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Toxins and Contaminants in Dietary Supplements both Elemental and Molecular species analyses using updated EPA Method 6800

H. M. “Skip” Kingston, Professor
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Pittsburgh PA 15229
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Toxins and Contaminants in Dietary Supplements both Elemental and Molecular species analyses using updated EPA Method 6800

H. M. “Skip” Kingston, Professor
Duquesne University
Pittsburgh PA 15229
Kingston@duq.edu
Newer direct mathematical method
SIDMS EPA Method 6900 (2012):
“NO Calibration Curves” – WHY?
Problematic in MS and Less Reliable
Can’t use calibration curves on active species
Method that does not use traditional calibration curves
2012 Method EPA 6800, RCRA Update V
Elemental and Molecular
Speciated Isotope Dilution Mass Spectrometry
- approved matrix and analyses

Excerpt from Item-1.5:
“Aqueous samples such as drinking water, groundwater, etc may be
directly spiked and analyzed. Solid samples such as soils, sludges, sediments,
industrial materials, biological tissues, botanicals, lysed cells, foods, including
dietary supplements and consumer products, mixed samples, blood, urine and
other samples containing solid matrices require spiking before, during or after
extraction or prior to analysis to solubilize and equilibrate the species before
introduction to the mass spectrometer. Animal and human clinical studies are
areas where the higher accuracy and precision provided by method 6800 yield
more statistically significant data than conventional methods. This method has
been and can be used to certify reference materials, for environmental forensic
analysis, and for detecting chemical and biological agents (specifically referred to
as CBRNE, Chemical Biological Radiological Nuclear and Explosives) in homeland
defense and homeland security applications.”
IDMS and SIDMS EPA Method 6800 to Certify SRMS and Analyze Toxins

- Universal new paradigm based on IDMS and SIDMS technology
- High confidence in results
- The highest accuracy and quality of data
- Legally defensible results (internationally recognized) – Required in the EU for court
- EPA codified (Method 6800) 2008 & 2012
- Proof statement: NIST SRM-2701

Table 4. Analytical Methods Used for Determination of Certified and Reference Values in SRM 2701

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPA Method 3060A [3] and speciated isotope dilution inductively coupled plasma</td>
</tr>
<tr>
<td></td>
<td>mass spectrometry (SID-ICP-MS) [4]</td>
</tr>
</tbody>
</table>

relative performance of the various EPA SW-846 methods for the determination of hexavalent chromium and total chromium in SRM 2701. The data should not be used for any other purpose. The certified values, provided in Table 1 of the Certificate of Analysis, are the best estimates of the true concentrations.
‘A species is a form of an element specified as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.’

- IUPAC

As referenced in:
Templeton, D; Ariese, F; Cornelis, R.; Danelsson, I. G.; Muntau, H; Van Leeuven, H. P.; Lobinski, R., *J. Pure Appl. Chem.* 2000, 72, 1453.
239,000 toxin measurements in 60 children
Metals,
PCBs,
Pesticides
VOCs
And Epigenetic damage

Acknowledgements: NSF Grant #s 0421252, & 0821401, Heinz Endowment, Richard King Mellon Endowment, Agilent Technologies, AIT,

Tests now commercially available from Applied Isotope Technologies (AIT) – SIDMS.COM
Most children with autism are on one to 10 supplements - their purity and level of contamination is important

- Children with Autism are prescribed supplements to bring their immune systems back into balance
- Many of the supplements are contaminated with metals, pesticides, industrial organics
- At present 80% of the raw materials for these supplements come from China
- We have been working selected companies to demonstrate that the quality and safety of dietary supplements can be improved by removing the toxins and contaminants
Dietary Supplements, are now a multi-billion dollar industry largely unregulated by FDA or any other agency.
Toxin & Toxicant Species

Cr(III) or Cr(VI) ?
Total Chromium does not inform us of toxicity or nutritional efficacy in dietary supplement only speciated data can do that

Hexavalent Chromium with Mass Balance in Dietary Supplement

Dr. Mizan Rahman, Ms. Naudia Martone, Professor Skip Kingston

Paper in Review
Method of IDMS (EPA Method 6800) analysis of total chromium in food supplements for Mass Balance

- **Sample preparation procedure**
  - Weight 1.0 g of homogenized food supplement directly into microwave digestion vessel
  - Add known amount of isotopically enriched chromium spike (\(^{50}\text{Cr}\)) whose concentration is known
  - Add 9 mL of concentrated HNO\(_3\) and 2 mL of H\(_2\)O\(_2\) (30%)
  - Add magnetic stir bar and seal microwave digestion vessels
  - Direct energy induction in a microwave system
  - Filter the digest
  - Store in cold room at 4 °C until analysis by ICP-MS

- **Microwave Decomposition Program**
  - **EPA RCRA Method 3052**
    - 1\(^{st}\) step: Time = 10 minutes; Temperature = Room temperature to 180 °C; Power = Full
    - Temperature feed back control
    - 2\(^{nd}\) step: Time = 10 minutes; Temperature = 180 ± 2 °C; Power = Full with temperature feedback control
    - Cool down and open in a hood
### Total chromium in food supplements by isotope dilution mass spectrometry (IDMS), EPA Method 6800, ICP-MS Analysis

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Unit type</th>
<th>Approximate amount of Total Cr /unit (µg)</th>
<th>Total Cr measured by IDMS / g sample (µg/g)</th>
<th>Total Cr measured by IDMS / unit (µg)</th>
<th>% Difference from reported total Cr / unit value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tablet</td>
<td>190</td>
<td>777 ± 5</td>
<td>209 ± 1</td>
<td>+10</td>
</tr>
<tr>
<td>2</td>
<td>Capsules</td>
<td>210</td>
<td>818 ± 11</td>
<td>231 ± 3</td>
<td>+10</td>
</tr>
<tr>
<td>3</td>
<td>Caplets</td>
<td>175</td>
<td>210 ± 5</td>
<td>227 ± 5</td>
<td>+30</td>
</tr>
<tr>
<td>4</td>
<td>Caplets</td>
<td>70</td>
<td>42 ± 3</td>
<td>58 ± 4</td>
<td>-17</td>
</tr>
<tr>
<td>5</td>
<td>Caplets</td>
<td>70</td>
<td>55 ± 1</td>
<td>74 ± 1</td>
<td>+6</td>
</tr>
<tr>
<td>6</td>
<td>Caplets</td>
<td>225</td>
<td>241 ± 2</td>
<td>209 ± 2</td>
<td>-7</td>
</tr>
<tr>
<td>7</td>
<td>Tablet</td>
<td>210</td>
<td>170 ± 2</td>
<td>198 ± 2</td>
<td>-6</td>
</tr>
<tr>
<td>8</td>
<td>Capsules</td>
<td>195</td>
<td>332 ± 19</td>
<td>193 ± 11</td>
<td>-1</td>
</tr>
<tr>
<td>9</td>
<td>Tablet</td>
<td>525</td>
<td>1,096 ± 22</td>
<td>489 ± 10</td>
<td>-7</td>
</tr>
<tr>
<td>10</td>
<td>Tablet</td>
<td>210</td>
<td>511 ± 7</td>
<td>221 ± 3</td>
<td>+5</td>
</tr>
<tr>
<td>11</td>
<td>Capsules</td>
<td>190</td>
<td>675 ± 15</td>
<td>271 ± 6</td>
<td>+43</td>
</tr>
</tbody>
</table>

PittCon 2010
Determination of hexavalent chromium in food supplements using EPA Methods 3060A and 6800

Start

Extract Cr(VI) from samples with 0.28 M Na₂CO₃ / 0.5 M NaOH (pH>12) at 90-95 °C for 1 hour

Filter / Centrifuge the extracts and store at 4 °C until analysis

Analyze the extract using IC-ICP-MS

Calculate Cr(VI) concentration using external calibration or SIDMS software

Samples were double spiked with isotopically labeled ⁵⁰Cr³⁺ and ⁵³Cr⁶⁺ before extraction
Agilent 7700 ICP-MS with Metrohm IC
IC-ICP-MS chromatogram of a food supplement sample spiked with $^{50}\text{Cr}^{3+}$ and $^{53}\text{Cr}^{6+}$

*Column:* PRP – X100 Anion Exchange (250 mm x 4.6 mm, 10 µm) [Hamilton, Metrohm];
*Eluent:* $A = 0.06\text{M HNO}_3$, pH 9.3; $B = 0.06\text{M HNO}_3$, pH 1.2; *Flow Rate:* 1.0 mL/min with gradient elution.

PittCon 2010
Total chromium and hexavalent chromium in food supplements by EPA Method 6800 (IDMS and SIDMS)

![Bar chart showing concentrations of total chromium and hexavalent chromium for different supplement IDs. The x-axis represents supplement IDs from 1 to 21, and the y-axis represents concentration in micrograms per gram (µg/g). Each bar is color-coded to represent either total Cr (blue) or Cr(VI) (red).]
Conversion of spiked Cr(III) to Cr(VI) during extraction with EPA Method 3060A

Conversion of Cr(III) to Cr(VI), %

Chromium Supplement ID

PittCon 2010
Cr(VI) to total Cr ratio in studied food supplements

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Cr(VI) to total Cr ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td>4</td>
<td>6.3</td>
</tr>
<tr>
<td>5</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>10.9</td>
</tr>
<tr>
<td>7</td>
<td>---</td>
</tr>
<tr>
<td>8</td>
<td>0.8</td>
</tr>
<tr>
<td>9</td>
<td>4.6</td>
</tr>
<tr>
<td>10</td>
<td>---</td>
</tr>
<tr>
<td>11</td>
<td>---</td>
</tr>
<tr>
<td>12</td>
<td>7.2</td>
</tr>
<tr>
<td>13</td>
<td>2.1</td>
</tr>
<tr>
<td>14</td>
<td>16.4</td>
</tr>
<tr>
<td>15</td>
<td>---</td>
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<td>16</td>
<td>---</td>
</tr>
<tr>
<td>17</td>
<td>---</td>
</tr>
<tr>
<td>18</td>
<td>---</td>
</tr>
<tr>
<td>19</td>
<td>3.2</td>
</tr>
<tr>
<td>20</td>
<td>14.2</td>
</tr>
</tbody>
</table>

PittCon 2010
Mass balance study procedure

Extract Cr(VI) using 0.28M Na₂CO₃ /0.5M NaOH (pH>12) at 90-95 °C for 1 hour

Filter / Centrifuge the extracts

Digest the residue from previous step using 9 mL HNO₃ + 2 mL H₂O₂ at 180 °C for 10 minutes

Analyze the extract with IC-ICP-MS for Cr(VI)

Determine Cr(VI) concentration using external calibration curve or SS-IDMS

Spike sample with isotopically enriched ⁵³Cr(VI)

Filter the digest and analyze with IC-ICP-MS for Cr(III)

Determine Cr(III) concentration using external calibration curve or SS-IDMS

Spike the residue with isotopically enriched ⁵⁰Cr(III)
Mass Balance, Paper in Press

![Graph showing concentrations of trivalent chromium, hexavalent chromium, and total chromium across different samples.](image)
Elemental Contamination in Nutritional Supplements Using Inductively Coupled Plasma-Mass Spectrometry

Gregory M. Zinn\textsuperscript{1}, G. M. Mizanur Rahman\textsuperscript{1}, H. M. Skip Kingston\textsuperscript{1}, Scott Faber\textsuperscript{2}, and Matt Pamuku\textsuperscript{3}

\textsuperscript{1}Duquesne University
\textsuperscript{2}The Children’s Institute
\textsuperscript{3}Applied Isotope Technologies
Study of Dietary Supplement Contaminants

• Dietary supplements are both prescribed by physicians and are available as over the counter products.

• In early 2010, a major dietary supplement company which markets its products to the autism community sold a supplement contaminated with antimony.

• An investigation of dietary supplements was launched.

• 4 different dietary supplement companies and 6 common types of dietary supplements (calcium, magnesium, zinc, multi vitamin, probiotic, and essential fatty acids) were investigated.
Sample Preparation

• 0.25 grams of sample
  – Tablets broken down with mortar and pestle
  – Capsule covers removed
  – Liquids shaken
• Samples stored in 50 mL centrifuge tubes
• 8 mL of ARISTAR® ULTRA HNO$_3$
• 2 mL of ARISTAR® ULTRA HCl
• 3 subsamples prepared per supplement
• Quality Assurance - Standard Reference Material (SRM) 3280 Multivitamin/Multielement Tablets
“Dietary Supplements” specifically prescribed for children with autism
Microwave Sample Decomposition Preparation

- Milestone UltraWAVE
- EPA Method 3052
  - 10 minute ramp to 180 °C
  - 10 minute hold at 180 °C
- Samples diluted to 20 mL using ARISTAR® ULTRA H₂O after microwaving
Instrumentation

- Agilent 7700 ICP-MS in Clean room
  - Plasma ionization
  - Elemental detector
  - Unit mass resolution
  - High sensitivity
  - H₂ and He collision gas

- Cetac ASX-520 Autosampler in Nitrogen Purged in Clean Room

- EPA Methods 6800 and 6020B
Quantification Method: EPA Method 6020A

• Three different additional dilutions were performed: 4x, 100x, and 10,000x.
• Matrix matched calibration standards prepared at 3 different levels
  – Low concentration- 0, 5, 10, 15, 20, and 25 ng/g
  – Medium concentration- 0, 10, 20, 30, 40, and 50 ng/g
  – High concentration- 0, 5, 10, 25, 50, 100 and 150 ng/g
• Calibration by external calibration curve
• Internal standard solution containing Bi, Ge, In, $^6$Li, Lu, Rh, Sc, and Tb ran at 1 µg/g
Reference SRM 3280 Results

All values shown in ng/g with n=12 and 95% confidence intervals.
Company 1 Results

All results are shown in ng/g with n=12 and 95% confidence intervals.
Company 2 Results

[Bar graph showing concentrations of various elements, including Al, As, Cd, Co, Cr, Mn, Mo, Ni, Pb, Sb, Se, Sn, U, and V. The graph indicates different concentration ranges for each element, with some elements having higher concentrations than others.]
Company 2 Results Scale #1

![Graph showing concentration of various elements for Company 2's results.]
Company 2 Results Scale #2

Concentration (ng/g)

- Company 2 Calcium Supplement Results
- Company 2 Magnesium Supplement Results
- Company 2 Zinc Supplement Results
- Company 2 Multivitamin Supplement Results
- Company 2 Essential Fatty Acids Supplement Results

Elements: Al, As, Cd, Co, Cr, Mn, Mo, Ni, Pb, Sb, Se, Sn, U, V
Company 2 Results Scale #3

![Graph showing various elements' concentration (ng/g) for different supplements and results scale. The graph includes data for Calcium, Magnesium, Zinc, Multivitamin, and Essential Fatty Acids supplements.](image-url)
Company 3 Results Scale #1

Concentration (ng/g)

- Company 3 Calcium Supplement Results
- Company 3 Magnesium Supplement Results
- Company 3 Zinc Supplement Results
- Company 3 Multivitamin Supplement Results
- Company 3 Essential Fatty Acids Supplement Results
- Company 3 Probiotic Supplement Results
Company 3 Results Scale #2

Company 3 Calcium Supplement Results
Company 3 Magnesium Supplement Results
Company 3 Zinc Supplement Results
Company 3 Multivitamin Supplement Results
Company 3 Essential Fatty Acids Supplement Results
Company 3 Probiotic Supplement Results

Concentration (ng/g)
Company 3 Results Scale #3

![Bar chart showing concentration levels of various elements in different categories of supplements.](image)
Company 3 Results Scale #4

- Company 3 Calcium Supplement Results
- Company 3 Magnesium Supplement Results
- Company 3 Zinc Supplement Results
- Company 3 Multivitamin Supplement Results
- Company 3 Essential Fatty Acids Supplement Results
- Company 3 Probiotic Supplement Results
Company 4 Results Scale #1

Concentration (ng/g)

Company 4 Calcium Supplement Results
Company 4 Magnesium Supplement Results
Company 4 Zinc Supplement Results
Company 4 Multivitamin Supplement Results
Company 4 Essential Fatty Acids Supplement Results
Company 4 Probiotic Supplement Results
Company 4 Results Scale #3
Company 4 Results Scale #4
EPA Method 6020A Results for Lead in Calcium Supplements

EPA Method 6020A results are shown with n=12 and 95% confidence intervals.
EPA Method 6020A results are shown with n=12 and 95% confidence intervals. Concentration for company 4 was below detection limit of 46.6 femtogram/gram.
Quantification of Toxins and Elements of Interest in Zinc Liquid Supplements

(Paper in Press)
Quantification of As, Cd, Pb, and Al from Zinc Liquid Supplement Samples from The Children’s Institute

![Bar chart showing concentrations of As, Cd, Pb, and Al in various sample numbers.](chart.png)
Acknowledgements: NSF Grant #s 0421252, & 0821401, Heinz Endowment, Richard King Mellon Endowment, Agilent Technologies, AIT,

Test now commercially available from Applied Isotope Technologies (AIT) – SIDMS.COM
Children with Autism, PCB 28 and 52 EPA Method 1625 results in ng/g serum with n=3 and 80% CIs

Note: Child 5 before cleanroom sample was below detection limit for PCB 52
*: U.S. 12 – 19 years old, years 1999 – 2002, Centers for Disease Control
Patient and Dietary Supplement Analyses in Autism Study
SRM 1598a, Inorganic Constituents in Animal Serum, Method 6020A and Method 6800 Comparison

EPA Method 6020A and EPA Method 6800 results are shown with n=8 and 95% confidence intervals.
EPA Method 6020A and EPA Method 6800A results are shown with n=8 and 95% confidence intervals.
SRM 1598a, Inorganic Constituents in Animal Serum, Clinical Commercial Laboratory Results

Certified values are shown with a coverage factor of 2, approximately 95% confidence intervals.
The plasma zinc/serum copper ratio for each individual patient separated by disease certification and graphed with reference of normal children.
What have we learned and what is the takeaway message

• Higher-accuracy methods such as 6800 are required to provide information for dozens of nutritionally important species and toxins Cr, Hg, As, Se, Fe…

• Some species can not use traditional calibration curves and requires newer methods such as 6800

• Without accurate measurements there is no quality control in dietary supplements

• Elements are biomarkers in some diseases
I will be in the Web Space for 15 minutes for Discussion of the new Speciated measurements applied to dietary supplements and - Applied to your interests

Copyright - all rights reserved on unpublished data
The Research Team
We apologize for this brief interruption but we are experiencing technical difficulties and will resume shortly.