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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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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).

INTERNATIONAL STANDARD
ISO 20647:2015(E)
IDF 234:2015(E)

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, NJ1).

NOTE KOH may contribute background levels of iodine.

3.2 Ammonium hydroxide (NH_4OH), 28 % to 30 % (m/m), certified ACS PLUS, Fisher Scientific1).

3.3 Sodium thiosulfate ($Na_2S_2O_3$), purity ≥ 99,99 %, metal basis, Fisher Scientific1).

3.4 Surfactant, e.g. Triton®X-100, Sigma, St. Louis, MO1).

3.5 Concentrated nitric acid (HNO_3), Optima, high purity, Fisher Scientific1).

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

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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.

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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.

4.3 Open-vessel microwave digestion unit, optional.

4.4 Analytical balance, capable of weighing to 0,0001 g.

4.5 Top-loader balance, capable of weighing to 0,01 g.

4.6 ICP-MS system, ELAN DRC II[3] Perkin Elmer, or equivalent.

4.7 Autosampler for ICP-MS, SC4-DX[3], Elemental Scientific, or equivalent.

4.8 Adjustable volumetric pipets, electronic or manual, capacity 100 µl to 5 000 µl.

4.9 Re-pipet volumetric dispensers, of adjustable volume.

4.10 Polypropylene or polytetrafluoroethylene (PTFE) bottles, for storage of reagents.

4.11 Disposable plastic syringes, e.g. capacity 10 ml, with Luer Lok connections.

4.12 Syringe filters, with 1 µm membrane, e.g. GMF-150[3] or PTFE.

4.13 Beakers, of assorted sizes.

4.14 Stir bars, 7,9 mm x 50 mm, or appropriate size.

4.15 Stir plate, adjustable speed.

4.16 Pump tubing, peristaltic, 0,76 mm inside diameter (id), two-stop polyvinyl chloride (PVC), used for introducing carrier solution (see 3.10.7).

4.17 Pump tubing, peristaltic, 0,38 mm id, two-stop PVC, used for introducing internal standard solution (see 3.11.6).

5 Procedure

5.1 Reconstitution

All powdered samples, with the exception of NIST SRM 1849a, are required to be analysed on a reconstituted basis. Do not reconstitute ready-to-feed (RTF) samples.

Accurately weigh 25 g ± 0,01 g of powdered test sample into an appropriate vessel (e.g. 400 ml beaker) and record the mass. Without zeroing the balance, add water to make approximately 225 g. Record the sample plus water mass. Place a stir bar in the mixture and stir on a stir plate to form a homogenous slurry/suspension. This reconstituted solution should be discarded after 24 h. Proceed to sample preparation.

5.2 Sample preparation

For reconstituted material, accurately weigh an aliquot of approximately 6 g of the reconstituted test sample into a 50 ml tube (4.1) or 12 g into a 100 ml tube (4.1).

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For the standard reference material, accurately weigh approximately 0,5 g of NIST SRM 1849a reference material into a 50 ml tube (4.1) or 1 g into a 100 ml tube (4.1).

For ready-to-feed (RTF) material, accurately weigh approximately 1 g of the RTF test sample into a 50 ml tube (4.1) or 2 g into a 100 ml tube (4.1).

The remaining RTF material should be transferred to a sealed, brown polypropylene container and held at refrigerated conditions between 2 °C to 8 °C. The solutions should be discarded after five days.

For the blank, designate at least one 50 ml tube (4.1) or a 100 ml tube (4.1) digestion vessel as the digest blank. The digestion blank(s) should be treated in the same manner as the samples.

Proceed to 5.3.

5.3 Addition of reagents

Add 10 ml of purified water to each 50 ml tube or 20 ml to each 100 ml tube. Add 5 ml of KOH solution (3.10.2) if the material was weighed into a 50 ml tube or add 10 ml of KOH solution (3.10.2) if the material was weighed into a 100 ml tube.

Seal the vessels and swirl or use a vortex apparatus to mix. Avoid inverting as this may allow the sample to adhere to the inner walls of the vessel above the level of digestion solution.

Proceed to 5.4 or to 5.5.

5.4 Oven digestion

Digest the samples in an oven (4.2) set to maintain 105 °C ± 5 °C until the dissolution of iodine is complete, approximately 1 h.

The digestion vessels may either be tightened completely or loosened slightly while in the oven. Carefully swirl by hand each digestion vessel at approximately halfway through the digestion/extraction procedure.

After removal of the samples from the oven, add 1 ml of stabilizer concentrate (3.10.3) to the 50 ml tube or add 2 ml if the material was weighed into a 100 ml tube. Allow the samples to cool to room temperature. Alternatively, allow samples to cool to room temperature first and then add the stabilizer concentrate.

If 50 ml or 100 ml vessels were used for digestion, bring the samples to a final volume of 50 ml or 100 ml respectively with purified water. Cap all vessels and then invert a minimum of three times to mix thoroughly.

5.5 Open vessel microwave digestion

Place the digestion vessels into the carousel of the open-vessel microwave digestion unit. If less than the maximum capacity is to be digested, distribute the vessels evenly throughout the carousel. Digest the samples in the microwave until the dissolution of iodine is complete. See Table 5 for suggested open-vessel microwave digestion parameters.

Using AOAC method iodine 2012.15[1] the parameters in Table 5, with the corresponding number of vessels, produced acceptable results for NIST SRM 1849a material for infant and adult nutritional formula. The microwave used was a CEM MARS 5[4)] or a CEM MARS 6[4)]. For each range of vessels, if fewer vessels than the minimum are placed in the microwave, overheating may occur resulting in loss of sample or injury. If a greater than the suggested number of vessels is placed in the microwave, the digestion may not be complete.

CAUTION — While using the microwave, ensure vessels do not completely seal (bursting hazard) or overheat (as melting may occur).

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Table 5 — Open vessel microwave digestion parameters

Number of 50 ml vessels	Power W	Power percentage %	Time min
6	400	10	5
	400	20	6
	400	20	7
12 to 18	400	25	10
	400	40	10
24	400	25	10
	400	40	10
	400	65	10

The vessel caps should be loosened slightly (from fully tightened) during the digestion procedure. Alternatively, instead of just loosening the caps, drill small holes (approximately 3 mm) in the caps. In this way the caps can be tightened, but venting (thus the 'open' vessel) can occur. The caps may be reused after acid washing.

After removal of samples from the microwave digestion unit, add 1 ml of stabilizer concentrate (3.10.3) to the 50 ml tube samples or add 2 ml if the material was weighed into a 100 ml tube. Allow the samples to cool to room temperature.

NOTE Alternatively, allow samples to cool to room temperature first, and then add the stabilizer concentrate.

If 50 ml or 100 ml vessels were used for digestion, bring the samples to a final volume of 50 ml or 100 ml respectively with purified water. Cap all vessels and then invert a minimum of three times to mix thoroughly.

5.6 Sample filtering

Filter each sample solution by filling a disposable syringe with the digested sample solution, attach a 1 µm membrane filter, then filter an adequate amount (e.g. at least 5 ml) into an appropriate vessel (e.g. 15 ml polypropylene centrifuge tube or autosampler vial) to be used for analysis.

Samples may be difficult to filter. Use of multiple filter membranes may be required. To ease filtration, allow the inverted sample digestates to rest for a period of time (e.g. 1 h) before filtering.

Digested sample solutions may be stored at ambient temperature. Samples may be stored at ambient temperature indefinitely, as long as the results for the applicable digest blank(s) and/or control sample(s) are acceptable when analysed.

5.7 Sample dilution

Aliquot 5 ml of each sample's filtrate into an appropriate volumetric vessel and then bring to a final volume of 10 ml with diluent (3.10.5). Analyse all samples diluted 5 ml to 10 ml.

5.8 Determination

The following parameters were applied satisfactorily when using an ICP-MS from Perkin Elmer, ELAN DRC II:

- Mode: Standard (STD);
- Gas: Argon (≥99,998 %, high purity);
- Rinse: 0,1 % Triton/1 % NH_4OH in purified water;

- Sweeps/readings: 20;
- Readings/replicate: 1;
- Replicates: 3;
- Nebulizer gas flow: optimized daily;
- Auxiliary gas flow: 1,2 l/min;
- Plasma gas flow: 15,00 l/min;
- Lens voltage: optimized daily;
- ICP radio frequency power: 1 500 W;
- Peristaltic pump: rate optimized.

Equivalent ICP-MS instrumentation may be used. Parameters of other manufacturer's instruments may be optimized accordingly to ensure the instrument's minimum daily performance requirements are met. All analysis shall be performed using STD mode (use of a reaction or collision gas is not required or allowed).

5.9 Conditioning

Condition the ICP-MS sample introduction system. Analyse the conditioning solution (3.10.6) while concomitantly introducing internal standard solution online (e.g. through a mixing block or T-piece) until conditioned (approximately 1 h). The internal standard solution is introduced via a peristaltic pump using 0,38 mm inside diameter two-stop polyvinyl chloride (PVC) pump tubing. After conditioning, begin to aspirate carrier solution (3.10.7) while continuing to add internal standard. Analyse samples using ICP-MS. Ensure the wash solution (rinse) (3.10.4) is available and ready for use to rinse out the sample lines and introduction system between each analysis.

If acidic sample solutions are typically analysed on the ICP-MS, perform a thorough cleaning of the entire sample introduction system prior to conditioning. Background counts for the iodine and the internal standard should be relatively stable (e.g. not ascending or descending). A dedicated set of cones (sampler and skimmer), if possible, is recommended. Analysis of acid type (e.g. HNO_3) matrices with the same set of cones used for iodine analysis may increase conditioning time or produce elevated background levels. Analysing several (e.g. at least six) digested samples (to further conditioning) prior to calibration is recommended.

Due to the nature of the digestion/extraction solution (i.e. KOH) and the amount of organic material in the sample solutions, additional maintenance may be required (as compared to typical acid matrix digestions/analysis). Lenses in instruments and/or lens stack assemblies may require more frequent cleaning. Once cleaned, a period of reconditioning may be required.

5.10 Calibration

In addition to a calibration blank, use working standards of 0,250 ng/ml, 0,500 ng/ml, 1,00 ng/ml, 10,0 ng/ml, 50,0 ng/ml and 100 ng/ml. Calibrate the ICP-MS using an autosampler or manually.

The curve type used should be linear, forced through the calibration blank. All standards shall be included in the calibration curve. Consistent background throughout the entire analytical run is imperative for a successful analysis. This will be evident based on the results obtained for the continuing calibration blank (see 5.12).

5.11 Sample analysis

Analyse a 5 ml to 10 ml dilution of each digested filtered sample using ICP-MS.

For the purpose of the collaborative study (see Annex A), a 5 ml to 10 ml dilution of the sample was analysed. Diluting the samples reduces the matrix load on the plasma and may reduce the frequency of maintenance (e.g. cleaning cones). For other applications, samples digested with 50 g/l KOH solution may be analysed directly or diluted (if necessary) so that the iodine concentration will fall within the calibration range. Alternative volume aliquots may be prepared by placing an aliquot of the filtrate into an appropriate volumetric vessel, then diluting to an appropriate final volume with diluent (3.10.5).

5.12 Data acceptability

The calibration curve shall include a calibration blank (as a calibration point). The calibration curve shall have a correlation coefficient (*r*) of ≥ 0,998 to be acceptable.

The individual back-calculated calibration standard concentrations shall be within 90 % to 110 % of the theoretical concentrations to be acceptable.

The 0,250 ng/ml signal shall be ≥ 1,5 times the calibration blank signal.

A continuing calibration blank (CCB) is analysed after calibration, at least every 10 samples, and after the last sample in the analysis batch to monitor background. A CCB should be of the same matrix as the standards used for calibration. Iodine levels ≤ 30 % of the lowest calibration standard are considered acceptable.

With each batch of samples, at least one digest blank should be prepared in the same manner as the samples. An iodine result of ≤ 30 % pf the lowest calibration standard is considered acceptable. A continuing calibration verification (CCV) standard solution containing iodine from a source other than that of the calibration standards is used to verify acceptable calibration and to evaluate the on-going performance of the instrument. The CCV should be analysed after calibration, at least every 10 samples, and after the last sample in the analysis. A CCV should be of the same matrix as the standards used for calibration. A CCV result if considered acceptable when the result is within 90 % to 110 % of the theoretical concentrations.

6 Calculation

If reconstitution was performed, use Formula (1) to determine the amount of iodine in the sample:

$$\rho_i = \frac{(c \times V) \times d}{m_s \times 10} \qquad (1)$$

where

ρ_i is the concentration of iodine in the sample, in µg/100 g reconstituted as fed basis;

c is the sample concentration, in ng/ml, sample solution reading on the curve;

V is the final volume after digestion, in ml;

d is the dilution factor (if not applicable, $d = 1$);

m_S is the mass of reconstitution aliquoted during sample preparation, in g.

If reconstitution was not performed, use Formula (2) to determine the amount of iodine in the sample:

$$\rho_i = \frac{(c \times V) \times d}{m \times 10} \qquad (2)$$

where

ρ_i is the concentration of iodine in the sample, in µg/100 g;

c is the sample concentration, in ng/ml, sample solution reading on the curve;