DRAFT AOAC SMPR 2011.XXX; Version 2; June 30, 2011

Method Name: Determination of Cr, Mo, and Se in Infant and Adult/Pediatric Nutritional Formula

Approved by: Stakeholder Panel for Infant Formula and Adult Nutritionals

Final version date: Effective date:

Intended Use:

1. Applicability:
   Determination of total Chromium (Cr), Molybdenum (Mo), and Selenium (Se) in all forms of infant, adult, and/or pediatric formula (powders, ready-to-feed liquids, and liquid concentrates).

2. Analytical Technique:
   Any analytical technique that measures all three analytes simultaneously and meets the following method performance requirements is acceptable.

3. Definitions:
   Adult/Pediatric Formula
   Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment¹, made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

   Infant formula
   Breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding², made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

   Limit of Detection (LOD)
   The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false positive risk and 5% false negative risk.

   Limit of Quantitation (LOQ)
   The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

   Repeatability
   Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period. Expressed as the repeatability standard deviation (SDr); or % repeatability relative standard deviation (%RSDr).

   Reproducibility
   The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SDR); or % reproducibility relative standard deviation (% RSDR).

¹ AOAC Stakeholders Panel for Infant Formula and Adult Nutritionals (SPIFAN); 2010.
² Codex Standard 72 – 1981.
Recovery
The fraction or percentage of spiked analyte that is recovered when the test sample is analyzed using the entire method.

4. Method Performance Requirements:

<table>
<thead>
<tr>
<th></th>
<th>Cr</th>
<th>Mo</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical range*</td>
<td>20 - 1600</td>
<td>20 - 1000</td>
<td>10 - 500</td>
</tr>
<tr>
<td>Limit of Detection (LOD)</td>
<td>7</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Limit of Quantitation (LOQ)</td>
<td>20</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Repeatability (RSD_r)</td>
<td>≤ 5%</td>
<td>≤ 5%</td>
<td>≤ 5%</td>
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<tr>
<td>Recovery Factor</td>
<td>90% to 110% of mean spiked recovery over the range of the assay.</td>
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<tr>
<td>Reproducibility (RSD_R)</td>
<td>≤ 15% over the analytical range</td>
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</tbody>
</table>

Concentrations apply to: a) "ready-to-feed" liquids "as is"; b) re-constituted powders (25 g into 200 g of water); and c) liquid concentrates diluted 1:1 by weight.

5. System suitability tests and/or analytical quality control:
Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range.

Example Protocol:
- Blank check samples (reagent blank levels <0.4 µg/L Cr, Mo; <0.2 µg/L Se)
- Calibration Verification standards at the midrange point of the calibration range (valid samples must be bracketed by CVs that agree within 5% of nominal)
- Calibration error must be no more than 5% at the BLANKCHECK concentration limits listed above (checked once), and all samples must have analytical solution concentrations above this lower linearity limit.
- The % RSD of duplicate results for Cr, Mo, and Se concentrations in each sample must be 10% or better (6% for the control sample). A control sample (NIST 1849 or equivalent) must be run with every set of samples. The mean of duplicate control results must be within certified limit and within local control limits, if a control chart is in place. The relative standard deviation (% RSD) of the mean for Cr, Mo, and Se as calculated from such control chart must be <5%.

6. Reference Material(s):
NIST Standard Reference Material® 1849; Infant/Adult Nutritional Formula - or equivalent. The SRM is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A unit of SRM 1849 consists of 10 packets, each containing approximately 10 g of material.

7. Validation Guidance:
Recommended level of validation: Official Methods of AnalysisSM

8. Maximum Time-To-Signal: 8 hrs. for all 3 nutrients.