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**Milk, milk products, infant formula and  
adult nutritionals — Determination  
of minerals and trace elements —  
Inductively coupled plasma mass  
spectrometry (ICP-MS) method**

*Lait, produits laitiers, formules infantiles et produits nutritionnels  
pour adultes — Détermination de la teneur en minéraux et en oligo-  
éléments — Méthode par spectrométrie de masse avec plasma à  
couplage inductif (ICP-MS)*

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## Foreword

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](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee 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 document is equivalent to the AOAC Official Method 2015.06: *Minerals and Trace Elements in Infant Formula*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

**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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This document was prepared by the IDF Standing Committee on Analytical Methods for Composition and 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 document is equivalent to the AOAC Official Method 2015.06: *Minerals and Trace Elements in Infant Formula*. All work was carried out by the ISO/IDF Action Team on C40 of the Standing Committee on Analytical Methods for Composition under the aegis of its project leader, Mr. H. Cruijsen (NL).

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# Milk, milk products, infant formula and adult nutritionals — Determination of minerals and trace elements — Inductively coupled plasma mass spectrometry (ICP-MS) method

## 1 Scope

This document specifies a method for the quantitative determination of calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), potassium (K), sodium (Na), zinc (Zn), chromium (Cr), molybdenum (Mo) and selenium (Se) using inductively coupled plasma and mass spectrometry (ICP-MS).

The method is applicable for the determination of all 12 elements in infant formula and adult nutritional products. The method is also applicable for milk, milk powder, whey powder, butter and cheese excluding the determination of Cr, because all Cr results were below the quantification limit and reproducibility could not be determined in these matrices<sup>[1]</sup>. The present method is an extension of ISO 20649 | IDF 235 (AOAC 2011.19<sup>[2]</sup>) which was validated only for Cr, Mo and Se in infant formula and adult nutritional products.

The ranges given in [Table 1](#) are in scope (see also [Table A.1](#)).

**Table 1 — Analytical ranges**

	Ca	Cu	Fe	Mg	Mn	P	K	Na	Zn	Cr	Se	Mo
Lower analytical range <sup>a</sup> , in mg/100 g	3	0,002	0,04	0,7	0,002	3	3	2	0,07	0,002	0,000 6	0,000 2
Upper analytical range <sup>a</sup> , in mg/100 g	1 280	1,2	20	110	1,0	800	2 000	850	18	0,16	0,05	0,10

<sup>a</sup> Concentrations apply to

— milk and "ready-to-feed" liquid as-is, using a typical sample size of 1 g per final analytical solution volume of 50 ml, and

— reconstituted milk powder, reconstituted infant formula powder and reconstituted adult nutritional powder (25 g into 200 g of water), using a typical sample size of 1,8 g of the reconstituted slurry per final analytical solution volume of 50 ml.

Ranges for non-reconstituted dairy ingredients (butter, cheese, whey powder, whey protein concentrate) are adjusted proportionally upward from these values based upon the sample size used for the ingredient. For example, if 0,3 g of cheese is digested the ranges will be 1 g/ 0,3 g = 3,3 × higher.

## 2 Normative references

There are no normative references in this document.

## 3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at <https://www.iso.org/obp>

— IEC Electropedia: available at <http://www.electropedia.org/>

## 4 Principle

Nitric acid, internal standard, and hydrogen peroxide are added to the sample in microwave vessels and the samples are digested at 200 °C using a pre-programmed temperature control digestion. The addition of hydrogen peroxide helps reduce carbon and nitrous oxide levels in the digestate. The presence of carbon in the samples causes signal enhancement of Se. Therefore, to matrix match the samples, carbon in the form of methanol is added to both the standard solutions and the digestate before analysis. An ionization buffer is not necessary because the multielement standards and samples always contain significant amounts of the easily ionized elements. Germanium (Ge, for 11 elements) and tellurium (Te just for Se) are used as internal standards. Analysis is performed by ICP-MS. Polyatomic interferences on the low mass elements are reduced or eliminated by analysing in He collision mode using kinetic energy discrimination (KED). For Se measurements the H<sub>2</sub> gas mode is preferred for increased sensitivity. Quantitation of 12 elements is achieved essentially simultaneously by comparing the analyte/internal standard response ratios in the unknown samples to a standard curve constructed from response ratios of calibration standards.

## 5 Reagents

**WARNING — The use of this document can involve hazardous materials, operations and reagents. This standard does not propose to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish safety and health practices.**

5.1 **Methanol**, purity ≥ 99,99 %, analytical reagent grade.

5.2 **Nitric acid (HNO<sub>3</sub>)**, concentrated, ultrapure reagent grade.

5.3 **Nitric acid (HNO<sub>3</sub>)**, concentrated, trace metal grade.

5.4 **H<sub>2</sub>O<sub>2</sub>, with a volume fraction of 30 %**, ACS reagent grade.

5.5 **Laboratory water**, metal-free, organic-free, pyrogen-free, filtered 18 MΩ-cm quality.

5.6 **Surfactant**, for example, Tergitol®<sup>1)</sup> Type 15-S-9, Sigma or equivalent (optional).

5.7 **Argon gas**, purity ≥ 99,996 %.

5.8 **Helium gas**, purity ≥ 99,999 9 %.

5.9 **Hydrogen gas**, purity ≥ 99,999 5 %, for Se analyses (recommended).

### 5.10 Multi-element standard stock solution.

NIST or NIST-traceable containing Se at mass concentration  $\rho = 20 \mu\text{g/l}$ ; Cr and Mo at 40  $\mu\text{g/l}$ ; Mn and Cu at 0,25 mg/l; Zn at 1 mg/l; Fe at 2,5 mg/l; Mg at 10 mg/l; P at 25 mg/l; Ca and K at 50 mg/l and Na at 25 mg/l in 2 % HNO<sub>3</sub> + trace hydrofluoric acid (HF). This stock standard solution expires on the date given by the manufacturer.

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1) Tergitol® 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 ISO or IDF of the product named.



### 5.11 Multi-element internal standard stock solution (ISTD stock).

NIST or NIST-traceable containing Ge and Te at 5 mg/l in 2 % HNO<sub>3</sub> + trace HF. This stock standard solution expires on the date given by the manufacturer.

### 5.12 Tuning and pulse/analogue (P/A) factor tuning stock solutions.

NIST or NIST-traceable containing various elements at concentration levels recommended by the manufacturer. Since this method determines the major elements at relatively high concentrations for an ICP-MS, it is important to understand the solutions needed and the procedure to obtain high quality calibration curves in which the detector is used in both pulse counting and analogue modes. A properly calibrated instrument will deliver the linearity requirements of the method; for example, that calibration residuals are < 4 %, see [Clause 9](#).

### 5.13 Quality control sample (QCS).

Standard Reference Material (SRM)<sup>2)</sup> 1849a<sup>2)</sup> milk-based hybrid infant/adult nutritional powder with certified values for Ca, Cu, Cr, Fe, Mg, Mn, Mo, P, K, Se, Na, and Zn. Supplied as a unit of 10 packets each containing approximately 10 g of material. This is the recommended control material for this analysis, but other suitable SRMs could be substituted.

## 6 Preparation of standards and solutions

### 6.1 Surfactant solution (optional, approximately 5 %)

Add about 700 ml laboratory water to a 1 l plastic bottle containing a polytetrafluoroethylene (PTFE)-coated stirring bar. Place the bottle on a magnetic stirrer and begin stirring at a moderate speed. Slowly add 50 ml surfactant from a graduated cylinder. When the surfactant is dissolved, fill the bottle to approximately 1 000 ml with laboratory water. Transfer to 1 l plastic bottle fitted with a PTFE-constructed dispenser with adjustable volume from 1 ml to 10 ml. This solution is added to the autosampler rinse solution to minimize residue build-up in the spray chamber. It does not otherwise affect the analysis. Expiration: 6 months. Store at room temperature.

### 6.2 Nitric acid rinse solution for autosampler rinse port, 2 %

Mix 20 ml of concentrated nitric acid ([5.2](#)) with 20 ml surfactant solution ([6.1](#)) and laboratory water to prepare a total volume of 1 000 ml. Expiration: 3 months. Store at room temperature.

### 6.3 P/A factor tuning working solution

Dilute and/or combine P/A factor tuning stock solutions (or equivalent) to the manufacturer's recommended dilution level with laboratory water for use at the instrument. Expiration: 6 months. Store at room temperature.

### 6.4 Calibration blank (Cal Blk) and preparation blank (PB) solution

Add approximately 15 ml laboratory water to a 50 ml volumetric flask. Dispense (using bottle dispenser or pipet) 5 ml nitric acid ([5.2](#)) into the same volumetric flask. Pipet (using calibrated digital pipet) 0,5 ml of the ISTD stock and 0,5 ml of methanol ([5.1](#)). Dilute to volume with laboratory water. This solution serves as both the calibration blank (Cal Blk) and preparation blank (PB). Use same lots of reagent for samples. Expiration: 2 days. Store at room temperature.

2) SRM 1849a is the trade name of a product supplied by the National Institute of Standards and Technology (NIST). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.

## 6.5 Calibration standard solution set

Prepare Cal Blk, Cal Std 1, Cal Std 2, Cal Std 3, and Cal Std 4 standard solutions by pipetting with a volumetric pipet (7.10) 0 ml, 1,00 ml, 5,00 ml, 20,00 ml and 40,00 ml, respectively, of the multi-element standard stock solution into separate 50 ml volumetric flasks or sample tubes. Add 0,5 ml of the ISTD with a volumetric pipet (7.10) or digital pipet calibrated at point-of-use to 0,8 % accuracy), 5 ml (using repipetter or PTFE bottle dispenser) of nitric acid (5.2), and 0,5 ml of methanol (5.1) to each flask. Fill the flasks to volume with laboratory water. Expiration: 2 days. Store at room temperature. The analyte and ISTD concentrations in the calibration standard solutions are shown in Table 2.

Table 2 — Concentrations of standards and ISTD in calibration standard solutions

	Calibration Blank	Calibration Standard 1	Calibration Standard 2	Calibration Standard 3	Calibration Standard 4	ISTD <sup>a</sup>
Na, mg/l	0	0,500	2,50	10,0	20,0	Ge
Mg, mg/l	0	0,200	1,00	4,00	8,00	Ge
P, mg/l	0	0,500	2,50	10,0	20,0	Ge
K, mg/l	0	1,00	5,00	20,0	40,0	Ge
Ca, mg/l	0	1,00	5,00	20,0	40,0	Ge
Cr, µg/l	0	0,800	4,00	16,0	32,0	Ge
Mn, mg/l	0	0,005 00	0,025 0	0,100	0,200	Ge
Fe, mg/l	0	0,050 0	0,250	1,00	2,00	Ge
Cu, mg/l	0	0,005 00	0,025 0	0,100	0,200	Ge
Zn, mg/l	0	0,020 0	0,100	0,400	0,800	Ge
Se, µg/l	0	0,400	2,00	8,00	16,0	Te
Mo, µg/l	0	0,800	4,00	16,0	32,0	Ge

<sup>a</sup> Internal standard (ISTD) added at 50 µg/l.

## 7 Apparatus

### 7.1 Inductively coupled plasma mass spectrometer.

It shall contain a quartz spray chamber, quartz torch, Ni/Pt sample cone, Ni/Pt skimmer cone, autosampler and printer. The ICP mass spectrometer shall have collision reaction cells (CRCs). In two separate interlaboratory studies, four different model ICP-MS instruments from three major vendors delivered equivalent performance[1][2].

### 7.2 Microwave oven.

Commercial microwave designed for laboratory use at 0 °C to 300 °C, with closed vessel system and controlled temperature ramping capability. Use vessels recommended by the manufacturer. In the interlaboratory study, five different microwave designs delivered equivalent performance[1].

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

### 7.3 Hydrogen generator, recommended for better Se sensitivity.

On-demand supply of > 99,999 % pure hydrogen at > 150 ml/min. Alternatively, a high pressure cylinder (99,999 % purity) may be used.

### 7.4 Magnetic stir plate and PTFE-coated magnetic stir bars.

### 7.5 Analytical balance.

**7.6 Fume hood.**

**7.7 Common laboratory glassware/plasticware.**

**7.8 Repipetter, 50 ml.**

**7.9 Bottle top dispenser, PTFE, adjustable volume 0,5 ml to 5 ml.**

**7.10 Volumetric pipets, Class A in accordance with ISO 1042[3], assorted sizes.**

**7.11 Digital pipets, 1 ml, adjustable, to deliver 500 µl with accuracy tolerance of better than 0,8 % and precision of better than 0,2 % relative standard deviation (RSD).**

## 8 Preparation of test sample

Make a single sample preparation to determine all 12 elements.

NOTE ISO 20649 | IDF 235[4] for Cr, Mo, and Se requires duplicate samples to be taken and their results averaged, but comparable reproducibility was obtained in a second interlaboratory study with the 12-element method described in this document in which only a single sample was analysed[1].

In sample vessels, weigh test portions to the nearest 0,000 1 g. For liquid products (including milk), the test portion size is 1,0 g. Liquid samples shall be thoroughly shaken (5 min in a mechanical shaker is appropriate), the container opened and the contents dumped into a plastic container into which a magnetic stir bar is placed. While stirring, remove the 1 g sample with a disposable pipet for weighing directly into the tared microwave vessel. For ingredients such as whey powder or whey protein concentrate use a direct mass of 0,3 g.

For powdered products, including whole milk powders, the test portion size is net 0,20 g of a powder sample, which should be taken from a 25 g powder and 200 g warm (60 °C) laboratory water reconstitution (i.e. 1,8 g of the 11,1 % reconstitution).

For butter or processed cheese (take a mould-free portion) use a direct mass of 0,3 g.

After weighing the sample, add 0,5 ml of ISTD stock using a digital pipet, 5 ml of nitric acid (5.2), and 2 ml of 30 % hydrogen peroxide.

The PB/Cal Blk solution prepared with the standards is the correct sample blank for this method. Specifically, do not microwave digest the sample blank, which can subject the blank to contamination[5]. The digital pipet used for the addition of ISTD solution shall be calibrated at point of use to ensure that it delivers a volume of 0,500 ml to a tolerance of better than 0,8 % and precision better than 0,2 % RSD.

Seal the vessels, and place into microwave oven. Execute a heating programme equivalent to that shown in Table 3, suitable for total digestion of the sample.

After digestion, place vessels in a fume hood. Unscrew the cap/venting nut slowly to gradually release the pressure. Then, completely remove the cap.

Slowly add approximately 20 ml of laboratory water to the contents of the vessel and transfer contents to a 50 ml sample vial. Add 0,5 ml methanol (5.1) to the sample vial and dilute to approximately 50 ml with laboratory water. Shake briefly. The transfer or the final volume does not need to be quantitative because internal standards were added prior to digestion. Therefore, the analyte/ISTD ratios will be constant.

**Table 3 — Microwave operating parameters**

Stage 1 sample digestion		
1	Power	100 % (1 600 W)
2	Ramp to temp., min	20
3	Hold time	20
4	Temp., °C	180
5	Cool down, min	20
Stage 2 sample digestion		
1	Power	100 % (1 600 W)
2	Ramp to temp., min	20
3	Hold time	20
4	Temp., °C	200
5	Cool down, min	20
Total, h		2

NOTE Stages 1 and 2 are operated sequentially, without removing vessels from the oven.

## 9 Determination

Using the appropriate tuning solutions, tune the instrument for optimal sensitivity in the kinetic energy discrimination mode and/or reaction mode according to the instrument design. Also, tune the instrument to find the P/A calibration factors that are needed for those calibration curves that will extend above roughly 100 µg/l (depends on instrument type). [Table 4](#) summarizes typical instrument parameters for analysis.

Analyse test solutions using an ICP-MS instrument standardized with the indicated standard solutions given in [Table 2](#). Ge is used as the ISTD for the 11 elements not including Se. Those 11 elements are determined in the He collision mode, employing KED. Te shall be used as the ISTD for Se determinations. 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 the other elements. Alternatively, verify in separate experiments that the practical limit of quantification (PLOQ) for Se is at or below 10 ng/g in the sample when using an alternate collision/reaction gas. One laboratory successfully completed the reproducibility study using helium, and another with ammonia gas<sup>[2]</sup>.

Typical calibration correlation coefficients are 0,999 5 or better for all analytes, but suitability is determined by calibration residuals as follows. Analyse Cal Std 3, or other suitable quality control solution, as a sample every 10 test portions to monitor for instrument drift and linearity. The result shall be within 4 % of the standard's nominal concentration. For good performance, include a method blank (run as a sample, its measured concentration shall be less than half of the lowest calibration standard) and known reference materials serving as control samples (recovery check within control or certified limits). Duplicate samples as required in the first multi-lab testing (MLT) study<sup>[2]</sup> are now optional, because they did not generally improve reproducibility<sup>[1]</sup>. If used, the mean result is reported and appropriate criteria based upon the data would be a relative percent difference within 10 % for Cr, 7 % for Se, and 5 % for all other elements. 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 (if used), and a repeated check standard.