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SIM.QM-S10

Supplementary Comparison for Trace elements in skim milk powder

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Abstract

SIM.QM-S10 was performed to assess the analytical capabilities of National Metrology Institutes (NMIs) and Designated Institutes (DIs) of SIM members (or other regions) for the accurate determination of trace metals in skim milk powder. The study was proposed by the coordinating laboratories National Research Council Canada (NRC) and INTI Argentina as an activity of Sistema Interamericano de Metrología (SIM) approved by the Inorganic Analysis Working Group (IAWG) of *Consultative Committee for Amount of Substance – Metrology in Chemistry and Biology* (CCQM). Participants included NMIs/DIs from twelve countries. No measurement method was prescribed by the coordinating laboratories. Therefore, NMIs used measurement methods of their choice. However, the majority of NMIs/DIs used closed vessel microwave system using a mixture of HNO_3 and H_2O_2 for the digestion and ICP-MS and ICP-OES for the determination of the measurands.

This SIM.QM-S10 Supplementary Comparison provides NMIs/DIs with the needed evidence for CMC claims for trace elements in skim milk powder and similar matrices.

1. Introduction and background

Skim milk powder is widely used as a food ingredient and has the same nutrition of fresh nonfat milk but with a longer shelf life. The determination of micronutrients and trace elements in skim milk powder is an important and commonly performed measurement responsibility to ensure the nutritional quality of milk powder and derivates products.

An earlier key comparison in this area was conducted in 2014 under the auspices of the CIPM as CCQM-K125, with the parallel pilot study CCQM-P159 (Iodine and other elements in infant formula). Since a few members from the SIM community did not participate in this comparison, the SIM regional comparison (SIM.QM-S10) was proposed to ensure the comparable and traceable measurement results for microelements and trace elements such as Ca, Fe, Se, and Zn in skim milk powder and similar matrices. This comparison provided NMIs with the needed evidence for CMC claims for trace elements in skim milk powder and similar matrices. Note that those laboratories wishing to utilize this exercise for support of CMC claims must register for this comparison. Although this is organized as a SIM regional comparison, it is open to other participants of the MRA throughout all RMOs. Results for the comparison are going to be registered on the BIPM Key and Supplemental Comparisons Database, the KCDB. The planned time scale of SIM.QM.S-10 is presented in Table 1.

Table 1. Timetable of SIM.QM-S10

Action	**Date**
Proposal agreed by SIM	August, 2019
Call for participating	September 23, 2019
Registration deadline	October 4, 2019
Shipment of the samples	Week of October 9, 2019
Deadline for report of results	January 10, 2020 (extended to January 31, 2020 as participants' requested)
Draft A circulation	April 2, 2020 (deadline for comments: May 22, 2020)
Draft B circulation	October, 2020
Presentation/discussion of results at IAWG meeting	November 4, 2020
Presentation/discussion of results at SIM meeting	November 10, 2020
Draft B Final report	November 30, 2020

Although this was organized as a SIM regional comparison, it was open to other participants of the MRA throughout all RMOs. The source of material was Canadian food-grade skim milk powder. The material was blended and packed into trilaminate stick-packs at a pharmaceutical manufacturing company. Long term storage of the material at NRC Canada is at -20 ^{o}C. Analyte

mass fractions are representing their natural levels, and four analytes were selected for this comparison. Participants may use any method of their choice. Table 2 summarizes the analytes and target mass fractions.

Table 2. Analytes and target mass fractions in SIM.QM-S10 Supplementary Comparison

Analyte	Target mass fraction
Ca	(0-20 000) mg/kg
Fe	(0-10) mg/kg
Se	(0-10) mg/kg
Zn	(0-100) mg/kg

2. Instruction to Participants

A technical protocol was sent to all participants to SIM.QM-S10 providing information about the approximate analyte contents, the sample handling and data submission form (in excel format). Appendix A presents the technical protocol for SIM.QM-S10.

Each participant received five identified trilaminate stick packs of the study sample, with each trilaminate stick pack containing approximately 2.5 g of skim milk powder.

Participants were requested to report results for the measurands in minimum triplicate as the element content mass fraction (mass/mass, mg/kg) on test aliquots drawn from the stick packs on a dry mass basis using their method of choice. Dry mass corrections were to be determined. No protocol for the dry mass correction was provided.

In order to allow a sufficient evaluation of the comparison, a complete description of the method(s) used, including sample preparation, calibration technique(s) along with their metrological traceability and uncertainty assessment in accordance with JCGM 100:2008 Evaluation of Measurement Data-Guide to the Expression of Uncertainty in Measurement, as well calibration standard, and reference materials used and any specific challenges encountered was also requested to be provided.

When the participant reported individual results from different methods, the reported values using the method with the lowest uncertainty was used as the official result for the reference value and degree of equivalence calculations.

3. Participants Institutes

In total, 12 participants (8 NMIs and 4 designated institutes (DIs)) registered for the SIM.QM-S10 supplementary comparison as listed in Table 3. Table 3 also present information regarding the analytes registered, sample delivery date, reporting date and analyte reported for each registered participant.

Table 3. Registered institutes, contacts, analytes registered, sample delivery date, reporting data and analyte reported.

Participant	Responsible	Country	Analytes registered	Sample delivery date	Reporting date	Analyte reported
INTI, Instituto Nacional de Tecnologia Industrial	Osvaldo Acosta; Mabel Puelles	Argentina	Ca, Fe, Se, Zn	Oct. 15, 2019	Jan. 13, 2020	Ca, Fe, Se, Zn
IBMETRO, Instituto Boliviano de Metrología	Mabel Delgado	Bolivia	Ca, Fe, Zn	Oct. 17, 2019	Jan. 10, 2020	Ca, Fe, Se
INMETRO, National Institute of Metrology, Quality and Technology	Rodrigo Caciano de Sena; Marcelo Dominguez de Almeida; Marcia Silva da Rocha	Brazil	Ca, Fe, Se, Zn	Oct.15, 2019	Dec. 27, 2019	Ca, Se, Zn
ISP, Public Health Institute of Chile	Soraya Sandoval; Claudia Núñez; Javier Vera	Chile	Fe, Zn	Nov. 10, 2019	Jan. 10, 2020	Fe, Zn
NIM China, National Institute of Metrology	Wei Chao; Li Xiao	China	Ca, Fe, Se, Zn	Dec 20, 2019	Jan. 25, 2020	Ca, Fe, Se, Zn
INMC Colombia, Instituto Nacional de Metrología de Colombia	Henry Torres Quezada; Gina A. Torres; Diego A. Garzón; Diego A. Ahumada	Colombia	Ca, Fe, Zn	Oct. 15, 2019	Jan. 10, 2020	Ca, Zn
LACOMET, Laboratorio Costarricense de Metrología	Jimmy Venegas Padilla; Katia Rosales Ovares, Bryan Calderón Jiménez	Costa Rica	Ca, Fe, Se, Zn	Oct. 15, 2019	Jan. 31, 2020	Ca, Zn
INEN, Servicion Ecuatoriano de Normalización	Evelyn Vasco	Ecuador	Ca, Fe, Zn	Nov. 14, 2019	Jan. 31, 2020	Fe, Zn
CENAM, National Metrology Institute of Mexico	Laura Regalado Contreras; Mariana Arce Osuna	Mexico	Ca, Fe, Se, Zn	Oct. 21, 2019	Feb. 2, 2020	Ca, Zn
JSI, Jozef Stefan Institute	Radojko Jacimovic; Tea Zuliani	Slovenia	Ca, Fe, Se, Zn	Oct. 16, 2019	Jan. 10, 2020	Ca, Fe, Se, Zn
NIMT, National Institute of Metrology	Nunnapus Laitip; Usana Thiengmanee; Nattikarn Ornthai; Pranee Phukphatthanachai, Suttinun Taebunpakul	Thailand	Ca, Fe, Se, Zn	Oct. 21, 2019	Jan. 10, 2020	Ca, Fe, Se, Zn
INRAP, National Institute of Research and Physical chemical Analysis	Hanen Klich	Tunisia	Ca, Zn	Oct. 16, 2019	Jan. 13, 2020	Zn

Samples were shipped to all participants by FEDEX international priority from October 9, 2019 to October 11, 2019. Samples were delivered between October 15, 2019 and November 14, 2019. There were some customs issues with shipping samples to China and Russia. For Russia, the material (skim milk powder) is banned to be import to the country and The Russian

Metrological Institute of Technical Physics and radio engineering requested to abandon the material. Thus the Russian Metrological Institute of Technical Physics and radio engineering was not able to participate in the SIM.QM-S10 comparison. For China, the packaged was detained during clearance and was informed by NIM China that FEDEX suggested to abandoned the package and send the samples by express mail service instead. Another set of samples were sent by express mail service to NIM China on December 20, 2019.

Samples were also submitted to two reference laboratories in Panama (Laboratorio de referencia de alimentos y aguas (ICGES) and Instituto Especializado de Ánalisis. To expedite the process, samples were shipped to Esther Santamaria (CENAMEP)) and she distributed to the participating laboratories. Results of those laboratories was not included in this report.

Participants were requested to inspect immediately the samples upon receipt and inform the coordinator if there were any issues with the condition of received samples. All laboratories reported that the samples arrived in good conditions.

4. Methods of measurement

Participants were free to use a method of their choice for both sample preparation and measurement method. A majority of the participants digested the samples using a closed vessel microwave system using a mixture of acid nitric (HNO_3) and hydrogen peroxide (H_2O_2) and measured the digested samples using ICP-MS or ICP-OES. Different calibration strategies were used ranging from external calibration and standard addition to isotope dilution. Table 4 summarized the sample preparation, measurement method (including calibration strategy) and sample mass used.

Table 4. Summary of sample preparation, measurement method and sample mass used.

Participant	Sample preparation (instrument)	Measurement method (instrument)	Sample mass (g)
INTI, Argentina	Microwave digestion (Ultrawave) (5 ml HNO_3 +0.5 ml HF)	Se: SA-ICP-MS, Ge as IS (Elan DRC II ICP-MS); Ca, Fe, Zn: SA-ICP-OES; Y as IS (Perkin Elmer Optima 7300 DV ICP-OES)	0.5
IBMETRO, Bolivia	Microwave digestion (Multiwave Pro) (8 ml HNO_3 + 2 ml H_2O_2)	Ca, Fe, Zn: EC- AAS, no IS (Perkin Elmer PinAACle 900T)	0.5
INMETRO, Brazil	Microwave digestion (Multiwave Pro) (4 ml HNO_3 + 2 ml H_2O_2)	Ca: EC- ICP-OES, no IS; Zn: SA-ICP-OES, no IS (Jobin Yvon Ultima 2 ICPOES); Se: SA-ICP-MS, no IS (Elan DRC II ICP-MS)	0.5
ISP, Chile	Microwave digestion (Multiwave Pro) (8 ml HNO_3 + 2 ml H_2O_2 + 1 ml H_2O)	Fe, Zn :SA-ICP-MS, Sc as IS (Agilent 7700 ICP-MS)	0.5
NIM China	Microwave digestion (CEM MARS 5) (5 ml HNO_3)	Ca, Fe, Zn : SA-ICP-OES, no IS (iCap 7400 ICP-OES); Se : ID-ICP-MS, reference isotope ^{80}Se, spiked isotope ^{78}Se (Agilent 8800 ICP-MS)	0.45 – 0.5

Participant	Sample preparation (instrument)	Measurement method (instrument)	Sample mass (g)
INMC Colombia	Microwave digestion (Multiwave Pro) (4 ml HNO_3 + 2 ml H_2O_2)	Ca, Zn: SA-ICP-MS, Tl and Rh as IS (ICP-MS NEXION 300D); Ca, Zn: EC-FAAS	0.5
LACOMET, Costa Rica	Microwave digestion (CEM MARS 6) (10 ml HNO_3)	Ca: EC-FAAS, no IS (PerkinElmer PiAAcle 900T); Zn: SA-FAAS, no IS (PerkinElmer PiAAcle 900T)	1
INEN, Ecuador	Dry Ashing (525^0C, 8 h, dissolved in 1M HNO_3)	Zn, Fe: EC-FAAS, no IS	1
CENAM, Mexico	Microwave digestion (Mars 6) (8 ml HNO_3 + 2 ml H_2O_2)	Ca: SA-ICP-MS, Y as IS (Thermo ICAP Q ICP-MS); Zn: SA-ICP-MS, Y as IS (Thermo ICAP Q ICP-MS)	0.5
JSI, Slovenia	Sample pelletized Microwave digestion MARS 6, CEM Corporation) (4 ml HNO_3 + 1 ml H_2O_2)	Ca, Se, Zn: k_0-INAA (250 kW TRIGA Mark II reactor, HPGe detector); Fe: EC-ICP-MS, Rh as IS (Agilent 7900x ICP-MS)	0.3-0.33 0.5
NIMT, Thailand	Microwave digestion (Multiwave 7000) (5 ml HNO_3)	Zn: ID-ICP-MS (reference isotope ^{66}Zn, spiked isotope ^{67}Zn) (Agilent 8800 ICP-MS) Ca: SA-ICP-MS, Rh as IS (Agilent 8800 ICP-MS); Ca: SA-ICP-OES, Rh as IS ; Fe: SA-ICP-OES, Y as IS (Perkin Elmer Avio 500); Se: SA-HR-ICP-MS, Rh as IS (Thermo Element XR, HR-ICP-MS)	0.25
INRAP, Tunisia	Microwave digestion (Milestone Start D) (8 ml HNO_3 + 2 ml H_2O_2)	Zn: EC-HR-ICP-OES, no IS (Analytik Jena, Plasma Quant 9000 Elite)	0.5

EC- external calibration; HR- high resolution; ID- isotope dilution; IS-internal standard, SA- standard addition

The primary standards as well the certified reference materials used are listed in Tables 5 and 6. Most participants used NIST standard solution as primary standards. NIM China used GBW primary standards for Ca and Zn and JSI used IRMM primary standards.

Regarding CRM used, all participants, except one (INRAP, Tunisia) used a CRM with similar matrix, i.e., milk powder or infant/nutritional formula. INRAP, Tunisia did not submit any results for CRM.

Table 5. Calibration Standards used as reported by the participants

Participant	Ca	Fe	Se	Zn
INTI, Argentina	NIST SRM 3109a	NIST SRM 3126	NIST SRM 3149	NIST SRM 3168a
IBMETRO, Bolivia	NIST SRM 3109a	NIST SRM 3126a	--	NIST SRM 3168a
INMETRO, Brazil	NIST SRM 3109	--	NIST SRM 3149	NIST SRM 3168a
ISP, Chile	--	NIST SRM 3126a	--	NIST SRM 3168a
NIM China	GBW(E)080118	GBW08616	NIST SRM 3149	GBW08620
INMC Colombia	NIST SRM 3109a	--	--	NIST SRM 3168a
LACOMET, Costa Rica	NIST SRM 3109a	--	--	NIST SRM 3168a

Participant	Ca	Fe	Se	Zn
INEN, Ecuador	--	NIST SRM 3126a	--	NIST SRM 3168a
CENAM, Mexico	NIST SRM 3109a	--	--	CENAM DMR-61d
JSI, Slovenia	IRMM-530R (Al-0.1% Au alloy)	NIST SRM 3126a	IRMM-530R (Al-0.1% Au alloy)	IRMM-530R (Al-0.1% Au alloy)
NIMT, Thailand	NIST SRM 3109a	NIST SRM 3126a	NIST SRM 3149	NIST SRM 3168a
INRAP , Tunisia	--	--	--	NIST SRM 3168a

Table 6. Certified reference materials used for quality assurance as reported by the participants

Participant	CRM used
INTI, Argentina	Ca, Fe, Se, Zn: NIST SRM 1849a (infant/adult nutritional formula)
IBMETRO, Bolivia	NP
INMETRO, Brazil	Ca, Se, Zn: NIST SRM 1849a (infant/adult nutritional formula)
ISP, Chile	Fe, Zn: NIST SRM 1849a (infant/adult nutritional formula)
NIM China	Ca, Fe, Zn: ERM BD-150 (skimmed milk powder) Se: GBW10115 (infant formula)
INMC Colombia	Ca, Fe, Zn: CENAM DMR-82c (Skim milk powder)
LACOMET, Costa Rica	Ca, Zn: NIST SRM 1869 (Infant/Adult Nutritional Formula II (milk/whey/soy-based)
INEN, Ecuador	Zn, Fe: NIST SRM 3234 (soy flour)
CENAM, Mexico	Ca, Zn: CENAM CMR-6300082d (Skim milk powder)
JSI, Slovenia	ERM-BD151 (Skimmed milk powder)
NIMT, Thailand	Ca, Fe, and Zn :NMIJ CRM 7512-a (milk powder) Se: SRM 1568b rice flour
INRAP , Tunisia	NP

NP: not provided

Table 7 presents the dry weight correction reported by each participants. INEN, Ecuador did not provide this information.

Table 7. Dry weight correction used as reported by the participants

Participant	Number of samples	Sample mass (g)	Correction for dry mass (% of weighted sample)[a]
INTI, Argentina	3	0.5	97.40 ± 0.32%
IBMETRO, Bolivia	5	0.5	97.34 ± 0.07%[b]
INMETRO , Brazil	3	1.0	96.43 ± 0.16%
ISP, Chile	4	1.0	96.605 ± 0.048 %
NIM China	4	0.4-0.7	98.00 ± 0.05%
INMC Colombia	3	0.25	97.10 ± 0.5%
LACOMET, Costa Rica	4	1.0	97.31 ± 0.18 %
INEN, Ecuador	NP	NP	NP
CENAM, Mexico	2	0.5	96.898 ± 0.095%
JSI, Slovenia	3	0.8-0.9	97.78 ± 0.003%
NIMT, Thailand	3	1	97.40 ± 0.10 %
INRAP , Tunisia	6	1	96.18 ± 0.07 %

[a] results presented as average ± uncertainty (k=1)
[b] performed for each sample separately. Results combined as average (100-dry weight)
NP: not provided.

5. Results and Discussion

5.1. General

The participants' results as reported to the coordinating laboratory are shown in Tables 8 to 15 and Figures 1 to 4. All measurement results were reported on a dry mass basis.

As documented in the technical protocol of SIM.QM-S10, the supplementary Comparison Reference Value (SCRV) was originally proposed to be assigned based on NRC results. However, after discussion with coordinators and participants, all data from participating NMIs (except identified outliers) were used to calculate the SCRV.

Results from each analyte is presented separately.

5.1.1. Calcium

Nine laboratories reported values for mass fraction of calcium. INMC, Colombia and NIMT Thailand reported two calcium results. INMC, Colombia used standard addition (SA) ICP-MS with Tl and Rh as internal standard (IS) and external calibration (EC) FAAS for the determination of mass fraction of Calcium. NIMT Thailand used both SA-ICP-MS and SA-ICP-OES (both using Rh as IS). For both laboratories, only the ICP-MS data was used for the calculation of the SCRV.

Table 8. Reported Results for mass fraction of Ca (dry mass basis) and their associated combined and relative expanded uncertainties, with the coverage factor *k* as reported by the participants **in the order of increasing mass fraction value.**

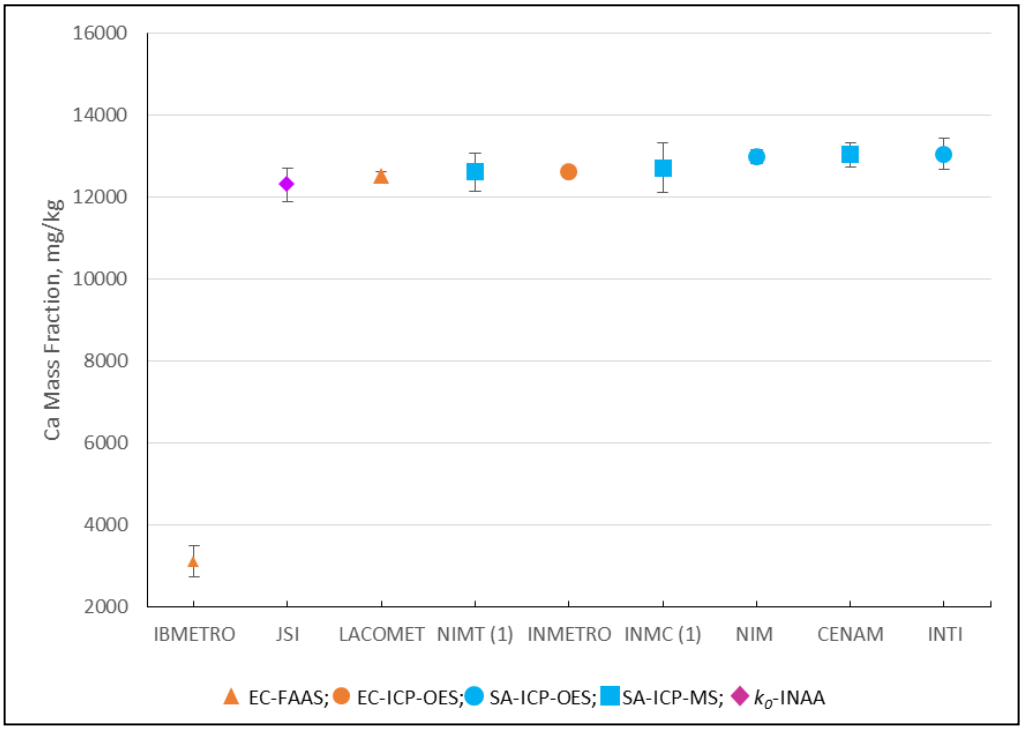
Participant	**Reported value mg/kg**	**u_c, mg/kg**	***k*** **(95% level confidence)**	**U, mg/kg**	**N[a]**	**Analytical Method/ Instrument**
IBMETRO, Bolivia	3099.74	375.89	2	751.78	5	EC-FAAS, no IS
JSI, Slovenia	12295	408	2	816	5	k_0-INAA
LACOMET, Costa Rica	12498	109	2	218	6	EC-FAAS, no IS
NIMT, Thailand [c]	12610 (12780)	465 (420)	2 2	930 (840)	5	SA-ICP-MS, Rh as IS SA-ICP-OES, Rh as IS
INMETRO, Brazil	12631	91	2	182	5	EC- ICP-OES, no IS
INMC Colombia[b]	12715 (12669)	612.8 (386.1)	1.97 1.97	1207 (761)	3 3	SA-ICP-MS, Tl & Rh as IS EC-FAAS
NIM China	12979	169	2	339	7	SA-ICP-OES, no IS
CENAM, Mexico	13033	292	2	583	5	SA-ICP-MS, Y as IS
INTI, Argentina	13053	379	2	758	7	SA-ICP-OES; Y as IS

[a] N Number of independent replicates

[b] Considered the ICP-MS value. Second value (in parenthesis) was determined with EC-FAAS

[C] Considered the ICP-MS value. Second value (in parenthesis) was determined with SA-ICP-OES

Figure 1. Calcium mass fraction ((dry mass basis) as reported by the participants. Error bars denote the combined uncertainty u_c for a coverage factor of k=1 as reported.



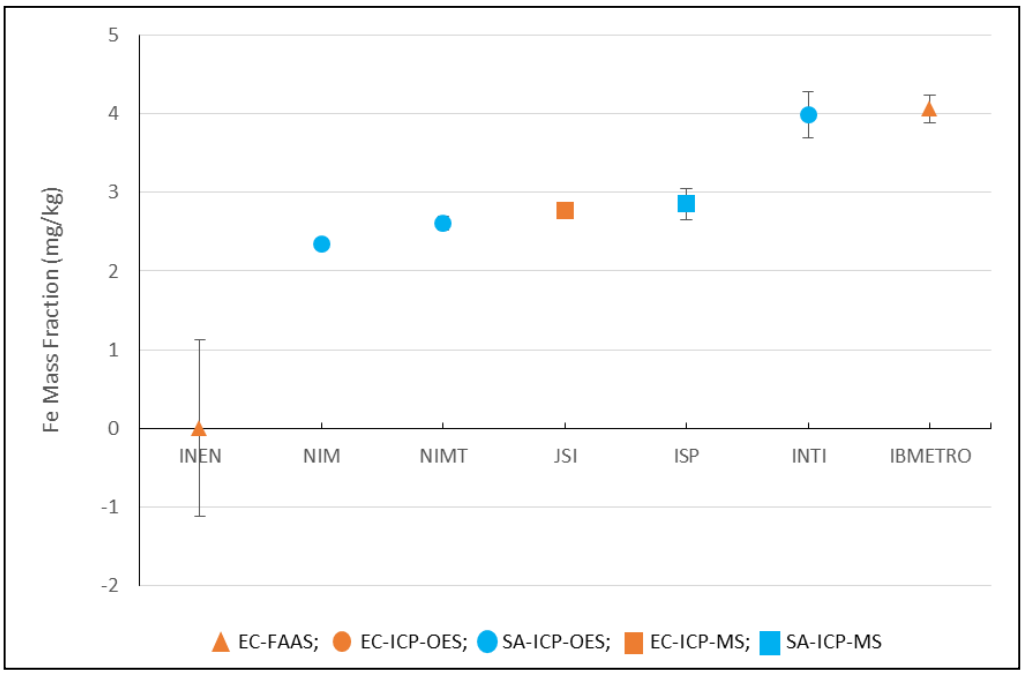
5.1.2. Iron

Seven laboratories reported values for mass fraction of iron. Results are presented in Table 9.

Table 9. Reported Results for mass fraction of Fe (dry mass basis) and their associated combined and relative expanded uncertainties, with the coverage factor k as reported by the participants **in the order of increasing mass fraction value.**

Participant	**Reported value mg/kg**	**u_c, mg/kg**	***k*** **(95% level confidence)**	**U, mg/kg**	**N[a]**	**Method**
INEN, Ecuador	0.002	1.12	2	2.24	3	EC-FAAS, no IS
NIM China	2.35	0.05	2	0.10	6	SA-ICP-OES, no IS
NIMT, Thailand	2.61	0.0859	2	0.18	3	SA-ICP-OES, Y as IS
JSI, Slovenia	2.77	0.03	2	0.06	5	EC-ICP-MS, Rh as IS
ISP, Chile	2.85	0.20	3.18	0.63	5	SA-ICP-MS, Sc as IS
INTI, Argentina	3.987	0.288	2	0.576	5	SA-ICP-OES; Y as IS
IBMETRO, Bolivia	4.06	0.17	2	0.34	5	EC-FAAS, no IS

Figure 2. Iron mass fraction (dry mass basis) as reported by the participants. Error bars denote the combined uncertainty u_c for a coverage factor of k=1 as reported.



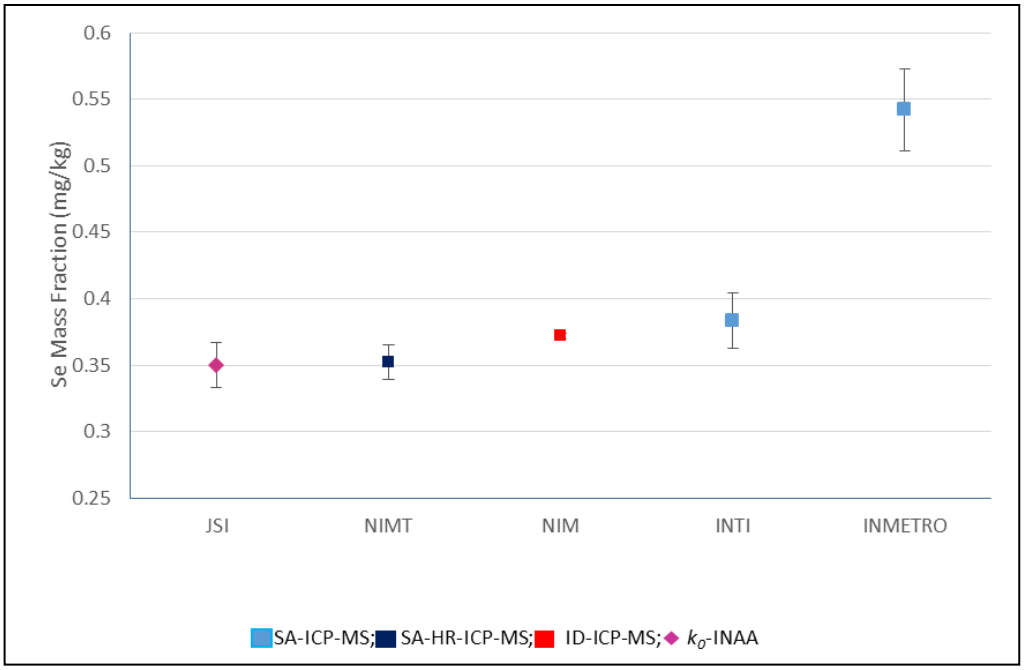
5.1.3. Selenium

Five laboratories reported values for mass fraction of selenium and results are presented in Table 10.

Table 10. Reported Results for mass fraction of Se (dry mass basis) and their associated combined and relative expanded uncertainties, with the coverage factor *k* as reported by the participants **in the order of increasing mass fraction value.**

Participant	**Reported value mg/kg**	**u_c, mg/kg**	***k*** **(95% level confidence)**	**U, mg/kg**	**N[a]**	**Method**
JSI, Slovenia	0.350	0.017	2	0.034	5	k_0-INAA
NIMT, Thailand	0.352	0.0130	2	0.027	4	SA-HR-ICP-MS, Rh as IS
NIM, China	0.372	0.002	2	0.004	6	ID-ICP-MS, reference isotope ^{80}Se, spiked isotope ^{78}Se
INTI, Argentina	0.3832	0.0207	2	0.0413	7	SA-ICP-MS, Ge as IS
INMETRO, Brazil	0.542	0.031	2	0.062	4	SA-ICP-MS, no IS

Figure 3. Selenium mass fraction (dry mass basis) as reported by the participants. Error bars denote the combined uncertainty u_c for a coverage factor of k=1 as reported.



5.1.4. Zinc

Twelve laboratories reported values for mass fraction of zinc. INMC, Colombia reported two zinc results. The first one used standard addition (SA) ICP-MS with Tl & Rh as internal standard (IS) and the second used external calibration (EC) FAAS for the determination of mass fraction of zinc. For both laboratories, only the ICP-MS data was used for the calculation of the SCRV. Results are presented in Table 11.

Table 11. Reported Results for mass fraction of Zn (dry mass basis) and their associated combined and relative expanded uncertainties, with the coverage factor k as reported by the participants **in the order of increasing mass fraction value.**

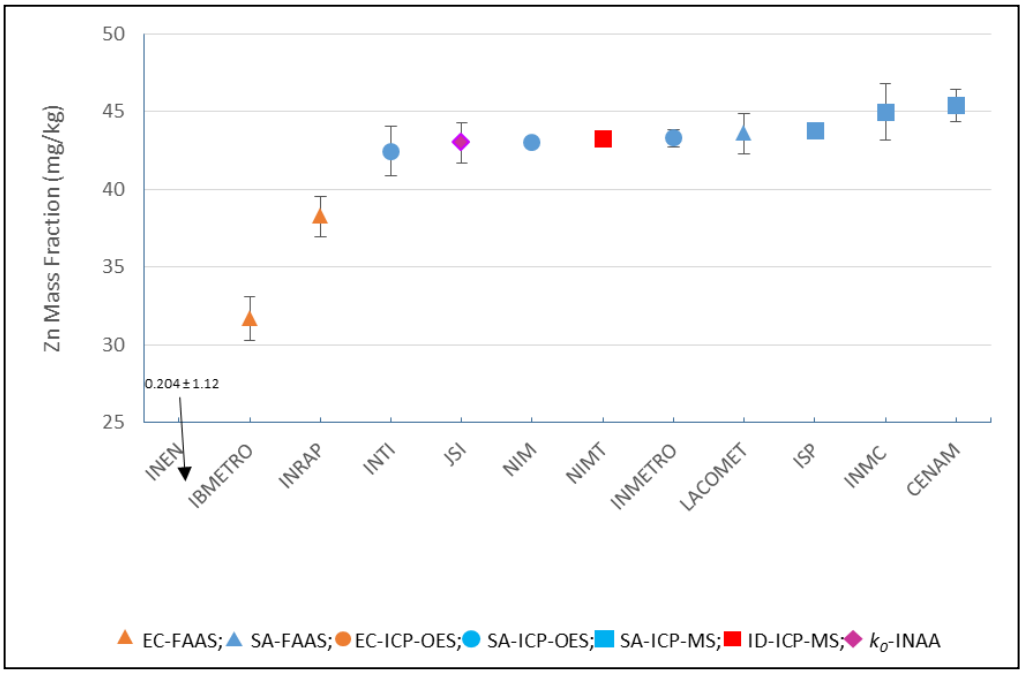
Institute/Country	**Reported value mg/kg**	**u_c, mg/kg**	***k*** **(95% level confidence)**	**U_c, mg/kg**	**N[a]**	**Method**
INEN, Ecuador	0.204	1.12	2	2.24	3	EC-FAAS, no IS
IBMETRO, Bolivia	31.70	1.39	2	2.80	5	EC-FAAS, no IS
INRAP, Tunisia	38.25	1.30	2	2.60	5	EC-HR-ICP-OES, no IS
INTI, Argentina	42.443	1.575	2	3.150	6	SA-ICP-OES; Y as IS
JSI, Slovenia	43.0	1.3	2	2.6	5	k_0-INAA

Institute/Country	Reported value mg/kg	u_c, mg/kg	*k* (95% level confidence)	U_c, mg/kg	N[a]	Method
NIM China	43.03	0.25	2	0.49	14	SA-ICP-OES, no IS
NIMT, Thailand	43.2	0.39	2	0.8	5	ID-ICP-MS (reference isotope ^{66}Zn, spiked isotope ^{67}Zn)
INMETRO, Brazil	43.3	0.56	2	1.1	5	SA-ICP-OES, no IS
LACOMET, Costa Rica	43.6	1.3	2	2.6	5	SA-FAAS, no IS
ISP, Chile	43.8	0.29	2.78	0.80	4	SA-ICP-MS, Sc ad IS
INMC Colombia[b]	45.0 (45.7)	1.8 (2.3)	1.97 (1.97)	3.6 (4.5)	3 3	SA-ICP-MS, Tl & Rh as IS EC-FAAS
CENAM, Mexico	45.40	1.03	2	2.07	5	SA-ICP-MS, Y as IS

[a] N Number of independent replicates

[b] Considered the ICP-MS value. Second value (in parenthesis) was determined with EC-FAAS

Figure 4. Zinc mass fraction (dry mass basis) as reported by the participants. Error bars denote the combined uncertainty u_c for a coverage factor of k=1 as reported.



5.2. Supplementary Comparison Reference Values (SCRVs)

The compile data for SIM.QM-S10 Supplementary Comparison for trace elements in skim milk powder was circulated among the participants on April 2, 2020 for checking any transcription and typographical errors. Participants were requested to review their data and provide comments by May 1, 2020, which was further extended as some participants have been serious affected with the COVID-19 situation in their countries and were not able to provide comments on time.

On May 4, 2020, IBMETRO requested to revise the reported results for zinc from 31.70 mg/kg (original results) to 42.45 mg/kg and for calcium from 3099.74 mg/kg (original results) to 13099.74 mg/kg informing that the correct value with CRM comparison was now included.

In this regard, those results (Ca and Zn) were and considered as outliers (see section below) and were not included in the calculation of SCRV.

On November 9, 2020, INTI informed that they found an error when applying the Grubbs test for their Fe data (7 results). They had wrongly discarded two results for the Fe measurements (i.e, only submitted 5 results). The results were re-checked applying two tests (Grubbs and Dixon) for outliers and had shown that the discarded results were not outliers. The revised data for Fe should be 3.637 ± 0.638 mg/kg instead of 3.987 ± 0.576 mg/kg.

In this regard, since the participant only informed at a latter stage of the comparison, no action was taken but mostly important with the mitigated action, the participant was able to improve their measurement capabilities.

Homogeneity uncertainty component was less than 0.8 % and considered insignificant compared with the spread between the results from all participants, thus were not carried in the future calculations.

5.3. *Screening the data for consistency and outlier rejection*

A preliminary inspection of the reported laboratory results show that few individual reported results are inconsistent with the majority of results. Consistency was checked using the chi-squared test and it was found that all datasets were mutually inconsistent, with chi-squared of 639.3, 145.8, 34.4 and 1536.8 for calcium, iron, selenium and zinc respectively (critical values were 15.5, 12.6, 9.5 and 16.9 respectively). Possible outliers were identified using a t-test and were based on DerSimonian-Laird mean calculation and 99 % confidence level. Calculation of the DerSimonian-Laird mean and associated standard uncertainty was performed according to section 3.4 of the Appendix 2 of the CCQM Guidance note 1. The t-test was applied to compare the di/U(di) (ratio of absolute difference between the individual value and the mean, and its expanded uncertainty) and the critical t value at 99 % of critical-99 % t for the purpose of identifying outliers. An individual value is considered as an outlier when di/U(di) is greater than the t critical-99% at given degree of freedom. All data are included and degree of freedom is calculated using n-1 (n: number of data). One low outlier (3099.74 ±375.89 mg/kg from

IBMETRO) was identified for calcium (see Table 8). Three low outliers (0.204 ± 1.12 mg/kg (INEN), 31.70 ± 1.39 mg/kg (IBMETRO) and 38.25 ± 1.30 mg/kg (INRAP) were identified for Zinc (see Table 11).. Repetitive outlier testing and rejection was used to identify multiple extreme values, but since no more than 20 % of the values in a data set should be rejected according to CCQM guidance, results from INRAP was considered for the calculations of the consensus estimators for zinc. No outliers were identified for iron and selenium.

The possible outliers were further investigated. Regarding digestion protocols, IBMETRO performed microwave digestion with HNO_3 and H_2O_2 and INEN used dry ashing (based on the AOAC official method 985.35) to digest the SIM.QM-S10 sample. The sample mass used by both laboratories are higher than the recommended mass. Both participants used FAAS as the measurement method and external calibration as the calibration strategy. They both used NIST Standard Solutions as calibration standards. IBMETRO did not included any quality control (QC) sample and INEN used NIST SRM 3234 (soy flour) as a QC sample but did not submit any data regarding the agreement between the results and QC samples. It was verified that IBMETRO only have CMCs for water and pH and INEN does not have any CMC. IBMETRO informed us that submitted values did not include the QC agreement. INEN informed that after investigation, they noticed some issues with contamination of the muffle (in common use with another area of the institution) and the quality of water used that may had caused the extreme value obtained.

5.4. *Determination of the Supplementary Comparison Reference Values (SCRV)*

Eight results were used for the calculation of the SCRV for calcium, seven for the calculation of the SCRV for iron, five for the calculation of the SCRV for selenium and ten for the calculation of the SCRV for zinc. All pilot study participants were excluded from the SCRV calculations as well the outliers as previously discussed. According to IAWG, the decision for proposed SCRV calculation should be based on the number of participants, with for more than 8 participants, the median should be used and for 7 or less participants, the arithmetic mean should be used.

Table 12 present consensus estimators based on arithmetic mean, median, as well uncertainty-weighted mean, uncertainty-weighted mean corrected for over-dispersion, and DerSimonian-Laird mean (DLS). These values are proposed in accordance with CCQM/13-22 Guidance note: Estimation of a consensus SCRV and associated Degrees of Equivalence.

Table 12. Consensus estimators for the measurand from SIM.QM-S10.

Consensus estimator	**SCRV**	***u*(SCRV)**	***U_{95}(SCRV)***
Ca, mg/kg[a] (n=8)			
Arithmetic mean	12727	97	194
Median	12673	158	316
Uncertainty-weighted mean	12656	61	121

Consensus estimator	**SCRV**	***u*(SCRV)**	***U_{95}(SCRV)***
Uncertainty-weighted mean (corrected for overdispersion)	12656	70	141
DerSimonian-Laird mean	12688	84	167
Fe, mg/kg (n=7)			
Arithmetic mean	2.66	0.51	1.02
Median	2.77	0.29	0.59
Uncertainty-weighted mean	2.69	0.02	0.05
Uncertainty-weighted mean (corrected for overdispersion)	2.69	0.12	0.24
DerSimonian-Laird mean	2.97	0.17	0.34
Se, mg/kg (n=5)			
Arithmetic mean	0.400	0.036	0.072
Median	0.372	0.017	0.033
Uncertainty-weighted mean	0.372	0.002	0.004
Uncertainty-weighted mean (corrected for overdispersion)	0.372	0.006	0.011
DerSimonian-Laird mean	0.389	0.018	0.035
Zn, mg/kg[a] (n=10)			
Arithmetic mean	43.10	0.61	1.22
Median	43.25	0.26	0.53
Uncertainty-weighted mean	43.30	0.16	0.31
Uncertainty-weighted mean (corrected for overdispersion)	43.30	0.26	0.52
DerSimonian-Laird mean	43.23	0.34	0.68

[a]after outliers removal

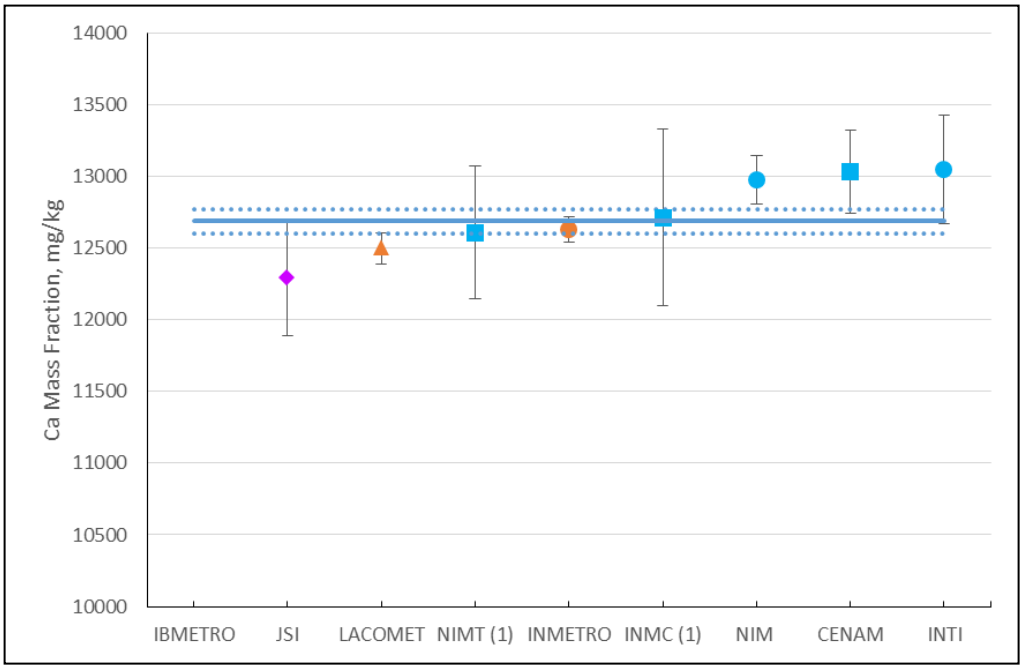
According to the CCQM Guidance note, the DerSimonian-Laird mean estimator is recommended to calculate the SCRV and respective uncertainty when a data set is lacking mutial consistency with no individual anomalous values (which is the case for Ca) or lacking mutial consistency with one or more anomalous values for dataset with 7 results or more (which is the case for Fe and Zn).

Since the DerSimonian-Laird (DSL) mean estimator also takes into account the uncertainties from participants' results and it handles the excess of variance given the suspected influence of random effects observed in the data, it was chosen for the final calculation of SCRV and related uncertainties for all analytes. Participants results are presented relative to the SCRV in Figures 5 to 8.

Table 13. Summary of DSL-mean SCRV and associated uncertainty.

	n	SCRV	*u*(SCRV)	$U_{95}(X)$
Ca, mg/kg	8	12688	84	167
Fe, mg/kg	7	2.97	0.17	0.34
Se, mg/kg	5	0.389	0.018	0.035
Zn, mg/kg	10	43.23	0.34	0.68

Figure 5. Plot of participant's results relative to the DSL-mean SCRV values for calcium. Uncertainties are standard uncertainties.

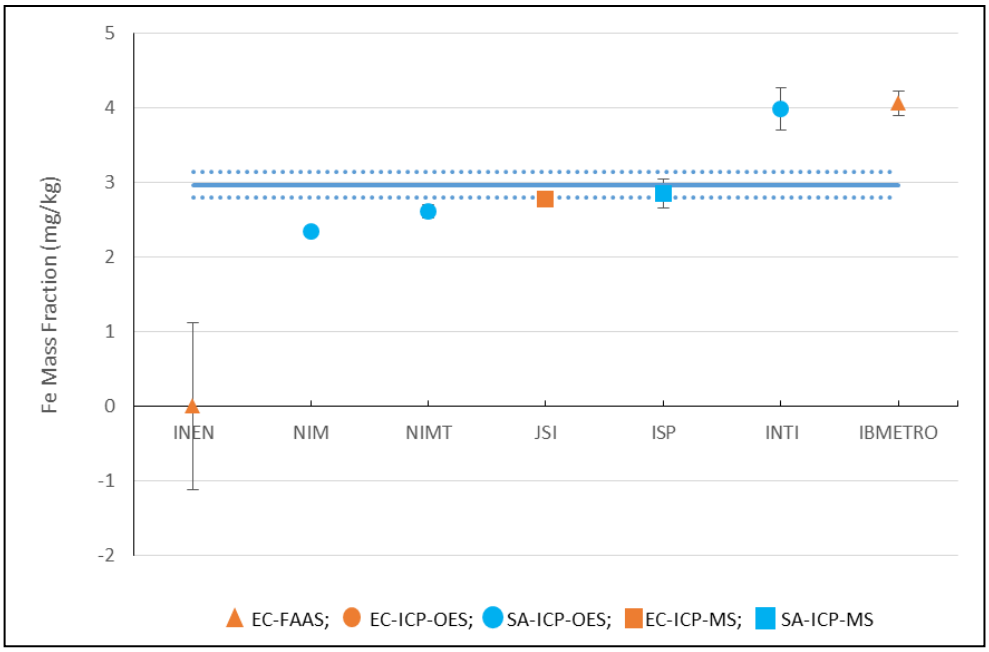


Notes:

(i) Error bars represent reported standard uncertainties. The solid horizontal blue line is the proposed SCRV (as DerSimonian-Laird mean) of the participant's results and the dashed lines show the standard uncertainty, *u*(SCRV).

(ii) The result submitted by IBMETRO were considered as an outlier and was not included in the calculation of SCRV. Please refer to Section 3.1.1

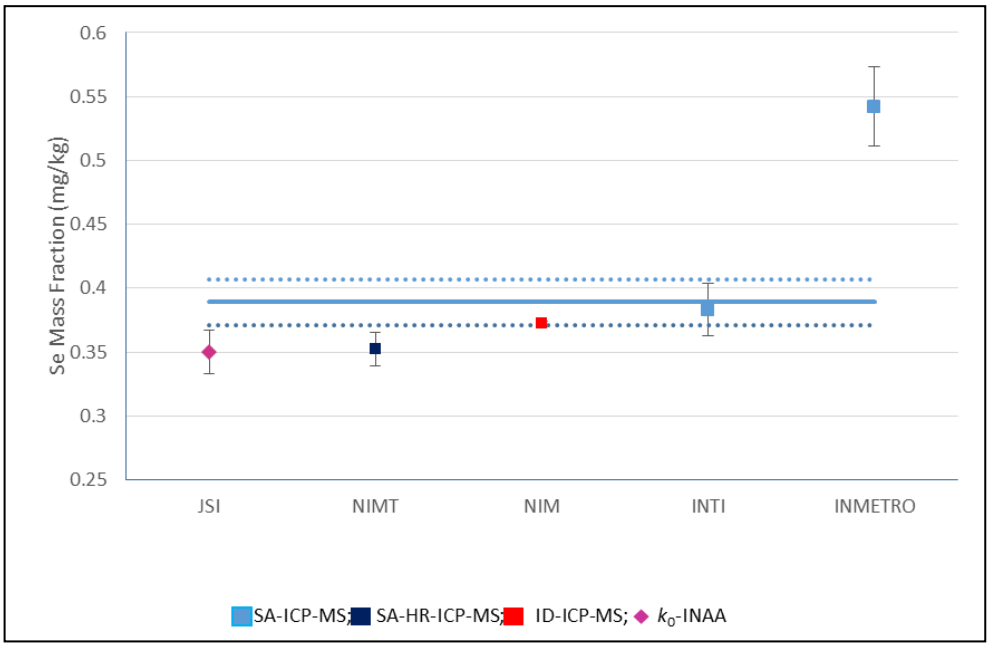
Figure 6. Plot of participant's results relative to the DSL-mean SCRV values for iron. Uncertainties are standard uncertainties.



Notes:

(i) Error bars represent reported standard uncertainties. The solid horizontal blue line is the proposed SCRV (as DerSimonian-Laird mean) of the participant's results and the dashed lines show the standard uncertainty, *u*(SCRV).

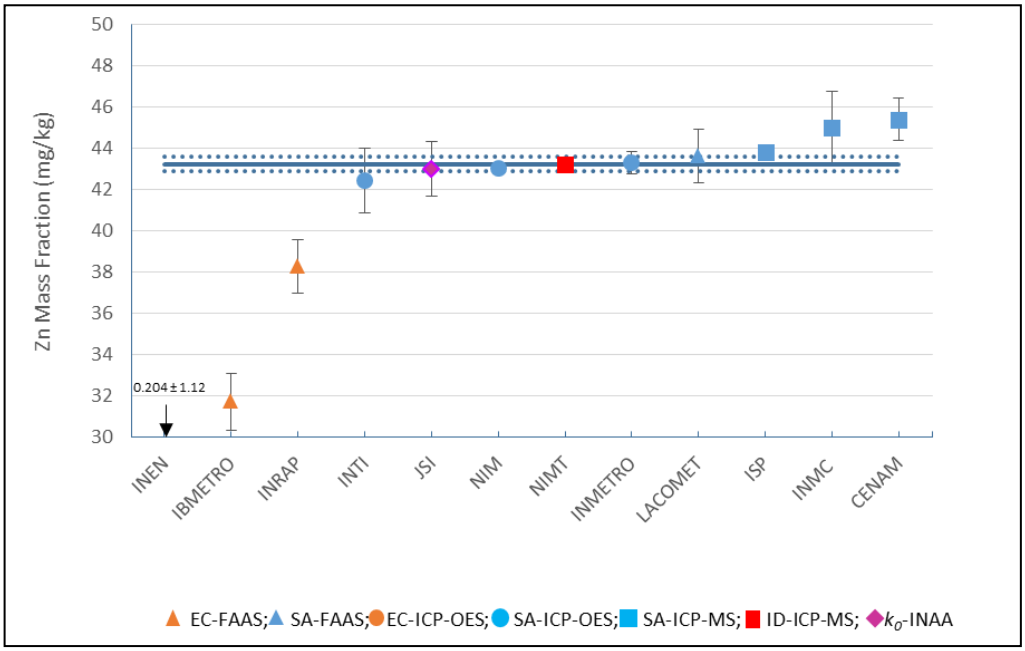
Figure 7. Plot of participant's results relative to the DSL-mean SCRV values for selenium. Uncertainties are standard uncertainties.



Notes:

(i) Error bars represent reported standard uncertainties. The solid horizontal blue line is the proposed SCRV (as DerSimonian-Laird mean) of the participant's results and the dashed lines show the standard uncertainty, *u*(SCRV).

Figure 8. Plot of participant's results relative to the DSL-mean SCRV values for zinc. Uncertainties are standard uncertainties.



Notes:

(i) Error bars represent reported standard uncertainties. The solid horizontal blue line is the proposed SCRV (as DerSimonian-Laird mean) of the participant's results and the dashed lines show the standard uncertainty, *u*(SCRV).

(ii)The result submitted by INEN and IBMETRO were considered as an outlier and was not included in the calculation of SCRV. Please refer to Section 3.1.1.

5.5. Degrees of equivalence and their associated uncertainties

Degrees of equivalence of each national measurement standard were calculated as its deviation from the SCRV values based on DSL mean estimator and the corresponding uncertainty of this deviation (at a 95% level of confidence) according to CCQM guidance note using the equation 1.

$$d_E = x_i - x_{SCRV} \qquad (1)$$

where X_{SCRV} is the calculated SCRV and x_i is the participant's result.

And corresponding uncertainty of the degree of equivalence (u (d_i) was calculated using equation 2 (when the value x_i was included in the calculation) or 3 (When the value x_i was not included in the calculation)

$$u^2(d_i) = u_i^2 + \lambda - u_{SCRV}^2 \quad (2)$$

$$u^2(d_i) = u_i^2 + \lambda + u_{SCRV}^2 \quad (3)$$

Where λ is the excess variance due to differences between submitted results from participating laboratories and its contribution was included in the uncertainty of degrees of equivalence.

Those values are listed in tables 14 to 17 and presented in Figures 9 to 12.

Table 14. Degrees of equivalence and their uncertainties (95% CI) for calcium in SIM.QM-S10.

Participant	d_E	$U(d_E)$	dE/U(dE)
IBMETRO*	-9588.5	803.00	-11.9
JSI	-393.2	829.94	-0.47
LACOMET	-190.2	265.47	-0.72
NIMT	-78.2	942.26	-0.08
INMETRO	-57.2	236.80	-0.24
INM	26.8	1234.93	0.02
NIM China	290.8	370.40	0.79
CENAM	344.8	603.33	0.57
INTI	364.8	772.99	0.47

*reported value not included in the calculation of SCRV

Figure 9. Degrees of equivalence estimates for calcium in SIM.QM-S10

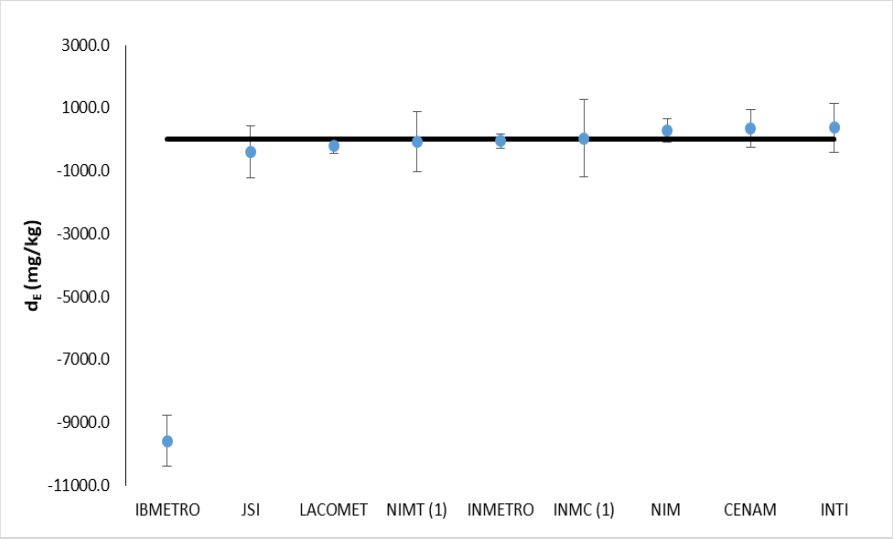


Table 15. Degrees of equivalence and their uncertainties (95% CI) for iron in SIM.QM-S10.

Participant	d_E	$U(d_E)$	dE/U(dE)
INEN	-3.0	2.35	-1.26
NIM	-0.6	0.72	-0.86
NIMT	-0.4	0.73	-0.49
JSI	-0.2	0.71	-0.28
ISP	-0.1	0.82	-0.15
INTI	1.0	0.92	1.11
IBMETRO	1.1	0.79	1.38

Figure 10. Degrees of equivalence estimates for iron in SIM.QM-S10.

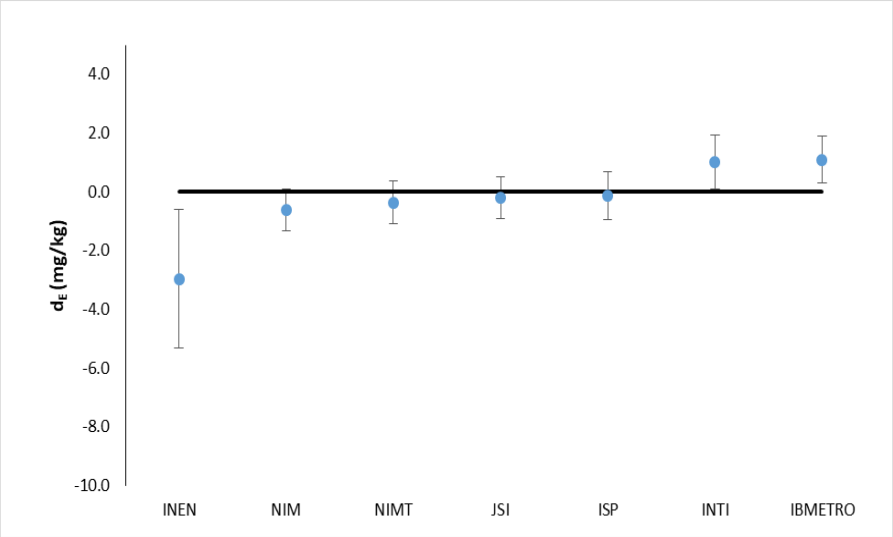


Table 16. Degrees of equivalence and their uncertainties (95% CI) for selenium in SIM.QM-S10.

Participant	d_E	$U(d_E)$	dE/U(dE)
JSI	-0.04	0.07	-0.56
NIMT	-0.04	0.07	-0.56
NIM	-0.02	0.06	-0.28
INTI	-0.01	0.07	-0.08
INMETRO	0.15	0.09	1.76

Figure 11. Degrees of equivalence estimates for selenium in SIM.QM-S10.

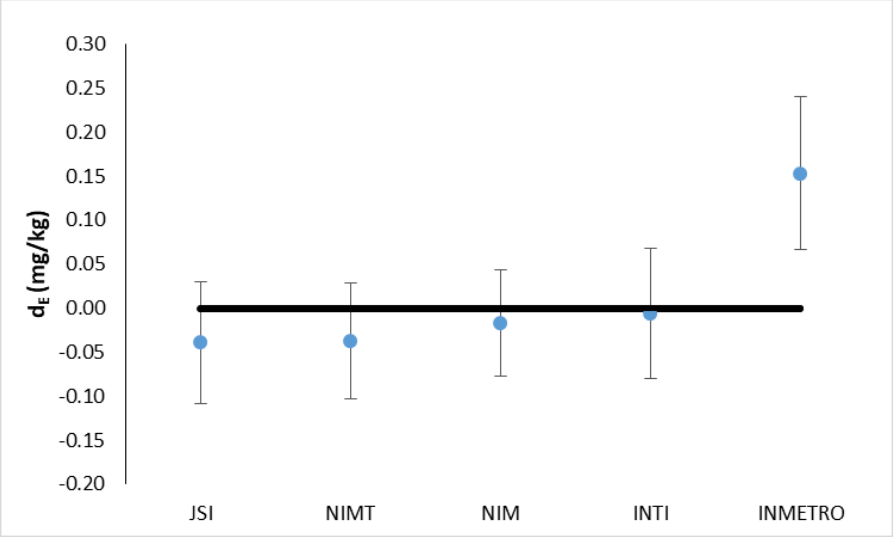
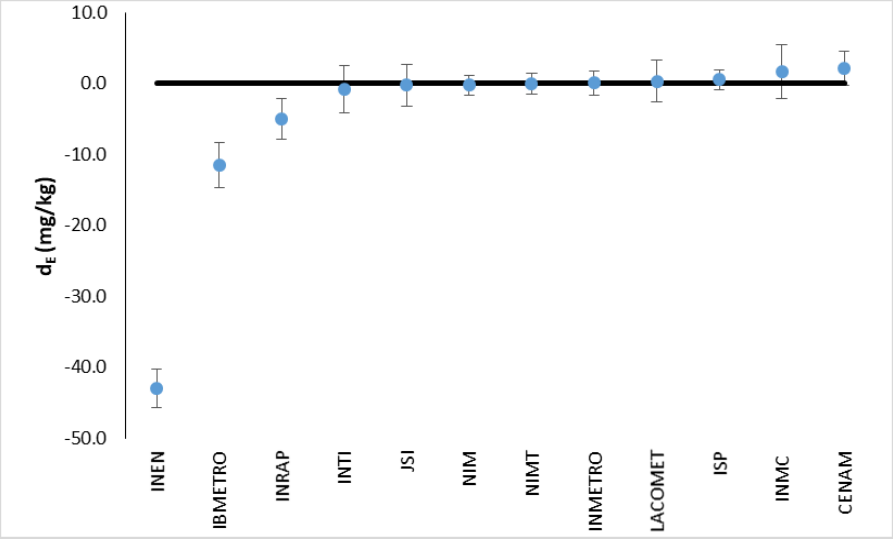


Table 17. Degrees of equivalence and their uncertainties (95% CI) for zinc in SIM. QM-S10.

Participant	d_E	$U(d_E)$	dE/U(dE)
INEN*	-43.0	2.75	-15.63
IBMETRO*	-11.5	3.21	-3.60
INRAP	-5.0	2.90	-1.72
INTI	-0.8	3.40	-0.23
JSI	-0.2	2.90	-0.08
NIM	-0.2	1.37	-0.15
NIMT	0.0	1.50	-0.02
INMETRO	0.1	1.70	0.04
LACOMET	0.4	2.90	0.13
ISP	0.6	1.40	0.40
INMC	1.7	3.82	0.46
CENAM	2.2	2.42	0.89

*reported value not included in the calculation of SCRV

Figure 12. Degrees of equivalence estimates for zinc in SIM.QM-S10.



5.6. Demonstrated Core capabilities – How far the light shines

Successful participation in SIM.QM-S10 demonstrates the following measurement capabilities in determining mass fraction of Ca, Fe, Se and Zn in a complex food matrix.

Calibration and Measurement Capabilities (CMCs) claim based on total elements may include other elements with similar core competencies in a wide range of food matrices at similar level of performance using the same measurement technique applied in this comparison.

6. Conclusion

Most participants used microwave digestion methods for sample preparation and ICP-MS or ICP-OES for determination of the proposed analytes in SIM.QM-S10. Participants used calibration materials, mostly from NIST, for traceability purposes.

Data was screnned for consistency using the chi-squared test and possible anomalous values were identified by t-test. Results for Ca and Zn from IBMETRO and for Zn from INEN were considered as outliers. Several approaches for the calculation of the consensus estimators (arithmetic mean, median, uncertainty weighed mean and DSL) and since all datasets were considered mutually inconsistent, the DLS values were proposed for the calculation of SCRV for Ca, Fe, Se and Zn in SIM.QM-S10.

In general, the majority of results from NMIs/DIs are in agreement with the SCRV with their expanded uncertainties, making the SIM.QM-S10 a successful supplementary comparison. Participants showed the measurement capabilities for Ca, Fe, Se and Zn in a complex food matrix.

7. Acknowledgements

The study coordinators thank the participating laboratories for providing the requested information used in this study. We also thank Dr. Mike Winchester for his hepful comments and suggestions.

8. Reference

CCQM Guidance note: Estimation of a consensus KCRV and associated degrees of Equivalence(2013) version 10 available from

https://www.bipm.org/cc/CCQM/Restricted/19/CCQM13-22_Consensus_KCRV_v10.pdf

Appendix A - Technical protocol

SIM.QM-S10 Supplementary Comparison for Trace elements in skim milk powder Technical Protocol

1. Background

The comparison is piloted by NRC Canada and INTI Argentina.

Skim milk powder is widely used as a food ingredient and has the same nutrition of fresh nonfat milk but with a longer shelf life. The determination of trace elements in skim milk powder is an important and commonly performed measurement responsibility.

An earlier Key comparison in this area was conducted under the auspices of the CIPM as CCQM-K125, with the parallel pilot study CCQM-P159 (Iodine and other elements in infant formula) in 2014. Since a few SIM members did not participate in this comparison, the purpose of SIM.QM-S10 is to ensure the comparable and traceable measurement results for trace elements such as Ca, Fe, Se, and Zn in skim milk powder and similar matrices. This comparison will provide NMIs with the needed evidence for CMC claims for trace elements in skim milk powder and similar matrices. Note that those laboratories wishing to utilize this exercise for support of CMC claims must register for this comparison. Although this is organized as a SIM regional comparison, it is open to other participants of the MRA throughout all RMOs. Results for the comparison are going to be registered on the BIPM Key and Supplemental Comparisons Database, the KCDB.

The SCRV for each element will be assigned based on NRC results, which are obtained by using both ID-ICP-MS (primary method) and standard addition ICP-MS for Fe, Se, and Zn, and standard additions calibration for Ca and Fe by ICPOES.

2. Material

The source of material was food-grade skim milk powder. The material was blended and packed into trilaminate stick-packs at a pharmaceutical manufacturing company.

Reference values are determined by primary measurement method (ID-ICPMS) and standard addition ICP-MS (SA-ICP-MS) for Fe, Se and Zn. Ca and Fe were determined by standard additions ICPOES. Bottle-to-bottle homogeneity was evaluated and determined to be fit for purpose.

Samples will be made available in stick-pack containing approximately 2.5 g of material.

3. Measurands

Element	Target Concentration
Ca	(0-20 000) mg/kg
Fe	(0-10) mg/kg
Se	(0-10) mg/kg
Zn	(0-100) mg/kg

4. Choice of Method / Procedure

Participants may use any method of their choice.

5. Test Sample Receipt / Handling

Samples will be distributed by courier to the participants. Each laboratory will receive five trilaminate stick packs.

Please inform the coordinator immediately if the test sample has been compromised in any way and arrives in questionable condition.

6. Reporting

A reporting form will be provided to participants after test materials are distributed. Results for each measurand should be reported in minimum triplicate as the element content mass fraction (mass/mass, mg/kg) on test aliquots drawn from the stick packs. All results shall be reported in a dry mass basis. Please state all the individual results, not only the final mean value. All analytical calibrations should be performed using metrologically traceable standards. Sources, purity and traceability of reference materials used for calibration purpose shall be provided.

Any participant that chooses to use multiple methods can decide only one composite result; e.g., an average value from different methods. If the participant decides to report the individual results from different methods as the reporting value(s) for each measurand, reported values using the method with the lowest uncertainty will be used, the others will be considered as information values.

Each laboratory shall provide a complete description of the method(s) used, including calibration technique(s) along with their metrological traceability and uncertainty assessment in accordance with JCGM 100:2008 Evaluation of Measurement Data-Guide to the Expression of Uncertainty in Measurement as well any specific challenges encountered.

6. Time Schedule

Registration deadline: October 4, 2019

Ship materials: October 11, 2019

Deadline for receipt of data: January 10 2020

Prepare/distribute draft A report: March 31, 2020

Discussion of the results and draft A report at CCQM IAWG: April, 2020

Finalize report: June 26, 2020

7. Coordinating laboratories

SIM.QM-S10 Supplementary Comparison for Trace elements in skim milk powder is coordinated by NRC Canada and INTI Argentina.

Patricia Grinberg NRC Canada

Registration Form

SIM.QM-S10 Supplementary Comparison for Trace elements in skim milk powder

Although this is a SIM comparison, the invitation to participate is extended to National Metrology Institutes (NMIs) and Designated Institutes (DIs) in all RMOs.

Indicate the element(s) for which you will be submitting results by inserting an **X** under the heading of the appropriate comparison.

Measurand	**SIM.QM-S10 Supplementary Comparison**
Ca	
Fe	
Se	
Zn	

Participant's Name	
Describe if it is a NMI or Designated Institute	
Name of the Institute	
Address	
Country	
E-Mail of contact	
Tel.-Number	
Fax-Number	

Shipping instructions:

Please indicate any special instructions (for importation) and the full shipping address and telephone number of a contact.

Please send the completed form by e-mail before **October 4, 2019** to:

Patricia Grinberg

patricia.grinberg@nrc-cnrc.gc.ca

If you do not receive an acknowledgement of your registration from us within 5 working days, please send us an email.

1. Coordinating laboratories

National Research Council of Canada, Metrology

Ottawa, Ontario, K1A 0R9, Canada

Tel. 613 991 5482

Fax. 613 993 2451

E-mail: patricia.grinberg@nrc-cnrc.gc.ca

Appendix B – Reporting form

The following form was available to all participants

Participating details SIM.QM.S10	
Supplementary Comparison for Trace Elements in Skim Milk Powder	
Data Submission Form	
Please complete all pages of the reporting form and submit it by email before January 10 2020 to: patricia.grinberg@nrc-cnrc.gc.ca	
Participating Laboratory	
Institute/ Laboratory:	
NMI/DI:	
Reporting date:	
Postal address:	
Contact person:	
E-mail:	

Report of Results
SIM.QM.S10

Results

Results should be reported as at least three replicates as the element content mass fraction (mass/mass, mg/kg) and reported on a dry mass basis.

Summary of Results sample SM.QM.S10 (dry mass basis)

	Ca		Fe		Se		Zn	
Mean value (mg/kg)								
Combined standard uncertainty (mg/kg)								
Coverage factor k (95% level of confidence)								
Expanded uncertainty (mg/kg)								

Individual Results sample SM.QM.S10 (Results should be reported as at least three replicates)

		Ca		Fe		Se		Zn	
	Identification stick pack	Mass Fraction (mg/kg)	Uncertainty	Mass Fraction (mg/kg)	Uncertainty	Mass Fraction (mg/kg)	Uncertainty	Mass Fraction (mg/kg)	Uncertainty
replicate # 1									
replicate # 2									
replicate # 3									
replicate # 4									
replicate # 5									

Summary of Results for Reference materials used

Reference Material used:

	Ca		Fe		Se		Zn	
	Mass Fraction (mg/kg)	Uncertainty	Mass Fraction (mg/kg)	Uncertainty	Mass Fraction (mg/kg)	Uncertainty	Mass Fraction (mg/kg)	Uncertainty
replicate # 1								
replicate # 2								
replicate # 3								
replicate # 4								
replicate # 5								
mean value								
standard deviation								
certified value, U								

Analytical Information
SIM.QM.S10

Description of the methodology used		
Instrumentation used		
Calibration method/design used		
For ID-ICP-MS, please indicate reference and spiked isotopes used		
Traceability (i.e., source, purity of calibration standards)		
Internal standards used (if applicable)		
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)		
Reference material used		
Sample amount used for analysis		
Sample amount used for dry mass correction		
Number of samples aliquots taken for dry mass correction.		
Correction for dry mass (% of weighted sample)		
Uncertainty for dry mass correction		
Additional Comments or Observations		

Appendix B – Summary of Participants' Analytical Information

Institute: INTI Argentina

Analytes	Ca, Fe, Se, Zn
QC sample	NIST 1849a
Description of the methodology used	Acid digestion by microwave 5ml HNO3 + 0,5ml HF Ca, Fe and Zn by ICP-OES Se by ICP-MS
Instrumentation used	SAMPLE DIGESTION BY ULTRAWAVE (MILESTONE) ICP-OES 7300 DV (PERKIN ELMER) ICP-MS ELAN DRC II (PERKIN ELMER)
Calibration method/design used	standard addition
For ID-ICP-MS, please indicate reference and spiked isotopes used	NA
Traceability (i.e., source, purity of calibration standards)	NIST STANDARD REFENCE MATERIAL 3109a CALCIUM NIST STANDARD REFENCE MATERIAL 3168a ZINC NIST STANDARD REFENCE MATERIAL 3126 IRON NIST STANDARD REFENCE MATERIAL 3149 SELENIUM
Internal standards used (if applicable)	
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	EXAMPLE FOR CALCIUM

Incertidumbre total					
Elemento	**Nist 1849a**		**S10**		
Promedio de replicados	5385,15	**5385,15**	13052,85	**13053**	
Desviacion estandar de los replicados	48,36	0,01	110,17	0,01	
Incertidumbre Curva	2,0174	0,0202	2,7159	0,0272	
u Humedad	0,0032	0,0032		0,0032	
Incertidumbre del MRC	51,0000	0,0049		0,0049	
Valor del MRC	5253,0000				
	combined	122,9736	combined	378,9019	c
	expanded	245,9473	expanded	757,8037	e
	expanded %	**4,57%**	expanded %	**5,81%**	ex
di = Xi - Xcertificado		132,15		13052,85	
Ui = 2* [Raiz(u2 (xi) + u2(Xcertif) - 2*cov(xi,xcertif)]		251,18		#¡VALOR!	
Grado de equivalencia = di / Ui		0,53		#¡VALOR!	
Recuperacion %		102,5		#¡DIV/0!	

Reference material used	NIST STANDARD REFENCE MATERIAL 1849a INFANT/ADULT NUTRITIONAL FORMULA I
Sample amount used for analysis	0,5 g OF SAMPLE
Sample amount used for dry mass correction	0,5 g OF SAMPLE
Number of samples aliquots taken for dry mass correction.	3
Correction for dry mass (% of weighted sample)	97,4%
Uncertainty for dry mass correction	0,32%
Additional Comments or Observations	

Institute: IBMETRO Bolivia

Analytes	Ca, Fe, Zn
QC sample	no QC sample
Description of the methodology used	The samples were dried at 102 ° C for 2hr to remove moisture and perform measurements on a dry basis.To determine the concentration of analytes, an approximate mass of 0,5 g of sample was weighed in an Anton Paar (Multiwave Pro) microwave oven for 30 min assisted by 8 ml of HNO3 and 2 ml of analytical grade H2O2. The digestate masses obtained were brought to a volume of 100 ml for measurement. The measurements were made by AAS, using lamps HCL and EDL Perkin Elemer brand. For the measurement of Fe a calibration curve of 0 to 1 mg / L was used. For the determination of Ca a calibration curve of 0 to 6 mg / L was used and for the measurement of Zn a calibration curve of 0 to 0,4 mg / L was used. The wavelengths and parameters of each analyte were determined based on supplier considerations. The traceability of the measurements were evaluated by MRC of INTI REDELAC milk PEA CPLLP milk powder, measured at the same time and conditions of the target samples. Sample masses and CRM were statistically treated to correct variations by environmental and air thrust factors. The values obtained in the measurements were statistically treated to calculate the uncertainties and amount of substance of the target analyte in each replica

	group. Replicas were performed for 3
Instrumentation used	AAS Perkin Elmer model PinAACle 900T, Microwave Anton Paar model Mutiwave Pro, Balance Mettler Toledo Model XS204, Hydrothermobarometer Extech, Stove Memmert, and mass set for calibration of the balance. For the Calibration curve used a CRM at NIST (Zn lot 120629, code 3168a, Ca lot 130213, code 3109a and Fe lot 140812 code 3126a) Finaly used a water desionizer MerckMilipore Ultrapure water equipment CE < 1,2 uS/cm
Calibration method/design used	Calibration curves were performed by gravimetric preparation from the CRM afore mentioned CRM The readings of the concentrations, as well as the determination of the optimal conditions of measurement was carried out by studying factors in a 2k experimental design using as responses of the process to the recovery and the characteristic concentration
For ID-ICP-MS, please indicate reference and spiked isotopes used	NA
Traceability (i.e., source, purity of calibration standards)	For the Calibration curve used a CRM at NIST (Zn lot 120629, code 3168a, Ca lot 130213, code 3109a and Fe lot 140812 code 3126a)
Internal standards used (if applicable)	Ge FOR SELENIUM Y FOR Ca, Zn and Fe
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	
Reference material used	For the Calibration curve used a CRM at NIST (Zn lot 120629, code 3168a, Ca lot 130213, code 3109a and Fe lot 140812 code 3126a)
Sample amount used for analysis	0,5 g for replicate (1,5 g for stick)

Sample amount used for dry mass correction	0,5 g for replicate (1,5 g for stick)
Number of samples aliquots taken for dry mass correction.	
Correction for dry mass (% of weighted sample)	36) 2,6271% 37)2,6379% 38) 2,6284% 39) 2,6986% 40) 2,6889%
Uncertainty for dry mass correction	36) 0,0480 % 37) 0,0850% 38)0,0450% 39) 0,0820% 40) 0,1210%
Additional Comments or Observations	

Institute: IMMETRO Brazil

Analytes	Ca, Zn, Se
QC sample	NIST 1849a
Description of the methodology used	A pool of three packs were homogeneized and after, five sub samples were weighed (0.5 g) and transferred to a teflon tube. Four mL of subboiling nitric acid and two mL of high purity hidrogen peroxide 30 % were added to the tubes. The samples were digested in a micro wave reaction sistem according the following program (300 W - ramp 8 minutes / 0W hold for 10 minutes/ 500 W - ramp 15 minutes / 0 W for 10 minutes / 1300 W - ramp 15 minutes / 1300 W for 15 minutes). The SRM 1849a was used as quality control and a reagent blank was running. The dry mass correction factor was calculated from three sub samples of 1 g that were dryed at 80 ºC for 60 h at -760 mmHg. Ca mass fraction was determined by external calibration and Zn and Se mass fraction were determined by standard addition method.
Instrumentation used	Ca/Zn - ICP OES Ultima 2 - Jobin Yvon Se - ICP-MS ELA DRC II - Perkin Elmer Sample Digestion - Micro wave reaction Sistem - Multiwave Pro - Anton Paar

Calibration method/design used	Ca - External Calibration Zn - Standard addition Se - Standard addition
For ID-ICP-MS, please indicate reference and spiked isotopes used	NA
Traceability (i.e., source, purity of calibration standards)	Ca - SRM 3109, Zn SRM 3168a , Se SRM 3149 from NIST
Internal standards used (if applicable)	Not applicable
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	External Calibration: w = w0 x df x frep x fdrymass, where w0 is the calcium mass fraction in the diluted solution, df is the dilution factor of the sample, frep is the factor of the instrumental repeatability and fdrymass is the dry mass correction factor. The main source of uncertainty are: calibration curve, dilution factor, repeatability, and dry mass factor. A typical contribution from these sources of uncertainty is: calibration curve (0.22 %) sample dilution (0.25 %), repeatability (0.55 %) and dry mass factor (0.16 %). Combined standard uncertainty is the square-root fo the linear sum of squared relative uncertainty components. The combined standard uncertainty ranged from 0.61 to 0.89 % relative to the calcium mass fraction in the sample. Gravimetric standard addition: "Uncertainty of standard addition experiments: a novel approach to include the uncertainty associated with the standard in the model equation" Anna-Lisa Hauswaldt• Olaf Rienitz• Reinhard Jahrling•Nicolas Fischer• Detlef Schiel• Guillaume Labarraque• Bertil Magnusson, Accred Qual Assur (2012) 17:129–138. DOI 10.1007/s00769-011-0827-5 w = 1/wdry * fexp * wx * dmx * dmz * dmi, where w is the Mass fraction of the analyte Zn or Se in the sample, wdry is the Dry mass correction—result of repeated measurements, fexp is Sampling, sample preparation and inhomogeneity, wx is the Result of the standard addition model equation, dmx is the Uncertainty contribution from the sample mass, dmz is Uncertainty contribution from the mass of standard added and dmi is the Uncertainty contribution from the mass of solutions measured.
Reference material used	SRM 1849a - Infant/Adult Nutritional Formula I (milk-based) was used as quality control. Normalized error was used to check the consistency between the measured and certified values.
Sample amount used for analysis	0.5 g

Sample amount used for dry mass correction	Approximately 1,0 g
Number of samples aliquots taken for dry mass correction.	Three sub samples
Correction for dry mass (% of weighted sample)	The dry mass factor correction is 0,9643 and the combined standard uncertainty is 0,0016. The uncertainty is a combination from the repeatability and the uncertainty from the sample mass.
Uncertainty for dry mass correction	The dry mass factor correction is 0,9643 and the combined standard uncertainty is 0,0016. The uncertainty is a combination from the repeatability and the uncertainty from the sample mass.
Additional Comments or Observations	

Institute:ISP Chile

Analytes	Fe, Zn
QC sample	NIST 1849a
Description of the methodology used	Digestion of food with nitric acid & hydrogen-peroxide in MW digestor. Quantification for ICP-MS with internal standard addition.
Instrumentation used	ICP MS Agilent Model 7700 - MW digestor Anton Para Model Multiwave PRO- Analytical Balance Sartorius Model LA320S
Calibration method/design used	Internal Standard Addition, preparation for gravimetric method.
For ID-ICP-MS, please indicate reference and spiked isotopes used	NA
Traceability (i.e., source, purity of calibration	NIST SRM® Fe 3126a ,NIST SRM® Fe 3168a , Metrology Designated Institute for mass of Chile CESMEC.

standards)	
Internal standards used (if applicable)	Scandium NIST 3148a
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	uncertainty contributions considered: Signal Ration Metal/Internal Standard Fraction of mass Calibration of metal mass sample dilution factor moisture, meaurement Precision $\omega_{smp} = \frac{R * W_{cal} * m_{cal-FB} * m_{sln}}{m_{sln-FB} * m_{smp}} * FD$
Reference material used	NIST CRM 1849a
Sample amount used for analysis	0,50 g +/- 0,05 g
Sample amount used for dry mass correction	1,00 g +/- 0,05 g
Number of samples aliquots taken for dry mass correction.	4 samples
Correction for dry mass (% of weighted sample)	Zn= 3,39 % & Fe = 3,39%
Uncertainty for dry mass correction	Colocar la inncertidumbre u , k=1
Additional Comments or Observations	Participants in the test analysis Claudia Núñez and Javier Vera. The report of Uncertaninty for Zn & Fe is 95%IC k=2,78. Review and statistical calculations Soraya Sandoval, Claudia Núñez and Javier Vera

Institute:NIM China

Analytes	Ca, Fe, Se, Zn
QC sample	ERM BD-150 (for Ca, Fe and Zn) & GBW10115 (for Se)
Description of the methodology used	Microwave digestion for sample preparation， 5mL HNO3 as digestion solvent ICP-OES, Std-Addtion Method for Fe, Zn and Ca Determination ICP-MS, IDMS Method for Se Determination
Instrumentation used	CEM Mars 5 Microwave Digestion System Thermofisher iCap 7400 ICP-OES Agilent 8800 ICP-MS
Calibration method/design used	Std-Addtion Method for Fe, Zn, Ca IDMS Method for Se
For ID-ICP-MS, please indicate reference and spiked isotopes used	80Se as reference isotope, 78Se as spiked isotope
Traceability (i.e., source, purity of calibration standards)	Ca, GBW(E)080118, 1000±5mg/L (992.8±5.0mg/g) Fe, GBW08616, 1000±2mg/L (990.5±2.0mg/g) Zn, GBW08620, 1000±1mg/L (996.6±1.0mg/g) Se, NIST SRM 3149
Internal standards used (if applicable)	None

Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	**Measurement equation and uncertainty budget** **Se** $C_y = \frac{R_x - R_b'}{R_b' - R_y} \cdot \frac{\sum_{i=1}^{n} R_{yi} M_i}{\sum_{i=1}^{n} R_{xi} M_i} \cdot \frac{m_z}{m_y} \cdot C_z$ $\quad C_x = \frac{R_y - R_b}{R_b - R_x} \cdot \frac{\sum_{i=1}^{n} R_{xi} M_i}{\sum_{i=1}^{n} R_{yi} M_i} \cdot \frac{m_y}{m_x} \cdot C$ **Ca, Fe & Zn** $C_x = \frac{m_{sol'}}{w \cdot m_x} \cdot \left(\frac{m_{sol'} + m_{std-add}}{m_{sol'}} \right) \cdot \left(\frac{y_x - b}{a} \right) - B$ **Ca** **Fe** **Zn**

Measurement equation and uncertainty budget

Parameter	Description	Value	Standard uncertainty	Unit	Type A/B
c_y	concentration of ^{77}Se spike	0.4683	0.0008	mg/kg	
R_x	measured isotope amount ratio in the milk powder sample $R_{78,80}$	0.4653	0.0010		
R_b	measured isotope amount ratio of blend b $R_{78,80}$	1.6132	0.0024		
R_b'	measured isotope amount ratio of blend b' $R_{78,80}$	1.7558	0.0032		
R_y	measured isotope amount ratio in the primary assay standard $R_{78,80}$	0.4578	0.0013		
R_z	measured isotope amount ratio in the spike $R_{78,80}$	2601	0.02		
c_z	amount content of the primary assay standard	10110	10	mg/kg	
m_x	mass fraction of sample in blend b	0.48233	0.00005	g	
m_y	mass fraction of spike in blend b	0.25020	0.00005	g	
$m_{y'}$	mass fraction of spike in blend b'	0.25248	0.00005	g	
m_z	mass fraction of primary assay standard in blend b'	0.50568	0.00005	g	
c_x	measured result of Se in milk powder sample	0.3724	0.0011	mg/kg	
w_x	dry mass of the weighted sample	98.00	0.05	%	
C_x	Mass fraction of Se	0.372	mg/kg		
u_c	Combined standard uncertainty	0.002	mg/kg		
k	Coverage factor	2			
U	Expanded uncertainty	0.004	mg/kg		

Ca

Parameter	Source of uncertainty	Typical value	Standard uncertainty	Unit	Ty
C_{Std}	concentration of CRM Solution	1000	2.5	mg/L	
w	dry mass correction	98.00%	0.05%		
m_x	mass of sample	0.48993	0.00005	g	
m_{sol}	mass of digestion solution	54.3198	0.00010	g	
$m_{std-add}$	mass of standard addition solution	4.10194	0.00010	g	
$m_{sol'}$	mass of measured digestion solution	1.02497	0.00010	g	
c_x	measured result of Ca in sample	12979	81	mg/kg	
$(y_x - b)/a$	concentration of Ca from sample in measured std-added solution	22.5	0.3	mg/kg	
B	procedure blank subtraction	0.4	0.1	mg/kg	
C_x	Mass fraction of Ca	12979	mg/kg		
u_c	Combined standard uncertainty	169	mg/kg		
k	Coverage factor	2			
U	Expanded uncertainty	339	mg/kg		

Fe

Parameter	Source of uncertainty	Typical value	Standard uncertainty	Unit	Type
C_{Std}	concentration of CRM Solution	1000	1	mg/L	B
w	dry mass correction	98.00%	0.05%		A
m_x	mass of sample	0.48993	0.00005	g	B
m_{sol}	mass of digestion solution	54.3198	0.00010	g	B
$m_{std-add}$	mass of standard addition solution	2.55745	0.00010	g	B
$m_{sol'}$	mass of measured digestion solution	5.11855	0.00010	g	B
c_x	measured result of Fe in sample	2.35	0.03	mg/kg	A
$(y_x - b)/a$	concentration of Fe from sample in measured std-added solution	13.54	0.18	mg/g	A
B	procedure blank subtraction	0.04	0.02	mg/kg	B
C_x	Mass fraction of Fe	2.35	mg/kg		
u_c	Combined standard uncertainty	0.05	mg/kg		
k	Coverage factor	2			
U	Expanded uncertainty	0.10	mg/kg		

Zn

Parameter	Source of uncertainty	Typical value	Standard uncertainty	Unit	Type
C_{Std}	concentration of CRM Solution	1000	0.5	mg/L	B
w	dry mass correction	98.00%	0.05%		A
m_x	mass of sample	0.48993	0.00005	g	B
m_{sol}	mass of digestion solution	54.3198	0.00010	g	B
$m_{std-add}$	mass of standard addition solution	2.55745	0.00010	g	B
$m_{sol'}$	mass of measured digestion solution	5.11855	0.00010	g	B
c_x	measured result of Zn in sample	43.03	0.11	mg/kg	A
$(y_x - b)/a$	concentration of Zn from sample in measured std-added solution	0.2487	0.0013	mg/kg	A
B	procedure blank subtraction	0.00007	0.00001	mg/kg	B
C_x	Mass fraction of Zn	43.03	mg/kg		
u_c	Combined standard uncertainty	0.25	mg/kg		
k	Coverage factor	2			
U	Expanded uncertainty	0.49	mg/kg		

Reference material used	ERM BD-150 (for Ca, Fe and Zn) & GBW10115 (for Se)

Sample amount used for analysis	0.45~0.55g
Sample amount used for dry mass correction	0.4~0.7g
Number of samples aliquots taken for dry mass correction.	4
Correction for dry mass (% of weighted sample)	98.01%, 97.97%, 98.07%, 97.95%
Uncertainty for dry mass correction	0.00053
Additional Comments or Observations	

Institute: INMC Colombia

Analytes	Ca, Zn Fe not reported
QC sample	
Description of the methodology used	Microwave assisted digestion was used. Samples were weight in a PFA digestion vessels, 4 mL of bisub-distilled nitric acid (69%) and 2 mL of hidrogen peroxide (30%) were added. The digestion was carried out to 900 W during 25 minutes with a predigestion step of 12 hours. After that, the extract were left to cool and diluted to final mass of 20 g with DIW.
Instrumentation used	- The digestion was carried out in the AntonPaar Multiwave PRO instrument. '- ICP-MS Perkin Elmer NEXION 300D was used with instrumental analytical.
Calibration method/design used	Measurement method by ICP-MS : Standard addition combined with internal standard. The Internal Standard used was Tl and Rh.
For ID-ICP-MS, please indicate reference and	NA

spiked isotopes used	
Traceability (i.e., source, purity of calibration standards)	For ICPMS measurements were used: - Zinc (Zn) Standard Solution (10.007 mg/g ± 0.020 mg/g) NIST SRM 3168a. The internal standards were Rh and Tl. - Calcium (Ca) Standard Solution (9.819 mg/g ± 0.019 mg/g) NIST SRM 3109a. The internal standards were Rh and Tl. - Iron (Fe) Standard Solution (10.013 mg/g ± 0.024 mg/g) NIST SRM 3126a. The internal standards were Rh and Tl. For FAAS measurements were used: - Zinc (Zn) Standard Solution (10.007 mg/g ± 0.020 mg/g) NIST SRM 3168a. The internal standards were Rh and Tl. - Calcium (Ca) Standard Solution (9.819 mg/g ± 0.019 mg/g) NIST SRM 3109a. The internal standards were Rh and Tl.
Internal standards used (if applicable)	To ICP-MS: The internal standard was Rh103, Tl 81
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	mass fraction (mg/kg) by ICP-MS measurement $$w_x = \frac{w_{x\ from\ standard\ model} m_{dilution}}{m_{aliquot} m_{sample} (1 - w_{moisture})} abc$$ mass fraction (mg/kg) by FAAS measurement $$w_x(\frac{mg}{kg}) = \frac{w_x\, EC(\frac{mg}{kg})}{(1 - w_{moisture})} ab$$ Uncertainty on measurements was evaluated based on a ISO-GUM approach, the main sources were: repeatability, calibration, CMRs and model regression (for addintion standard method). In addition, for sodium measurements were include: dilution factor and sample mass The uncertainty sources for the calibrants were: calibration balance, resolution and CRMs certificates. These sources were estimated and integrated in the estimation of the total combined uncertainty. Then, the most the contributions were obtained from the statistical analysis of repeated measurements to estimate the combined uncertainty. After the estimation of all sources of uncertainty, they were combined according to the law of propagation of uncertainties, obtaining the combined standard uncertainty. The expanded uncertainty, U, is obtained by multiplying relative uncertainty by a coverage factor k, assuming a normal distribution of the measurand.
Reference material used	1) NIST SRM 3109a, Calcium (Ca) Standard Solution 2) NIST SRM 3126a, Iron (Fe) Standard Solution 3) NIST SRM 3168a, Zinc (Zn) Standard Solution 4) DMR-82c CENAM, Leche descremada en polvo.

Sample amount used for analysis	0.5g
Sample amount used for dry mass correction	0.25g
Number of samples aliquots taken for dry mass correction.	3
Correction for dry mass (% of weighted sample)	Between 2.6% to 3.2%
Uncertainty for dry mass correction	2.5% (relative)
Additional Comments or Observations	The iron measurement result in the sample SM.QM.S10 was not reported, because we had problems in the measurement.

Institute: LACOMET Costa Rica

Analytes	Ca, Zn
QC sample	NIST 1869
Description of the methodology used	1,0 g test portions taken from SIMQM-S10 packets. Samples were digested using a high purity nitric acid in a microwave oven. The remaining acid after digestion was evaporated to a volume between (2 and 3) ml. All samples were mass diluted with desionized water with resistance ≥ 18 MΩ· cm and COT ≤ 5 ppm.
Instrumentation used	For calcium and zinc a flame atomic absorption spectrometry PerkinElmer PinAAcle 900T with hollow cathode lamps.
Calibration method/design used	For calcium external calibration. For zinc standard addtion calibration.
For ID-ICP-MS, please indicate reference and spiked isotopes used	NA
Traceability (i.e.,	For Ca NIST, SRM 3109a.

source, purity of calibration standards)	For Zn NIST, SRM 3168a.
Internal standards used (if applicable)	none
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	$\bar{y}_{Ca_i} = \bar{y}_{regression_{Ca_i}} \cdot \frac{m_{solution_i}}{\bar{m}_{alicuot_i}} \cdot \frac{m_{extract_i}}{\bar{m}_{dry\ mass\ sample_i}} \cdot rep + C_{Recovery}$ $\bar{y}_{Zn_i} = -b_{intercept\ of\ the\ inverse\ regression_{Ca_i}} \cdot \frac{m_{solution_i}}{\bar{m}_{alicuot_i}} \cdot \frac{m_{extract_i}}{\bar{m}_{dry\ mass\ sample_i}} \cdot rep + C_{Recovery}$ m = mass measure; C= correction of recovery; extract= diluted extract of digestion solution; rep= method repeatability Uncertainties mass measurements: resolution and balance calibration certificate. External calibration: Uncertainties from linear least squares calibration. Mass dry: resolution and balance calibration certificate. Uncertainty type B from SRM 3109 and SRM 3168a. Uncertainty type A from repeatibility method. For mass dry basis the uncertainty was estimated using the Kragten method. For Ca and Zn concentration estimation the uncertainty was estimated using the "Guide to the Expression of Uncertainty in Measurement (GUM).
Reference material used	NIST, SRM 1869
Sample amount used for analysis	1g
Sample amount used for dry mass correction	1g
Number of samples aliquots taken for dry mass correction.	4
Correction for dry mass (% of weighted sample)	% Total Solids: (97,31±0,18) %
Uncertainty for dry mass correction	0.18%

Additional Comments or Observations	

Institute: INEN Equador

Analytes	Fe, Zn
QC sample	no QC sample
Description of the methodology used	Organic matrix is destroyed by dry ashing in muffle furnace at 525 °C during a time no longer than 8 hours. The remaining ash is dissolved in diluted nitric acid 1M and the analyte is determined by atomic absorption spectrophotometry - flame method (AAS).
Instrumentation used	Porcelain Crucibles Hot plate Glassware Atomic absorption spectrophotometer Muffle furnace Drying oven
Calibration method/design used	EC-FAAS: External calibration-Atomic flame absorption spectrometry
For ID-ICP-MS, please indicate reference and spiked isotopes used	NA
Traceability (i.e., source, purity of calibration standards)	NIST SRM 3126A Iron Standard Solution NIST SRM 3168a Zinc Standard Solution NIST SRM 3234 Soy flour.
Internal standards used (if applicable)	

Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	$w_M = w_{(x-B)} * fd * fd_n$ $m_x = m * \frac{100}{(100 - w_{H_2O})}$
Reference material used	NIST SRM 3234 Soy flour.
Sample amount used for analysis	1 g
Sample amount used for dry mass correction	
Number of samples aliquots taken for dry mass correction.	
Correction for dry mass (% of weighted sample)	
Uncertainty for dry mass correction	
Additional Comments or Observations	

Institute: CENAM Mexico

Analytes	Ca, Zn
QC sample	CMR082d

Description of the methodology used	Three aliquots of 0.5 g were accurately weighed for the five samples into microwave vessels, 8 mL of HNO3 and 2 mL of H2O2 were added to each vessel and the contents were digested using a MARS 6 microwave digestor. The digested samples were transferred to a 250 mL PTFE beakers and the contents were evaporated on a hot plate to near dryness. The contents were transferred to a 50 mL low-density polyethylene (LDPE) tube and diluted to 30 g with 1 % HNO3. A 3.6 g aliquot of each sample was weighed into a 125 mL LDPE bottle and diluted to 120 g with 1 % HNO3. The control CMR082d was similarly treated.
Instrumentation used	A Thermo Scientific ICAP Q inductively coupled plasma mass spectrometer (ICP-MS) was used for calcium and zinc measurements with the follow conditions: KED mode, RF power 1550 W, Nebuliser gas flow 0.91 L/min, Auxiliary Argon flow 0.8 mL/min, Colision gas He flow of 5.368 mL/min for Ca and 5.355 mL/min for Zn
Calibration method/design used	A 9.75 g aliquot subsample containing internal standard (Y) was transferred into a 15 mL tube, 250 mg aliquot of a solution containing 300.99 µg/g of Calcium was added to the vial to constitute a spiked sample for the purpose of quantification by the method of standard addition. A 9.2 g aliquot subsample containing internal standard (Y) was transferred into a 15 mL tube, 700 mg aliquot of a solution containing 4.26 µg/g of Zinc was added to the vial to constitute a spiked sample for the purpose of quantification by the method of standard addition.
For ID-ICP-MS, please indicate reference and spiked isotopes used	NA
Traceability (i.e., source, purity of calibration standards)	NIST SRM 3109a Calcium Standard Solution was employed for calcium measurements CENAM Certified Reference Material DMR-61d Zinc spectrometric solution was employed for zinc measurements
Internal standards used (if applicable)	Y spectrometric solution was used as internal standard
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	Standard addition method was used for calcium and zinc measurements. The uncertainty budget included: dry mass correction, measurement replication, calibrant and dilutions. The expanded uncertainty U= ku was calculated using the uncertainty propagation law where k is the coverage factor of 2 for a 95 % confidence level. The components of uncertainty for calcium and zinc measurements are described as follow For calcium- dry mass correction: 0.095 g/100 g, measurement replications: 139 mg/kg, calibbrant: 291 µg/kg, dilutions: 0.00009 g For zinc- dry mass correction: 0.095 g/100 g, measurement replications: 0.5314 mg/kg, calibbrant: 0.689 µg/kg, dilutions: 0.00009 g

Reference material used	CENAM Certified Reference Material CMR082d Skim milk powder was used as control
Sample amount used for analysis	0.5 g of sample were used for calcium and zinc analysis
Sample amount used for dry mass correction	0.5 g were used for dry mass correction
Number of samples aliquots taken for dry mass correction.	Duplicated aliquots of 0.5 g for each sample were used for dry mass correction
Correction for dry mass (% of weighted sample)	3.102 g/100 g
Uncertainty for dry mass correction	0.095 g/100 g
Additional Comments or Observations	Due to technical problems during sample preparation of sample 61, for calcium only two results are reported

Institute: JSI Slovenia

Analytes	Ca, Fe, Se, Zn
QC sample	ERM-BD151
Description of the methodology used	For k0-INAA, an aliquot varied from 0.30 to 0.33 g was pelletized using manual hydraulic press in diameter 10 mm and 3 mm high. An aliquot and standard Al-0.1%Au alloy (IRMM-530R) were stacked together, fixed in the polyethylene vial in sandwich form and irradiated for 20 hours in the carousel facility (CF) of the TRIGA reactor with a thermal neutron flux of 1.1E+12 cm-2 s-1. 5 aliquots were taken in this study. This technique is non-destructive. ICP-MS: About 0.5000 g of samples were weighted into Teflon tubes. Then concentrated HNO3 and H2O2 were added. The tubes were closed and subjected to closed vessel microwave-assisted digestion for 1 hour. After the samples were cooled down, they were quantitatively transferred into 30 mL PE tubes and filler with MilliQ to 20 mL. Before measurement, the samples were diluted 10 times.
Instrumentation used	250 kW TRIGA Mark II reactor, HPGe detector ICP-MS 7900x, Agilent Technology

Calibration method/design used	k0-standardization method of INAA. Reference material used for calibration: IRMM-530R (Al-0.1%Au alloy). k0-INAA technique is non-destructive. ICP-MS: External calibration
For ID-ICP-MS, please indicate reference and spiked isotopes used	N/A
Traceability (i.e., source, purity of calibration standards)	IRMM-530R, Al-0.1%Au alloy (1.003±0.012 g/kg, k=2) ICP-MS NIST SRM 3126a
Internal standards used (if applicable)	ICP-MS: Rh
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	The uncertainty budget of k0-INAA includes the following uncertainties: literature values for T1/2, Ēr, Q0 and k0; the irradiation, decay and measuring times; true-coincidence correction factor (COI); Au composition in Al-0.1%Au alloy; masses of sample and standard (Al-0.1%Au alloy); dry mass correction; previously determined neutron flux parameters (f and α) using Cd-ratio method; and detection efficiency. Combined standard uncertainty of k0-INAA in this study is calculated as: $u_c = \sqrt{\frac{St.dev.^2}{n} + u_{method}^2}$ where St.dev. is standard deviation of independent measurements (n=5) and umethod is combined standard uncertainty of the method used (k=1). Expanded uncertainty is calculated as: $U = 2 \times u_c$ ICP-MS: Measurement uncertainty was estimated on the basis of Eurachem Guide "Quantifying Uncertainty in Analytical Measurement" (3rd Edition, 2012). Combined measurement uncertainty was calculated by the following equations: where m is sample mass; V is the final volume; c is the element concentration as measured by ICP-MS, f(rep) is the repeatability of the method and f(moisture) is the factor of sample moisture. $\gamma = \frac{c \times V}{m} \times f_{repeatability} \times f_{moisture}$ $\frac{u(\gamma)}{\gamma} = \sqrt{\left(\frac{u(c)}{c}\right)^2 + \left(\frac{u(V)}{V}\right)^2 + \left(\frac{u(m)}{m}\right)^2 + \left(\frac{u(f_{rep})}{f_{rep}}\right)^2 + \left(\frac{u(moisture)}{moisture}\right)^2}$ $U = 2 \times u(\gamma)$

Reference material used	ERM-BD151
Sample amount used for analysis	K0-INAA: from 0.30 to 0.33 g; ICP-MS 0.5g
Sample amount used for dry mass correction	from 0.8 to 0.9 g
Number of samples aliquots taken for dry mass correction.	n=3
Correction for dry mass (% of weighted sample)	Correction dry mass factor of 1.0227 was used corresponding to moisture content of 2.22 %.
Uncertainty for dry mass correction	Standard uncertainty of moisture content is about 0.003% and negligible contribute to the uncertainty budget of the method used.
Additional Comments or Observations	

Institute: NIMT, Thailand

Analytes	Ca, Fe, Se, Zn
QC sample	SRM 1568b (for Se). NMIJ CRM 7512-a Trace Elements in Milk Powder (for Ca, Fe and Zn).

Description of the methodology used	Se: Through GSA-HR-ICPMS method, SIM.QM-S10 sample weighed out accurately 0.25 g with the addition of Rh as an internal standard was digested with 5 mL HNO3 using Multiwave 7000 Microwave. Temperature program was set up to 250 degree celsius. This condition made it possible to obtain clear digests. The digestate was then made up with deionized water by approximately 25 g. Then a series of standard addition solutions was prepared and monitored at m/z 82 (Medium resolution). Ca:Through GSA-ICPOES and GSA-ICPMS method, SIM.QM-S10 sample weighed out accurately 0.25 g with the addition of Rh as an internal standard was digested with 5 mL HNO3 using Multiwave 7000 Microwave. Temperature program was set up to 250 degree celsius. This condition made it possible to obtain clear digests. The digestate was then made up with deionized water by approximately 25 g. Then a series of standard addition solutions was prepared and monitored at m/z 42,43, and 44 for ICP-MS.Calcium measurement was monitored at wavelength 317.933 nm and Ca 315.887 nm for ICP-OES. Zn: Isotope Diluton-ICP-MS was uesd and the target mole ratio was aimed at 0.7. 66Zn was an analyte ion and 67Zn was spike ion. 0.25g of SIM.QM.S10 sample was microwave acid digested with 5 mL of HNO3 using multiwave 7000 microwave. The digestion condition was ramp to 250 °C for 30 min and hold for 30 min and then cool down to room temperature. This condition made it possible to obtain clear digests. The digestate was then made up with deionized water to 25 mL. This solution was diluted 8 times. Then, they were analsed utilizing ICP-MS for Zn quantitation. 66Zn and 67Zn were monitored. Fe: Through GSA-ICPOES method, SIM.QM-S10 sample weighed out accurately 0.25 g with the addition of Y as an internal standard was digested with 5 mL HNO3 using Multiwave 7000 Microwave. The digestion condition was ramp to 250 °C for 30 min and hold for 30 min and then cool down to room temperature. This condition made it possible to obtain clear digests. The digestate was then made up with deionized water by approximately 25 g. Then a series of standard addition solutions was prepared and monitored at wavelength 238.204 nm and 239.562 nm.
Instrumentation used	Se: High Resolution ICP-MS (Element XR, Thermo Fisher Scientific) Ca: ICP-MS Triple Quad (8800, Agilent) and ICP-OES (Avio 500,PerkinElmer) Fe: ICP-OES (Avio 500, PerkinElmer) Zn: ICP-MS Triple Quad (8800, Agilent)
Calibration method/design used	Se: Gravimetric Standard Addition ICP-MS Ca: Gravimetric Standard Addition(GSA) ICP-MS, GSA-ICP-OES Fe: Gravimetric Standard Addition(GSA)-ICP-OES Zn: ID-ICPMS
For ID-ICP-MS, please indicate reference and spiked isotopes used	Reference isotope was 66Zn and spiked isotope was 67Zn.
Traceability (i.e., source, purity of	SRM 3149 Lot No. 100901, purchased from NIST, was used as primary calibrations standard for Se.

calibration standards)	SRM 3109a Lot No. 130213, purchased from NIST, was used as primary calibrations standard for Ca. SRM 3126a Lot No. 140812, purchased from NIST, was used as primary calibrations standard for 56Fe. SRM 3168a Lot No. 120629, purchased from NIST, was used as primary calibrations standard for 66Zn. Stable enriched 67Zn isotope batch no. 217901, purchased from Oak Ridge, was used as an isotropic spike.
Internal standards used (if applicable)	Rhodium (Rh) SRM 3144 Lot No. 070619 for Se, Ca Yttrium (Y) SRM 3167a Lot No. 120314 was purchased from NIST. (for Fe)
Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	The equation for the calculation of the mass fraction of Zn $C_x = f_{H_2O} \cdot f_P \cdot f_B \cdot f_D \cdot C_z \cdot \frac{M_y \cdot M_{zc}}{M_x \cdot M_{yc}} \cdot \frac{R_y - R_b}{R_b - R_x} \cdot \frac{R_{bc} - R_z}{R_y - R_{bc}}$

Parameter	Source of unceratinty	Typical value	Standard uncertainty	Unit	Type
R_b	Isotopic ratio in sample blend	0.6877	0.0013	-	A
R_{bc}	Isotopic ratio in calibration blend	0.6843	0.0015	-	A
Rx	Isotopic ratio in sample	6.8638	0.0024	-	B
Rz	Isotopic ratio in standard	6.8638	0.0024	-	B
f_D	Digestion factor	1.0000	0.0050	-	B
f_B	Blank correction factor	1.0000	0.0003	mg kg^{-1}	B
f_P	Method Precision	1.0000	0.0018	-	A
Cz	Calibration solution	20.0711	0.0356	mg kg^{-1}	B
Ry	Isotopic ratio in spike	0.0206	0.0001	-	B
M_x	Mass of sample in sample blend	0.2475	0.0004	g	B
M_y	Mass of spike in sample blend	0.1071	0.0004	g	B
M_{yc}	Mass of spike in calibration blend	0.1078	0.0004	g	B
M_z	Mass of standard in calibration blend	0.5230	0.0004	g	B
f_{H2O}	Moisture content factor	1.0000	0.0014	-	B

C_x	Mass fraction of total Zn	43.2	mg kg^{-1}
u_c	Combined standard uncertainty	0.39	mg kg^{-1}
k	Coverage factor	2	
U	Expanded unceratinty	0.8	mg kg^{-1}

The equation for the calculation of the mass fraction of Fe

$c_x = P.B.D.C_0.DF.100/(100-\%moisture)$

Parameter	Source of unceratinty	Typical value	Standard uncertainty	Unit	Type
P	Precision	1	0.0194	-	A
Co	Regression	1	0.0095		B
Cal Std	Calibration standard	1	0.0052	mg kg^{-1}	B
moisture	Dry mass	1	0.0010	%	B
DF sample digest	Dilution factor for sample digest	1	0.0032	-	B
DF sample solution	Dilution factor for sample solution	1	0.0040	-	B
B	Blank factor	1	0.0232	-	B
D	Digestion	1	0.00500	-	B

C_x	Mass fraction of total Fe	2.61	mg kg^{-1}
u_c	Combined standard uncertainty	0.09	mg kg^{-1}
k	Coverage factor	2	
U	Expanded unceratinty	0.18	mg kg^{-1}

ICP-MS

The equation for the calculation of the mass fraction of Ca

$c_x = P.B.D.C_o.DF.100/(100-\%moisture)$

Parameter	Source of unceratinty	Typical value	Standard uncertainty	Unit	Type
P	Precision	1	0.0353	-	A
Co	Regression	1	0.0084		B
Cal Std	Calibration standard	1	0.0013	mg kg^{-1}	B
moisture	Dry mass	1	0.0010	%	B
DF sample digest	Dilution factor for sample digest	1	0.0033	-	B
DF sample solution	Dilution factor for sample solution	1	0.0014	-	B
B	Blank factor	1	0.0006	-	B
D	Digestion	1	0.00500	-	B

C_x	Mass fraction of total Ca	12610	mg kg^{-1}
u_c	Combined standard uncertainty	465	mg kg^{-1}
k	Coverage factor	2	
U	Expanded unceratinty	930	mg kg^{-1}

The equation for the calculation of the mass fraction of Se

$c_x = P.B.D.C_o.DF.100/(100-\%moisture)$

Parameter	Source of unceratinty	Typical value	Standard uncertainty	Unit	Type
P	Precision	1		-	A
Co	Regression	1			B
Cal Std	Calibration standard	1		mg kg^{-1}	B
moisture	Dry mass	1		%	B
DF sample digest	Dilution factor for sample digest	1		-	B
DF sample solution	Dilution factor for sample solution	1		-	B
B	Blank factor	1		-	B
D	Digestion	1		-	B

C_x	Mass fraction of total Se	0.352	mg kg^{-1}
u_c	Combined standard uncertainty	0.0130	mg kg^{-1}
k	Coverage factor	2	
U	Expanded unceratinty	0.027	mg kg^{-1}

Reference material used	NMIJ CRM 7512-a Trace Elements in Milk Powder was used as matrix reference material as QC sample.
Sample amount used for analysis	0.25 g of SIM.QM S-10 sample for each analysis.
Sample amount used for dry mass correction	1 g of SIM.QM S-10 samples (three saperate SIM.QM S10 sample) were put in chamber. They were kept in VirTis wizard 2.0 lyophilizer controller freeze dryer (SP SCIENTIFIC) using vacuum mode at 50 mmHg and at room temperature (20 °C) for 24 h. Then, they were weighed. The process was repeated for every 24 h. to a constant mass.
Number of samples aliquots taken for dry mass correction.	Three separate SIM.QM S-10 samples aliquots (Sample No. 21, No. 22 and No. 23)

Correction for dry mass (% of weighted sample)	97.4% (moisture 2.61%)
Uncertainty for dry mass correction	0.10%
Additional Comments or Observations	

Institute: INRAP, Tunisia

Analytes	Zn
QC sample	no QC sample
Description of the methodology used	Acid Digestion by Microwave (Milestone), Using 8 mLhigh pur Nitric Acid HNO3 (67%) and 2 mL Hydrogen peroxide H2O2 (37%), final volume: 40 mL, parameters of Microwave: Power 1000 Watts, Temperature 120°C, Time 30 min
Instrumentation used	HR-ICP-AES (Analytik Jena)
Calibration method/design used	Standard calibration method
For ID-ICP-MS, please indicate reference and spiked isotopes used	
Traceability (i.e., source, purity of calibration standards)	Zinc (Zn)Standard solution (NIST) SRM 3168a, Certified Zinc Mass Fraction : 10,007 mg/g ± 0,02 mg/g
Internal standards used (if applicable)	

Measurement equation and uncertainty budget (please include breakdown of the budget, describing individual uncertainty contributions and how they were combined)	Mass fraction of Zinc (mg/Kg) = [CZn (mg/L)*final Volume(mL)]/sample mass(g) ; Standard solution(u = 0,01 mg/Kg), Trueness(u = 0,13),Reproducibility (u = 1,27)
Reference material used	
Sample amount used for analysis	0.5 g
Sample amount used for dry mass correction	1g
Number of samples aliquots taken for dry mass correction.	6
Correction for dry mass (% of weighted sample)	96,18 % ; Humidity (3,82 %)
Uncertainty for dry mass correction	0.07
Additional Comments or Observations	