
**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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

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.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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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).

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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.^[1] 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 Ω /cm.

3.2 Concentrated nitric acid (HNO₃), 65 % to 70 %, trace metal grade.

3.3 Hydrogen peroxide (H₂O₂), 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, $\rho_{Cr} = 2$ mg/l, $\rho_{Mo} = 2$ mg/l and $\rho_{Se} = 1$ mg/l. High-Purity™ Standards¹⁾, or equivalent.

3.6.2 Ge/Te multi-element stock standard solution in nitric acid, $\rho_{Ge} = 5$ mg/l and $\rho_{Te} = 5$ mg/l. High-Purity™ Standards¹⁾, 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 HNO₃ 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 HNO₃. 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

1) 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.

(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 HNO₃ followed by 2 ml H₂O₂ 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 HNO₃. 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 NO₂ is very toxic.

Add 1 ml of H₂O₂ 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.