

BS ISO 20649:2015



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# Infant formula and adult nutritionals — Determination of chromium, selenium and molybdenum — Inductively coupled plasma mass spectrometry (ICP-MS)

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**National foreword**

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**Infant formula and adult  
nutritional — Determination of  
chromium, selenium and molybdenum  
— Inductively coupled plasma mass  
spectrometry (ICP-MS)**

*Formules infantiles et produits nutritionnels pour adultes —  
Détermination de la teneur en chrome, sélénium et molybdène —  
Spectrométrie de masse avec plasma à couplage inductif (ICP-SM)*





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## Forewords

**ISO (the International Organization for Standardization)** is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products* and the International Dairy Federation (IDF), in collaboration with AOAC INTERNATIONAL. It is being published jointly by ISO and IDF and separately by AOAC INTERNATIONAL. The method described in this International Standard is equivalent to the AOAC Official Method 2011.19: *Chromium, selenium, and molybdenum in infant formula and adult nutritional products, inductively coupled plasma-mass spectrometry*.

**IDF (the International Dairy Federation)** is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

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ISO 20649 | IDF 235 was prepared by the IDF Standing Committee on Analytical Methods for Composition and the ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with AOAC INTERNATIONAL. It is being published jointly by ISO and IDF, and separately by AOAC INTERNATIONAL. The method described in this International Standard is equivalent to the AOAC Official Method 2011.19: *Chromium, selenium, and molybdenum in infant formula and adult nutritional products, inductively coupled plasma-mass spectrometry*.

All work was carried out by the ISO-IDF Project Group C38 of the Standing Committee on *Analytical Methods for Composition* under the aegis of its project leader, Mr Erik Konings (CH).





# Infant formula and adult nutritionals — Determination of chromium, selenium and molybdenum — Inductively coupled plasma mass spectrometry (ICP-MS)

**WARNING** — The use of this International Standard can involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

## 1 Scope

This International Standard specifies a method for the quantitative determination of chromium, selenium and molybdenum in infant formula and adult nutritional formula.<sup>[1]</sup> The method has been validated in an interlaboratory study on seven different matrices.

## 2 Principle

A test portion is heated with nitric acid in a closed vessel microwave digestion system at 200 °C. The digested test solution, or an appropriate dilution, is presented to the inductively coupled plasma-mass spectrometer (ICP-MS) instrument standardized with acid matched standard calibrant solutions. An ionization buffer (potassium) is used to minimize easily ionizable element (EIE) effects, methanol is added to normalize the carbon content, and germanium and tellurium are used as internal standards. It is permissible to combine the analysis of Cr/Mo/Se with simultaneous determination of any or all of these elements: Na, K, P, Mg, Ca, Fe, Zn, Cu, Mn. An ionization buffer would not be added if the calibration standards already contained Na, K, Mg, and/or Ca.

## 3 Reagents and materials

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity. Equivalent chemicals and reagents may be used.

**3.1 Purified water**, 18 M $\Omega$ /cm.

**3.2 Concentrated nitric acid (HNO<sub>3</sub>)**, 65 % to 70 %, trace metal grade.

**3.3 Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)**, 30 % ACS reagent grade.

**3.4 Methanol**, purity  $\geq$  99,99 %, analytical reagent grade for matrix matching.

**3.5 Potassium solution**, mass concentration  $\rho = 10\,000$  mg/l in nitric acid for matrix matching.

The potassium solution may be replaced by multi-element standards that contain K, if major minerals are determined simultaneously.

### 3.6 Standards

**3.6.1 Cr/Mo/Se multi-element stock standard solution in nitric acid**,  $\rho_{Cr} = 2 \text{ mg/l}$ ,  $\rho_{Mo} = 2 \text{ mg/l}$  and  $\rho_{Se} = 1 \text{ mg/l}$ . High-Purity™ Standards<sup>1)</sup>, or equivalent.

**3.6.2 Ge/Te multi-element stock standard solution in nitric acid**,  $\rho_{Ge} = 5 \text{ mg/l}$  and  $\rho_{Te} = 5 \text{ mg/l}$ . High-Purity™ Standards<sup>1)</sup>, or equivalent.

**3.6.3 Standard reference material SRM 1849a**, from the National Institute of Standards and Technology (NIST) or other suitable standard reference material to serve as a control for this analysis.

### 3.7 Preparation of standard solutions

Prepare intermediate standards from commercial stock standards at 40 ng/ml for Cr and Mo and at 20 ng/ml for Se. Custom blended multi-element stock standard in HNO<sub>3</sub> is acceptable. Prepare three multi-element working standards containing 0,8 ng/ml, 4,0 ng/ml and 20 ng/ml Cr and Mo and 0,4 ng/ml, 2,0 ng/ml and 10 ng/ml Se, plus a blank, with both 50 ng/ml Ge and Te internal standards, in HNO<sub>3</sub>. Ge is used as the internal standard for both Cr and Mo, and Te shall be used for Se.

## 4 Apparatus

Usual laboratory glassware and equipment and, in particular, the following.

**4.1 Microwave oven.** Use a commercial microwave designed for laboratory use at 0 °C to 300 °C, with a closed vessel system and a controlled temperature ramping capability. Select the vessel design that will withstand the maximum possible pressure, since organic material, and also carbonates if not given sufficient time to predigest, will generate significant pressure during digestion. Vent according to the manufacturer's recommendation.

**CAUTION — Microwave operation involves hot pressurized acid solution. Use appropriate face protection and laboratory clothing.**

**4.2 ICP-MS.** ICP mass spectrometer with collision reaction cells and hydrogen and helium source. In the collaborative study, five different mode ICP-MS instruments from three major vendors delivered equivalent performance.

**4.3 Various plasticware and pipets.** Disposable plastic tubes/autosampler tubes to hold sample solutions, Class A volumetric pipets for standard preparation, adjustable 1 000 µL micropipette or fixed 500 µl pipet to add internal standards, calibrated at point of use.

**4.4 Analytical balance.** The balance shall read to 0,0001 g.

## 5 Procedure

### 5.1 Sample preparation

Prepare powder samples by reconstituting approximately 25 g of sample in 200 ml warm purified water (60 °C). Accurately weigh approximately 1,8 g reconstituted test portion into the digestion vessel. This represents approximately 0,2 g original powder sample. SRM 1849a is weighed at 0,2 g directly into a microwave vessel. Fluid samples may be prepared by accurately weighing approximately 1 g test portion directly into the digestion vessel after mixing. For the recommended one step digestion

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(two stages in microwave programme), add 0,5 ml of 5 000 ng/ml Ge and Te internal standard solution with a micropipette calibrated at point-of-use to deliver with at least 0,8 % accuracy. To take maximum advantage of the internal standard, and thereby improve the robustness of this method, do not add the internal standards online. Add 5 ml trace metal-grade HNO<sub>3</sub> followed by 2 ml H<sub>2</sub>O<sub>2</sub> to the microwave digestion vessels. Seal the vessels according to manufacturer's directions and place in the microwave. Ramp temperature from ambient to 180 °C in 20 min, and hold for 20 min in stage 1. In stage 2, the microwave will automatically ramp to 200 °C in 20 min, and hold for 20 min, see [Table 1](#).

**Table 1 — Microwave operating parameters**

Stage 1 – Sample digestion		
1	Power	100 % (1 600 W)
2	Ramp to temperature	20 min
3	Hold time	20 min
4	Temperature	180 °C
5	Cool down	20 min
Stage 2 – Sample digestion		
1	Power	100 % (1 600 W)
2	Ramp to temperature	20 min
3	Hold time	20 min
4	Temperature	200 °C
5	Cool down	20 min

For microwave ovens without the 2-stage programme and where it is more convenient, use the 2-step digestion. Add 0,5 ml of 5 000 ng/ml Ge and Te internal standard solution (with calibrated micropipet as above) and 5 ml trace metal-grade HNO<sub>3</sub>. Do not add the internal standards online. With power settings appropriate to microwave model and number of vessels, ramp temperature from ambient to 200 °C in 20 min. Hold at 200 °C for 20 min. Cool vessels according to manufacturer's directions, approximately 20 min. Slowly open the microwave vessels, venting the brownish nitrogen dioxide gases.

**CAUTION — Venting shall be performed in a hood because NO<sub>2</sub> is very toxic.**

Add 1 ml of H<sub>2</sub>O<sub>2</sub> and redigest samples by ramping the temperature from ambient to 180 °C in 15 min. Hold at 180 °C for 15 min and cool for 20 min.

## 5.2 Preparation of the test solution

Add approximately 20 ml of purified water to the contents of the vessel with the digested samples and transfer to a 50 ml sample vial. Rinse the vessel and transfer the rinsate into the sample vial. Add 0,5 ml methanol to the sample vial and dilute to 50 ml with purified water. Alternatively, the methanol may be added online at 1 % volume percentage.

## 5.3 Determination

[Table 2](#) summarizes typical instrument parameters for analysis. Analyse test solutions using an ICP-MS instrument standardized with the indicated standard solutions. Ge is used as the internal standard for both Cr and Mo (helium mode), and Te shall be used for Se (hydrogen mode).

Hydrogen mode is recommended for the determination of low levels of Se in infant formula and, depending on the instrument model, it may not be possible to easily switch between helium and hydrogen mode. In such a case, follow the instructions of the instrument manufacturer for changing from helium to hydrogen mode and analyse Se separately from Cr and Mo. Alternatively, verify in separate experiments that the practical limit of quantification (PLOQ) for Se is at or below 10 ng/g when using an alternate collision/reaction gas. One laboratory successfully completed the reproducibility study using helium, and another with ammonia gas.

Analyse a 4 ng/ml Cr and Mo working standard solution and 2 ng/ml Se working standard or other suitable quality control solution every 10 test portions to monitor for instrument drift and linearity. The result shall be within 4 % of the standard's nominal concentration. The inclusion of a method blank (run as a sample, its measured concentration shall be < half of the lowest calibration standard), a duplicate sample (relative percent difference (RPD) ≤ within 10 % for Cr, 7 % for Se and 5 % for Mo) and known reference materials serving as control samples (recovery check within control limits) are mandatory for good method performance. If any of these quality control checks fails, results should be considered invalid. The order of analysis should be calibration standards, followed by rinse, blank check, check standard, control sample, sample, sample duplicate (up to 10 samples), and finally check standard.

**Table 2 — ICP-MS parameters**

Typical operating conditions	
RF power, W	1 600
RF matching voltage, V	1,8
Sample depth, mm	9
Extract 1 lens voltage, V	0
Carrier gas flow, l/min	0,9
Make up gas flow, l/min	0,2
Nebulizer (glass concentric)	MicroMist® <sup>a</sup>
Spray chamber temperature, °C	2
Interface cones	Ni
He cell gas flow rate, ml/min	4,5
H <sub>2</sub> cell gas flow rate, ml/min	4,2
Nebulizer pump rate, s <sup>-1</sup>	0,1 (= 0,5 ml/min)
Analyte/internal standard/gas mode	<sup>52</sup> Cr, <sup>95</sup> Mo/ <sup>72</sup> Ge in He mode <sup>78</sup> Se/ <sup>130</sup> Te in H <sub>2</sub> mode
<sup>a</sup> This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by either ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.	

## 6 Calculation

Response ratios of analyte to internal standard were measured in the calibration standards and the calibration curve slope and intercept were automatically calculated by the software using a non-weighted least squares linear regression calibration analysis to produce a best-fit line. Response ratios of analyte to internal standard were then measured in the sample solutions and the calculated analytical solution concentration was multiplied by an appropriate dilution factor to arrive at the final concentration of analyte in the product.

## 7 Method validation

### 7.1 Linearity

All calibration curves were prepared using a non-weighted least-squares linear regression analysis, and correlation coefficient (r) values were calculated with each calibration curve. Each calibration curve was prepared with four multi-element standard solutions, including the blank standard solution. It should be noted that all analyte concentrations in samples were within linear range of the calibration curve and above the established lower linearity limit.

## 7.2 Limit of quantification (LOQ)

The LOQ is the lowest concentration of the analyte in the sample that can be reliably quantitated by the instrument. The method LOQ is typically determined by multiplying the average SD of 10 digested blanks by a factor of 10, and the instrument LOQ by multiplying the instrument LOD by 3 (see Reference [2]). However, in this method the useful LOQ, or practical LOQ (PLOQ), was determined to be the lower linear limit value of the calibration curve because the accuracy and precision of sample measurements below that value would be uncertain. Almost all mineral-fortified nutritional products can be prepared with a dilution factor such that Cr, Se, and Mo will be present in the analytical solution above the PLOQ.

## 7.3 Matrix matching with methanol

The presence of carbon (organic compounds) in analytical solutions causes signal enhancement of Se during analysis by ICP-MS (see References [3],[4],[5]). To determine the optimum concentration of methanol (source of carbon) needed to compensate for Se signal enhancement, various concentrations of methanol were added to both calibration standards and digested samples.

## 7.4 Effects of easily ionizable elements (EIEs)

Many nutritional products contain significant levels of EIEs, such as Ca, Na, K and Mg. Therefore, blank solutions and solutions containing 4 ng/ml Cr and Mo and 2 ng/ml Se were analysed both with and without EIEs to determine any changes in concentrations of the analytes.

## 7.5 Specificity

Specificity of the method is its ability to accurately measure the analyte in the presence of other components in the sample matrix that might cause spectral interferences. To demonstrate the specificity of the method, undigested blank solutions were spiked with multi-element solutions at concentrations that are representative of nutritional products in samples for ICP-MS analysis. The typical H<sub>2</sub> gas mode for Se, and He gas mode for Cr and Mo, were used.

## 7.6 Accuracy

Accuracy was demonstrated by analysing three standard reference materials (SRMs) from NIST on two independent days, measuring spike recoveries in 10 nutritional products on three different days, and comparing results for 10 nutritional products obtained by this method to results obtained by other in-house validated ICP-AES and atomic fluorescence spectrometry (AFS) methods. The spike levels of the analytes added to the products were between 50 % and 200 % of the analyte concentrations in each product.

## 7.7 Precision

Both within- and between-day relative standard deviation (RSD) values were determined by analysing several nutritional products and two in-house laboratory control samples. Within-day precision was determined by analysing the samples in duplicate on each day, and between-day precision was measured by using the mean results of the duplicate samples analysed on each day on 10 different days.

## 7.8 Ruggedness and robustness

To determine the ruggedness of the method, laboratory control samples were analysed by two analysts on 10 different days. Also, NIST SRM 1849 was analysed in triplicate with varying sample weights and with different internal standards.

## 7.9 Reproducibility

Eight laboratories completed an interlaboratory testing protocol with this method on seven samples submitted as blind duplicates (14 total samples analysed plus the SRM 1849a control, which was not blinded). Represented were four countries, and five models of ICP-MS from three major vendors. Results showed an average  $RSD_R$  of 9,3 % for Cr, 5,3 % for Mo and 6,5 % for Se, with an average HorRat value of 0,35 across all three analytes and samples.

## Annex A (informative)

### Precision data

The data given in [Table A.1](#) were obtained in an interlaboratory study and published in 2015,<sup>[6]</sup> in accordance with ISO 5725-2<sup>[7]</sup> and AOAC-IUPAC Harmonized Protocol for collaborative study procedures, to assess precision characteristics of a method of analysis.<sup>[8]</sup> The method exhibited excellent performance across eight laboratories, seven times two samples and five different models of ICP-MS instruments. A nominal eight laboratories participated in this study, although for some analytes in some matrices, there may be less than eight results because of outlier rejection (Cr had three matrices with seven laboratories, Mo had two with seven laboratories and Se had all eight laboratories reporting at least one replicate result for all matrices).

More information on the validation of the method can be found at <http://standards.iso.org/iso/20649>

**Table A.1 — Precision data for chromium, molybdenum and selenium**

Sample <sup>h</sup>	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>c</sup>	4 <sup>d</sup>	5 <sup>e</sup>	6 <sup>f</sup>	7 <sup>g</sup>	Mean
<b>Chromium (PLOQ = 20 ng/g)</b>								
Mean result (ng/g)	16	48	140	130	30	24	< < 20	
Repeatability relative standard deviation, RSD <sub>r</sub> , %	3,4	4,7	2,1	7,0	5,5	3,8	< PLOQ	4,4
Reproducibility relative standard deviation, RSD <sub>R</sub> , %	12,1	7,1	5,8	8,1	9,2	13,4	< PLOQ	9,3
HorRat value	0,57	0,39	0,27	0,37	0,48	0,67	< PLOQ	0,46
<b>Molybdenum (PLOQ = 20 ng/g)</b>								
Mean result (ng/g)	33	63	190	150	30	18	20	
Repeatability relative standard deviation, RSD <sub>r</sub> , %	1,0	1,6	1,2	1,0	3,3	1,7	3,3	1,9
Reproducibility relative standard deviation, RSD <sub>R</sub> , %	7,9	3,1	3,8	3,0	4,6	7,9	6,7	5,3
HorRat value	0,42	0,18	0,19	0,14	0,24	0,38	0,33	0,27
<b>Selenium (PLOQ = 10 ng/g)</b>								
Mean result (ng/g)	24	30	133	93	24	23	27	
Repeatability relative standard deviation, RSD <sub>r</sub> , %	6,1	5,9	4,7	2,3	3,8	6,4	2,4	4,5
Reproducibility relative standard deviation, RSD <sub>R</sub> , %	6,1	7,2	5,0	8,1	7,3	9,3	2,5	6,5
HorRat value	0,31	0,37	0,23	0,36	0,37	0,46	0,13	0,32
<sup>a</sup> Adult milk protein, <sup>b</sup> Adult powder, low fat, <sup>c</sup> Adult RTE, high fat, <sup>d</sup> Adult nutritional RTE, high protein, <sup>e</sup> Child formula powder, <sup>f</sup> Infant elemental, <sup>g</sup> Infant milk powder, <sup>h</sup> Sample concentrations are indicated on an as-fed basis (25 g of powder diluted + 200 ml of water).								

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