Determination of Trivalent and Hexavalent Chromium with Mass Balance in Dietary Supplements Using Speciated Isotope Dilution Mass Spectrometry

Naudia Martone

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DETERMINATION OF TRIVALENT AND HEXAVALENT CHROMIUM WITH
MASS BALANCE IN DIETARY SUPPLEMENTS USING SPECIATED ISOTOPE
DILUTION MASS SPECTROMETRY

A Thesis
Submitted to the Bayer School of Natural and Environmental Sciences

Duquesne University

In partial fulfillment of the requirements for
the degree of Master of Science

By
Naudia R. Martone

December 2011
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By

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ABSTRACT

DETERMINATION OF TRIVALENT AND HEXAVALENT CHROMIUM WITH MASS BALANCE IN DIETARY SUPPLEMENTS USING SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY

By

Naudia Martone

December 2011

Dissertation supervised by Dr. H. M. “Skip” Kingston

In order to assess the benefit or toxicity of chromium in dietary supplements, trivalent chromium and hexavalent chromium must be measured and verified with mass balance (sum of both species equaling total chromium). This is necessary because dietary supplements report trivalent chromium, an essential trace element, as an ingredient, but hexavalent chromium, a toxic carcinogen, may also be present. Because trivalent chromium is stable in acidic conditions and hexavalent chromium in alkaline conditions, interconversions between species occur and increase the difficulty of quantification. Therefore, EPA Method 3060A was first performed to extract hexavalent chromium. Then, EPA Method 3052 was performed on the residue to digest the remaining trivalent chromium. Speciated Isotope Dilution Mass Spectrometry (SIDMS) with Ion-Exchange Chromatography-Inductively Coupled Plasma-Mass Spectrometry (IC-ICP-MS) was used
to account for interconversions as well as determination of trivalent and hexavalent chromium concentrations in the studied samples. Mass balance indicated that the analyzed supplements contained hexavalent chromium ranging from 0 to 16% of the total chromium content.
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## LIST OF ABBREVIATIONS

AIT ............................................. Applied Isotope Technologies, Inc.
COPR ........................................ Chromite Ore Processing Residue
DDI ............................................ Double deionized
DL .............................................. Detection limit
DPC ............................................ Diphenylcarbazide
DPCO .......................................... Diphenylcarbazone
EDTA .......................................... Ethylenediaminetetraacetic acid
EPA ............................................. Environmental Protection Agency
FDA ............................................ Food and Drug Administration
IC ................................................ Ion-Exchange Chromatography
IC-ICP-MS .................................... Ion-Exchange Chromatography-Inductively
                                            Coupled Plasma-Mass Spectrometry
ICP-MS ........................................ Inductively Coupled Plasma-Mass Spectrometry
IDMS .......................................... Isotope Dilution Mass Spectrometry
NTP ............................................ National Toxicology Program
ppb ............................................. parts per billion; ng/g, µg/L
ppm ............................................. parts per million; µg/g, mg/L
ppt ............................................. parts per trillion; pg/g, ng/L
RoHS .......................................... Restriction of the Use of Certain Hazardous
                                            Substances in Electrical and Electronic Equipment
SIDMS ......................................... Speciated Isotope Dilution Mass Spectrometry
TFM........................................... Tetrafluoromethane
CHAPTER 1: Introduction

Chromium is an element that is important in different ways depending on its valence state. It is a trace metal that is naturally-occurring on earth, found in rocks, plants, and animals. Chromium is used as a metal for various applications, such as the manufacturing of stainless steel. Cigarette smoke can also release chromium and create high indoor chromium levels. Chromium content in the air can vary depending on the area. The values for air exposure of chromium in rural areas are less than 10 ng/m$^3$ compared to urban areas with 0 to 30 ng/m$^3$. Chromium is generally stable as trivalent chromium [chromium (III)] and hexavalent chromium [chromium (VI)]. Although these species are from the same element, they have very different effects on human health. As a result, knowledge of only total chromium content will not provide enough information to make assessments about risks or benefits. Therefore, in determination of chromium, it is important to quantify both trivalent and hexavalent chromium and to be able to verify these values.

Previous research on chromium speciation in dietary supplements is limited. The research that is available on chromium has some focus on the health effects from different species. However, the conclusions can vary from study to study. Also, different techniques have been researched for hexavalent chromium determination and on attempts to determine both trivalent and hexavalent chromium. A large part of the previous research involves less complex matrices, such as water samples or standards created in the laboratory. This research will expand the literature to include actual consumer products in the more complex matrix of supplements.
1.1 Trivalent Chromium

In general, trivalent chromium is considered essential. It can be found in foods, such as meat and whole-grains, typically around 2 µg per serving. An adequate intake value for chromium was established in 2001 and varies with age groups and sex. For adults 19 to 50 years old, males should receive 35 µg/day and women 25 µg/day. Trivalent chromium is generally considered essential for insulin, glucose, and lipid metabolism; and its deficiency may be linked to diabetes. It is often added to supplements because of these benefits. Trivalent chromium is not absorbed into cells as readily as hexavalent chromium. Therefore, it would not cause the same damaging effects as hexavalent chromium. However, there is some controversy over the actual benefits of trivalent chromium consumption.

The studies that support the benefits of trivalent chromium often focus on glucose and insulin metabolism. R. A. Anderson has written many papers discussing how trivalent chromium is essential. One paper notes that diets often attribute to 60% of the adequate intake of trivalent chromium. If trivalent chromium is essential, this could support the use of chromium supplements. Deficiency is said to result in problems similar to diabetes. Benefits from trivalent chromium intake are emphasized as being dependent on the amount and form of chromium and as improving those who are chromium deficient. The benefits noted are improved lean body mass and improvement for people with glucose or insulin problems. Another review with meta-analysis of findings concluded that chromium supplements improved glucose levels in those with diabetes. It also noted that further studies should be done to make any strong conclusions about chromium supplements. Even if trivalent chromium is facilitating in metabolism, the
mechanism is still unknown. One study proposes biochemistry to explain how it is acting as an essential trace metal. The study suggests that chromodulin, or glucose tolerance factor (GTF), is involved in the receptors of insulin.

However, other studies suggest that trivalent chromium is not an essential trace metal. A review by Stearns states that an essential metal should be naturally-occurring in the body and have a specific function. The review argues that trivalent chromium does not meet these conditions. Another requirement for being essential is that a deficiency would cause impairment. However, Stearns found that there is little evidence for actual cases of trivalent chromium deficiency. Also, although the adequate intake values for trivalent chromium were decided, no recommended daily allowance was determined because of the lack of data. Another study specifically evaluates chromium picolinate used as a dietary supplement. It is noted that this form of chromium supplement is generally dosed at 200-600 µg of chromium per day and is absorbed more efficiently than trivalent chromium that would be consumed in the diet. This means that people would be consuming trivalent chromium at levels much higher than the adequate intake value. Their review showed that chromium picolinate consumption showed no changes in “body composition or glucose or insulin responses in healthy individuals.” They also mentioned possible mutagenic effects from its consumption.

However, the view that chromium picolinate, or any form of trivalent chromium, is actually a harm to the body is not fully supported. The Institute of Medicine did not find enough evidence to conclude any harmful effects from chromium picolinate. The National Toxicology Program (NTP) conducted a two year study on rats and mice that also observed no toxicity from exposure to chromium picolinate. The Agency for Toxic
Substances and Disease Registry concluded there was no significant evidence that trivalent chromium was carcinogenic.¹ These and other studies leave uncertainties as to the actual benefits from trivalent chromium. Some of the studies even suggest possible deleterious effects. Overall, however, trivalent chromium is considered to be beneficial at some level and, unlike hexavalent chromium, is not found to be carcinogenic.

1.2 Hexavalent Chromium

Hexavalent chromium is a known carcinogen through inhalation. It has been used in dye pigments, electroplating, and other industrial applications. These workplaces often contain elevated levels of hexavalent chromium relative to the environment. This causes a higher occupational hazard. Hexavalent chromium can also be formed from the hot temperatures at coal-fired power plants and be released in the fly ash. Inhalation of hexavalent chromium is known to be carcinogenic, specifically causing lung cancer. Direct dermal contact is capable of producing negative effects as well; and there is evidence for carcinogenicity and toxicity from ingestion. Less severe effects can include other respiratory reactions, such as coughing and irritation. Dermal and allergic responses, including nasal perforations and skin rashes, are results of exposure as well. Hexavalent chromium is also the cause of more severe health problems, such as kidney and liver damage.

Different organizations, nationally and internationally, have recognized the risks from exposure to hexavalent chromium. The Occupational Safety and Health Administration (OSHA) has set occupational permissible exposure limits in the air for hexavalent chromium at 5 µg/m³ over 8 days.¹² The International Agency for Research
on Cancer (IARC) has classified hexavalent chromium in Group 1 as a human carcinogen. Hexavalent chromium is included in California’s Proposition 65 (Safe Drinking Water and Toxic Enforcement Act of 1986) which identifies chemicals that cause cancer, birth defects, or reproductive problems. Proposition 65 requires businesses to notify the public if one of these chemicals, including hexavalent chromium, is within their products. This would extend to the dietary supplements in this study.

Hexavalent chromium is known to be a carcinogen through the inhalation route of exposure and is mostly encountered in an occupational environment. It is very reactive and causes damage to macromolecules. The main mode of toxicity is thought to be from hexavalent chromium’s conversion to trivalent chromium after absorption into the cells. The carcinogenicity or health problems from consumption of hexavalent chromium in supplements would be occurring through ingestion. However, the data on carcinogenicity from ingestion is less prevalent or conclusive. One hypothesis is that hexavalent chromium is reduced to the less toxic trivalent chromium when ingested. This reduction would decrease the risk of harm leading to the conclusion that hexavalent chromium ingestion is not carcinogenic. There are studies that agree with this hypothesis. However, many of the studies with this conclusion were with short-term exposure. Also, they determined hexavalent chromium absorption by measuring chromium in the body or in urine without the inclusion of bone or soft tissues.

There are other studies which showed that hexavalent chromium is absorbed and carcinogenic by ingestion. The NTP performed a study on sodium dichromate dihydrate ingestion by rats and mice. The study animals were exposed to drinking water with varying amounts of sodium dichromate dihydrate for two years. This was meant to
determine the carcinogenicity and toxic effects from ingestion of hexavalent chromium in drinking water. The results were rats with carcinomas in the mouth concluding that ingestion of hexavalent chromium caused oral cancer in rats. The mice had an increased risk of small intestine tumors concluding ingestion was cancer-causing in mice. Another study on rats by Stern concluded that ingestion of hexavalent chromium is “likely to be carcinogenic to humans.”21 Tumors in the small intestines of the rats were observed, and a human equivalent cancer slope factor of 0.5 (mg of hexavalent chromium/kg-body weight/day)1 was also determined. It is important to understand that this is an estimate from studies performed on rodents. Rodents have a much shorter lifespan than humans; and the mechanism of carcinogenicity in rodents differs from humans. Another study with potassium dichromate exposure in water resulted in increased ultraviolet-induced skin tumors in hairless mice.22 The results showed that increased doses of potassium dichromate resulted in increased number of the induced tumors. They concluded with their concern for hexavalent chromium exposure through drinking water.

Data from human exposure through oral intake is limited. One set of data is from a Chinese province with hexavalent chromium contaminated well water. This data showed an increase in stomach cancer in contaminated areas.23 One review of different types of evidence for the effects of oral ingestion included toxicity and mechanism studies and animal and human studies.24 The review concluded that there is evidence that some of the ingested hexavalent chromium will enter cells and cause DNA damage. The overall conclusion is that hexavalent chromium looks to be carcinogenic through oral exposure. Other toxic effects from ingestion have also been observed. These are mainly in the gastrointestinal tract, including ulcers, but can also affect the blood with anemia.1
The concerns for ingestion of hexavalent chromium are not agreed upon by all studies. However, with the data that does show harmful effects and the known carcinogenicity from inhalation, it is best to be on the side of caution with regard to ingestion of hexavalent chromium.

### 1.3 Challenges with Chromium Speciation and its Importance

If trivalent and hexavalent chromium were stable under similar conditions, separation and analysis would be more straightforward. However, interconversions between the species introduce difficulties in determining the concentration of each species in the sample. These interconversions (trivalent chromium to hexavalent chromium and vice versa) can occur throughout sample preparation and analysis. This is influenced by the stability of each species at different pH and Eh values. Trivalent chromium is stable at lower pH and Eh values. On the other hand, hexavalent chromium is stable at higher pH and Eh values.
Figure 1: Phase diagram for chromium species at different pH and Eh values

The phase diagram for chromium, Figure 1, allows visualization of these stabilities. The lower the pH of a solution, the more likely it is for the species of chromium to be trivalent chromium or to be converted to trivalent chromium. Therefore, if sample preparation involves using an acid, the hexavalent chromium present in the original media could be converted to trivalent chromium because of the conditions. Being able to track and correct for these interconversions to obtain the correct values is important for analysis.

Chromium is found within different matrices through natural and anthropogenic means. It can be found at some level in drinking water. This causes a concern if the species that is being consumed is hexavalent chromium. There is an EPA drinking water maximum contaminant level for total chromium of 100 parts per billion (ppb).
Currently there are no national standards for hexavalent chromium in drinking water. However, California has implemented a Public Health Goal of 0.02 ppb which is believed not to cause any adverse human health effects. A Public Health Goal is meant to be used as a start to creating a future maximum contaminant level in drinking water. This very low concentration for a particular species of chromium needs to be quantifiable in order to assess drinking water’s compliance. Having a method and an instrument that is sensitive enough to be able to detect this level would be needed.

Another important application of chromium speciation is in dietary supplements. Because trivalent chromium is believed to facilitate glucose metabolism, it is often a component of dietary supplements and vitamins. The supplements in which it is present are often promoted to induce weight loss. The trivalent chromium is listed in different forms, including chelates. Examples include chromium picolinate, chromium nicotinate, and chromium chloride. It should not be problematic if these forms of chromium are present in the supplements. However, if there are impurities or there are interconversions during supplement production, hexavalent chromium may also be present within these supplements. The supplement and vitamin industry is not regulated under the Food and Drug Administration (FDA). This means that FDA approval is not needed for the marketing of the products. Older methods of analysis may be used or no testing may be done at all.

These supplements often contain many other ingredients as well as the chromium. This can create a complex solid or liquid matrix that may consist of cellulose or silica filler, vitamins, and gelatin coating. These components can create different environments that may be more acidic or alkaline. Because different supplements contain varying
combinations which may promote interconversions, there can be difficulties in chromium speciation. The challenge is to be able to determine a correct concentration for trivalent and hexavalent chromium before sample preparation or analysis was performed on the supplements.

1.4 Methods to Determine Chromium Species

Current analysis of chromium often involves only the determination of total chromium, hexavalent chromium, or the use of an unreliable method. In terms of chromium analysis techniques, EPA Method 7196A and EPA Method 7199 are older colorimetric methods that are still used extensively.²⁹,³⁰ Often, new protocols for chromium determination are tested with the use of aqueous prepared standards. However, dietary supplements and other solid samples will create new difficulties, especially in terms of sample preparation. Some studies also implement the complexing agent ethylenediaminetetraacetic acid (EDTA). The main methods used for this thesis research are approved by the EPA and have been used in various studies. EPA Method 3060A is often used for solid samples with hexavalent chromium.³¹ EPA Method 3052 is used for many metals for digestion from solid samples.³² EPA Method 6800A has proven to be able to obtain correct values unlike Method 7196A and other methods that do not take interconversions into account.³³

Method 7196A relies on detection of the absorption of a violet color that is representative of hexavalent chromium. The reaction of diphenylcarbazide (DPC) with hexavalent chromium results in the reduction of hexavalent chromium to trivalent chromium. Diphenylcarbazide is oxidized to diphenylcarbazone (DPCO) and complexes
with the newly formed trivalent chromium. This complex creates the violet color that is measured as absorbance at 540 nm. The hexavalent chromium concentration in the samples can then be determined from a calibration curve. It was used in several studies that involved hexavalent chromium remediation.\textsuperscript{34,35} Method 7199 is a method used for hexavalent chromium in drinking water, groundwater, and wastewater. It involves adjustment of pH to 9 to 9.5. Ion chromatography is used to separate hexavalent chromium. This resulting solution is then reacted with DPC for hexavalent chromium detection by the resulting color formation. In the development of the hexavalent chromium soil standard reference material, laboratories were asked to use Method 3060A and then an analysis method.\textsuperscript{36} In total for the two studies, there were fourteen uses of Method 7196A, fourteen uses of Method 7199, and six uses of Method 6800A. There was evidence that Method 7196A and Method 7199 were giving low values for hexavalent chromium which was corrected if using Method 6800.

The problems with these older techniques are that they do not allow for tracking of interconversions or measurement of trivalent chromium. Method 7199 is also specified for water samples, not for the application of the solid supplements. The inability to measure trivalent chromium from both of these methods does not allow for mass balance to verify the obtained hexavalent chromium value. Also, detection limits are greater for these methods. Therefore, smaller amounts of hexavalent chromium would not be detected.

The complexation of trivalent chromium with EDTA has also been performed in some studies. One study used EDTA to first complex with trivalent chromium and then introduced the solution into chromatography.\textsuperscript{37} This study used prepared stock solutions
made with DDI water. Another study used IC-ICP-MS with EDTA to determine both species in aqueous standards.\textsuperscript{38} Detection limits around 100 parts per trillion (ppt) were obtained. Chromatography was also used in another study demonstrating the use of EDTA for chromium speciation in aqueous standards.\textsuperscript{39} An acidic pH value of 4 was found to be optimal for Cr(III)-EDTA complexation. The studies claim no or little conversions between species in their methods. However, this cannot be verified from their results, and the use of acidic conditions may be promoting conversions from hexavalent to trivalent chromium. None of these studies implemented Method 6800A to track any interconversions. They also did not analyze any solid matrices. There was one study that did involve EDTA using a solid sample of chromium picolinate.\textsuperscript{40} The picolinate supplements were ground into a powder, dissolved in water, and filtered. EDTA was then added to the solution and heated. Chromatography was used to separate the species. However, none of the samples that were analyzed showed any hexavalent chromium. The use of water to dissolve the supplements does not implement the microwave chemistry used in Method 3060A. The solvent and conditions used are not likely to be able to extract the hexavalent chromium into solution.

Extraction of hexavalent chromium has been performed with Method 3060A in soils and other media. Performing only this sample preparation method does not allow for the mass balance that is being performed in this research project. However, it has been proven to be efficient at minimizing interconversions and accurately determining hexavalent chromium. In a study of five methods for the extraction of hexavalent chromium from soils, using a mixture of sodium hydroxide and sodium carbonate solution with heating was the best.\textsuperscript{41} Method 3052 being performed separately is a
commonly used EPA method for total digestion of sample matrices. Another study compared four methods for the digestion of soil to measure trace metals.⁴² This study showed that Method 3052 had greater recovery for chromium than the other four methods. This method has been used specifically for elemental chromium, including the digestion of soils.⁴³ However, when it is the only method used, it will not provide chromium speciation.

EPA Method 6800A, Speciated Isotope Dilution Mass Spectrometry (SIDMS), has been applied to trivalent and hexavalent chromium speciation. The method was investigated in a study that proved its accuracy and applicability, specifically with chromium species.⁴⁴ It is theoretically possible to correct up to 90% species conversion using SIDMS. Method 6800A was used in another study on sand and soil extracts and chromite ore processing residue.⁴⁵ Method 3060A was first performed for extraction of hexavalent chromium. Comparison to Method 7196A showed that all of the hexavalent chromium was not fully recovered for sand and soil extracts in Method 7196A. This is less accurate than the values obtained from corrections through Method 6800A. The method was also used on different coal-fired combustion by-products, including fly ash and leachate.⁴⁶ One conclusion was that the combustion of the coal leads to oxidation of trivalent chromium to hexavalent chromium. However, this study was not focused on mass balance. Studies using Method 6800A have shown that it gives more accurate values than other methods by correcting for interconversions. Although, Method 6800A has yet to be applied to dietary supplements as it was in this research project.
1.5 Summary of Project Goals

The main goal of the current research project involved determining trivalent, hexavalent, and total chromium in a variety of dietary supplements. It was predicted that the use of microwave-enhanced chemistry and SIDMS would provide more accurate values than older methods. The main technique was to apply two different EPA sample preparation methods (Method 3060A and Method 3052) to the same sample to determine hexavalent chromium followed by trivalent chromium. These methods involve extractions and digestions performed in a laboratory microwave. This allows the species to be present in a solution. Ion-Exchange Chromatography (IC) was then used to separate the two chromium species from solution. The detector was Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). These instruments and methods allow for more sensitive detection of analytes. SIDMS was also applied which allows for tracking and correction of interconversions between the two chromium species. This involves double spiking of the samples with isotopically-enriched chromium species of $^{50}$Cr(III) and $^{53}$Cr(VI). Total chromium was determined using microwave digestion and analysis with ICP-MS. Isotope Dilution Mass Spectrometry (IDMS) was used to determine accurate concentrations even with partial loss of analyte.

The goal was to determine the mass balance of both trivalent and hexavalent chromium in the dietary supplements. The sum of the trivalent and hexavalent values being equal to the total chromium value allows for mass balance. This confirms that the values for the individual species are correct and action can be taken if potential harm is being caused from hexavalent chromium’s presence.
Literature shows that the popular methods for hexavalent chromium analysis are not the best choice for use in analyzing dietary supplements. Comparison to older analysis methods, specifically Method 7196A, involved the use of colorimetry. Method 3060A, 3052, and 6800A separately prove to be effective in sample preparation and analysis. However, there has yet to be any studies to specifically and accurately address chromium speciation in the supplements with the ability to correct for interconversions.

The goal was also to examine other matrices and methods for hexavalent chromium. The research investigated chromium speciation detection limit with the IC-ICP-MS. In attempts to achieve mass balance, water samples were analyzed using calibration curve, IDMS, and SIDMS. Hexavalent chromium in soil, electronic parts, and chromite ore processing residue was also analyzed using EPA methods.

Because of the differences in the effects of the chromium species, this research allows more information to be gained about the chromium in different media. Proper analysis of dietary supplements is important because of the popularity of dietary supplements and their lack of FDA regulation. The risks from ingestion of hexavalent chromium are also high enough to cause concern of possible contamination in these supplements. With new concern for hexavalent chromium content in water, the ability to quantify at low levels and the knowledge of the species is valuable. A verified hexavalent chromium value with mass balance in matrices is important because of the risk of negative health effects from hexavalent chromium.
CHAPTER 2: Materials & Methods

The methods that were used to successfully obtain mass balance with chromium speciation were based on EPA methods. Appendix A.1 contains a table comparing the methods used with some of their advantages and disadvantages. These include Method 3060A for hexavalent chromium extraction, Method 3052 for digestion, Method 6020A for calibration curve, and Method 6800A for species interconversion corrections. Other methods were also used and compared, including Method 7196A for hexavalent chromium. Variations on these methods in order to optimize the technique were also implemented.

The isotope abundances of the chromium isotope spikes (Applied Isotope Technologies, Inc., Pittsburgh, PA) that were used throughout sample preparation and analysis are shown in Table 1. On Earth, $^{52}\text{Cr}$ is greater in abundance (83.79%) than the other isotopes of chromium. However, sample preparation and analysis may involve adding isotopically-enriched spikes that are more abundant in either $^{50}\text{Cr}$ or $^{53}\text{Cr}$.

Sample preparation often involved an Ethos-1 microwave to prepare the sample to be analyzed (Milestone, Shelton, CT). Metrohm’s Ion-Exchange Chromatography (IC) system with 818 IC Pumps and 838 Advanced Sample Processor was used for separation of chromium species (Metrohm USA, Riverview, FL). For some of the work with water samples, Metrohm’s 850 IC Pumps and 838 Advanced Sample Processor were used. A Hamilton PRP-X100 IC anion-exchange column (PEEK 150 mm x 4.6 mm or 150 mm x 4.6 mm, 10 µm) was used with both models of the Metrohm IC system. Detection was
performed on Agilent’s HP-4500 and 7700 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Agilent Technologies, Santa Clara, CA). Liquid argon was used as the nebulizer carrier gas (Airgas Inc., Radnor, PA). Table 2 shows the conditions for the Ion-Exchange Chromatography-Inductively Coupled Plasma-Mass Spectrometry (IC-ICP-MS).

Table 1: Isotope Abundances of Standards and Spikes

<table>
<thead>
<tr>
<th>Isotope</th>
<th>natCr (%)</th>
<th>50Cr(III) (%)</th>
<th>53Cr(VI) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50Cr</td>
<td>4.345</td>
<td>97.30</td>
<td>0.03</td>
</tr>
<tr>
<td>52Cr</td>
<td>83.79</td>
<td>2.40</td>
<td>2.19</td>
</tr>
<tr>
<td>53Cr</td>
<td>9.501</td>
<td>0.20</td>
<td>97.7</td>
</tr>
<tr>
<td>54Cr</td>
<td>2.365</td>
<td>0.10</td>
<td>0.08</td>
</tr>
</tbody>
</table>

The “nat” superscript represents “natural isotopic abundance”

Table 2: Operating Conditions for IC-ICP-MS

<table>
<thead>
<tr>
<th>Plasma</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma flow rate (L/min)</td>
<td>15.0</td>
</tr>
<tr>
<td>Auxiliary gas flow rate (L/min)</td>
<td>1.0</td>
</tr>
<tr>
<td>Radio frequency power (W)</td>
<td>1450</td>
</tr>
<tr>
<td>Sample cone</td>
<td>Nickel, 1.1 mm orifice</td>
</tr>
<tr>
<td>Skimmer cone</td>
<td>Nickel, 0.89 mm orifice</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measurement Parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis mode</td>
<td>Time resolved analysis (TRA)</td>
</tr>
<tr>
<td>Analysis isotopes</td>
<td>50Cr, 52Cr, and 53Cr</td>
</tr>
<tr>
<td>Nebulizer gas flow rate (L/min)</td>
<td>0.93-1.00</td>
</tr>
<tr>
<td>Peristaltic pump rate (rpm)</td>
<td>0.50</td>
</tr>
<tr>
<td>Integration time per point(s)</td>
<td>0.10</td>
</tr>
<tr>
<td>Total analysis time (s)</td>
<td>840</td>
</tr>
<tr>
<td>Eluent flow rate (mL/min)</td>
<td>1.0</td>
</tr>
</tbody>
</table>
2.1 Total Chromium in Dietary Supplements

The dietary supplements investigated were those that can be purchased from online stores and drug stores over the counter. Some of the supplements were sent with limited supplement information from a consumer lab, and the remaining samples were purchased directly online or in store from a local market. Weight-loss, chromium, and vitamin supplements are some of the main products that were analyzed. These included solid samples of capsules and tablets with the exception of one liquid sample. Another type of supplement that was tested was the raw chromium compound, such as chromium chelates, that are used to create the dietary supplements. These raw materials cause difficulties during analysis because they contain a much higher content of chromium.

For a solid matrix, such as supplements, the chromium needed to be extracted or digested into a solution in order to introduce it into the uv-vis spectrophotometer, IC, and/or ICP-MS. In order to have homogeneous sub-samples, the solid supplements were ground with a mortar and pestle into powder which was then used for the EPA methods. The supplement samples were stored in polypropylene centrifuge tubes at room temperature. Analytical techniques were performed in a class-1000 clean-room or on a class-100 clean-bench.

To achieve mass balance, total chromium values needed to be determined. A summary of EPA Method 3052 can be seen in Appendix A.2. Table 3 shows the approximate amounts of chemicals that were used for Method 3052. This involved the traditional techniques in Method 3052 with a solution of concentrated nitric acid (Trace metal, Fisher Scientific, Pittsburgh, PA) and 30% hydrogen peroxide (Ashland Chemicals, Columbus, OH). Total chromium analysis used IDMS which involved
spiking with $^{50}\text{Cr(III)}$ at a known isotopic ratio that differs from the natural isotopic ratio. The sample and reagents were added, along with a magnetic stirring bar, to a high pressure microwave vessel made of TFM (tetrafluoromethane). These TFM vessels are a thermally resistant fluoropolymer (Milestone’s Model SK-10). Three sub-samples were created for each supplement sample. A blank, without any sample, was also created for each set of analysis. The masses of the sample and spike were recorded for each sub-sample. These closed vessels were sealed and placed on a rotor. Equilibration of the spike with the sample occurred through Method 3052 microwave irradiation. The temperature was increased to 180 °C (microwave is accurate within ±2 °C) for 10 minutes and then held at that temperature for 10 minutes. The samples were then cooled to room temperature.

**Table 3**: Approximate sample and reagent amounts for Method 3052

<table>
<thead>
<tr>
<th>Sample (g)</th>
<th>$^{50}\text{Cr(III)}$ [725 ppm] (g)</th>
<th>Nitric Acid (mL)</th>
<th>Hydrogen Peroxide (30%) (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.25</td>
<td>9.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

After microwave digestion, the digests were filtered with 0.45 µm glass fiber filters using a Milestone FAM–40 vacuum unit (Sorisolet, Italy). The filtered solutions were decanted into 50 mL polypropylene centrifuge tubes and adjusted to equal volumes using 18MΩ double deionized (DDI) water from a Barnstead Nanopure water purification system (Dubuque, IA). The tubes were weighed before and after the extract addition. This allowed for the calculation of extract mass. The digests were stored in a cold room at 4 °C until analysis.
A summary of the IDMS method can be found in Appendix A.3. In ICP-MS analysis, mass bias correction was performed using *nat* Cr(III) standards and DDI water. Mass bias is a positive or negative instrumental bias of the measured isotope ratio from the true isotope ratio. Each of the extracts was diluted 100-fold (100 µL extract and 9.9 mL DDI water). There were four replicate runs in spectrum mode on the HP-4500 ICP-MS for each of the extracts. The species was not taken into account for IDMS because the digest was analyzed for elemental chromium with the ICP-MS. If chromium was lost after equilibration, the original concentration could still be determined through the IDMS spiking with known isotope ratios. ICP-MS obtains an isotope ratio that was then used, along with known natural and spike isotope ratios, to determine the chromium concentration in the sample. AIT’s IDMS software was used for calculations. Appendix A.4 contains the equations used for IDMS.

### 2.2 Chromium Speciation in Dietary Supplements

One technique to measure both trivalent and hexavalent chromium from a sample was to perform an EPA sample preparation method followed by a separate method on the remaining sample. First, Method 3060A was used to extract the hexavalent chromium from the supplements. Appendix A.5 contains a summary of Method 3060A. This involved the preparation of an alkaline digestion solution containing 5.0 g sodium hydroxide (Certified ACS, Fisher Scientific) and 7.5 g sodium carbonate (Certified ACS, Fisher Scientific) dissolved in 250 mL of DDI water. A phosphate buffer was also made using 3.40 g monobasic (Fisher Scientific) and 4.35 g dibasic potassium phosphate (Aldrich Chemicals, Milwaukee, WI) dissolved in 50 mL of DDI water. The sample,
digestion solution, phosphate buffer (pH=7.0), and magnesium chloride were added to a polypropylene centrifuge tube along with a stirring bar. Magnesium chloride is used to suppress oxidation from trivalent to hexavalent chromium during sample extraction using a microwave. The approximate amounts of reagents and sample are shown in Table 4. Masses of the sample and spikes were recorded for each sub-sample.

Previously, TFM microwave vessels were used for microwave extraction. However, inconsistent data with the vessels led to the use of polypropylene centrifuge tubes. The TFM vessels are re-usable and were cleaned using an acid solution. When using these vessels for Method 3060A, acid leaching from the vessels caused immediate conversion of the added spikes before equilibration. Polypropylene tubes were used because they are disposable and did not cause leaching. Figure 2 shows the polypropylene centrifuge tubes placed in the microwave. Holes were punctured in the lids of these tubes to allow for pressure release. The data that is presented in the results is from sample preparation with the polypropylene tubes. Three sub-samples from each supplement and a blank were heated in the microwave to 90 ºC for 60 minutes after ramping to that temperature for 10 minutes. These samples were also allowed to cool to room temperature.

<table>
<thead>
<tr>
<th>Table 4: Approximate sample and reagent amounts for Method 3060A</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>METHOD 3060A</strong></td>
</tr>
<tr>
<td>Sample (g)</td>
</tr>
<tr>
<td>0.50</td>
</tr>
</tbody>
</table>
During the sample preparation to these samples, interconversions can occur. EPA Method 6800A, SIDMS, was used to correct for these. A summary of this method can be found in Appendix A.6. If Method 6800A was being used, the solution was double spiked with both $^{50}\text{Cr(III)}$ and $^{53}\text{Cr(VI)}$. Table 4 shows the amounts of spikes that were added. For the use of SIDMS throughout this project, the amount of spike could vary depending on the concentration of the spike and the expected amount of trivalent and hexavalent chromium in the sample. The spikes that are added should create an isotope ratio ($^{53}\text{Cr} : ^{52}\text{Cr}$ and $^{50}\text{Cr} : ^{52}\text{Cr}$) of approximately 1:1. The isotope ratio ranges of the samples must be within 0.1:10 and 10:1 to minimize error. Optimization of the spiking procedure was also performed. Double spiking of both $^{50}\text{Cr(III)}$ and $^{53}\text{Cr(VI)}$ before Method 3060A extraction was tested. Spiking with $^{53}\text{Cr(VI)}$ before Method 3060A extraction, and then spiking the remaining residue with $^{50}\text{Cr(III)}$ before Method 3052 was also performed.
Method 6800A relies on isotopically-enriched chromium standards where the species of those isotopes matters. Equilibration with the sample occurred through microwave-enhanced chemistry. By knowing the natural isotopic ratios and the enriched isotopic ratios for each of the spikes and their species, changes through interconversions can be tracked and corrected.

Following microwave extraction, the polypropylene tubes were centrifuged at 3,500 revolutions per minute for 10 minutes in a Sorvall RC-5B Refrigerated Superspeed Centrifuge (DuPont Instruments). The solution was then decanted into another tube with the extracted hexavalent chromium now in solution. DDI water was added to the extracts to make each the same volume. Weighing the tubes with and without the extract allowed the extract mass to be calculated. Because this solution is alkaline, hexavalent chromium was the only stable species, and trivalent chromium precipitated out of solution.

In order to determine the trivalent chromium, Method 3052 was used to digest the remaining, precipitated residue. The same Method 3052 procedures were followed as used in total chromium sample preparation. However, the residue was considered to be the sample. Spike was only added at this point if it was not doubly spiked before performing Method 3060A. The acidic solution of 9.0 mL nitric acid and 2.0 mL hydrogen peroxide (30%) was added along with the residue into TFM microwave vessels with stirring bars. This was digested in the microwave at 180 °C for 10 minutes. The resulting solution was then filtered and decanted into centrifuge tubes. Both Method 3060A and Method 3052 solutions were stored in the cold room at 4 °C until analysis.

The first extract containing hexavalent chromium from Method 3060A was diluted 100-fold. A calibration curve was created for analysis using Method 6020A if no
spikes were added. Appendix A.7 contains a summary of Method 6020A. This involved preparation of different levels of natural abundance chromium standards. If using Method 6800A, this was not necessary; and instead a mass bias correction standard was prepared by spiking DDI water with both naturally abundant trivalent and hexavalent chromium. These diluted extracts were run through the IC with a 100 µL sample loop to separate the trivalent and hexavalent chromium. Four replicate runs were performed on each of the extracts and the blank. A gradient was used by changing between the use of an acidic and alkaline mobile phase through the Hamilton PRP-X100 IC anion-exchange column. The reagents and conditions of the mobile phases are shown in Table 5. The ammonium hydroxide was used to adjust the pH of Eluent A. The thulium (High Purity Standards, Inc, Charleston, SC) was used to create optimum column separation of the two chromium peaks. The gradient time program using the 200 mm IC column is shown in Table 6. Some of the samples were analyzed using a 150 mm column which reduced the time for analysis. The signal from the two chromium species was detected on the HP-4500 ICP-MS in Time Resolved Analysis mode and shown through a chromatogram. The chromatogram data was used to determine the hexavalent and trivalent chromium concentrations. Calibration curve was used if Method 6020A was performed. If mass balance using SIDMS was performed, AIT’s SIDMS software was used to calculate values. The algorithms, assumptions, and calculations involved in SIDMS can be seen in Appendix A.8.
**Table 5:** Approximate reagent amounts for the Alkaline and Acidic IC Eluents in approximately 2 L of water

<table>
<thead>
<tr>
<th>Eluent</th>
<th>Concentrated Nitric Acid (mL)</th>
<th>Thulium [10 ppm] (mL)</th>
<th>Ammonium Hydroxide (mL)</th>
<th>Approximate pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluent A</td>
<td>7.8</td>
<td>2.0</td>
<td>Approximately 15.0</td>
<td>9.3</td>
</tr>
<tr>
<td>Eluent B</td>
<td>7.8</td>
<td>2.0</td>
<td>0</td>
<td>2.2</td>
</tr>
</tbody>
</table>

**Table 6:** Gradient time program using a Hamilton PRP-X100 IC anion-exchange column (PEEK 250 mm x 4.6 mm, 10 µm)

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Flow (mL/min)</th>
<th>% Eluent A</th>
<th>% Eluent B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>1.0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>1.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>1.0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

The second solution of digested chromium from Method 3052 was analyzed using IDMS. Direct aspiration of the digests through the ICP-MS was performed after a 100-fold dilution with DDI water. Each digest was analyzed with four replicates in Spectrum Mode. The chromium value acquired from AIT’s IDMS software represented the precipitated trivalent chromium.

Hexavalent and trivalent chromium values obtained from sample preparation with Method 3060A and Method 3052 were summed. This total was compared to the total chromium value obtained using Method 3052 and IDMS. If these two values were the same, the values were considered to be verified by mass balance.
Another component of the dietary supplements are the raw chromium compounds. Preliminary experiments were performed on four different samples of this material without mass balance. Raw chromium compounds contained very high amounts of chromium; and Method 3052 was not performed because of the large amount of spike that would be required for Method 6800A. Therefore, these materials were only analyzed for hexavalent chromium. Only Method 3060A was used for sample preparation with the same procedures as the dietary supplements. These samples were analyzed using Method 6020A, SIDMS, and Method 7196A for comparisons.

2.3 Alternative Methods with Dietary Supplements: Hot Water & EDTA

Other experiments to attempt simultaneous determination of both species involved variations on these methods. One approach at simultaneously determining both species was to extract the species with a solution that is more neutral in pH value. Hot water was used instead of Method 3060A’s alkaline digestion solution or Method 3052’s acidic solution that both allow only one species to be stable. Table 7 shows the amount of sample and reagents used for this variation. The procedure for Method 3060A was followed except there was a substitution of DDI water in place of digestion solution. Also, these experiments were performed with the conventional use of TFM microwave vessels and not the polypropylene tubes. Analysis was also performed using IC-ICP-MS.

**Table 7**: Approximate sample and reagent amounts for Hot Water Extraction

<table>
<thead>
<tr>
<th>Sample (g)</th>
<th>DDI water (mL)</th>
<th>$^{50}$Cr(III) [725 ppm] (g)</th>
<th>$^{51}$Cr(VI) [90 ppm] (g)</th>
<th>Phosphate Buffer (mL)</th>
<th>Magnesium Chloride (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>19.0</td>
<td>0.30</td>
<td>0.30</td>
<td>0.50</td>
<td>0.050 g</td>
</tr>
</tbody>
</table>
Another variation was the addition of EDTA (Fisher Scientific, Pittsburgh, PA) to form a complex with trivalent chromium and keep it in the same solution as hexavalent chromium. This was done by performing Method 3060A with the addition of an amount of EDTA. Actual dietary supplement samples were used, with and without spiking, to evaluate this method. Sample amount was 0.50 g with addition of approximately 0.10 or 0.036 g of EDTA. The procedure was also performed by addition of chromium spikes and 0.036 g EDTA without any supplement samples. Different temperatures were tested that were greater than Method 3060A’s 90 ºC. These temperatures ranged from 125 to 200 ºC. Analysis involved procedures similar to the other experiments performed on IC-ICP-MS.

2.4 Method 7196A with Dietary Supplements

The widely-used Method 7196A was also performed in this project for comparisons. A summary of this method is found in Appendix A.9. This was only performed on selected raw material samples. Method 3060A was used as sample preparation for the dietary supplements. The prepared extract was evaluated using Method 7196A. Method 7196A involved the preparation of a DPC solution by adding 250 mg of DPC (Certified ACS, Fisher Scientific) in 50 mL of acetone within a brown bottle. A solution of 10% sulfuric acid was also prepared with 10 mL of DDI water and 1.0 mL concentrated sulfuric acid (Trace metal, Fisher Scientific). The DPC and acid solution were added to a vial along with the sample extract. Table 8 shows the approximate amounts of sample and reagent used for this method.
Table 8: Approximate sample and reagent amounts for Method 7196A

<table>
<thead>
<tr>
<th>Method 3060A Extract (mL)</th>
<th>DPC Solution (mL)</th>
<th>Sulfuric Acid Solution (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.050; 0.50; 1.0 *</td>
<td>2.0</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*Differed depending on the samples

Because this is a light-sensitive reaction that causes a color change, the analysis was performed shortly following the reactions. The pH values of the solutions were checked with pH paper to verify that the samples were acidic with pH values around 2.0. A Cary 100 1E UV Visible spectrophotometer was used to measure the absorbance of the violet color, indicative of hexavalent chromium, at 540 nm. The uv-vis was zeroed with DDI water, and DDI water was also used as the reference. A calibration curve with hexavalent chromium standards (0, 25, 50, 100, and 200 ppb) was created to determine the hexavalent chromium concentrations in the samples. The solutions were each added to a cuvette before analysis. The absorbance for each sample was recorded.

2.5 Detection Limit of Chromium Species for IC-ICP-MS and Analysis of Water Samples

In addition to the main focus of chromium in dietary supplements, other matrices were evaluated for comparison of methods and results. Because of the interest and concern for chromium in drinking water, the detection limit for water was investigated with IC-ICP-MS. This involved both qualitative and quantitative analysis. Analysis involved 818 and 838 IC instruments and the Metrohm 850 Pumps and 858 Advanced
Sample Processor. The HP-4500 and 7700 ICP-MS were also both used. Calibration curve, IDMS, and SIDMS were all used for water samples.

Chromium speciation was performed with several variations on experiments. Different samples were analyzed for the speciation study, including DDI water, tap water supplied from different water authorities, and bottled water supplied from different companies. Calibration curve was created for analysis. SIDMS was also performed using both high and low ratio spiking. Chromium speciation was performed without any sample preparation with microwave or acid addition. IC-ICP-MS was used for separation and analysis. These samples were separated using the newest 850 and 858 IC instrumentation and 7700 ICP-MS. SIDMS using the 7700 ICP-MS involved the use of a helium collision cell (flow rate of 4.0 mL/min). This eliminates the polyatomic ions, such as argon carbide and argon oxide, that would normally interfere at the same mass as the analytes. These larger ions collide with the helium gas and lose the energy needed to pass through the ion filter.

Mass balance was also attempted by determining total chromium values. Calibration curve and IDMS were both performed. IDMS was performed under different conditions. Experiments involved no acid addition or microwave chemistry. Other experiments involved heating in the microwave with and without the addition of nitric acid. Studies also involved spiking at different ratios and using No Gas and Helium mode in the ICP-MS.

This study is currently on-going with the objective of achieving mass balance. Thus far, the procedures performed will facilitate in determining the most successful
methods. Methods that will give stable and similar values for the sum of species and total chromium value will then be perfected.

2.6 Hexavalent Chromium and Chromium Speciation in Other Matrices

In addition to dietary supplements and water samples, other matrices were evaluated for chromium. These included a solid standard reference material for hexavalent chromium, electronic parts, and chromite ore processing residue. These studies involved the same EPA methods used for dietary supplements.

Chromium Standard Reference Material (SRM) 2701 for Hexavalent Chromium in Soil was evaluated using Method 3060A. The soil SRM was prepared using approximately 0.25 g of the SRM sample in three sub-samples for each of the two procedures. One procedure was sample preparation using Method 3060A without spiking. These extracts were analyzed by 100-fold dilution and the use of a hexavalent chromium calibration curve for Method 6020A with IC-ICP-MS. Method 3060A was also performed with spiking of $^{50}\text{Cr(III)}$ and $^{53}\text{Cr(VI)}$ to create appropriate isotope ratios. These spiked extracts were diluted 100-fold and analyzed using SIDMS with IC-ICP-MS. Procedures for Method 3060A, Method 6020A, and SIDMS used in the other experiments were also used for this matrix.

In addition to the SRM sample, computer parts were evaluated using only Method 3060A to determine if hexavalent chromium was present. This was to determine if they are compliant with the European Union’s Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment (RoHS). The samples were mainly motherboard components, including transistors. These electronics were previously
prepared into a powder, and an amount of 0.50 g was used for each sub-sample. SIDMS spiking and Method 3060A were then performed. These extracts were diluted 10-fold. Analysis was similar to the previous experiments using SIDMS on the IC-ICP-MS.

Chromite Ore Processing Residue (COPR) was another matrix where hexavalent chromium may be present. COPR is the residue after the manufacturing of chromium compounds from chromium-containing ores. COPR often contains chromium species and other metals. The COPR samples that were analyzed included four samples that were treated for the removal of hexavalent chromium and a sample that was left untreated. The means of treatment are unknown. Method 3060A was used for sample preparation of three sub-samples per sample. Approximately 0.25 g of the COPR sample was used along with $^{50}$Cr(III) and $^{53}$Cr(VI) spikes. SIDMS was performed with IC-ICP-MS.

Although dietary supplements are the main focus of the current research of the chromium speciation study, other samples give some comparisons of matrices and methods. The implementation of different EPA Methods shows the limitations and capabilities of the preparation and analysis of hexavalent chromium or chromium speciation in different situations. Modification and combinations of methods present new techniques for analysis of chromium species. Furthermore, mass balance is useful in verifying each chromium species concentration.
CHAPTER 3: Results

3.1 Mass Balance in Dietary Supplements

In general, the total chromium values from IDMS were similar to those reported on the supplement packages. These range anywhere from 3 to 1000 parts per million (ppm) depending on the supplement. Table 9 compares the total chromium values from IDMS with those obtained using label information. Because some of the supplements analyzed were sent from a consumer lab, the chromium and serving size values were not explicitly given and are not reported. The total chromium concentration from label information was determined from the reported total chromium mass divided by the average unit mass. This data shows measured values are similar to reported values. However, some values are noticeably different such as Samples 19, 20, and 21. The reported values for Samples 19 and 21 are nearly double the chromium concentrations that were actually found in the supplements. For Sample 20, the reported value was approximately half of the total chromium concentration that was actually measured in the supplement.
Table 9: Comparison of total chromium with IDMS and total chromium from label information

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Total Chromium by IDMS (µg/g)</th>
<th>Total Chromium Calculated by Label Information (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>205.52 ± 2.92</td>
<td>190.056</td>
</tr>
<tr>
<td>13</td>
<td>229.38 ± 4.60</td>
<td>188.141</td>
</tr>
<tr>
<td>14</td>
<td>302.78 ± 28.08</td>
<td>227.162</td>
</tr>
<tr>
<td>15</td>
<td>243.28 ± 4.04</td>
<td>222.499</td>
</tr>
<tr>
<td>18</td>
<td>3.09 ± 0.03</td>
<td>1.830</td>
</tr>
<tr>
<td>19</td>
<td>561.1 ± 11.2</td>
<td>1150.536</td>
</tr>
<tr>
<td>20</td>
<td>860.9 ± 5.6</td>
<td>413.078</td>
</tr>
<tr>
<td>21</td>
<td>58.9 ± 1.6</td>
<td>177.333</td>
</tr>
</tbody>
</table>

Uncertainties are at 95% CL with n = 12

Method 3060A using polypropylene tubes resulted in complete extraction of the hexavalent chromium into solution. Preliminary experiments used the TFM microwave vessels. However, the results were inconsistent and showed high hexavalent chromium values. This is from the leaching of acid from the microwave vessels. However, the polypropylene tubes and stirring bars worked well for extraction. Values were consistent and were more realistic between the different samples.

The approach that proved to give the most accurate results was double spiking at the beginning of sample preparation. Addition of $^{53}$Cr(VI) before Method 3060A and then addition of $^{50}$Cr(III) during preparation for Method 3052 was not effective. It did not allow for tracking of conversion from trivalent to hexavalent chromium. Therefore, the procedure used to obtain the trivalent and hexavalent values was double spiking of $^{50}$Cr(III) and $^{53}$Cr(VI) when the sample was prepared for Method 3060A. This allows for proper tracking of interconversions throughout entire sample preparation. Figure 3 shows a chromatogram from the diluted Method 3060A extract solution with SIDMS spiking. More examples of chromatograms are shown in Appendix A.10. Hexavalent chromium is
shown in the first peak, before 600 seconds, and trivalent chromium is represented in the second peak, after 800 seconds. This shows good separation between the two peaks. There are minimum interferences with only a small argon carbide (ArC) peak around 300 seconds that is most noticeable in $^{52}\text{Cr}$.

Figure 3: IC-ICP-MS chromatogram for Sample 8 spiked with $^{50}\text{Cr(III)}$ and $^{53}\text{Cr(VI)}$ standards [Flow rate: 1.0 ml/min with gradient elution; Eluent: A = 0.06 M HNO$_3$, pH = 9.3, and B = 0.06 M HNO$_3$, pH 2.2; Column: Hamilton PRP-X100 Anion Exchange (250 mm x 4.6 mm, 10µm)]

Table 10 shows the results from the mass balance study. Total chromium with IDMS is included to compare to the sum of trivalent and hexavalent chromium values. These separate values are from Method 3060A and Method 3052 using SIDMS and IDMS. Many of the samples have total values that are essentially identical (ex: Samples
6, 7, and 14) confirming mass balance. The remainder of the samples, with few exceptions, are very similar in total values with only slight variation. These samples were also considered to have successful mass balance. Appendix A.10 contains two chromatograms from samples that achieved mass balance, Samples 4 and 20. SIDMS calculations also were able to determine conversions that were occurring. For example, Samples 4 and 14 produced approximately 7% conversion from trivalent to hexavalent chromium that was then corrected in calculations.

Table 10: Results from dietary supplement mass balance for trivalent and hexavalent chromium

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Total Chromium by IDMS (µg/g)</th>
<th>Mass Balance Study using SIDMS and IDMS (µg/g)</th>
<th>Cr(VI) to Total Cr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Chromium by IDMS (µg/g)</td>
<td>Trivalent Chromium (IDMS)</td>
<td>Hexavalent Chromium (SIDMS)</td>
</tr>
<tr>
<td>1</td>
<td>777.1 ± 4.6</td>
<td>692.0 ± 20.2</td>
<td>26.4 ± 2.5</td>
</tr>
<tr>
<td>2</td>
<td>818.0 ± 10.9</td>
<td>790.5 ± 8.2</td>
<td>&lt; DL</td>
</tr>
<tr>
<td>3</td>
<td>209.6 ± 5.0</td>
<td><strong>720.8 ± 47.1</strong></td>
<td><strong>17.0 ± 2.3</strong></td>
</tr>
<tr>
<td>4</td>
<td>41.6 ± 3.2</td>
<td>31.3 ± 1.8</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>55.1 ± 1.1</td>
<td>58.5 ± 1.4</td>
<td>&lt; DL*</td>
</tr>
<tr>
<td>6</td>
<td>240.9 ± 2.2</td>
<td>215.2 ± 12.4</td>
<td>26.3 ± 3.3</td>
</tr>
<tr>
<td>7</td>
<td>170.5 ± 1.9</td>
<td>161.8 ± 9.6</td>
<td>&lt; DL*</td>
</tr>
<tr>
<td>8</td>
<td>331.8 ± 19.2</td>
<td>341.9 ± 15.7</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>9</td>
<td>1,095.9 ± 21.5</td>
<td>1,012.5 ± 29.2</td>
<td>50.5 ± 2.1</td>
</tr>
<tr>
<td>10</td>
<td>510.8 ± 7.0</td>
<td>513.7 ± 24.9</td>
<td>&lt; DL*</td>
</tr>
<tr>
<td>11</td>
<td>674.9 ± 15.1</td>
<td>723.8 ± 29.4</td>
<td>&lt; DL*</td>
</tr>
<tr>
<td>12</td>
<td><strong>205.52 ± 2.92</strong></td>
<td><strong>497.5 ± 55.7</strong></td>
<td><strong>14.87 ± 2.60</strong></td>
</tr>
<tr>
<td>13</td>
<td><strong>229.38 ± 4.60</strong></td>
<td><strong>618.1 ± 77.3</strong></td>
<td><strong>4.86 ± 0.54</strong></td>
</tr>
<tr>
<td>14</td>
<td><strong>302.78 ± 28.08</strong></td>
<td><strong>262.3 ± 7.2</strong></td>
<td>49.7 ± 8.0</td>
</tr>
<tr>
<td>15</td>
<td><strong>243.28 ± 4.04</strong></td>
<td><strong>247.4 ± 7.0</strong></td>
<td>&lt; DL*</td>
</tr>
<tr>
<td>16</td>
<td><strong>187.52 ± 7.67</strong></td>
<td><strong>158.4 ± 2.6</strong></td>
<td>&lt; DL*</td>
</tr>
<tr>
<td>17</td>
<td><strong>548.34 ± 5.59</strong></td>
<td><strong>506.5 ± 16.6</strong></td>
<td>&lt; DL*</td>
</tr>
<tr>
<td>18</td>
<td><strong>3.09 ± 0.03</strong></td>
<td><strong>3.8 ± 0.4</strong></td>
<td>&lt; DL*</td>
</tr>
<tr>
<td>19</td>
<td><strong>561.1 ± 11.2</strong></td>
<td><strong>620.11 ± 11.83</strong></td>
<td><strong>18.48 ± 1.89</strong></td>
</tr>
<tr>
<td>20</td>
<td><strong>860.9 ± 5.6</strong></td>
<td><strong>820.3 ± 8.2</strong></td>
<td><strong>122.39 ± 13.01</strong></td>
</tr>
<tr>
<td>21</td>
<td><strong>58.9 ± 1.6</strong></td>
<td><strong>59.0 ± 0.6</strong></td>
<td>&lt; DL*</td>
</tr>
</tbody>
</table>

DL – 0.5 ng/g Cr(VI) in sample; Uncertainties are at 95% CL with n = 12 <DL* - sample is strongly reducing. The entire spiked 53Cr(VI) was lost during extraction and no Cr(VI) peak was observed in the chromatogram. The lost 53Cr isotope from the 53Cr(VI) was found to be present in the acid digest.
Figure 4 shows a visualization of select samples, excluding those samples that did not achieve mass balance and those that produced false values using SIDMS. The confidence intervals are also shown for each of the values. Figure 4 allows mass balance to be seen and shows that hexavalent chromium is a noticeable amount of some of the samples.

![Bar graph showing trivalent and hexavalent chromium values obtained from speciation using SIDMS and IDMS compared to total chromium values obtained from IDMS. Error bars show 95% confidence intervals.](image)

**Figure 4:** Trivalent and hexavalent chromium values obtained from speciation using SIDMS and IDMS compared to total chromium values obtained from IDMS; Error bars show 95% confidence intervals

Some of the samples (including Samples 5 and 11) have a value of below detection limit (<DL*) for hexavalent chromium. These samples had high conversions.
from trivalent to hexavalent chromium. Also, some of the samples were so reducing that the spiked isotopically-enriched hexavalent chromium was reduced to trivalent chromium and precipitated out of solution. Therefore, a hexavalent chromium isotopic ratio was produced that was similar to the natural abundance ratio. As a result of this, these samples’ chromatographic data could not be analyzed using SIDMS software because incorrectly high values for hexavalent chromium would be obtained. Appendix A.10 contains chromatograms from Samples 11 and 15 demonstrating the reduction of much of the $^{53}$Cr(VI) spike.

Three of the samples (Samples 3, 12, and 13) have a large difference in the two values for total chromium and show a lack of mass balance. These samples had high conversion of spiked trivalent chromium to hexavalent chromium during alkaline extraction. Therefore, less of the isotopically-enriched trivalent chromium was available in the precipitated residue which produced lower isotope ratios than expected. The matrix of the supplement could have an influence on the large differences in total chromium values. A chromatogram of Sample 13 (found in Appendix A.10) shows the $^{50}$Cr(III) spike that remained in the alkaline solution.

All measurements of total chromium by IDMS and total chromium by the summation of trivalent and hexavalent chromium, except for those displayed in bold font, are within 5 to 10% of each other. The results show that many of the samples do contain hexavalent chromium levels in the ppm range. The highest concentration is over 100 ppm.

Further analysis of raw chromium compound that are added to the supplements was also performed. Because of the large amount of chromium within these samples,
other methods were used for comparisons. SIDMS, calibration curve, and Method 7196A showed the differences in method results. Table 11 allows comparison of SIDMS and calibration curve which showed different values for Samples RM1, RM2, and RM3. Sample RM3’s values for those methods were also significantly different but compared to the other samples were relatively close for the two methods. For RM2, the SIDMS value was below detection limit. However, there was a large value of 545 ppm with Calibration Curve. The RM4 values are both very high, but they also differ greatly.

Table 11: Comparison of hexavalent chromium values in supplement raw materials using three methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>SIDMS (ppm)</th>
<th>Calibration Curve (ppm)</th>
<th>7196A (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM1</td>
<td>63 ± 2</td>
<td>155.5406 ± 13.72</td>
<td>0.8796</td>
</tr>
<tr>
<td>RM2</td>
<td>&lt;DL</td>
<td>545.0810 ± 49.96</td>
<td>3.2644</td>
</tr>
<tr>
<td>RM3</td>
<td>161 ± 14</td>
<td>143.6128 ± 14.30</td>
<td>0.8491</td>
</tr>
<tr>
<td>RM4</td>
<td>4683 ± 226</td>
<td>1196.8288 ± 71.59</td>
<td>14.2806</td>
</tr>
</tbody>
</table>

DL for SIDMS – 0.5 ng/g Cr(VI) in sample; Uncertainties are at 95% CL with n = 12

3.2 Method 7196A

Qualitative results from Method 7196A were a color change in the solutions. The calibration curve standards and samples had a pink tint to them. Calibration curve can be seen in Figure 5. Data points for 100 and 200 ppb were removed to create a more linear graph. Table 11 also contains the data from Method 7196A. Comparing these values to calibration curve and SIDMS, Method 7196A is less than 1% of the other values. Calibration Curve and SIDMS are more similar. However, they are not significantly the same.
Figure 5: Calibration curve for Method 7196A

3.3 Variations: Hot Water Extraction and EDTA

Other attempts at quantitative chromium speciation were preliminary experiments to find alternatives to mass balance. Because these were preliminary experiments, qualitative results were often used to determine if the method should be developed further. Also, these experiments were performed with TFM microwave vessels which caused intrinsic problems of their own. Therefore, results that obtained mass balance with polypropylene tubes should be used for dietary supplement values.

Hot water extraction chromatogram results showed unsuccessful hexavalent chromium extraction. Although sample and spikes were added along with the reagents, Method 3060A solution did not result in a hexavalent chromium peak. No further tests were done because of the lack of extraction.
The EDTA complexation results shown in Table 12 are from extracts of 0.50 g of Sample 14 and 0.10 g EDTA. These values are from the use of TFM vessels instead of polypropylene tubes. Therefore, they are not expected to match with values shown in Table 11. Although the results are sub-samples of the same supplement, there are differences between the values. There is not a linear correlation with the temperature. The highest hexavalent chromium value was seen at 150 °C, and the lowest was at 200 °C. These results also showed that there was not sufficient complexation with trivalent chromium in Method 3060A extraction. SIDMS calculations concluded that there was no trivalent chromium present. The conversion from trivalent to hexavalent chromium with SIDMS is shown to be around 100% in some cases.

**Table 12:** Results for hexavalent chromium using Method 3060A and SIDMS with EDTA addition and temperature variations on Sample 14

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Hexavalent Chromium (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>200.5865 ± 4.4763</td>
</tr>
<tr>
<td>150</td>
<td>254.2724 ± 17.4571</td>
</tr>
<tr>
<td>175</td>
<td>190.2358 ± 18.0870</td>
</tr>
<tr>
<td>200</td>
<td>107.9119 ± 12.7827</td>
</tr>
</tbody>
</table>

Uncertainties are at 95% CL with n = 12

**3.4 Detection Limit and Water Analysis**

In water analysis using the 100 uL sample loop and the Metrohm 818 and 838 IC instrumentation, hexavalent chromium could be detected up to 5 ppb with a distinct peak for hexavalent chromium. This was determined from visualizing the peaks of the
chromatograms using the 7700 IC-ICP-MS. The sample loop was changed to 1 mL to attempt to lower the detection limit.

Results from the 850 and 858 IC instrumentation and 7700 ICP-MS were obtained using both calibration curve, SIDMS, and IDMS. The detection limit itself was considered to be the background equivalent concentration (BEC) which was 15.53 ppt for trivalent chromium and 1.48 ppt for hexavalent chromium. There were many different experiments performed on the various water samples. The results are promising in terms of mass balance. However, it is essential to obtain mass balance and reproducible data to validate the procedures. Preliminary results show hexavalent chromium in some of the samples in the ppt range with other samples showing no hexavalent chromium. Trivalent chromium appears to be present in the ppt range around 200 to 500 ppt. Acid addition experiments produced values that were slightly higher than other values. The total values from IDMS and from the summation of trivalent and hexavalent chromium values are similar. However, they are not yet significantly the same for all of the samples.

3.5 Other Matrices

The data from the SRM allows comparison to the certified value with the two methods that were used (Table 13). Calibration curve data does not provide any information on interconversions or provide corrections for this matrix. SIDMS corrects for these interconversions, and the value obtained is significantly the same as the certified value. The value from the calibration curve alone gives a systematic underestimate of hexavalent chromium by nearly 70 ppm.
Table 13: Results for hexavalent chromium in 2701 SRM using calibration curve and SIDMS

<table>
<thead>
<tr>
<th>Mode of Analysis</th>
<th>Cr(VI) Concentration (µg/g)</th>
<th>Conversion from Cr(III) to Cr(VI) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration Curve</td>
<td>478.87 ± 4.20</td>
<td>----</td>
</tr>
<tr>
<td>SIDMS</td>
<td>539.20 ± 3.77</td>
<td>1.94 ± 0.50</td>
</tr>
<tr>
<td>Certified value* (Using SIDMS)</td>
<td>551.2 ± 34.5</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Uncertainties are at 95% CI with n = 12

The lack of any hexavalent chromium peak in RoHS samples (computer parts) demonstrated that no hexavalent chromium was present. SIDMS calculations verified the lack of hexavalent chromium. Trivalent chromium values could not be determined because an alkaline solution was used (Method 3060A).

The treated and untreated COPR sample results all showed relatively high hexavalent chromium content. Table 14 shows the hexavalent chromium values for the samples. Although the treated samples contain less hexavalent chromium than the untreated COPR samples, the values still range from 360 to 700 ppm. The untreated sample was around 2,500 ppm. Sample T1 and the untreated sample show the greatest difference in hexavalent chromium values.
Table 14: Results for hexavalent chromium for four treated and one untreated COPR samples using SIDMS

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Hexavalent Chromium (mg/kg)</th>
<th>Conversion from Cr(III) to Cr(VI) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>361 ± 58</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>T2</td>
<td>704 ± 65</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>T3</td>
<td>544 ± 40</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>T4</td>
<td>403 ± 18</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>U1 (Untreated)</td>
<td>2,390 ± 75</td>
<td>10.0 ± 0.9</td>
</tr>
</tbody>
</table>

DL – detection limit is 5 ng/g in sample. Uncertainties are at 95% CL with n = 12
CHAPTER 4: Discussion

4.1 Dietary Supplements

The dietary supplement market in the United States is the largest in the world reaching $28 billion in 2010.\textsuperscript{50} The popularity of these products and the different health implications from trivalent and hexavalent chromium make the mass balance results important for companies and consumers. Companies need to realize the importance of proper sample preparation and analysis techniques to test their products. Regulators and citizens should realize the importance of stricter regulations for dietary supplements. Consumers also have the right to know that their products are being properly tested and the ingredients listed are correct.

The dietary supplement analyses supported the hypothesis that sample preparation involving microwave-enhanced chemistry and SIDMS analysis would give more sensitive and selective hexavalent chromium results than older methods. Method 7196A gave hexavalent chromium results with extreme differences from Method 6020A and SIDMS results. For most of the samples, Method 7196A results are less than 1\% of the results from Method 6020A and from SIDMS. SIDMS calculations indicated that conversion was occurring; and without the use of SIDMS, biased hexavalent chromium values would be obtained. Most of the dietary supplements’ analyses showed mass balance. Although trivalent chromium was reported as an ingredient within the supplements, hexavalent chromium in the ppm range was determined in some samples. Results from variations on the EPA methods showed that the procedures were ineffective.
The use of polypropylene tubes was a successful modification of method materials. They prevented the acid leaching that the TFM tubes produced. This prevented unnecessary conversions of spikes or of chromium within the samples. It was important that the stirring bars fit into the tubes and allowed sufficient stirring to extract all sample and create a homogeneous solution. Also, in order to prevent immediate spike conversion upon contact with the sample matrix, the chromium spikes were deliberately added as the last reagent.

Issues were encountered because some of the dietary supplements provided a highly reducing, as well as oxidizing, matrix. This resulted in reduction of the $^{53}$Cr(VI) spike to trivalent chromium during extraction with Method 3060A. This spike was then precipitated with the trivalent chromium instead of being extracted with the hexavalent chromium. The isotope ratios after spiking were similar to the natural ratios causing the calculation of a high hexavalent chromium value. The natural 50/52 ratio is around 0.05, and the natural ratio for 53/52 is around 0.11. Samples 10 and 15 had 53/52 hexavalent chromium ratios around 0.20 which is close to the natural abundance. However, the theoretical ratio should be higher. This created the false hexavalent chromium values in the calculations. High trivalent to hexavalent chromium conversions were also calculated with SIDMS. For example, Sample 7 and 21 had conversions around 40%. Because of the reducing qualities and the other ingredients within these supplements, the SIDMS software was not used to calculate the hexavalent chromium value. Instead, it was determined from the chromatogram data that no hexavalent chromium was present in the samples. Therefore, these samples are said to have hexavalent chromium concentrations below detection limit (<$\text{DL}$). If the SIDMS software were used, hexavalent chromium
values would be inaccurately high. Therefore, it is important to have an expert in the sample preparation technique, chromatograms, and calculations to determine the correct values from the analysis.

Other values were not indicative of mass balance because of highly oxidizing samples. Trivalent chromium values in Samples 3, 12, and 13 exceeded the value for total chromium. For Sample 12, there was around a 50% conversion of trivalent to hexavalent chromium. The ratio of 50/52 of hexavalent chromium from Method 3060A extract was also much higher than theoretical. Samples 3, 12, and 13 had a 50/52 Cr(VI) isotope ratio averaging around 7.0. This high oxidation is an obvious sign that the trivalent chromium values are incorrect. These samples also had 50/52 isotope ratios in the Method 3052 extract that were lower than the theoretical isotope ratio. This caused a very high value of trivalent chromium to be calculated. These samples are most likely oxidizing causing the $^{50}\text{Cr(III)}$ spike to be converted to hexavalent chromium and present in alkaline solution.

Being able to compare the mass balance data to the serving size and daily dose that would be consumed is also important for making conclusions about health effects. Table 15 shows this data for the supplements that had the necessary nutritional information. This gives the amount of hexavalent chromium that would be consumed daily if one were to take the recommended serving size.

If the oral route of exposure does cause cancer, there will not be a threshold for toxicity. Any level of a carcinogen is thought to cause some level of risk. However, the human cancer potency for oral route of exposure of hexavalent chromium was determined to be 0.5 $(\text{mg/kg-bodywt/day})^{-1}$. The state of California used this and other studies to determine an estimated dose in humans for a 10% increase in tumors. For
ingestion, they found the lower limit to be 0.17 mg/kg-day.\textsuperscript{28} For the example of a 70 kg human, 11,900 µg of hexavalent chromium consumed daily would result in a 10% increase in the risk of tumors. Although those samples reported in Table 15 are well below this value, it does not mean that there will not be any adverse health effects.

Acceptable Daily Doses were also calculated in the California Public Health Goal report. For non-cancer outcomes, these ranged from 0.0002 to 0.0025 mg/kg-day.\textsuperscript{28} Therefore, a 70 kg human would have a safe daily hexavalent chromium ingestion of 14 µg to 175 µg in terms of non-cancer endpoints of toxicity. Table 15 results show supplements that would be within the range of that safe limit or exceeding it. For example, if a non-cancer outcome is in the lower end of the range (14 µg), Sample 20 is nearly four times higher at 59.26 µg hexavalent chromium. These values for acceptable daily doses and lower dose limits are mostly based upon drinking water. However, they can give some idea as to the potential harm from consuming supplements that contain hexavalent chromium. It also shows the uncertainties for ingestion through drinking water or through solid matrices.

\textbf{Table 15:} Amount of hexavalent chromium per serving size of select supplements

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Serving Size</th>
<th>Mass of serving size (g)</th>
<th>Cr(VI) concentration (ug/g)</th>
<th>Cr(VI) daily dose (ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1 caplet</td>
<td>1.05232</td>
<td>14.87</td>
<td>15.65</td>
</tr>
<tr>
<td>13</td>
<td>1 caplet</td>
<td>1.06303</td>
<td>4.85</td>
<td>5.16</td>
</tr>
<tr>
<td>14</td>
<td>1 caplet</td>
<td>0.88043</td>
<td>49.7</td>
<td>43.76</td>
</tr>
<tr>
<td>15</td>
<td>1 caplet</td>
<td>1.1236</td>
<td>&lt;DL</td>
<td>--</td>
</tr>
<tr>
<td>18</td>
<td>30 mL</td>
<td>32.7846</td>
<td>&lt;DL</td>
<td>--</td>
</tr>
<tr>
<td>19</td>
<td>1 caplet</td>
<td>0.4346</td>
<td>18.48</td>
<td>8.03</td>
</tr>
<tr>
<td>20</td>
<td>1 tablet</td>
<td>0.4842</td>
<td>122.39</td>
<td>59.26</td>
</tr>
<tr>
<td>21</td>
<td>1 tablet</td>
<td>1.12782</td>
<td>&lt;DL</td>
<td>--</td>
</tr>
</tbody>
</table>

\textbf{DL (Detection Limit)} – 0.5 ng/g Cr(VI) in sample
Method 7196A results verified its weakness in proper quantification compared to the main methods used. The color change or reaction with DPC could be affected by the other ingredients within the supplements. The method requires the addition of sulfuric acid to create a low pH value. However, this could be causing immediate conversion to trivalent chromium which may affect the detection of hexavalent chromium. Also, the extract from Method 3060A already had a colored tint to most of the samples. Figure 6 is a photograph showing the coloration of some of the extracted samples using Method 3060A. Method 7196A also lists possible interferences from hexavalent molybdenum, mercury salts, vanadium, and iron. These are not an issue with analysis using ICP-MS.

**Figure 6:** Photo displaying the color of Method 3060A extracts in some dietary supplements

The raw material data shows uncertain conclusions. There were significant differences between the results from the three methods. Further testing must be performed to achieve mass balance to actually verify the results. This is a case that shows the importance in having mass balance. Raw material analysis is important because it can
give some insight into where the hexavalent chromium contamination could be originating.

For the EDTA data presented, temperature showed possible conversions. The lower hexavalent chromium value at a higher temperature suggests that conversion from hexavalent chromium to trivalent chromium occurred in higher temperatures (175 °C and above). The lack of successful complexation of trivalent chromium with EDTA also demonstrated the complexity of sample preparation with a solid matrix. Dietary supplements contain many different ingredients depending on the purpose. EDTA does not selectively complex with trivalent chromium. It chelates to other metal ions as well, including calcium, sodium, and iron. In the supplements, there are other ingredients that could interact more readily with EDTA. These may inhibit trivalent chromium complexation. The trivalent chromium may also not be free to interact with EDTA.

Further studies on dietary supplements could be done to gain more information about possible hexavalent chromium contamination. Because the stabilities of the chromium species are important for analysis and transformations, further investigation into reduction potential (Eh) contributions could be helpful. Also, chromium speciation and mass balance within different bottles and lots of the same supplement would lead to further conclusions. This could determine if the company is consistent in its production processes in each lot. Also, it could possibly show that contamination was only within one lot.
4.2 Water Samples

Recently, the presence of heavy metals and other toxins in drinking water has been a concern. In particular, the Environmental Working Group (EWG) has published a report that claims that hexavalent chromium was found in 31 of 35 tested cities and that hexavalent chromium was often the majority of the chromium species. This would cause concern for the safety of our drinking water. The report used an older method of analysis, Method 218.6. This method is specifically for analyzing hexavalent chromium in waters. However, it requires the adjustment of the sample to a pH level from 9 to 9.5, more alkaline than most natural waters. During this pH adjustment, the native trivalent chromium can convert into hexavalent chromium and will produce false positive results for native hexavalent chromium. This is an important step that affects the accuracy of the results.

Although Method 218.6 was not used in this project, the results obtained again stress the importance of using proper methods. The methods that were used provide results that limit the possible interferences and can account for interconversions. The instruments allow accurate measurements and detection at low levels. Method 6800A also creates lower background chromium levels and eliminates polyatomic interferences. Although argon is generally unreactive, the high temperatures of the ICP-MS can create compounds such as argon carbide (ArC\(^+\)) and argon oxide (ArO). These polyatomic interferences are eliminated by collision with helium gas in the 7700 ICP-MS.
Mass balance needs to be obtained for results to be verified and reliable. Because the concentrations are extremely low, stabilities and contamination are of particular importance. For example, in the experiment with the addition of acid for sample preparation, chromium values were slightly higher. This could be from chromium contamination within the nitric acid creating a false chromium value for the water. Slight changes in chemistry or stability can also affect the accuracy of the results. As a result, this study is still in progress with the goal of achieving mass balance.

Because California is proposing a new level of 0.02 ppb hexavalent chromium in drinking water, the methods used must be able to detect at this level. The implications are that this water would be unsafe to drink. However, if the majority of drinking water is above the proposed safe level, it would be unrealistic to have all of the drinking water comply with the new level.

Water sample analysis is somewhat different than the dietary supplement analysis. Water samples do not require extensive sample preparation before chromium species separation and analysis. If SIDMS was being applied, the only preparation was spiking. This is much less involved than the complex matrix of supplements which require extractions and digestions. Only one sample was required to simultaneously evaluate trivalent and hexavalent chromium with water samples compared to the two solutions for solid supplements. However, some sample preparation may be needed to create the best method for analysis. This could include raising the temperature through the microwave or pre-concentration of the analytes on a solid phase extraction column or on the analytical column before analysis.
Because this information is important for the entire country, including California, further drinking water analysis could provide comparisons between different drinking water facility conditions and regions. Also, comparisons to local rivers, streams, and lakes could provide information on similarities between source water and drinking water or possible contamination. If different treatment processes are used to remove hexavalent chromium contaminated water, analysis could also be done for a comparison of treated and untreated water. This would determine which treatment processes are successful. However, the proper methods and procedures must be determined before further samples and conclusions can be made.

4.3 Other Matrices

The SRM values can be compared to the hexavalent chromium values reported as the reference. This is another matrix, soil, that was analyzed using the same sample preparation and SIDMS procedures as the dietary supplements. However, soil and sediments are more extensively researched in the past. This study verified the ability for SIDMS to obtain more accurate results than calibration curve.

The small study on electronic parts for hexavalent chromium showed that no hexavalent chromium was present in the samples. Since RoHS specifies that no carcinogenic compound can be above a safe limit in European products, testing those products for compliance is important. In this case, these electronic products would pass for hexavalent chromium content. Because this restriction is specifically for hexavalent, not trivalent, chromium, it is important that speciation can correctly be performed. Companies need proper techniques to produce reliable data to know if their products are
acceptable. They also need to know if there is a problem and if their products will be not be approved.

The COPR samples allow comparison of the treatment that was used. This shows that hexavalent chromium levels in this type of matrix are high. It also showed that there was some elimination of the hexavalent chromium by using the treatment. However, all of the hexavalent chromium was not removed which is shown by the high concentrations. This treatment could not be the sole or preferred technique to remove hexavalent chromium from this media. Although information on the specific type of treatment was not given, the analysis could be helpful to the company to improve their technique.

These other matrices were only a small number of the possible matrices that could be studied. Many other materials and products contain amounts of chromium. The most important would be consumer products, especially those being consumed by, or in contact with, more susceptible individuals, such as children and pregnant women. Other food products and food additives would be an interesting continuation of this work. Proper method development would reveal if other matrices would provide the same difficulties and complexities as dietary supplements or would be more straightforward. As with the RoHS samples, the methods developed for chromium speciation in dietary supplements and the use of mass balance could be important in areas of compliance.
CHAPTER 5: Conclusions

Although some methods may be widely-used in the scientific and industrial/commercial community, they may now be outdated with the development of sophisticated and powerful new analytical tools. Method 7196A’s reliance on color absorbance proved to be a gross underestimate of actual values. It only allowed hexavalent chromium measurement without the ability to measure trivalent chromium. This prevented verification with mass balance or correction of interconversions that is essential if there is a need for defensible data.

With the concern for hexavalent chromium, especially in California, undisclosed distribution of a product containing detectable hexavalent chromium is dangerous and could result in legal action. These supplements are consumed for a benefit but may be causing harm. Even results from only total chromium analysis showed that the amount of chromium printed on the labels was at times a gross underestimate or overestimate. Because the FDA is not currently involved in regulating supplements, action needs to be taken to make testing more rigorous. Products with hexavalent chromium need to be removed from the market, and the problem needs to be corrected with accurate verification of the fix. The dietary supplement results show legally-defensible data from proper methods. Although some of the results showed that the matrix was altering the accuracy of the calculated chromium species, these values were able to be identified as incorrect data. The procedure used for the dietary supplements in this research project can be expanded to other areas where there are complex matrices.
Chromium speciation is important in various other matrices, such as food additives, foods, and medications. This research stresses the importance of thorough and appropriate testing of consumer products. Unlike Method 7196A, the instrumentation and methods used for mass balance created little interferences that could add to error. The underlying chemistry involved in chromium speciation was needed to choose the proper sample preparation. Even with appropriate sample preparation, the stability of chromium species changes, and interconversions did occur. This emphasizes the importance of using a method that tracks and corrects interconversions. This procedure also allowed the identification of incorrect data because of the conditions of the matrix.

The other matrices provided support for the methods used in the dietary supplements. SIDMS was accurately able to reproduce reference data for the soil SRM. This method was more successful than calibration curve. Speciation also provided information about treatment processes for COPR and that hexavalent chromium was not completely eliminated. Water data showed that some samples may contain some level of hexavalent chromium. The analysis also demonstrates the difficulties of working with small concentrations and the need to be certain of the conclusions that are made from data.

Although this project was focused on the analytical aspect of chromium species, it is also important for further studies to be done on the health effects and toxicity from each of the chromium species. If hexavalent chromium is carcinogenic through the oral route of exposure, the sooner the effects are shown through different studies and data then the better off people will be. People can sooner become aware of the hazards, and safety measures can be put into effect for protections through different media.
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APPENDICES

A.1 Comparison of Methods

Table 16: Comparison of Method 7196A, Method 3052, Method 3060A, SIDMS, IDMS, and Method 6020A

<table>
<thead>
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<th>METHOD</th>
<th>INSTRUMENTATION</th>
<th>PURPOSE</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method 7196A</td>
<td>Ultraviolet-visible spectrometer</td>
<td>Cr(VI) quantification</td>
<td>Inexpensive; Quick</td>
<td>Interferences from iron, hexavalent molybdenum, vanadium, mercury salts; Acid addition; Higher detection limit; Only detects Cr(VI); Cannot correct for interconversions</td>
</tr>
<tr>
<td>Method 3052</td>
<td>Microwave</td>
<td>Digestion of complex matrices</td>
<td>Quick; Complete digestion of matrix</td>
<td>Does not preserve the species</td>
</tr>
<tr>
<td>Method 3060A</td>
<td>Microwave</td>
<td>Cr(VI) extraction</td>
<td>Quick; Complete Cr(VI) extraction</td>
<td>Only extracts Cr(VI)</td>
</tr>
<tr>
<td>SIDMS (Method 6800A)</td>
<td>IC-ICP-MS</td>
<td>Quantification</td>
<td>Tracks and corrects for interconversions</td>
<td>Assumption that there is equilibration with spike and sample analytes; Complex mathematics</td>
</tr>
<tr>
<td>IDMS (Method 6800A)</td>
<td>ICP-MS</td>
<td>Quantification</td>
<td>Corrects for partial analyte loss</td>
<td>Assumption that there is equilibration with spike and sample analytes</td>
</tr>
<tr>
<td>Calibration Curve (Method 6020A)</td>
<td>ICP-MS (or IC-ICP-MS)</td>
<td>Quantification</td>
<td>Quick</td>
<td>Does not correct for interconversions</td>
</tr>
</tbody>
</table>
A.2 Summary of EPA Method 3052

METHOD 3052
MICROWAVE ASSISTED ACID DIGESTION OF SILICEOUS AND ORGANICALLY BASED MATRICES

2.0 SUMMARY OF METHOD

2.1 A representative sample of up to 0.5 g is digested in 9 mL of concentrated nitric acid and usually 3 mL hydrofluoric acid for 15 minutes using microwave heating with a suitable laboratory microwave system. The method has several additional alternative acid and reagent combinations including hydrochloric acid and hydrogen peroxide. The method has provisions for scaling up the sample size to a maximum of 1.0 g. The sample and acid are placed in suitably inert polymeric microwave vessels. The vessel is sealed and heated in the microwave system. The temperature profile is specified to permit specific reactions and incorporates reaching 180 ± 5 ºC in approximately less than 5.5 minutes and remaining at 180 ± 5 ºC for 9.5 minutes for the completion of specific reactions. After cooling, the vessel contents may be filtered, centrifuged, or allowed to settle and then decanted, diluted to volume, and analyzed by the appropriate SW-846 method.

Procedure is found in Section 7.0 of Method 3052
A.3 Summary of EPA Method 6800A: Isotope Dilution Mass Spectrometry (IDMS)

METHOD 6800A
ELEMENTAL AND SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY

2.0 SUMMARY OF METHOD

2.1 IDMS method

2.1.1 Samples may require a variety of sample preparation procedures, depending on sample matrices and the isotope ratio measurement methods. One primary purpose of sample preparation is to solubilize the analyte of interest and equilibrate the spike isotopes with sample isotopes. Solids, slurries, and suspended material must be subjected to digestion after spiking using appropriate sample preparation methods (such as Method 3052). Water samples may not require digestion when ICP-MS is used as a detection method because ICP can destroy elemental species and thus many species are indistinguishable for ICP-MS.

2.1.2 A representative measured sample is thoroughly mixed with a measured amount of the isotopic spike. If a digestion procedure is required, the spiked sample is then digested to equilibrate the spikes and samples. The sample solutions are then measured with mass spectrometry such as ICP-MS to obtain the altered isotope ratios. Method 6020 can be used as a reference method for ICP-MS detection. In addition to Method 6020, dead time correction and mass bias correction must be included in the measurement protocol. The equations described in Sec. 12.1 are used to calculate the concentrations.

Procedure is found in Section 11.0 of Method 6800A
A.4 Calculations for IDMS

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 IDMS calculations

Data were corrected for deadtime and mass bias and the isotope ratio of $^{53}\text{Cr}/^{52}\text{Cr}$ in Cr(VI) species, ($R_{53/52}^{\text{VI}}$), was calculated for each sample. The final concentration of Cr(VI) was then determined from the following IDMS equations.

$$
\begin{align*}
C_x^{\text{VI}} &= C_{\text{std},s}^{\text{VI}} / M_x \\
C_s^{\text{VI}} &= \frac{C_x^{\text{VI}} W_x}{W_s} \left( \frac{^{53}A_x - R_{53/52}^{\text{VI}} ^{52}A_x}{R_{53/52}^{\text{VI}} ^{52}A_x - ^{53}A_x} \right) \\
C_{\text{spike}}^{\text{VI}} &= C_s^{\text{VI}} M_s \\
\end{align*}
$$

Eqn. A4-1

where, $C_s^{\text{VI}}$ and $C_x^{\text{VI}}$ are the concentrations of Cr(VI) in the isotope-enriched spike and natural standard in $\mu\text{mol/g}$, respectively. $M_s$ and $M_x$ are the average atomic weights of the spike and the natural standard in g/mol, respectively. $^{53}A_x$ and $^{53}A_s$ are the atom fractions of $^{53}\text{Cr}$ for the spike and natural standard, respectively. $^{52}A_x$ and $^{52}A_s$ are the atom fractions of $^{52}\text{Cr}$ for the spike and natural standard, respectively.
A.5 Summary of EPA Method 3060A

METHOD 3060A
ALKALINE DIGESTION FOR HEXAVALENT CHROMIUM

2.0 SUMMARY OF METHOD

2.1 This method uses an alkaline digestion to solubilize both water-insoluble and water soluble Cr(VI) compounds in solid waste samples. The pH of the digestate must be carefully adjusted during the digestion procedure. Failure to meet the pH specifications will necessitate redigestion of the samples.

2.2 The sample is digested using 0.28M Na CO /0.5M NaOH solution and heating at 90-95°C for 60 minutes to dissolve the Cr(VI) and stabilize it against reduction to Cr(III).

2.3 The Cr(VI) reaction with diphenylcarbazide is the most common and reliable method for analysis of Cr(VI) solubilized in the alkaline digestate. The use of diphenylcarbazide has been well established in the colorimetric procedure (Method 7196), in rapid-test field kits, and in the ion chromatographic method for Cr(VI) (Method 7199). It is highly selective for Cr(VI) and few interferences are encountered when it is used on alkaline digestates.

Procedure is found in Section 7.0 of Method 3060A
A.6 Summary of EPA Method 6800A: Speciated Isotope Dilution Mass Spectrometry (SIDMS)

METHOD 6800A
ELEMENTAL AND SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY

2.0 SUMMARY OF METHOD

2.2 SIDMS method

2.2.1 Speciated samples generally require sample preparation specific to the sample matrices, species, and the isotope ratio measurement method. The purpose of sample preparation is to solubilize the species of interest and to equilibrate the natural and spiked species, creating a homogeneous solution. Solids, slurries, and suspended material must be subjected to extraction before or after spiking, using appropriate sample preparation methods (such as Method 3060A for the determination of Cr(VI) in soils and 3200 for mercury species in food, blood and tissues). Method 3546 and other sample preparation methods in SW-845 Chapters Three and Four are applicable environmental health and other toxicants in the environment and human health. Water samples may not need extraction. In contrast to total element analysis, efforts must be taken to avoid the destruction of the species in SIDMS. For example, in molecular species analysis, reduced glutathione in blood, plasma and/or serum is transformed by oxygen in the air during the analysis to oxidized glutathione (dimer species). Sample preparation, spiking, and spike equilibration must be carried out in the absence of oxygen prior to ESI-MS, nano-ESI-MS or MALDI-MS analysis. Species of mercury-glutathione complex appear to be more stable to oxygen but methylation and demethylation of these species can occur during sample preparation and analysis.
2.2.2 Although SIDMS is a general method applicable to many elements in various species forms, such environmental samples, such as water samples or soil, extracts, containing chromium species, Cr(III) and Cr(VI), will be used for demonstration purposes. Two isotopic spikes are prepared and characterized as follows: $^{50}\text{Cr}(\text{III})$ spike enriched in $^{50}\text{Cr}$ and $^{53}\text{Cr}(\text{VI})$ spike enriched in $^{53}\text{Cr}$. The dominant natural isotope for Cr is $^{52}\text{Cr}$, at 83.79% ($^{50}\text{Cr}$, 4.35%; $^{53}\text{Cr}$, 9.50%; $^{54}\text{Cr}$, 2.36%). A measured amount of a representative aqueous sample is mixed well with an appropriate amount of $^{50}\text{Cr}(\text{III})$ and $^{53}\text{Cr}(\text{VI})$ spike solutions. The spiked sample is then separated into Cr(III) and Cr(VI) using chromatography or another separation method (Figure 3). Four isotope ratios are measured: $^{50}\text{Cr}(\text{III})/^{52}\text{Cr}(\text{III})$, $^{53}\text{Cr}(\text{III})/^{52}\text{Cr}(\text{III})$, $^{50}\text{Cr}(\text{VI})/^{52}\text{Cr}(\text{VI})$, and $^{53}\text{Cr}(\text{VI})/^{52}\text{Cr}(\text{VI})$. The concentrations of the species are determined from speciated isotope dilution calculations.

2.2.3 SIDMS may be called upon to analyze multiple species of interest that require selection of different isotopically enriched species. For example, if reduced and oxidized glutathione are being observed, the two isotopic forms of enriched reduced and oxidized glutathione are distinguishable. However, if the mercury species (or other species) of inorganic (Hg$^{2+}$) and methylmercury (CH$_3$Hg$^+$) forms of glutathione are being quantified, then enriched C-13, N-15, O-17 or O-18, or Hg-196 through Hg-204 enriched isotopic fractions are potential alternative enriched species-spikes for the glutathione and mercury species quantification.

*Procedure is found in Section 11.0 of Method 6800A*
A.7 Summary of EPA Method 6020A

METHOD 6020A
INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, samples should be solubilized or digested using the appropriate sample preparation methods (see Chapter Three). When analyzing groundwater or other aqueous samples for dissolved constituents, acid digestion is not necessary if the samples are filtered and acid preserved prior to analysis (refer to Sec. 1.1).

2.2 This method describes the multi-elemental determination of analytes by ICP-MS in environmental samples. The method measures ions produced by a radio-frequency inductively coupled plasma. Analyte species originating in a liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and extracted through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a mass spectrometer. The ions transmitted through the mass spectrometer are quantified by a channel electron multiplier or Faraday detector and the ion information is processed by the instrument’s data handling system. Interferences must be assessed and valid corrections applied or the data qualified to indicate problems. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix.

Procedure is found in Section 11.0 of Method 6020A
A.8 Algorithms, Assumptions and Calculations for SIDMS

In EPA Method 6800A, the algorithms for Cr(III) and Cr(VI) in aqueous sample have been demonstrated and are summarized below in Equations A8-1 to A8-4. Their derivation is based on these assumptions: spike isotopes and natural isotopes are equilibrated before species transformations; there are no selective losses of the species; and each isotopic spike has been converted completely to a single species (in this case, all Cr in $^{50}\text{Cr}$Cr(III) spike is in Cr(III) form; and all Cr in $^{53}\text{Cr}$Cr(VI) spike is in Cr(VI) form).

(1) The following equations are solved simultaneously, using a Microsoft Excel spreadsheet.

\[
R_{50/52}^{\text{III}} = \frac{(50\;A_x\;C_{x-x}^{\text{III}}\;W_x + 50\;A_x^{\text{III}}\;C_{x-x}^{\text{III}}\;W_x)}{(52\;A_x\;C_{x-x}^{\text{III}}\;W_x + 52\;A_x^{\text{III}}\;C_{x-x}^{\text{III}}\;W_x)}(1 - \alpha) + \frac{(50\;A_x\;C_{x-x}^{\text{VI}}\;W_x + 50\;A_x^{\text{VI}}\;C_{x-x}^{\text{VI}}\;W_x)}{(52\;A_x\;C_{x-x}^{\text{VI}}\;W_x + 52\;A_x^{\text{VI}}\;C_{x-x}^{\text{VI}}\;W_x)}\beta
\]

Eqn. A8-1

\[
R_{53/52}^{\text{III}} = \frac{(53\;A_x\;C_{x-x}^{\text{III}}\;W_x + 53\;A_x^{\text{III}}\;C_{x-x}^{\text{III}}\;W_x)}{(52\;A_x\;C_{x-x}^{\text{III}}\;W_x + 52\;A_x^{\text{III}}\;C_{x-x}^{\text{III}}\;W_x)}(1 - \alpha) + \frac{(53\;A_x\;C_{x-x}^{\text{VI}}\;W_x + 53\;A_x^{\text{VI}}\;C_{x-x}^{\text{VI}}\;W_x)}{(52\;A_x\;C_{x-x}^{\text{VI}}\;W_x + 52\;A_x^{\text{VI}}\;C_{x-x}^{\text{VI}}\;W_x)}\beta
\]

Eqn. A8-2

\[
R_{50/52}^{\text{VI}} = \frac{(50\;A_x\;C_{x-x}^{\text{III}}\;W_x + 50\;A_x^{\text{III}}\;C_{x-x}^{\text{III}}\;W_x)}{(52\;A_x\;C_{x-x}^{\text{III}}\;W_x + 52\;A_x^{\text{III}}\;C_{x-x}^{\text{III}}\;W_x)}\alpha + \frac{(50\;A_x\;C_{x-x}^{\text{VI}}\;W_x + 50\;A_x^{\text{VI}}\;C_{x-x}^{\text{VI}}\;W_x)}{(52\;A_x\;C_{x-x}^{\text{VI}}\;W_x + 52\;A_x^{\text{VI}}\;C_{x-x}^{\text{VI}}\;W_x)}(1 - \beta)
\]

Eqn. A8-3

\[
R_{53/52}^{\text{VI}} = \frac{(53\;A_x\;C_{x-x}^{\text{III}}\;W_x + 53\;A_x^{\text{III}}\;C_{x-x}^{\text{III}}\;W_x)}{(52\;A_x\;C_{x-x}^{\text{III}}\;W_x + 52\;A_x^{\text{III}}\;C_{x-x}^{\text{III}}\;W_x)}\alpha + \frac{(53\;A_x\;C_{x-x}^{\text{VI}}\;W_x + 53\;A_x^{\text{VI}}\;C_{x-x}^{\text{VI}}\;W_x)}{(52\;A_x\;C_{x-x}^{\text{VI}}\;W_x + 52\;A_x^{\text{VI}}\;C_{x-x}^{\text{VI}}\;W_x)}(1 - \beta)
\]

Eqn. A8-4

where,

$R_{50/52}^{\text{III}}$ is the measured isotope ratio of $^{50}\text{Cr}$ to $^{52}\text{Cr}$ of Cr(III) in the spiked sample

$R_{53/52}^{\text{III}}$ is the measured isotope ratio of $^{53}\text{Cr}$ to $^{52}\text{Cr}$ of Cr(III) in the spiked sample

$R_{50/52}^{\text{VI}}$ is the measured isotope ratio of $^{50}\text{Cr}$ to $^{52}\text{Cr}$ of Cr(VI) in the spiked sample
$R_{53/52}^{VI}$ is the measured isotope ratio of $^{53}\text{Cr}$ to $^{52}\text{Cr}$ of Cr(VI) in the spiked sample

$^{50}A_x$ is the natural relative isotopic abundance of $^{50}\text{Cr}$ in the sample

$^{52}A_x$ is the natural relative isotopic abundance of $^{52}\text{Cr}$ in the sample

$^{53}A_x$ is the natural relative isotopic abundance of $^{53}\text{Cr}$ in the sample

$^{50}A_{x}^{\text{III}}$ is the relative isotopic abundance of $^{50}\text{Cr}$ in the $^{50}\text{Cr(III)}$ spike

$^{52}A_{x}^{\text{III}}$ is the relative isotopic abundance of $^{52}\text{Cr}$ in the $^{50}\text{Cr(III)}$ spike

$^{53}A_{x}^{\text{III}}$ is the relative isotopic abundance of $^{53}\text{Cr}$ in the $^{50}\text{Cr(III)}$ spike

$^{50}A_{x}^{\text{VI}}$ is the relative isotopic abundance of $^{50}\text{Cr}$ in the $^{53}\text{Cr(VI)}$ spike

$^{52}A_{x}^{\text{VI}}$ is the relative isotopic abundance of $^{52}\text{Cr}$ in the $^{53}\text{Cr(VI)}$ spike

$^{53}A_{x}^{\text{VI}}$ is the relative isotopic abundance of $^{53}\text{Cr}$ in the $^{53}\text{Cr(VI)}$ spike

$C_{x}^{\text{III}}$ is the concentration of Cr(III) in the sample ($\mu\text{mol/g}$, unknown)

$C_{x}^{\text{VI}}$ is the concentration of Cr(VI) in the sample ($\mu\text{mol/g}$, unknown)

$W_x$ is the weight of the sample (g)

$C_{s}^{\text{III}}$ is the concentration of Cr(III) in the $^{50}\text{Cr(III)}$ spike ($\mu\text{mol/g}$)

$W_{s}^{\text{III}}$ is the weight of the $^{50}\text{Cr(III)}$ spike (g)

$C_{s}^{\text{VI}}$ is the concentration of Cr(VI) in the $^{53}\text{Cr(VI)}$ spike ($\mu\text{mol/g}$)

$W_{s}^{\text{VI}}$ is the weight of the $^{53}\text{Cr(VI)}$ spike (g)

$\alpha$ is the proportion of Cr(III) oxidized to Cr(VI) after spiking (unknown)

$\beta$ is the proportion of Cr(VI) reduced to Cr(III) after spiking (unknown)
For Method 3060A extraction, several simplifying assumptions have been employed to aid the solution of the algorithms. These assumptions are based on the extreme stability afforded chromium species by the pH, as seen in the species chromatogram given in Chapter 1.3 Figure 1. We have referred to these assumptions as one-way species degradations, and they assist in analytical method development by reducing the bidirectionality of dynamic species to unidirectional degradation probabilities. Accordingly, we treat first $\beta=0$, because Cr(VI) is stable in alkaline solution and there is little Cr(VI) reduced to Cr(III) in the Method 3060A hot alkaline extraction. Second, because $^{50}$Cr(III) spike is the dominant soluble Cr(III) species in pH 9.3 solutions and determines the isotopic ratio for soluble Cr(III) species in the final extract solution, we treat $C_{x}^{III} = 0$ due to its suppression by the isotopically labeled spike.

Thereby, equations A8-1 through A8-4 are simplified to:

$$R_{50/52}^{VI} = \frac{\left( ^{50}A_{s}^{III} C_{s}^{III} W_{s}^{III} \right) \alpha + \left( ^{50}A_{s}^{VI} C_{s}^{VI} W_{s}^{VI} + ^{50}A_{s}^{IV} C_{s}^{IV} W_{s}^{IV} \right)}{\left( ^{52}A_{s}^{III} C_{s}^{III} W_{s}^{III} \right) \alpha + \left( ^{52}A_{s}^{VI} C_{s}^{VI} W_{s}^{VI} + ^{52}A_{s}^{IV} C_{s}^{IV} W_{s}^{IV} \right)}$$

Eqn. A8-5

$$R_{53/52}^{VI} = \frac{\left( ^{53}A_{s}^{III} C_{s}^{III} W_{s}^{III} \right) \alpha + \left( ^{53}A_{s}^{VI} C_{s}^{VI} W_{s}^{VI} + ^{53}A_{s}^{IV} C_{s}^{IV} W_{s}^{IV} \right)}{\left( ^{52}A_{s}^{III} C_{s}^{III} W_{s}^{III} \right) \alpha + \left( ^{52}A_{s}^{VI} C_{s}^{VI} W_{s}^{VI} + ^{52}A_{s}^{IV} C_{s}^{IV} W_{s}^{IV} \right)}$$

Eqn. A8-6

The four unknown factors in these two equations are the isotopic ratios of 50/52 and 53/52 for Cr(VI) species, $C_{s}^{VI}$ and $\alpha$ in the final extract solution. We can measure the isotopic ratios of 50/52 and 53/52 for Cr(VI) species, by using IC-ICP-MS. Although some of the Cr(VI) may transform to Cr(III) during the chromatographic separation and measurement, the isotopic ratios of Cr(VI) species are constant because no Cr(III) spike transforms to Cr(VI) in the acidic eluent. There remain only two unknown variables, $C_{s}^{VI}$ and $\alpha$. Equations A8-5 and A8-6 then become two equations in two unknowns and can
be solved easily for the concentration of Cr (VI) in the samples, $C_{VI}^x$, and the fraction of Cr(III) transformed to Cr(VI), $\alpha$. The algorithm solutions and assumptions are an extension of EPA Method 6800A specific to solid samples where equilibrium between the Cr(III) from the sample and the Cr(III) from isotopic spike usually is not achieved.
A.9 Summary of EPA Method 7196A

METHOD 7196A
CHROMIUM, HEXAVALENT (COLORIMETRIC) 29

2.0 SUMMARY OF METHOD

2.1 Dissolved hexavalent chromium, in the absence of interfering amounts of substances such as molybdenum, vanadium, and mercury, may be determined colorimetrically by reaction with diphenylcarbazide in acid solution. A redviolet color of unknown composition is produced. The reaction is very sensitive, the absorbancy index per gram atom of chromium being about 40,000 at 540 nm. Addition of an excess of diphenylcarbazide yields the red-violet product, and its absorbance is measured photometrically at 540 nm.

Procedure is found in Section 7.0 of Method 7196A
A.10 Select Chromatograms from Mass Balance in Dietary Supplements

The following chromatograms are with the use of Metrohm 818 IC Pumps and 838 Advanced Sample Processor for separation of species (MetrohmUSA, Houston TX). Detection was on an HP-4500 ICP-MS (Agilent, Santa Clara CA). Conditions were as follows: Flow rate: 1.0 ml/min with gradient elution; Eluent: A = 0.06M HNO₃, pH = 9.3, and B = 0.06M HNO₃, pH=2.2; Column: Hamilton PRP-X100 Anion Exchange (150 mm x 4.6 mm, 10µm).

![Chromatogram](image)

**Figure 7:** IC-ICP-MS chromatogram for Sample 4 spiked with $^{50}$Cr(III) and $^{53}$Cr(VI)

Sample 4 contained 2.6 ± 0.5 µg/g of hexavalent chromium and achieved mass balance. This is an example of a sample with hexavalent chromium in a smaller concentration.
Figure 8: IC-ICP-MS chromatogram for Sample 20 spiked with $^{50}$Cr(III) and $^{53}$Cr(VI)

Sample 20 contained 122.39 ± 13.01 µg/g of hexavalent chromium and achieved mass balance. This is an example of a sample with a higher concentration of hexavalent chromium.
Sample 11 did not contain hexavalent chromium (<DL*) and achieved mass balance. This was one sample where SIDMS software was not used for hexavalent chromium determination. In this case, the $^{53}$Cr(VI) spike was reduced to trivalent chromium. Therefore, SIDMS software would have calculated a false value for hexavalent chromium.
Sample 15 also did not contain hexavalent chromium (<DL*) and achieved mass balance. This was another sample where SIDMS software was not used for hexavalent chromium determination. In this case, the $^{53}\text{Cr(VI)}$ spike was reduced to trivalent chromium. Therefore, SIDMS software would have calculated a false value for hexavalent chromium.

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**Figure 10**: IC-ICP-MS chromatogram for Sample 15 spiked with $^{50}\text{Cr(III)}$ and $^{53}\text{Cr(VI)}$
Sample 13 did not achieve mass balance. This was one sample where the trivalent chromium value was larger than the value for total chromium. The $^{50}\text{Cr(III)}$ spike did not precipitate into the residue. It can still be seen in the alkaline solution. Therefore, IDMS calculated a false value for trivalent chromium.