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Infant formula and adult nutritionals — Determination of total iodine — Inductively coupled plasma mass spectrometry (ICP-MS)

Formules infantiles et produits nutritionnels pour adultes — Détermination de la teneur en iode total — Spectrométrie de masse avec plasma à couplage inductif (ICP-SM)

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B • B-1030 Brussels

Tel. + 32 2 325 67 40
Fax + 32 2 325 67 41
info@fil-idf.org
www.fil-idf.org

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Forewords

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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 wit the editorial rules of 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 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 2012.15: *Total iodine in infant formula and adult/pediatric nutritional formula – 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 20647 | IDF 234 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 2012.15: *Total iodine in infant formula and adult/pediatric nutritional formula – inductively coupled plasma-mass spectrometry*.

All work was carried out by the ISO-IDF Project Group C37 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 total iodine — 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 total iodine in infant formula and adult nutritional formula.[1] The method is applicable to the measurement of total iodine in infant formula and adult nutritional formula from 0,5 µg/100g to 1 500 µg/100g reconstituted final product and for ready-to-feed products from 2,5 µg/100 g to 1 000 µg/100 g using ICP-MS.

Using various infant formula and adult nutritional products, the method was subjected to an interlaboratory study. Levels obtained ranged from 3,47 µg/100 g to 124 µg/100 g. For all precision data related to the interlaboratory study, see Table A.1 located in Annex A.

2 Principle

Digestion occurs using a potassium hydroxide (KOH) solution in an oven or open-vessel microwave system. Iodine is stabilized with ammonium hydroxide and sodium thiosulfate after digestion. The solution is brought to volume followed by filtration. The filtrate is analysed directly or after dilution by inductively coupled plasma mass spectrometry (ICP-MS).

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 KOH pellets, certified ACS grade, e.g. Fisher Scientific, Fairlawn, NJ[1)].

NOTE KOH may contribute background levels of iodine.

3.2 Ammonium hydroxide (NH_4OH), 28 % to 30 % (m/m), certified ACS PLUS, Fisher Scientific[1)].

3.3 Sodium thiosulfate ($Na_2S_2O_3$), purity ≥ 99,99 %, metal basis, Fisher Scientific[1)].

3.4 Surfactant, e.g. Triton®X-100, Sigma, St. Louis, MO[1)].

3.5 Concentrated nitric acid (HNO_3), Optima, high purity, Fisher Scientific[1)].

3.6 Perchloric acid ($HClO_4$), 70 % (m/m).

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.

3.7 Purified water, 18 MΩ/cm.

3.8 Reference standards

3.8.1 Iodide standard solution in water, mass concentration ρ = 1 000 µg/ml, SPEX CertiPrep[1)].

3.8.2 Iodide standard solution in 1 % triethylamine (TEA), ρ = 1 000 µg/ml, Inorganic Ventures[1)].

3.8.3 Standard Reference Material (SRM), National Institute of Standards and Technology (NIST) SRM 1849a, Infant/Adult Nutritional Formula.

Either stock iodide reference solutions may be used for intermediate and working standard solutions preparation. The remaining source may be used as a continuing calibration verification (CCV) standard. Equivalent reference standards may be substituted. 'Iodide' may be referred to as 'iodine' throughout this International Standard.

3.9 Internal standards

3.9.1 Praseodymium (Pr) standard solution in 5 % HNO_3, ρ = 10 µg/ml, Inorganic Ventures[1)].

Individual values of iodine are reported for each test sample using praseodymium as the internal standard. Equivalent stock internal standard solutions may be substituted.

3.10 Preparation of reagent solutions

3.10.1 General

Prepare all reagent solutions as recommended by either mass per volume or volume per volume. Adjusting for purity and/or concentration is not required.

3.10.2 KOH solution, ρ = 50 g/l

Dissolve 25 g of KOH pellets in an appropriate amount of purified water, then dilute to 500 ml with purified water. This solution may be added using a re-pipet volumetric bottle top dispenser. Store this solution at room temperature. Reagent expires 6 months after preparation date.

3.10.3 Stabilizer concentrate

Dissolve 5 g of $Na_2S_2O_3$ in an appropriate amount of purified water, add 50 ml of NH_4OH, then dilute to 500 ml with purified water. The resulting concentration is 10 % NH_4OH and 1 % $Na_2S_2O_3$ in purified water. Store this solution at room temperature. Reagent expires 6 months after preparation date.

3.10.4 Wash solution (rinse)

Dissolve 2 g of surfactant (3.4) in an appropriate amount of purified water, add 20 ml of NH_4OH, then dilute to 2 000 ml with purified water. The resulting concentration is 1 % NH_4OH and 0,1 % surfactant in purified water. This solution may be added using a re-pipet volumetric bottle top dispenser. Store this solution at room temperature. Reagent expires 6 months after preparation date.

3.10.5 Diluent

Dissolve 10 g of KOH pellets and 0,4 g $Na_2S_2O_3$ in an appropriate amount of purified water, add 4 ml NH_4OH, then dilute to 2 000 ml with purified water. Store this solution at room temperature. Reagent expires 6 months after preparation date.

Alternatively, for a smaller volume, dilute 50 ml of 5 % KOH and 10 ml of stabilizer concentrate (3.10.3) to 500 ml with purified water. The resulting concentrations for both preparations are 0,5 % KOH, 0,2 % NH_4OH, and 0,02 % $Na_2S_2O_3$ in purified water. Store this solution at room temperature. Reagent expires 6 months after preparation date.

3.10.6 Conditioning solution

Prepare by aliquoting 25 ml of KOH solution (3.10.2), then dilute to 250 ml with purified water. Use this solution to prepare the instrument for analysis. The resulting concentration is 5 g/l KOH. Store this solution at room temperature. Reagent expires 6 months after preparation date.

3.10.7 Carrier solution

This solution is equivalent to the wash solution (3.10.4). The carrier solution is used to deliver the sample solution to the nebulizer through the ICP-MS autosampler introduction system. The carrier solution is introduced via a peristaltic pump using 0,76 mm inside diameter (id) two-stop polyvinyl chloride pump tubing. Store this solution at room temperature. Reagent expires 6 months after preparation date.

3.11 Preparation of standard solutions

3.11.1 General

Stock solutions are stable until the end date indicated on the certificate of analysis. Intermediate stock standard, calibration standard, continuing calibration verification and internal standard solutions are stable at room temperature until the earliest expiration date of all components used to prepare the solution. These solutions are ready to use for analysis. Do not subject them to any of the various procedures used to prepare actual samples (i.e. infant formulas and adult nutritionals) for analysis.

3.11.2 Stock iodine and praseodymium solutions

Purchase of stock iodine and praseodymium standard solutions with accompanying certificates of analysis is recommended.

3.11.3 Intermediate stock standard (ISS) iodine solutions

Prepare the intermediate stock standard iodine solutions according to Table 1.

These ISS solutions are used for calibration standard preparation and are typically prepared according to Table 1. The ISS concentrations presented are nominal. Using the stock iodine concentration found on the certificate of analysis, determine the exact concentration of each ISS. An electronic adjustable volume pipet, capable of delivering 100 µl to 5 000 µl should be used.

Table 1 — Preparation of intermediate stock standard (ISS) iodine solutions

Iodine standard solution ID	ID of solution used for preparation	Initial iodine concentration ng/ml	Aliquot volume ml	Final volume ml	Final iodine concentration ng/ml
10 000 (ISS)	Stock	1 000 000	0,5	50	10 000
1 000 (ISS)	10 000 (ISS)	10 000	5	50	1 000
10,0 (ISS)	1 000 (ISS)	1 000	0,5	50	10,0
Aliquot the appropriate amount of iodine standard solution into a single use 50 ml tube (4.1) and add 5 ml of stabilizer concentrate (3.10.3), fill to the 50 ml mark on the tube with water, cap the tube and then mix thoroughly. The resulting matrix concentration is 1 % NH_4OH and 0,1 % $Na_2S_2O_3$ in water.					

3.11.4 Calibration standard (CS) iodine solutions

Prepare the intermediate stock standard iodine solutions according to Table 2.

Typical CS standard concentrations are nominally 0,250 ng/ml, 0,500 ng/ml, 1,00 ng/ml, 10,0 ng/ml, 50,0 ng/ml and 100 ng/ml iodine and are typically prepared according to Table 2. The calibration blank is the zero point of the curve. The curve type used, if using a Perkin Elmer[2)] ICP-MS with ELAN[2)] software, should be linear through zero. If using an Agilent[2)] or Thermo[2)] ICP-MS, force the curve through the calibration blank. The calibration curve shall have a correlation coefficient (r) of ≥ 0,998 to be acceptable. Determine the exact concentration of each CS (traceable back to the certificate of analysis) and assign these values to the curve points used to generate the final results. An electronic adjustable volume pipet, capable of delivering 100 µl to 5 000 µl should be used.

Table 2 — Preparation of calibration standard (CS) iodine and calibration blank (CB) solutions

Iodine standard solution ID	ID of solution used for preparation	Initial iodine concentration ng/ml	Aliquot volume ml	Final volume ml	Final iodine concentration ng/ml
100 (CS)	1 000 (ISS)	1 000	5	50	100
50,0 (CS)	1 000 (ISS)	1 000	2,5	50	50,0
10,0 (CS)	1 000 (ISS)	1 000	0,5	50	10,0
1,00 (CS)	10,0 (ISS)	10,0	5	50	1,00
0,500 (CS)	10,0 (ISS)	10,0	2,5	50	0,500
0,250 (CS)	10,0 (ISS)	10,0	1,25	50	0,250
Blank (CB)	n.a.[a]	n.a.[a]	n.a.[a]	50	0
Aliquot the appropriate amount of iodine standard solution into a single use 50 ml tube (4.1) and add 5 ml of 5 % KOH and 1 ml of stabilizer concentrate (3.10.3), fill to the 50 ml mark on the tube with water, cap the tube and then mix thoroughly. The resulting matrix concentration is 0,5 % KOH and approximately 0,2 % NH_4OH and approximately 0,02 % $Na_2S_2O_3$ in water.					
[a] n.a. is not applicable					

3.11.5 Intermediate continuing calibration verification (ICCV), continuing calibration verification (CCV) iodine solutions and continuing calibration blank (CCB) solution

A CCV solution shall be prepared from a source other than that used for the CS solutions. For example, if a stock solution from SPEX CertiPrep[2] was used to prepare the CS solutions, do not use this same solution to prepare the CCV solution. Instead use a stock solution from Inorganic Ventures[2] (or another suitable manufacturer) to prepare the CCV solution.

Prepare the intermediate continuing calibration verification, continuing calibration verification standards solutions and continuing calibration blank according to Table 3.

ICCV solutions are used for preparation of the CCV standard solution and are typically prepared according to Table 3. The ICCV and CCV concentrations presented are nominal. Using the stock iodine concentration found on the certificate of analysis (from the second source), determine the exact concentration of each ICCV. With this information, determine the exact concentration of the CCV standard. An electronic adjustable volume pipet, capable of delivering 100 µl to 5 000 µl should be used.

2) 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.

Table 3 — Preparation of intermediate continuing calibration verification (ICCV), continuing calibration verification (CCV) iodine solutions and continuing calibration blank (CCB) solution

Iodine standard solution ID	ID of solution used for preparation	Initial iodine concentration ng/ml	Aliquot volume ml	Final volume ml	Final iodine concentration ng/ml
10 000 (ICCV)	Stock	1 000 000	0,5	50	10 000
1 000 (ICCV)	10 000 (ICCV)	10 000	5	50	1 000
10,0 (CCV)	1 000 (ICCV)	1 000	0,5	50	10,0
Blank (CCB)	n.a.[a]	n.a.[a]	n.a.[a]	50	0
Aliquot the appropriate amount of iodine standard solution into a single use 50 ml tube (4.1), fill to the 50 ml mark on the tube with diluent (3.10.5), cap the tube and then mix thoroughly. The resulting matrix concentration is 0,5 % KOH, approximately 0,2 % NH_4OH and approximately 0,02 % $Na_2S_2O_3$ in water. For the blank (CCB), fill a single use 50 ml tube (4.1) to the 50 ml mark on the tube with diluent (3.10.5), cap the tube and then mix thoroughly. [a] n.a. is not applicable					

3.11.6 Internal standard (IS) solution

Prepare the internal standard solutions according to Table 4.

The IS concentration typically used for analysis is 30 ng/ml Pr. Table 4 outlines a typical preparation scheme for the internal standard concentration.

Table 4 — Preparation of internal standard (IS) solution

Standard solution ID	ID of solution used for preparation	Initial concentration ng/ml	Aliquot volume ml	Final volume ml	Final iodine concentration ng/ml
30,0 (Pr)	Stock	10 000	1,5	500[a]	30,0
[a] After aliquoting the 10000 ng/ml Pr solution into the 500 ml vessel, add approximately 100 ml of water, 10 ml of HNO_3, 0,5 ml of $HClO_4$, 0,05 g of Triton® X-100 and then bring to volume with water and mix thoroughly. The resulting concentration is 2 % HNO_3, 0,1 % $HClO_4$ and 0,01 % Triton® X-100 in water.					

NOTE As some ICP-MS instruments provide greater sensitivity, the concentration of Pr may be adjusted accordingly to provide intensities similar to the intensity generated by the 50,0 ng/ml iodine standard.

4 Apparatus

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

Equivalent apparatus may be used. All laboratory plasticware should be single-use whenever possible. If reuse is necessary, wash using 10 % nitric acid, then rinse thoroughly with purified water prior to use. When needed, general laboratory acid-washed glassware may also be used.

Filter membranes < 1 µm (e.g. 0,25 µm or 0,45 µm) may be used. Adherence as close as possible to the recommended inside diameters of the pump tubing is critical. The ratio of the pump tubing inside diameter (id) (0,76 mm) used for the carrier solution (see 3.10.7) to the pump tubing id (0,38 mm) used for the internal standard solution (see 3.11.6) may be used as a guideline (0,76/0,38 = 2). For best performance, the ratio should remain as close to 2 as possible. Vast differences in id between the carrier solution pump tubing and the internal standard solution pump tubing (e.g. 1,02/0,19, respectively) may result in poor accuracy.

4.1 Polypropylene tubes, capacity 50 ml and 100 ml.

4.2 Oven, e.g. warming or drying oven, set to maintain 105 °C ± 5 °C.