Excel for further processing and statistical workup.

4.3.1 TOTAL CHROMIUM ANALYSIS

The quantitation of total chromium in twenty independent dietary supplement formulations was performed according to EPA Method 3052 and EPA Method 6800 by ICP-MS (sample preparation outlined in section 4.2.5.1) using the instrument parameters provided in Table 4.2. All sample formulations were multimineral/multivitamin dietary supplements, which were marketed for general supplementation, prenatal support, or men/women-specific nutritional supplementation. The dietary supplements were provided as solid dose tablets, solid dose caplets, and flavored gummies. Assessment of the method suitability was provided by the analysis of NIST multivitamin/multielement tablets standard reference material (NIST SRM 3280). An aliquot from each individual formulation was subsampled three times and analyzed with four replicate measurements (n = 12). The results of the total chromium analysis are summarized in Table 4.7. The table provides a description of the supplement unit form, average unit mass, daily serving size as number of units, total chromium claimed on the bottle label, total chromium found (μg/g) with 95% confidence interval, total chromium found (μg/daily serving size) with 95% confidence interval, and percent difference between the labelled chromium content and the experimentally determined chromium content. A graphical representation of the

data is provided in Figure 4.4. This figure compares the total chromium content that was determined using the experimental analytical methodology and the chromium content as provided by the product label. The experimentally determined chromium values are provided with error bars that indicate the 95% confidence intervals.

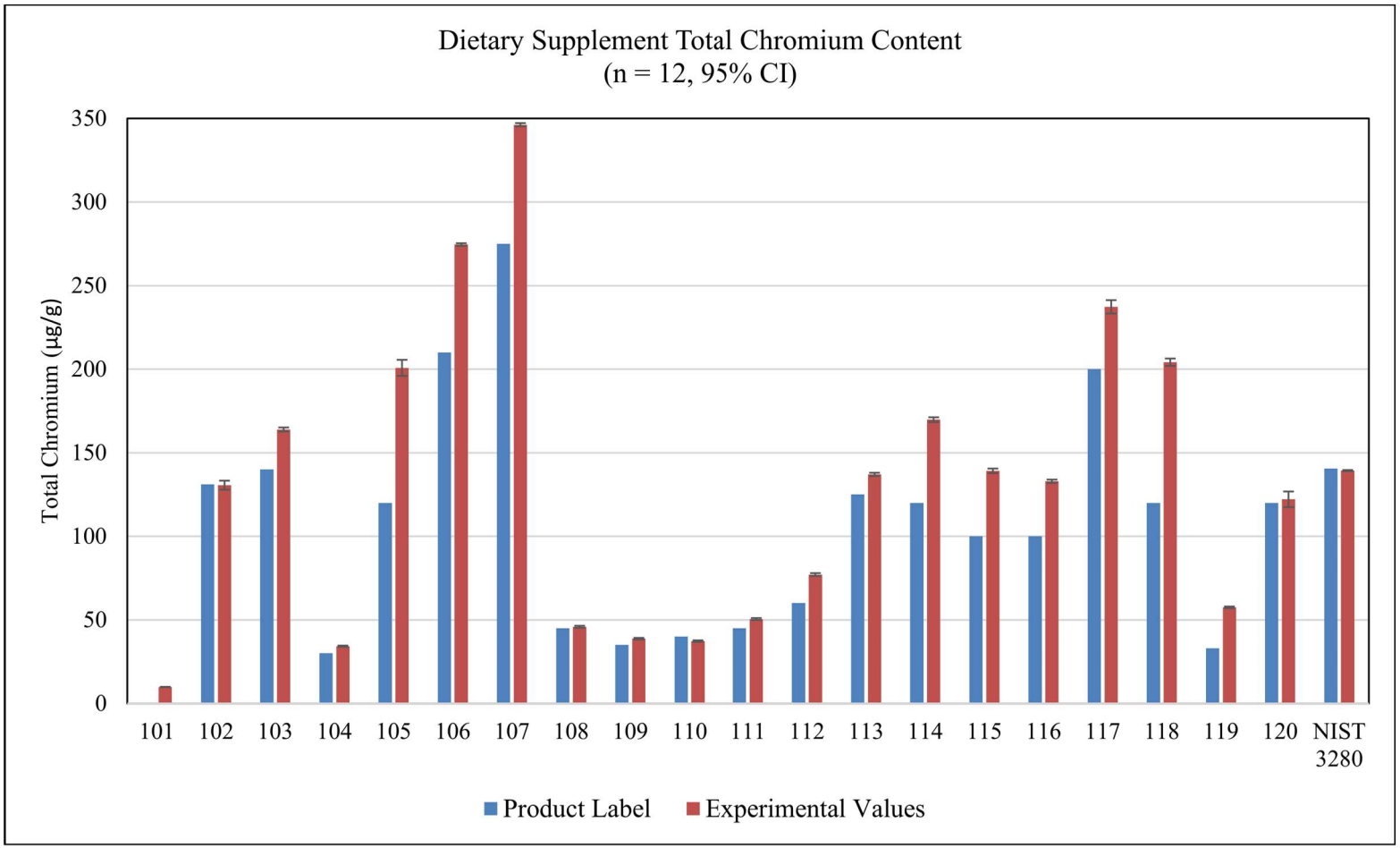
The NIST SRM 3280 formulation was found to have an average total chromium concentration of 92.89 ± 0.2 μg/g (139.34 ± 0.3 μg/serving size). When compared to the certified total chromium value (140.55 ± 2.7 μg/serving size), the experimentally determined chromium concentration difference was -0.9% (99.1% certified value recovery). This recovery indicates system suitability for determination of total chromium in the remaining supplement formulations. The experimentally determined total chromium content for several formulations provided close agreement with the values provided by the product labels. For example, the experimentally determined chromium concentrations for products 108 and 120 were within 2% agreement of their respective labelled value. Yet, considerable difference was found for many of the formulations. The total chromium content for product 119 was found to be nearly 75% different than the labelled amount (175% recovery). Also, approximately 10 μg/g of chromium was found in product 101, even though chromium was not included on the bottle label. When percent differences for the twenty formulations were averaged, the experimentally determined total chromium found in the supplements was 24.0% different than the labelled amounts (124% recovery). Since the analytical methodology provided 99.1% of total chromium in NIST SRM 3280, the method is appropriate for total chromium quantitation. Furthermore, previously published studies have found that the amounts of dietary supplement components varied between 8% to 177% of the declared labelled values [10, 27]. These results indicate that many dietary supplement manufactures do not have sufficient control of the total chromium content of their

formulations. Improved manufacturing practices and product quality control testing would help ensure that consumers are not exposed to unexpected concentrations of elemental supplementation.

Table 4.7: Total chromium analysis of twenty independent dietary supplement formulations. Assessment of the method suitability was provided by the analysis of NIST multivitamin/multielement tablets standard reference material (NIST SRM 3280). For each formulation, fifteen (15) units were subsampled and homogenized, along with determination of the average unit mass. Capsule products were subsampled, emptied, and mixed to provide a representative sample of the capsule contents. The samples were prepared in triplicate using EPA Method 3052 and analyzed with ICP-MS according to EPA Method 6800 (IDMS), with four replicate measurements for each sample (n = 12). The 95% confidence interval is provided for each assessment. The percent difference compares the labeled claim and experimentally determined chromium concentration.

Product ID	Description	Unit Mass (g)	Daily Serving Size (units)	Cr-Total Claimed (µg/daily size)	Cr-Total Found (µg/g)	Cr-Total Found (µg/daily size)	Difference (%)
NIST 3280	Tablet	1.50	1	140.55 ± 2.7	92.89 ± 0.2	139.34 ± 0.3	-0.9
101	Tablet	2.24	1	0	4.4 ± 0.1	9.9 ± 0.1	NA
102	Tablet	1.34	1	131	97.7 ± 2.0	130.6 ± 2.7	-0.3
103	Capsule	1.02	1	140	159.7 ± 1.2	163.3 ± 1.2	16.6
104	Tablet	1.57	1	30	21.8 ± 0.3	34.3 ± 0.4	14.3
105	Tablet	1.27	3	120	52.7 ± 1.3	200.2 ± 4.8	66.8
106	Capsule	0.45	1	210	616.0 ± 1.7	274.6 ± 0.8	30.8
107	Tablet	1.69	1	275	204.7 ± 23.9	346.2 ± 4.1	25.9
108	Caplet	1.56	2	45	14.7 ± 0.2	45.9 ± 0.6	2.0
109	Tablet	1.29	1	35	30.1 ± 1.4	38.7 ± 1.8	10.7
110	Tablet	1.26	1	40	29.5 ± 0.2	37.3 ± 0.3	-6.8
111	Caplet	1.53	2	45	16.5 ± 0.2	50.3 ± 0.6	11.9
112	Tablet	1.74	1	60	44.3 ± 0.5	77.1 ± 0.9	28.4
113	Capsule	0.87	1	125	157.8 ± 3.8	137 ± 3.3	9.6
114	Caplet	1.50	2	120	56.6 ± 0.5	170.2 ± 1.4	41.8
115	Tablet	1.04	3	100	44.6 ± 0.4	139.0 ± 1.4	39.0
116	Capsule	0.86	1	100	154.3 ± 25.4	133 ± 2.2	33.0
117	Tablet	1.28	6	200	30.9 ± 0.5	237.6 ± 4.0	18.8
118	Gummy	2.49	2	120	41.0 ± 0.5	204.1 ± 2.2	70.1
119	Capsule	0.79	1	33	72.7 ± 7.3	57.5 ± 5.8	74.3
120	Tablet	1.97	1	120	62.0 ± 2.4	122.1 ± 4.7	1.7

Figure 4.4: Graphical comparison of total chromium analysis of twenty independent dietary supplement formulations. Assessment of the method suitability was provided by the analysis of NIST multivitamin/ multielement tablets standard reference material (NIST SRM 3280). For each formulation, fifteen (15) units were subsampled and homogenized, along with determination of the average unit mass. Capsule products were subsampled, emptied, and mixed to provide a representative sample of the capsule contents. Samples were prepared in triplicate using EPA Method 3052 and analyzed with ICP-MS according to EPA Method 6800 (IDMS), with four replicate measurements for each sample (n = 12). The 95% confidence interval is provided for each assessment. The percent difference compares labeled claim and the experimentally determined chromium.



4.3.2 SPECIATED HEXAVALENT CHROMIUM ANALYSIS

The quantitation of speciated hexavalent chromium in the twenty independent dietary supplement formulations was performed using a 50 mM EDTA alkaline extraction solution and EPA Method 6800 by IC-ICP-MS (sample preparation outlined in section 4.2.5.2) using the instrument parameters provided in Table 4.3 and Table 4.4. A Metrosep A Supp 5 PEEK ion chromatography column (Metrohm) containing polyvinyl alcohol with quaternary ammonium groups, 250 x 4.0 mm, 5 µm particle size, pH range 3 to 12 with Metrosep A Supp 5 guard column (5 x 4.0 mm, 5 µm particle size) were used for the speciated chromium analysis. An aliquot of the homogenized formulation was subsampled three times and analyzed with four replicate measurements (n = 12). The results of the speciated hexavalent chromium analysis are summarized in Table 4.8. The table provides a description of the supplement unit form, average unit mass, daily serving size as number of units, total chromium claimed on the bottle label, hexavalent chromium found (µg/g) with 95% confidence interval, hexavalent chromium found (µg/daily serving size) with 95% confidence interval, and the percent of the experimentally determined chromium content corresponding to hexavalent chromium. A graphical representation of the data is provided in Figure 4.5. This figure compares the speciated chromium content that was determined using the experimental analytical methodology and the chromium content as provided by the product label. The speciated chromium values are provided as their representative fractions of the experimentally determined total chromium content.

Thirteen formulations were found to contain hexavalent chromium, with concentrations that ranged from approximately 4.22 µg/daily size to 107.17 µg/g. Twelve samples were found to have hexavalent chromium levels that exceed the maximum allowable dose level (MADL) of

8.2 µg per day established by California Proposition 65 [22]. Four manufacturers produced the hexavalent chromium-containing products, and seven of the formulations were marketed for prenatal support. The use of these prenatal support consumer products would result in both mother and child being chronically exposed to an established genotoxic and carcinogenic substance. The hexavalent chromium concentrations in five of the formulations were greater than 50% of the measured total chromium content, with one formulation having approximately 90% hexavalent chromium content.

For the purposes of this study, the trivalent chromium concentrations of the dietary supplements represented in Figure 4.5 were calculated by subtracting the hexavalent chromium concentrations determined using IDMS equations from the experimentally determined total chromium content. This approach was taken since the use of SIDMS equations produced large, negative trivalent chromium values. If the solid trivalent chromium in the dietary supplement formulations was not readily soluble in the alkaline extraction solution, then equilibrium with the aqueous trivalent isotope spike standard solution may not have occurred completely. When the 50 mM EDTA extraction solution was introduced to the sample spiked with the aqueous 50-Cr(III) isotope standard, it is likely that the 50-Cr(III) was immediately chelated with the aqueous EDTA solution. Therefore, the 50-Cr(III) formed a disproportionately low amount of solid 50-Cr(III) precipitate when compared to the insoluble Nat-Cr(III) of the sample. This would generate a system that would produce a large negative bias for trivalent chromium when the sample extracts were analyzed using IC-ICP-MS. As a result, the chromatographic data for Cr(III) was not processed using SIDMS equations. The apparent lack of equilibrium between the 50-Cr(III) from aqueous standard solution and the solid indigenous Nat-Cr(III) of the sample also prevented accurate quantitation of trivalent chromium in the solid residues by mass balance

calculations, since there would be a disproportionately large amount of 52-Cr(III) in the solid residue samples. This accounts for the extremely large Cr(III) concentrations found when mass balance calculations were attempted on the speciated sample precipitate residues using EPA Method 3052 with EPA Method 3800. To further investigate the role that the sample formulations had on the sample processing and species equilibrium, a Mettler Toledo SevenCompact pH/Ion meter S220 equipped with an InLab Expert Pro-ISM pH probe and InLab Redox ORP probe to measure the sample pH, temperature, and Eh values. These values are compared to Eh-pH diagram references found in literature to predict the most probable, thermodynamically stable chromium species in the sample preparations in order to provide insight into the expected solution chemistry. The results from this experiment are superimposed onto Eh-pH diagrams found in literature and provided in Figure 4.6. The stability diagrams predict the formation of a solid Cr_2O_3 species, which would promote the stabilization of the solid Cr(III) in the samples.

Table 4.8: Speciated chromium analysis of twenty independent dietary supplement formulations. For each formulation, fifteen (15) units were subsampled and homogenized, along with determination of the average unit mass. Capsule products were subsampled, emptied, and mixed to provide a representative sample of the capsule contents. The samples were prepared in triplicate using a 50 mM EDTA alkaline extraction and analyzed with IC-ICP-MS according to EPA Method 6800 (IDMS) using a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase. Each sample was prepared in triplicate with four replicate measurements (n = 12). The 95% confidence interval is provided for each assessment. The Cr(VI) content (%) indicates the percentage of experimentally determined chromium that is hexavalent chromium. ND = not detected; NA = not applicable.

Product ID	Description	Unit Mass (g)	Daily Serving Size (units)	Cr-Total Claimed (µg/daily size)	Cr(VI) Found (µg/g)	Cr(VI) Found (µg/daily size)	Cr(VI) Content (%)
101	Tablet	2.24	1	0	ND	ND	NA
102	Tablet	1.34	1	131	77.22 ± 10.65	77.22 ± 10.65	79.0
103	Capsule	1.02	1	140	103.6 ± 18.74	103.60 ± 18.74	64.8
104	Tablet	1.57	1	30	ND	ND	NA
105	Tablet	1.27	3	120	ND	ND	NA
106	Capsule	0.45	1	210	9.47 ± 0.59	4.22 ± 0.26	1.5
107	Tablet	1.69	1	275	63.36 ± 22.09	107.17 ± 37.36	31.0
108	Caplet	1.56	2	45	16.41 ± 0.98	51.20 ± 3.06	111.6
109	Tablet	1.29	1	35	19.61 ± 2.83	25.24 ± 3.64	65.1
110	Tablet	1.26	1	40	ND	ND	NA
111	Caplet	1.53	2	45	12.55 ± 1.13	38.32 ± 3.46	76.1
112	Tablet	1.74	1	60	ND	ND	NA
113	Capsule	0.87	1	125	69.88 ± 5.12	60.68 ± 4.44	44.3
114	Caplet	1.50	2	120	11.36 ± 0.85	34.05 ± 2.55	20.1
115	Tablet	1.04	3	100	ND	ND	NA
116	Capsule	0.86	1	100	39.26 ± 10.78	33.85 ± 9.28	25.4
117	Tablet	1.28	6	200	10.52 ± 1.28	80.85 ± 9.86	34.0
118	Gummy	2.49	2	120	1.87 ± 0.16	9.295 ± 0.81	4.6
119	Capsule	0.79	1	33	33.15 ± 2.48	26.23 ± 1.96	45.6
120	Tablet	1.97	1	120	ND	ND	NA

Figure 4.5: Graphical representation of speciated chromium analysis for twenty independent dietary supplement formulations. For each formulation, fifteen (15) units were subsampled and homogenized, along with determination of the average unit mass. Capsule products were subsampled, emptied, and mixed to provide a representative sample of the capsule contents. The samples were prepared in triplicate using a 50 mM EDTA alkaline extraction and analyzed with IC-ICP-MS according to EPA Method 6800 (IDMS) using a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column with 2 mM EDTA mobile phase. Each sample was prepared in triplicate with four replicate measurements (n = 12). The labelled chromium content is compared to the calculated speciated chromium content.

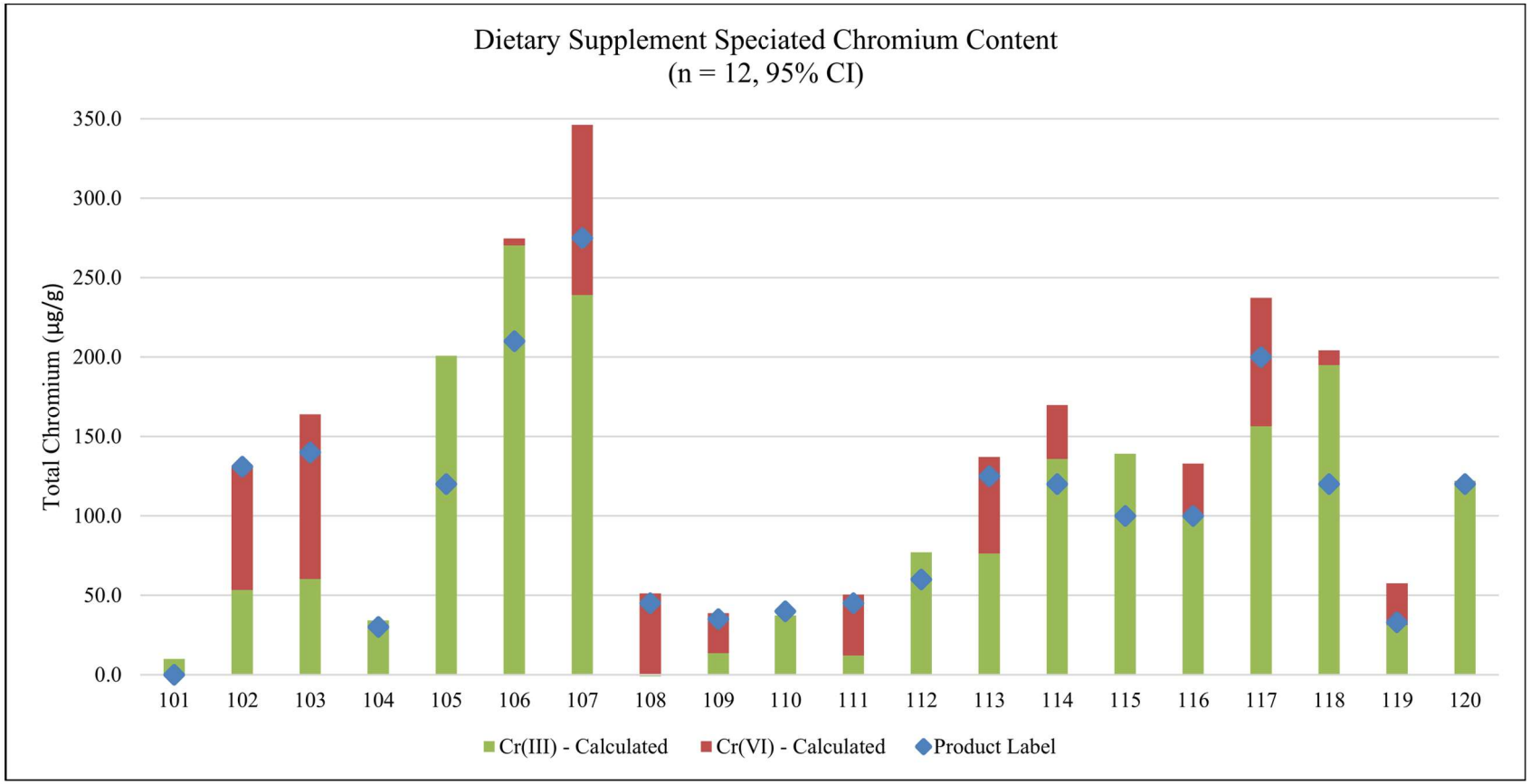


Figure 4.6: Evaluation of sample pH and Eh values using a Mettler Toledo SevenCompact pH/Ion meter S220 equipped with an InLab Expert Pro-ISM pH probe and InLab Redox ORP probe. A 50 mM EDTA alkaline extraction solution was used to prepare twenty dietary supplement products. The pH and Eh values were measured after the samples were centrifuged and diluted to their final concentrations. These values are superimposed onto Eh-pH diagram references found in literature to predict the most probable, thermodynamically stable chromium species in the sample. The results indicate that the formation of the solid Cr_2O_3 species is expected during sample preparation. The four Eh-pH diagrams provide a comparison of thermodynamic databases as part of an open source project from the Research Center for Deep Geological Environments, Geological Survey of Japan. The diagrams are emended and from the Atlas of Eh-pH diagrams; National Institute of Advanced Industrial Science and Technology, Research Center for Deep Geological Environments, Geological Survey of Japan, Open File Report No. 419; Pages 78-79; May 2005. [28] The ranges of the measured pH and Eh values for the dietary supplement formulations are represented in orange on the stability diagrams.

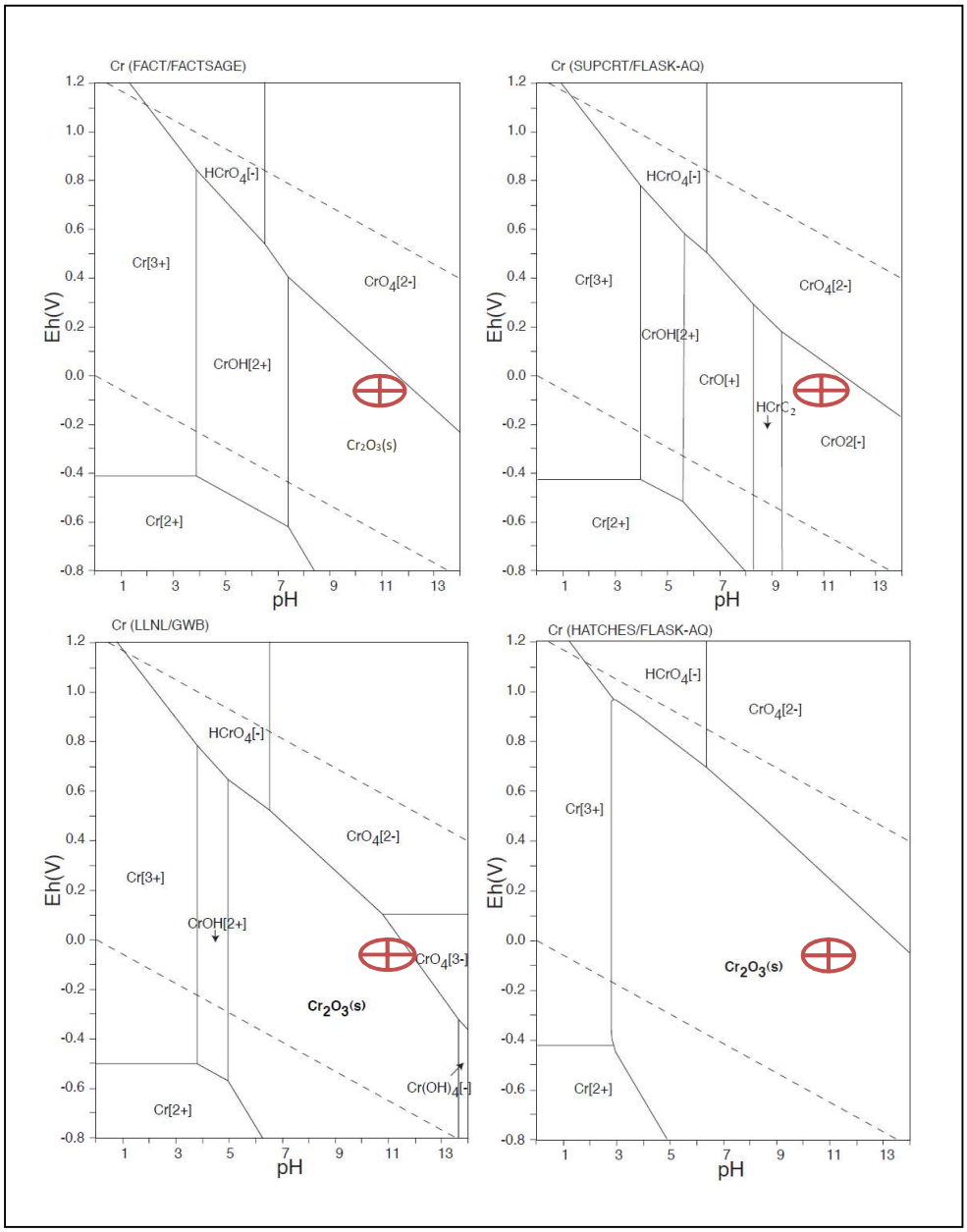


Figure 4.7 provides example chromatograms for speciated chromium analysis of several dietary supplement samples using a 50 mM EDTA alkaline extraction solution with IC-ICP-MS and a Metrosep A Supp 5 column. The retention time for Cr(III) was found to be approximately 1.5 minutes and the retention time for Cr(VI) was found to be approximately 4.3 minutes. The solution blank example chromatogram illustrates 50-Cr(III) and 53-Cr(VI) peaks that correspond to the respective isotope standards. A chromatogram for the 53-Cr(VI) identification standard is provided for the expected retention time for Cr(VI). The chromatograms for sample 111 and sample 118 are typical for dietary supplement formulations that contain quantifiable hexavalent chromium. The chromatogram for sample 104 illustrates a typical chromatographic result for formulations that contain hexavalent chromium below the limit of detection.

Figure 4.7: Example chromatograms for speciated hexavalent chromium analysis of dietary supplements. A 50 mM EDTA alkaline extraction solution was used to prepare the samples, which were analyzed using IC-ICP-MS with a Metrosep A Supp 5 (250/4.0 mm, 5 µm) ion chromatography column and 2 mM EDTA mobile phase. Examples of the resulting chromatograms are provided: (A) solution blank spiked with 50-Cr(III) and 53-Cr(VI) isotope standards, (B) 53-Cr(VI) identification standard, (C) sample 111, (D) sample 118, and (E) sample 104. The retention time for Cr(III) was found to be approximately 1.5 minutes and the retention time for Cr(VI) was found to be approximately 4.3 minutes. The example chromatogram includes the ion count for each of the major isotopes of chromium.

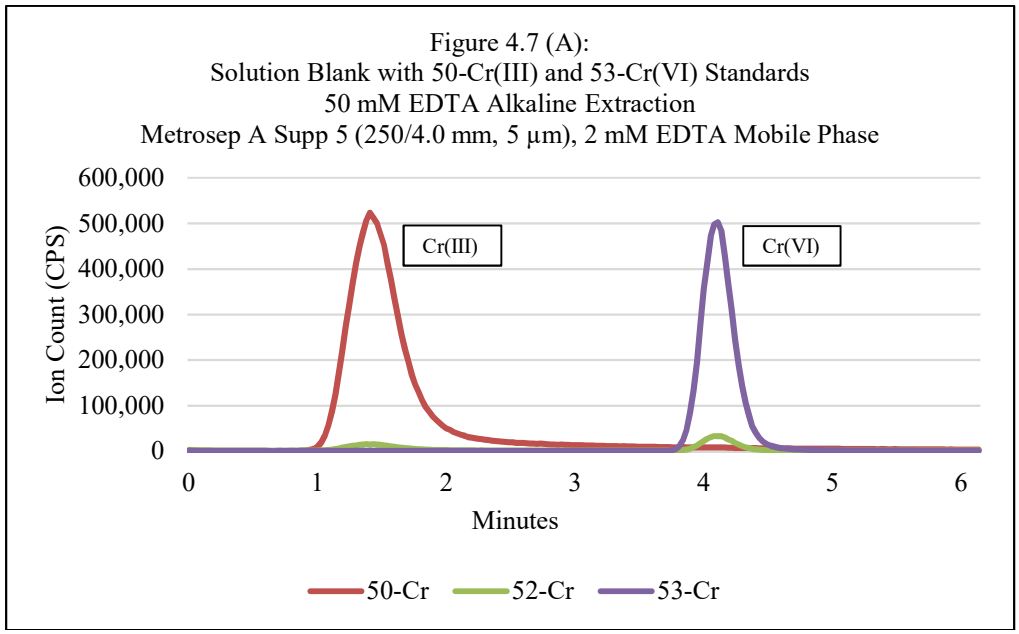


Figure 4.7 (B):
53-Cr(VI) Identification Standard
50 mM EDTA Alkaline Extraction
Metrosep A Supp 5 (250/4.0 mm, 5 µm), 2 mM EDTA Mobile Phase

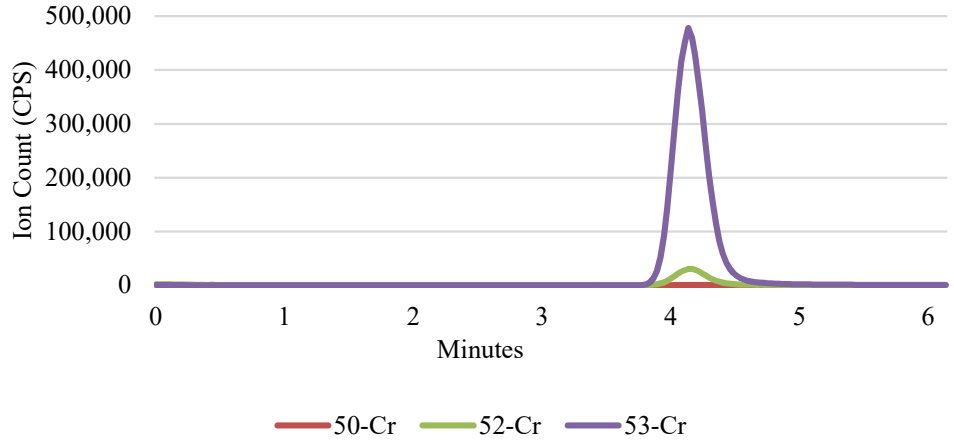
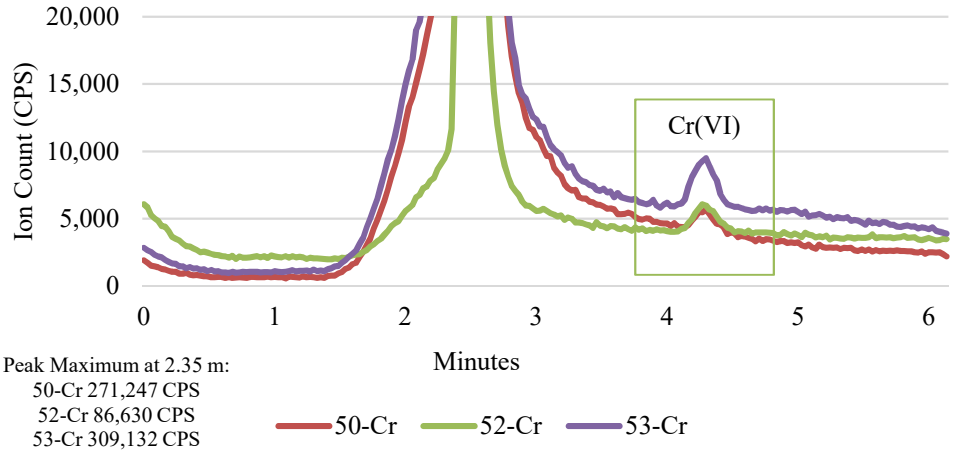
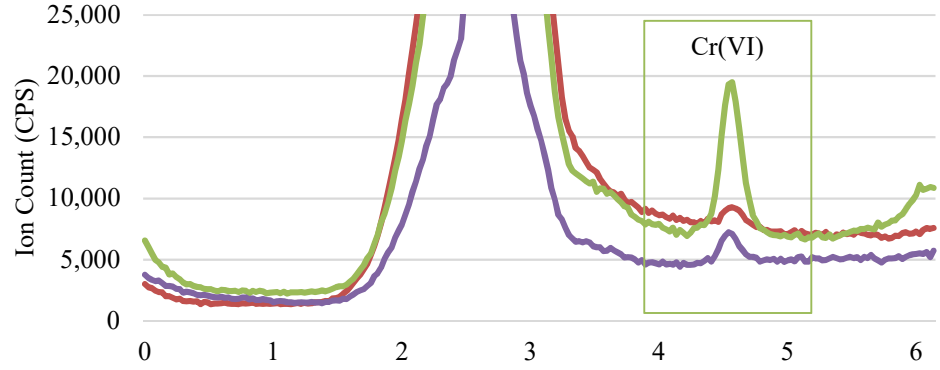


Figure 4.7 (C):
Sample 111
50 mM EDTA Alkaline Extraction
Metrosep A Supp 5 (250/4.0 mm, 5 µm), 2 mM EDTA Mobile Phase



Peak Maximum at 2.35 m:
50-Cr 271,247 CPS
52-Cr 86,630 CPS
53-Cr 309,132 CPS

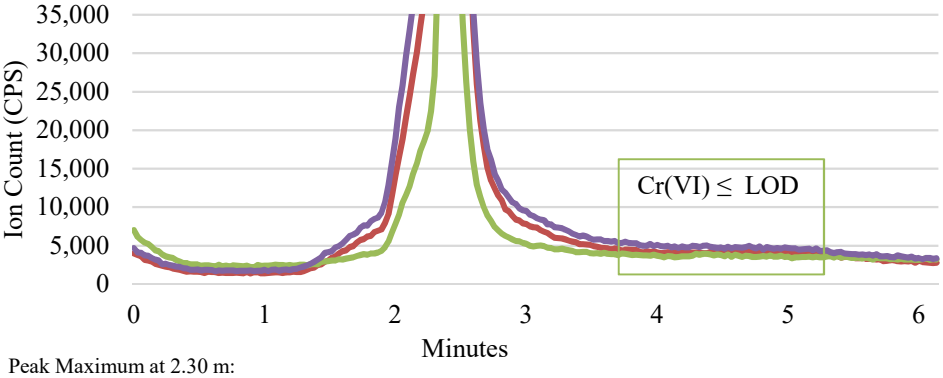
Figure 4.7 (D):
Sample 118
50 mM EDTA Alkaline Extraction
Metrosep A Supp 5 (250/4.0 mm, 5 µm), 2 mM EDTA Mobile Phase



Peak Maximum at 2.50 m:
50-Cr 216,434 CPS
52-Cr 180,155 CPS
53-Cr 89,424 CPS

50-Cr 52-Cr 53-Cr

Figure 4.7 (E):
Sample 104
50 mM EDTA Alkaline Extraction
Metrosep A Supp 5 (250/4.0 mm, 5 µm), 2 mM EDTA Mobile Phase



Peak Maximum at 2.30 m:
50-Cr 163,251 CPS
52-Cr 73,243 CPS
53-Cr 193,419 CPS

50-Cr 52-Cr 53-Cr

4.4 CONCLUSION

EPA Method 6800 was used to effectively quantitate the total chromium and speciated chromium content of twenty dietary supplement formulations. Accuracy, precision, linearity, specificity and selectivity, limit of quantitation, and limit of detection of the sample preparations and analytical methods were fully validated. For determination of total chromium content, EPA Method 3052 was used for acid digestion of the samples before quantitation by EPA Method 6800 with IDMS. Speciated chromium content was determined using a 50 mM EDTA alkaline extraction solution before quantitation by EPA Method 6800 with IDMS/SIDMS.

For total chromium content, the resulting experimentally determined chromium concentrations were compared to the amount provided by the product labels. To verify system suitability, NIST Standard Reference Material 3280 was prepared according the method outlined for total chromium analysis and was found to have a total chromium concentration of 92.89 ± 0.2 μg/g. The experimentally determined value represents a 99.1% recovery of the NIST certified value and indicates that the methodology is suitable for the intended analysis. The experimentally determined total chromium content for several formulations provided close agreement with the values provided by the product labels. However, the experimentally determined total chromium content for the majority of the formulations were significantly different than the labelled amounts. When the percent differences for the twenty formulations were averaged, the experimentally determined total chromium found in the supplements was 24% different than the labelled amounts (124% recovery).

Speciated hexavalent chromium determinations were made using a 50 mM EDTA alkaline extraction solution with a Metrosep A Supp 5 ion chromatography column. Thirteen formulations were found to contain hexavalent chromium, with concentrations that ranged from

approximately 4.22 µg/daily size to 107.17 µg/daily size. These levels exceed the maximum allowable dose level (MADL) of 8.2 µg per day established by California Proposition 65 for twelve of the samples. This is especially a concern since seven of the formulations were marketed for prenatal support. Furthermore, the hexavalent chromium concentrations in five of the formulations were greater than 50% of the measured total chromium content, with one formulation having approximately 90% hexavalent chromium content.

The use of the 50 mM EDTA extraction solution for speciated chromium analysis of actual dietary supplement samples generated a large negative bias for trivalent chromium when the sample extracts were analyzed using IC-ICP-MS with SIDMS quantitation. This is likely due to the availability of the aqueous 50-Cr(III) isotope standard for immediate chelation with EDTA in the extraction solution. Thus, a disproportionately low amount of solid 50-Cr(III) precipitate is formed when compared to the insoluble Nat-Cr(III) of the sample. Therefore, Cr(III) was not processed using SIDMS equations. This also prevented accurate quantitation of trivalent chromium in the solid residues by mass balance calculations, since there would be a disproportionately large amount of 52-Cr(III) in the solid residue samples when compared to the 50-Cr(III) isotope standard. The Eh and pH values for each sample were measured and compared to reference stability diagrams, which indicate that the formation of a solid Cr_2O_3 species is thermodynamically favored. As a result, the extraction appears to stabilize the solid Cr(III) in the dietary supplement samples.

These results indicate that many dietary supplement manufactures do not have sufficient control of the total chromium content of their formulations. Improved manufacturing practices and product quality control testing would help ensure that consumers are not exposed to unexpected concentrations of elemental supplementation. Also, twelve of the tested

formulations had hexavalent chromium concentrations above regulatory limits. Since several of these formulations are marketed for prenatal support, these results indicate that both mother and child would be chronically exposed to a genotoxic and carcinogenic substance. Today, there is rapid growth of the multi-billion dollar dietary supplement industry, with evidence of increased risk from unsafe and adulterated products. To ensure the quality and safety of chromium-containing dietary supplement products, manufactures should be compelled to adopt routine analytical testing and controls for hexavalent chromium. The developed methods provide techniques for accurately measuring total chromium and hexavalent chromium concentrations in a robust variety of dietary supplement sample formulations. The use of these methods by dietary supplement manufactures would ensure assessment of hexavalent chromium, which would enhance the quality control, quality assurance, and safety of their consumer products. Given the number of incorrectly and insufficiently labelled dietary supplements, and the prevalence of hexavalent chromium in multivitamin/multimineral vitamins, the routine use of these methods is recommended for quality assessment prior to the release of the finished products to the commercial marketplace.

4.5 REFERENCES

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CHAPTER FIVE:

CONCLUSION

Nearly a quarter-century has passed since the town of Hinkley, California was awarded hundreds of millions of dollars from a class-action lawsuit against Pacific Gas & Electric (PG&E). The film *Erin Brockovich* portrays how the legal clerk investigated the utility company and found that it dumped carcinogenic hexavalent chromium [Cr(VI)], used to suppress rust formation, into an unlined pond in the 1950s and 1960s. This activity resulted in contamination of the town's groundwater, severe and chronic health problems, and deceptive company practices. By establishing health effects with the hexavalent chromium contamination, Brockovich was able to confront the powerful PG&E lawyers. Yet, today, residents in the town are still engaged in an ongoing battle to remediate the environmental damage caused by hexavalent chromium. The town of Hinkley is not alone. In 2002, residents in Garfield, New Jersey found that hexavalent chromium-contaminated groundwater infiltrated their basements. This later evaporated to leave behind a toxic carcinogenic dust made from chromate crystals. The source of this contamination was found to be a corroded underground tank at a nearby electroplating company. There are countless numbers of similar examples, all of which illustrate how anthropogenic activities and industries can greatly impact the environment and human health.

The ability to perform accurate, repeatable, and defensible speciated chromium analysis is immensely significant for measurements that support human health, environmental science, and industry. This is especially true since Cr(III) is necessary for proper nutrition, while Cr(VI) is extremely toxic, genotoxic, and carcinogenic. The dichotomous nature of chromium toxicity

requires the use of an accurate analytical method that is capable of specific quantification of both Cr(III) and Cr(VI). Yet, the main challenges associated with speciated analysis are related to reactive species that are continuously transformed or converted to other species during sample processing. Due to this complexity, accurate determination of the concentrations and stabilities of the Cr(III) and Cr(VI) species require a method that is capable of monitoring and correcting for interconversion, bias, and instrumental error.

This dissertation examined the use of molecular speciated isotope dilution mass spectrometry (SIDMS), which is codified in EPA Method 6800, as a powerful technique that allows for the accuracy, precision, and robustness needed to correct Cr(III)/Cr(VI) species interconversions. In order to investigate the use of SIDMS methodology, it was necessary to first prepare isotopically enriched standard solutions. To generate these solutions, guidance provided in EPA Method 6800 was followed for the preparation of isotopically enriched standards. The new isotopically-enriched speciated chromium standards were synthesized and characterized to allow for further studies and assessment of chromium species in various research materials and projects.

My research described the development and certification of a Sigma-Aldrich hexavalent chromium standard reference material in a soil matrix, which will provide the scientific community with a standard material that supports quality assurance and quality control of the analytical methodology used for hexavalent chromium testing. Considering the expected growth in chromium ore excavation and processing, this new standard will be a valuable addition to the analytical materials used for performing ambient level Cr(VI) background assessment measurements. This type of assessment will undoubtedly be used in the future to help mitigate the impact of mineral processing on the surrounding environment and assist in monitoring

remediation of hexavalent chromium-containing waste materials produced during industrial activities.

During this research, limitations of this methodology were examined. Potential method bias and method error were evaluated using low-concentration isotope standards. With these standards and optimized instrument parameters, it was possible to achieve improved mass balance determinations. The results indicate that a thorough investigation of the error propagation factor is necessary to achieve the most accurate quantitation. Full certification of this new standard reference material will be dependent on the ability of additional laboratories generating repeatable results.

My research also examined the development of methodology to determine the amount of hexavalent chromium in a range of dietary supplement sample formulations. In addition to speciated chromium analysis, the total chromium content of the dietary supplement formulation was examined. The research indicates that many dietary supplement manufactures do not have sufficient control of the total chromium content of their formulations. Also, most of the tested formulations had hexavalent chromium concentrations above regulatory limits. To ensure the quality and safety of chromium-containing dietary supplement products, manufactures should be compelled to adopt routine analytical testing and controls for hexavalent chromium. However, dietary supplement manufacturers have been hesitant to adopt the advanced analytical techniques required for speciated chromium analysis. Changes to the regulation of dietary supplements are needed to ensure that consumers are not exposed to toxic, carcinogenic, and genotoxic products.

As analytical capabilities improve, it is reasonable to expect that speciated analysis will become more routine and informative. Targeted analysis of speciated impurities in food, nutritional supplements, cosmetics, and pharmaceuticals will undoubtably become more routine.

Novel toxicological, metabolic, and pharmaceutical clinical studies will benefit from the resolution provided by corrected speciated analysis. Future studies must include the validation of these methods before they are used for routine analysis.

APPENDIX ONE:

REAGENTS AND MATERIALS CERTIFICATE OF ANALYSIS (COA) REFERENCES

A.1 **NIST 136e COA:** National Institute for Standards and Technology, Certificate of Analysis, Standard Reference Material 136e, Potassium Dichromate, Oxidimetric Standard, April 2000.

A.2 **NIST 136f COA:** National Institute for Standards and Technology, Certificate of Analysis, Standard Reference Material 136f, Potassium Dichromate, Oxidimetric Standard, April 2008.

A.3 **ORNL 50-Cr COA:** Oak Ridge National Laboratory, Certificate of Analysis, Chromium Metal, Isotopically Enriched in 50-Chromium, Batch 144980, May 2005.

A.4 **ORNL 53-Cr COA:** Oak Ridge National Laboratory, Certificate of Analysis, Chromium Oxide, Isotopically Enriched in 53-Chromium, Batch 177090, August 2004.

A.1 NIST 136e COA

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 136e

Potassium Dichromate
($K_2Cr_2O_7$)
Oxidimetric Standard

This Standard Reference Material (SRM) consists of high-purity potassium dichromate and is intended primarily for use in oxidimetric standardization. It conforms to the American Chemical Society specifications for analytical reagent grade material and meets the primary standard criteria of the Analytical Chemistry Section of the International Union of Pure and Applied Chemistry [Analyst 90, 251 (1965)].

The certification of this lot of potassium dichromate was a cooperative effort of the National Institute of Standard and Technology (NIST) and the U.S. Department of Energy New Brunswick Laboratory (NBL), Argonne, Illinois.

The certified value is the weighted mean of results from three different analytical methods (see Analysis). The weights were computed according to the iterative procedure described in Paule and Mandel (NBS Journal of Research 87 1982, pp. 377-385).

Certified Value

Oxidimetric Assay 99.984 ± 0.010 weight percent

The uncertainty is the half-width of an approximate 95 % confidence interval for the certified value plus an allowance for bias among the analytical techniques.

The molecular weight of $K_2Cr_2O_7$ used in all calculations was 294.18 and the density used in computing its mass in vacuum was 2.69 g X cm^{-3}

The experimental sequence was developed by S.B. Schiller of the Statistical Engineering Division, who also statistically evaluated the results. Analytical measurements were performed by G. Marineako of the Inorganic Analytical Research Division.

The cooperative measurement effort at NBL was coordinated by N.M. Trahey, Manager, NBL Reference Materials Program. The measurement plan for titrimetric assay was developed by P.M. Santoliquido, Safeguards Instrumental Measurement Division; the high-precision version of NBL-modified titrimetric method for uranium was performed by W. Nichiporuk, Safeguards Measurement and Development Division; and the statistical scheme and calculations were generated by M.M. Smith, Operations Support Division.

The technical and support aspects involved in the procurement, certification, and issuance of this SRM were coordinated through the Standard Reference Materials Program by R.W. Seward.

Willie E. May, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899

Nancy M. Trahey, Chief

ANALYSIS

Coulometric Assay: The coulometric assay of SRM 136e is based on the reduction of the dichromate ion in 500 mg samples. The coulometric procedure used in this analysis has been described by G. Marineako and J.K. Tavior, High Precision Titration of Potassium Dichromate, J. Res. Nat. Bur. Stand. (U.S.). 67A(5), pp. 453-4591, 1963 (September-October). The value of the Faraday constant used in the calculation was 96486.0 C_{NIST} mol^{-1}.

Titrimetric: Assay SRM 136e was analyzed at NBL using the high-precision version of the NBL-modified titrimetric method for uranium. NBL CRM 112-A (formerly SRM 960), Uranium Metal Standard, was used in the analysis of ten bottles of 136e in duplicate or triplicate along with samples of CRM 99 (NBL Potassium Dichromate Standard) and SRM 136c (a previous lot of the Potassium Dichromate Standard), which were used for controls.

Gravimetric Titration Assay: Six of the same bottles of 136c used for the coulometric assay were intercompared with SRM 136c by a weight titration with ferrous ammonium sulfate. While this method is not as precise as either the coulometric method or the NBL-modified titrimetric method, it does provide an independent assay method.

Homogeneity: This SRM lot of potassium dichromate is homogeneous within the bounds of the random error uncertainty of the measurement process.

Drying: This material may be used as received or after drying. However, it is recommended that this material be dried at 110 °C for 2 h.

Stability: SRM 136e is stable and the certification is valid for 5 years from the date of shipment, when stored in its original container with the top tightly closed. Should any change occur before the expiration of the certification, NIST will notify purchasers.

This lot of potassium dichromate was obtained from Mallinckrodt, Inc, St. Louis, Missouri.

A.2 NIST 136f COA

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 136f

Potassium Dichromate
(Oxidimetric Standard)

This Standard Reference Material (SRM) is certified as a chemical of known assay and is intended for use as a primary oxidimetric standard. A unit of SRM 136f consists of 60 g of highly purified potassium dichromate ($K_2Cr_2O_7$) in a clear glass bottle.

Certified Value: The certified mass fraction, $w_{K_2Cr_2O_7}$, of total oxidizing capacity expressed as $K_2Cr_2O_7$ is listed in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST [1].

Table 1. Certified Value[a] for SRM 136f Potassium Dichromate

$w_{K_2Cr_2O_7}$	99.9954 %	±	0.0044 %

[a] The certified value is expressed as the value ± its expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where k is the coverage factor and u_c is the combined standard uncertainty calculated according to the ISO "Guide to the Expression of Uncertainty in Measurement" [2] and NIST Technical Note 1297 [3]. The value of u_c represents the combined uncertainty in the certified value, at the level of one standard deviation, arising from material homogeneity and from all sources of uncertainty inherent to the coulometric and titrimetric assay techniques. The value of k controls the approximate level of confidence associated with U. For this SRM, $k = 2.00$. This value corresponds to a level of confidence of approximately 95 %. The value of k is obtained from the Student's t-distribution with effective degrees of freedom, $\nu_{eff} > 300$.

Expiration of Certificate: The certification of SRM 136f is valid, within the measurement uncertainties specified, until **01 February 2023**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Notice and Warnings to Users" and "Instructions for Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the technical measurements leading to the certification of SRM 136f was provided by K.W. Pratt of the NIST Analytical Chemistry Division.

Coulometric and titrimetric analyses were performed in the NIST Analytical Chemistry Division by K.W. Pratt.

Statistical consultation was provided by W.F. Guthrie of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 10 April 2008

Robert L. Watters, Jr., Chief
Measurement Services Division

NOTICE AND WARNINGS TO USERS

Stability and Storage: This SRM should be stored in its original bottle at room temperature. It must be tightly re-capped after use and protected from moisture and organic fumes.

Homogeneity: Tests indicate that this SRM is homogeneous within the uncertainty limits for sample sizes greater than 250 mg. The use of samples of mass less than 250 mg is not recommended.

Density: The density of SRM 136f was taken as 2.686 g/cm^3 in the correction for air buoyancy associated with weighing the material.

INSTRUCTIONS FOR USE

Drying Instructions: Dry for 2 hours at 110 °C. Store the dried material over anhydrous magnesium perchlorate.

OTHER INFORMATION

Source of Material: The potassium dichromate used for this SRM was obtained from a commercial company. The material was examined for compliance with the specification for reagent grade $K_2Cr_2O_7$ as specified by the American Chemical Society [4]. The material was found to meet or exceed the minimum requirements in every respect.

Assay Techniques: Coulometric assays were performed by an automated procedure [5] using electrogenerated iron (II) [6]. Titrimetric assays were performed by reaction with excess arsenious oxide (SRM 83d), with the excess arsenic (III) back-titrated with a standard cerium (IV) solution [7]. The certified value was obtained as a weighted mean of the results from the two techniques. Results were calculated based on a value of 96 485.336 coul/mol for the Faraday constant [8] and 294.1846 g/mol for the molar mass of $K_2Cr_2O_7$ [9].

REFERENCES

[1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements;* NIST Special Publication 260-136, U.S. Government Printing Office: Washington, DC (2000); available at http://ts.nist.gov/MeasurementServices/ReferenceMaterials/upload/sp260-136.pdf.

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[6] Marinenko, G.; Taylor, J.K.; *Precise Coulometric Titrations of Potassium Dichromate*; J. Res. Natl. Bur. Stand., Vol. 67A, No. 5, pp. 453–459 (1963).

[7] Willard, H.H.; Young, P.; *Standardization of Potassium Dichromate*; Ind. Eng. Chem. Anal. Ed., Vol. 7, No. 1, pp. 57–58 (1935).

[8] Mohr, P.J.; Taylor, B.N.; *CODATA Recommended Values of the Fundamental Physical Constants: 2002*; Rev. Mod. Phys., Vol. 77, pp. 1–107 (2005).

[9] IUPAC, Commission on Isotopic Abundances and Atomic Weights; *Atomic Weights of the Elements 2005*; Pure Appl. Chem., Vol. 78, No. 11, pp. 2051–2066 (2006).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at telephone (301) 975-6776; fax (301) 926-4751; email srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

A.3 ORNL 50-Cr COA

ASSAY

Element: Chromium
Symbol: Cr
Isotope: 50
Series: LZ
Batch: 144980

ISOTOPIC ANALYSIS

Isotope	Atomic Percent	Precision plus/minus
50	96.05	0.04000
52	3.66	0.04000
53	0.24	0.01000
54	0.05	0.00500

The limits quoted above are an expression of the precision of this measurement only. The error is estimated at less than 1% from known sources of systematic errors.

Internal Use Only
Date Entered: 04/21/1998
Last Change: 08/09/2005 12:28:50 PM
By: E1H

SPECTROGRAPHIC ANALYSIS (SSMS)

Element: ppm	Element: ppm	Element: ppm	Element: ppm
Ag:	I:	Rh:	Tc:
Al:	In:	Ru:	Te:
As:	Ir:	S: 70	Th:
Au:	K:	Sb:	Ti:
B:	Li:	Sc:	Tl:
Ba:	Mg:	Se:	U:
Be:	Mn:	Si:	V:
Bi:	Mo:	Sn:	W:
Br:	N:	Sr:	Zn:
C:	Na:	Ta:	Zr:
Ca:	Nb:		
Cb:	Ni:		
Cd:	O:		
Cl:	Os:		
Co:	P:		
Cr:	Pa:		
Cs:	Pb:		
Cu:	Pd:		
F:	Pm:		
Fe:	Po:		
Ga:	Pt:		
Ge:	Ra:		
Hf:	Rb:		
Hg:	Re:		

LANTHANIDES and ACTINIDES

Am:	La:
Bk:	Lu:
Ce:	Md:
Cf:	Nd:
Cm:	Np:
Dy:	Pr:
Er:	Pu:
Es:	Sm:
Eu:	Tb:
Fm:	Y:
Gd:	Yb:
Ho:	Tm:

* Symbols: M - major; T - trace; I - interference; < - less than; </= less than/equal to; ~ approximately; nd - not detected.
* Elements listed above without values were not detected or would calculate less than 10 ppm.
* This analysis reflects impurities prior to conversion/fabrication.
* Request No. 34597; Requisition No.9773; special work authorization 82559.
* Note: residual oxygen should be expected in all chromium metal powders.
* <- No spectrum line visible. Probably absent, definitely less than value given.
* <T- Present but less than value given.
* The spectrographic results reported herein are semi-quantitative estimates and should not be interpreted or construed to be precise quantitative determinations.

A.4 ORNL 53-Cr COA

ASSAY

Element: Chromium
Symbol: Cr
Isotope: 53
Series: OO
Batch: 177090

ISOTOPIC ANALYSIS

Isotope	Atomic Percent	Precision plus/minus
50	0.03	0.00500
52	2.65	0.02000
53	97.20	0.02000
54	0.12	0.00500

The limits quoted above are an expression of the precision of this measurement only.
The error is estimated at less than 1% from known sources of systematic errors.

Internal Use Only
Date Entered: 02/20/1992
Last Change: 08/10/2004 10:20:38 AM
By: E1H

SPECTROGRAPHIC ANALYSIS (SSMS)

Element: ppm	Element: ppm	Element: ppm	Element: ppm
Ag:	I:	Rh:	Tc:
Al:	In:	Ru:	Te:
As:	Ir:	S: 800	Th:
Au:	K:	Sb:	Ti:
B: 90	Li:	Sc:	Tl:
Ba:	Mg:	Se:	U:
Be:	Mn:	Si: 280	V:
Bi:	Mo:	Sn:	W:
Br:	N:	Sr:	Zn:
C:	Na: 20	Ta:	Zr:
Ca:	Nb:		
Cb:	Ni:		
Cd:	O:		
Cl:	Os:		
Co:	P:		
Cr: MAJOR	Pa:		
Cs:	Pb: 50		
Cu:	Pd:		
F:	Pm:		
Fe:	Po:		
Ga:	Pt:		
Ge:	Ra:		
Hf:	Rb:		
Hg:	Re:		

LANTHANIDES and ACTINIDES

Am:	La:
Bk:	Lu:
Ce:	Md:
Cf:	Nd:
Cm:	Np:
Dy:	Pr:
Er:	Pu:
Es:	Sm:
Eu:	Tb:
Fm:	Y:
Gd:	Yb:
Ho:	Tm:

* Symbols: M - major; T - trace; I - interference; < - less than; </= less than/equal to; ~ approximately; nd - not detected.
* Elements listed above without values were not detected or would calculate less than 10 ppm.
* Request No. 34591; Requisition No. 9767; Notebook No. A-100325, pg. 120
* Note: residual oxygen should be expected in all chromium metal powders.
* <- No spectrum line visible. Probably absent, definitely less than value given.
* <T- Present but less than value given.
* The spectrographic results reported herein are semi-quantitative estimates and should not be interpreted or construed to be precise quantitative determinations.