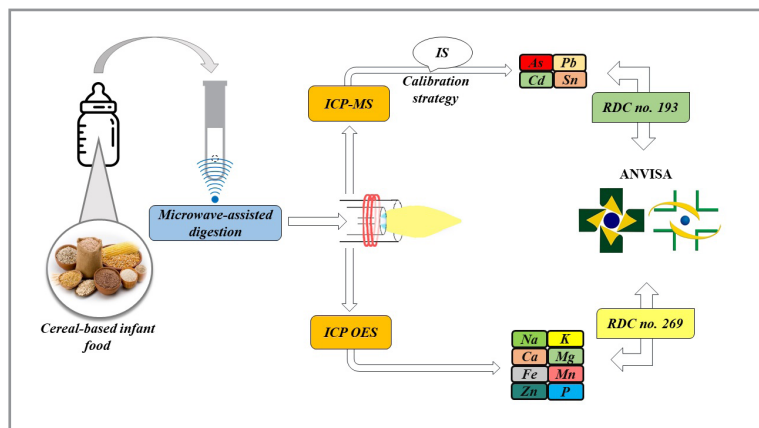


ARTICLE

Determination of Macro, Micro and Toxic Elements using Argon-Based Plasma Spectroanalytical Methods in order to support Brazilian Regulations on Inorganic Constituents in Infant Foods

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Children's cereal-based foods and supplements are widely consumed by newborn and infant population. The Resolution RDC no. 193/2017 of the Brazilian National Health Surveillance Agency (ANVISA) established maximum tolerated limits (MTL) for toxic elements in infant foods aiming to control health risks. Complementarily, the determination of macro and microelements based on values of recommended daily intake (RDI) is regulated by Resolution RDC no. 269/2005.

Two analytical procedures were developed

using inductively coupled plasma mass spectrometry (ICP-MS) for determination of As, Cd, Sn and Pb and inductively coupled plasma optical emission spectrometry (ICP OES) for determination of Ca, Fe, K, Mg, Mn, Na, P and Zn attending both Brazilian regulations. Microwave-assisted acid digestion of solid samples was carried out in closed vessels using 7 mol L⁻¹ HNO₃. Two rice flours reference materials as well as addition and recovery experiments were applied to check accuracy. Recoveries ranged from 70 to 128% for ICP OES and from 82 to 128% for ICP-MS using internal standardization. The analytical procedure presented LOQs lower than maximum limits allowed by both regulations. Fifteen samples of cereal-based infant foods were analyzed and all analyte concentrations were lower than the respective MTLs recommended by RDC no. 193, except to Pb concentration in one milk flour sample. According to the RDI established by RDC no. 269 and the average concentrations of Ca, Fe, K, Mg, Mn, P and Zn in samples, it was possible to assign a minimum mass value of food that meets the recommendations of the Brazilian resolution for different population groups.

Keywords: Sample preparation, ANVISA Resolution 193, ANVISA Resolution 269, ICP-MS, ICP OES.

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INTRODUCTION

Good nutrition is vital for human health. In this sense, it is known that cereal grains are important in human health and also a good source of energy and dietary fibers as well as proteins and essential elements, especially for children and newborns during development phase [1]. Despite the nutritional important roles of cereal grains in the human diet, cereal-based foods, commonly present in the diet of children and newborns, are a possible source of exposure to contaminants, such as As, Cd, Pb and Sn, also known as potentially toxic elements (PTEs) [2-7].

Contamination by some PTEs, even in low concentrations, can be harmful to health, causing diseases and irreversible damage to the body. Because children differ regarding their physiology and metabolism, the contamination risk can be more severe [8,9]. Exposure to environmental threats at these sensitive stages of the child's life may negatively influence the growth and development and also cause irreversible damage, such as injury to the liver, kidneys, bones, lungs, central nervous system, cardiovascular diseases and even cancerous effects [10].

Associated with the control of PTEs in foods, the interest in the determination of As, Cd, Sn and Pb in infant foods is also related to the fulfillment of Brazilian legislations, including the Resolution RDC no. 193, of December 2017, which establishes Maximum Tolerated Limits (MTLs) of these contaminants in infant foods and cereal-based lactating foods [11]. In addition to RDC no. 193, the Resolution no. 269, of September 2005, recommends the determination of macro and microelements based on values of recommended daily intake (RDI) of proteins, vitamins and minerals for individuals in different population groups [12].

According to Damodaran et al. [13] the variation in the deficiency of some elements, rare or practically nonexistent in the human body, is due to socio-economic issues and geographical divisions. However, plant-based foods are the main sources of macro and microelements to satisfy nutritional needs. In biological systems, elemental nutrients are separated into two categories: macroelements (elements in higher concentrations in human body, such as Ca, K, Mg, Na and P) and microelements (elements present in lower concentrations in human body, but which perform important functions for normal metabolism of human beings, such as Co, Cr, Cu, Fe, Mn and Zn) [3,13].

Spectrochemical methods with atomization-excitation-ionization in argon plasmas are widely used for elemental determination providing multi-element analysis, high sensitivity, robustness and relatively simple operation of equipment [14-16]. Due to the high sensitivity and low detection limits argon-based plasma methods are intensely used for determination of inorganic contaminants at trace concentrations in several types of food samples [15-17]. Additionally, the combination of microwave-assisted digestion and closed vessels has clear advantages compared to traditional acid digestion using conventional heating and open vessels in terms of better recoveries for volatile elements, lower contamination, lower volume of reagents, better reproducibility and a better working environment [18].

In this context, this study proposed a microwave-assisted digestion procedure using dilute nitric acid solutions for determination of As, Cd, Sn and Pb using ICP-MS and Ca, Fe, K, Mg, Mn, Na, P and Zn using ICP OES in several types of children's foods and supplements in order to meet simultaneously both Brazilian resolutions: RDC no. 193 and RDC no. 269. Instrumental strategies for correcting spectral interferences as well as calibration methods for non-spectral interferences were evaluated in order to improve accuracy and precision of the analytical procedure using either ICP-MS or ICP OES. Fifteen samples of cereal-based infant foods were analyzed and all analyte concentrations were lower than the respective MTLs recommended by RDC no. 193, except to Pb concentration in one milk flour sample. According to the RDI from RDC no. 269 and the average concentrations of Ca, Fe, K, Mg, Mn, P and Zn in samples, it was possible to assign a minimum mass value of food that meets the recommendations of the Brazilian resolution for four different population children groups.

MATERIALS AND METHODS

Samples and sample preparation

Fifteen samples of children's foods and supplements (coded from A1 to A15) were analyzed. Samples A1, A2 and A3 were purchased in commercial establishments (as normally sold in commercial packaging) in São Carlos, SP, Brazil, and all other samples were supplied by the Laboratory Exata located in Jataí, GO, Brazil also as they are normally sold. Samples, types and their nutritional indication are presented in Table I. These samples were produced by several food companies (Danone, Maisena, Nestle, Sustagen and Yoki) in four Brazilian states. One sample came from Buenos Aires, Argentina.

Table I. Samples of children's foods and supplements and their food indication according to the commercial label of each food

Sample	Type of infant food	Indication
A1	Infant Cereal – Rice	Children over 6 months
A2	Corn Starch with Rice Flour	Children over 2 years
A3	Rice Cream	Children over 2 years
A4	Milk Flour	Children over 2 years
A5	Infant Formula	Infants from 0 to 6 months
A6	Milk Flour	Children over 2 years
A7	Infant Cereal – Rice and Oats	Children over 6 months
A8	Infant Cereal – Corn	Children over 6 months
A9	Infant Formula	Infants over 5 months
A10	Infant Cereal – Multicereals	Children over 6 months
A11	Sustagen	Children over 6 months
A12	Infant Formula	Infants over 9 months
A13	Powdered Milk	Children over 1 year
A14	Powdered Milk	Children over 1 year
A15	Infant Formula	Infants from 0 to 6 months

Masses of approximately 500 mg of each sample were weighed in perfluoroalkoxy-alkane (PFA) digestion vessels and digested in a single reaction chamber (SRC) system (UltraWave™, Milestone, Sorisole, BG, Italy). Volumes of 150 mL of water and 5 mL of concentrated nitric acid were inserted into the SRC and the chamber was pressurized with nitrogen gas to 40 bar (99.9%, White Martins-Praxair, Sertãozinho, SP, Brazil) as recommended by the manufacturer. Volumes of 5 mL of HNO₃ in three different concentrations (1; 2 and 7 mol L⁻¹) were tested for digestion of food samples. The microwave heating program was applied as follows [18]: (1) 2.5 min to reach 140 °C, (2) 2.5 min hold at 140 °C, (3) 2.5 min to reach 180 °C, (4) 2.5 min hold at 180 °C, (5) 10 min to reach 220 °C, (6) 10 min hold at 220 °C. Temperature was controlled by an internal temperature sensor.

Subsequently, digests were diluted to 25.0 mL with distilled-deionized water and an aliquot of each solution was appropriately diluted with deionized water, followed by quantification by ICP OES (10-fold dilution) using external calibration and by ICP-MS (2-fold dilution) using internal standardization.

Reagents and standard solutions

Experiments were performed using HNO_3 (Synth, Diadema, SP, Brazil) purified in a sub-boiling distillation apparatus Distillacid™ BSB-939-IR (Berghof, Eningen, Germany) and ultrapure water with resistivity higher than $18.2 \text{ M}\Omega \text{ cm}$ (Milli-Q® Bedford, MA, USA). All glass and polypropylene bottles were washed and kept in an acid bath (HNO_3 10% v/v) for 24 h. The standard solutions used for calibration and for addition and recovery experiments were prepared by adequate dilution of mono-elementar stock solutions containing 1000 mg L^{-1} of As, Ca, Cd, Fe, K, Mg, Mn, Na, P, Pb, Sn and Zn (Qhemis, Jundiaí, SP, Brazil) in 0.7 mol L^{-1} HNO_3 medium. The internal standards evaluated (ISs) were also prepared by adequate dilution of mono-elementar stock solutions containing 1000 mg L^{-1} of Ge, Pd and Y. These elements were evaluated as IS based on previous studies [18–20].

The concentrations for analytical calibration solutions used in ICP-MS were 0, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and $20.0 \text{ }\mu\text{g L}^{-1}$ of As, Cd, Sn and Pb prepared in 0.14 mol L^{-1} HNO_3 medium. Addition and recovery experiments were performed at two levels of addition: 0.50 and $1.0 \text{ }\mu\text{g L}^{-1}$ for all analytes in four different samples: A1 (Infant cereal of rice); A3 (Rice cream); A9 (Infant formula) and A13 (Powdered Milk). The standards were added before sample digestions. To correct for matrix effects, the evaluated ISs Ge, Pd and Y were added at $1.0 \text{ }\mu\text{g L}^{-1}$ to analytical calibration solutions, analytical blanks, and sample digests. For addition and recovery experiments the ISs were added at same concentration for both addition levels.

For ICP OES, the concentrations for analytical calibration solutions were 0, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10, 25 and 50 mg L^{-1} of Ca, Fe, K, Mg, Mn, Na, P and Zn prepared in 0.70 mol L^{-1} HNO_3 medium. Matrix effects were not observed using ICP OES and, consequently, ISs were not added. Addition and recovery experiments were not performed for ICP OES analysis because both reference materials have certified values for all analytes. The certified reference material (CRM) of rice flour NIST 1568a (National Institute of Standard and Technology, Gaithersburg, MD, EUA) and the reference material (RM) also of rice flour Agro AR-01/2015 (Embrapa Pecuária Sudeste, São Carlos, SP, Brazil) were used for optimization of experimental procedures and evaluation of accuracy for ICP OES and ICP-MS. For ICP-MS, addition and recovery experiments were also considered because both reference materials did not inform reference concentrations for Pb and Sn. CRM NIST 1568a has certified values for As and Cd. On the other hand, RM Agro AR-01/2015 only has certified value for As.

Instrumentation

Measurements were performed using an ICP OES model iCAP6000 (Thermo Fisher Scientific, Waltham, MA, USA) operated under robust conditions and axial viewing mode and an ICP-MS model Agilent 7800 Quadrupole (Agilent Technologies, Tokyo, JHS, Japan) without using collision cell (*standard mode*) or using collision cell technology (*He mode*) pressurized with He gas (99.999%, White Martins-Praxair, Sertãozinho, SP, Brazil) both applying the aerosol dilution strategy, i.e. the aerosol was diluted with argon under adjusted flow rates (aerosol dilution gas flow rate of 0.62 L min^{-1} and carrier gas flow rate of 0.40 L min^{-1} , thus 1.02 L min^{-1} of total flow rate [20]. Argon (99.999%, White Martins-Praxair) was used in all measurements for both instruments. Plasma operating conditions adopted in ICP-MS and ICP OES are presented in Table II.

Table II. Instrumental parameters adopted in ICP OES and Quadrupole ICP-MS

Instrumental Parameter	ICP OES	ICP-MS
RF applied power (kW)	1.20	1.55
Plasma gas flow rate (L min^{-1})	12	15
Auxiliary gas flow rate (L min^{-1})	0.5	1.0
Carrier gas flow rate (L min^{-1})	0.50	1.02
Carrier gas flow rate in <i>HMI mode</i> (L min^{-1})	NA	0.40

Table II. Instrumental parameters adopted in ICP OES and Quadrupole ICP-MS (Continuation)

Instrumental Parameter	ICP OES	ICP-MS
HMI gas flow rate (L min ⁻¹)	NA	0.62
Sampling depth (mm)	NA	8.0
He flow rate in collision cell (mL min ⁻¹)	NA	4.5
Integration time (s)	15	3.0
Nebulizer	V-Groove	Mira-Mist
Spray chamber	Cyclonic	Double-pass
Number of replicates	3	3
Analyte	Emission Line (nm)	Isotope (m/z)
As	NA	75
Ca	184.006	NA
Cd	NA	114
Fe	238.204	NA
K	769.896	NA
Mg	279.079	NA
Mn	259.373	NA
Na	330.237	NA
P	178.284	NA
Pb	NA	208
Sn	NA	120
Ge	NA	70
Pd	NA	104
Y	NA	89
Zn	202.548	NA

NA: Not applicable.

RESULTS AND DISCUSSION

Microwave-assisted sample preparation

The preliminary assessment of the digestion procedure was carried out visually since the goal was to reach digestion without residual solids for all analyzed samples. Residual solids were observed for samples digested using 1 and 2 mol L⁻¹ HNO₃. Thus, a solution containing 7 mol L⁻¹ HNO₃ was selected for further experiments since complete and clear digests were obtained for all samples without adding hydrogen peroxide. The sample preparation procedure using only 1+1 v/v dilute nitric acid solution is attractive because the use of hydrogen peroxide may sometimes imply in the addition of contaminants when not using a high purity reagent [16,18,21].

Analytical performance for ICP OES and ICP-MS

Limits of detection (LOD) and quantification (LOQ) were calculated considering standard deviation (SD) for 10 measurements of a blank solution divided by slope of analytical curve multiplied by 3 (LOD) and 10 (LOQ), and then multiplied by the dilution factor [22]. For ICP-MS, measured isotopes, mode of acquisition, linear correlation coefficient and LOQs are shown in Table III. The LOQs obtained for all analytes were lower than the respective MTL suggested by RDC no. 193 [11], inferring that the developed procedure has sufficient detectability to meet this resolution.

Table III. Maximum tolerable limits (MTL) and figures of merit for determination of As, Cd, Pb and Sn by ICP-MS using external calibration and internal standardization

Isotope	⁷⁵ As ⁺	¹¹⁴ Cd ⁺	¹²⁰ Sn ⁺	²⁰⁸ Pb ⁺
Acquisition mode	<i>He</i>	<i>Standard</i>	<i>Standard</i>	<i>Standard</i>
MTL ^a (mg kg ⁻¹)	0.15	0.050	50	0.050
External Calibration				
Sensitivity	956	2076	4464	4842
R ²	0.9999	0.9998	0.9999	0.9997
LOD (mg kg ⁻¹)	0.0031	0.00050	0.0069	0.0087
LOQ (mg kg ⁻¹)	0.010	0.0020	0.023	0.029
Internal Standardization				
Internal Standard	Y	Y	Y	Y
R ²	0.9993	0.9992	0.9994	0.9990
LOD (mg kg ⁻¹)	0.0038	0.00050	0.0076	0.0093
LOQ (mg kg ⁻¹)	0.013	0.0020	0.025	0.031

^a Resolution no. 193, of December 12, 2017 [11].

For ICP-MS measurements, sample dilution is often necessary to keep the total dissolved solids (TDS) below 0.1% m/v. To overcome this limitation and improve the sensitivity, ICP-MS was operated using aerosol dilution strategy. This instrumental strategy enabled the introduction of digests with TDS of up to 0.5% m/v and residual acidity up to 1% v/v, which eliminates possible contamination associated with manual dilution, saves time and reduces the volume of waste compared to conventional dilution using a liquid diluent [18,20].

ICP-MS is susceptible to spectral and non-spectral interferences due to matrix effects associated with transport, nebulization, and/or energetic effects in argon plasma [15,16]. Consequently, internal standardization was used as calibration strategy to correct matrix effects in the determination of all analytes. Furthermore, the instrumental strategy of collision cell technology (CCT) with kinetic energy discrimination (KED) was adopted to correct spectral interferences when determining As. The CCT is an instrumental strategy used for removing spectral interferences caused by polyatomic species [23]. The collision cell mode was used only for determination of As, due to possible interferences caused in the mass/charge 75, such as ⁴⁰Ca³⁵Cl⁺ and ⁵⁹Co¹⁶O⁺. For all other elements, adequate accuracy was obtained using the standard mode acquisition.

Measurements using ICP OES were performed in axial configuration for improving sensitivity. Table IV presents the reached limits of quantification (LOQ) for determination of Ca, Fe, K, Mg, Mn, Na, P and Zn using ICP OES.

Table IV. Analytical parameters for determination of Ca, Fe, K, Mg, Mn, Na, P and Zn by ICP OES using external calibration

Element	Sensitivity	R2	LOD (mg kg ⁻¹)	LOQ (mg kg ⁻¹)
Ca	1019	0.9999	3.5	12
Fe	95	0.9999	0.060	0.19
K	13077	0.9998	0.48	1.6
Mg	7306	0.9998	0.51	1.7
Mn	54727	0.9998	0.013	0.042
Na	1497	0.9999	8.7	29
P	129	0.9998	1.9	6.3
Zn	24067	0.9996	0.55	1.8

Evaluation of accuracy for ICP OES and ICP-MS procedures

For ICP-MS measurements, the accuracy of the developed analytical procedure was evaluated by addition and recovery experiments at two-levels (0.50 and 1.0 µg L⁻¹) applied to four samples with different compositions (infant cereal, rice cream, infant formula and powdered milk) and also by analysis of CRM NIST 1568a and RMAgroAR-01/2015, both composed by flour rice. The addition levels of spike experiments were performed based on the specific MTL for each analyte by means of RDC no. 193 [11] (Table III). Spikes were added before microwave-assisted digestion. Table V presents the recoveries obtained for addition and recovery experiments using external calibration and internal standardization. Considering the CRM and RM analyzed, only As (0.29 ± 0.03 and 0.112 ± 0.015 mg kg⁻¹, respectively) and Cd (0.022 ± 0.002 mg kg⁻¹ for the CRM) presented certified values. For As, recoveries of 101% (0.30 ± 0.03 mg kg⁻¹ of As for CRM) and 106% (0.12 ± 0.01 mg kg⁻¹ of As for RM) were obtained for both materials. For Cd, only the CRM contained the certified content, and a 70% recovery was obtained (0.015 ± 0.001 mg kg⁻¹ of Cd).

Table V. Addition and recovery experiments (recovery (%), RSD (%), n = 3) by ICP-MS using external calibration and internal standardization

Isotope	Adition (µg L ⁻¹)	External Calibration			
		A1	A3	A9	A13
⁷⁵ As ⁺	0.5	51 (4)	79 (8)	84 (6)	86 (3)
	1	63 (7)	79 (2)	83 (2)	79 (4)
¹¹⁴ Cd ⁺	0.5	85 (7)	95 (9)	72 (2)	74 (6)
	1	83 (4)	91 (2)	73 (4)	75 (6)
¹²⁰ Sn ⁺	0.5	88 (5)	90 (4)	105 (6)	82 (3)
	1	87 (7)	88 (1)	95 (9)	80 (7)
²⁰⁸ Pb ⁺	0.5	94 (8)	101 (9)	113 (6)	94 (7)
	1	89 (4)	103 (10)	94 (6)	93 (25)

Table V. Addition and recovery experiments (recovery (%), RSD (%), n = 3) by ICP-MS using external calibration and internal standardization (Continuation)

Isotope	Addition ($\mu\text{g L}^{-1}$)	Internal Standardization			
		A1	A3	A9	A13
$^{75}\text{As}^+$	0.5	85 (6)	84 (4)	86 (4)	96 (4)
	1	83 (7)	86 (1)	82 (3)	90 (3)
$^{114}\text{Cd}^+$	0.5	91 (5)	108 (5)	82 (2)	85 (7)
	1	90 (4)	97 (3)	85 (6)	87 (5)
$^{120}\text{Sn}^+$	0.5	94 (6)	97 (5)	96 (7)	92 (4)
	1	94 (7)	94 (1)	87 (8)	91 (5)
$^{208}\text{Pb}^+$	0.5	102 (7)	104 (8)	97 (6)	98 (6)
	1	96 (3)	108 (11)	87 (9)	102 (23)

The choice of IS was evaluated by addition and recovery experiments and also by analysis of CRM NIST 1568a and RM Agro AR-01/2015. Among the ISs evaluated (Ge, Pd and Y), the best recoveries, ranging from 82 to 108%, were obtained for all analytes when using Y as IS, but satisfactory recoveries were also obtained when using $^{70}\text{Ge}^+$ as internal standard for $^{75}\text{As}^+$, $^{120}\text{Sn}^+$ and $^{208}\text{Pb}^+$, as also observed in previous studies [18,20]. Since RDC no. 193 [11] does not specify a validation parameter, recoveries ranging from 80 to 120% were considered satisfactory.

The improvement in recoveries using internal standardization can be explained due to the correction of matrix effects caused during transport and/or ionization of analytes. In general, it is accepted that a good IS should have physico-chemical properties similar to the analytes, such as similar isotopic mass between the IS and the analyte [24] or the first ionization energy [25]. However, recent studies have demonstrated divergences in the literature related to IS selection criteria in ICP-MS [19,20,26,27]. According to Olesik and Jiao [26,27] and Barros et al. [19,20] in current ICP-MS instruments is not always necessary to have analyte and IS with similar masses probably due to the different behavior of space charge effects in current ion lenses design. This new assumption might explain why a single IS, sometimes with highly different mass and physico-chemical parameters, is effective for correcting matrix effects, as also observed here.

For ICP OES measurements, recoveries ranged from 70 to 128% for the RM of rice flour Agro AR-01/2015 and from 85 to 123% for the CRM NIST 1568a inferring that the analytical procedure developed for determination of macro and micro elements in baby food samples using ICP OES is accurate. Some elements i.e., Ca, Na and K, presented recoveries slightly out of range considered quantitative (from 80 to 120%), however, for just one of the evaluated RMs. No significant matrix effects were observed for ICP OES measurements, i.e. matrix effects observed in ICP OES were reduced in comparison to ICP-MS. Therefore, internal standardization was not required. Table VI shows certified and determined contents obtained for both reference materials.

Along with the contents shown in Table VI, F-test with 95% of confidence and paired Student's *t*-test were applied to evaluate agreement between certified and determined values for each element in both reference materials. The *t*-test was performed assuming unequal variances for Zn on RM Agro and Fe, K, Mg and Na on NIST SRM and equal variances for the others elements according to the F-test previously made. Under the calculated conditions (n = 3), with 95% confidence ($t_{95\%} = 2.78$), Mg and P had shown significant differences (3.19 and 3.08, respectively) for the RM Agro AR-01/2015, and also K and Zn had shown significant differences (36.4 and 5.44, respectively) for the CRM NIST 1568a. Adopting 99% confidence level ($t_{99\%} = 4.60$), only K had shown significant difference for the CRM NIST 1568a.

Table VI. Certified and determined contents (mean \pm standard deviation, mg kg⁻¹, n = 3), recoveries (%), Student's t-test obtained for RM Agro AR-01/2015 and CRM NIST 1568a using ICP OES

Element	RM Agro AR-01/2015			NIST SRM 1568a		
	Certified value	Determined value	t value	Certified value	Determined value	t value
Ca	90 \pm 32	114 \pm 3 (128)	0.75	118 \pm 6	106 \pm 1 (91)	1.97
Fe	17 \pm 1	17.32 \pm 0.05 (102)	0.32	7.4 \pm 0.9	6.2 \pm 0.3 (87)	1.26
K	1852 \pm 319	1908 \pm 20 (103)	0.18	1280 \pm 8	1591 \pm 3 (123)	36.4
Mg	1258 \pm 116	1620 \pm 20 (120)	3.08	560 \pm 20	513 \pm 3 (92)	2.32
Mn	62 \pm 4	67.0 \pm 0.6 (109)	1.24	20 \pm 2	16.86 \pm 0.07 (85)	1.57
Na	117 \pm 28	82 \pm 3 (70)	1.24	<LOD	<LOD	NA
P	3037 \pm 184	3631 \pm 28 (119)	3.19	1530 \pm 80	1454 \pm 15 (96)	0.93
Zn	19 \pm 3	23 \pm 1 (119)	1.26	19.4 \pm 0.5	16.66 \pm 0.6 (86)	3.51

NA: Not applicable.

Sample analysis

After optimizing the analytical parameters for determination of macro, micro and toxic elements using argon-based plasma spectrochemical methods, fifteen samples of infant foods and supplements were analyzed (Tables VII and VIII). For toxic elements (Table VII), the concentration ranges determined in the samples were 0.026 to 0.14 mg kg⁻¹ for As and 0.029 to 0.097 mg kg⁻¹ for Sn. For Cd, only two samples (A6 and A7 contained 0.002 and 0.009 mg kg⁻¹ of Cd, respectively) contained concentrations higher than the respective LOQ. For Pb, only one sample (A6, 0.07 mg kg⁻¹) was higher than the LOQ established for Pb. However, all determined concentrations for these elements were lower than the limits proposed by RDC no. 193 [11], except to Pb for sample A6.

Table VII. Determination of As, Cd, Sn and Pb in samples of infant foods and supplements (mean \pm standard deviation, mg kg⁻¹, n = 3) using ICP-MS

Sample	⁷⁵ As ⁺	¹¹⁴ Cd ⁺	¹²⁰ Sn ⁺	²⁰⁸ Pb ⁺
A1	0.14 \pm 0.02	<0.0020	<0.025	<0.031
A2	<0.013	<0.0020	<0.025	<0.031
A3	0.1333 \pm 0.0005	<0.0020	<0.025	<0.031
A4	<0.013	<0.0020	<0.025	<0.031
A5	<0.013	<0.0020	0.03 \pm 0.01	<0.031
A6	<0.013	0.002 \pm 0.001	<0.025	0.07 \pm 0.03
A7	0.09 \pm 0.01	0.009 \pm 0.001	<0.025	<0.031
A8	<0.013	<0.0020	<0.025	<0.031
A9	<0.013	<0.0020	0.097 \pm 0.002	<0.031
A10	<0.013	<0.0020	<0.025	<0.031
A11	<0.013	<0.0020	<0.025	<0.031
A12	<0.013	<0.0020	0.060 \pm 0.005	<0.031

Table VII. Determination of As, Cd, Sn and Pb in samples of infant foods and supplements (mean \pm standard deviation, mg kg⁻¹, n = 3) using ICP-MS (Continuation)

Sample	⁷⁵ As ⁺	¹¹⁴ Cd ⁺	¹²⁰ Sn ⁺	²⁰⁸ Pb ⁺
A13	0.027 \pm 0.002	<0.0020	<0.025	<0.031
A14	0.026 \pm 0.007	<0.0020	<0.025	<0.031
A15	<0.013	<0.0020	0.085 \pm 0.009	<0.031

Table VIII. Determination of Ca, Fe, K, Mg, Mn, Na, P and Zn in samples of infant foods and supplements (mean \pm standard deviation, mg kg⁻¹, n = 3) using ICP OES

Sample	Ca	Fe	K	Mg	Mn	Na	P	Zn
A1	2577 \pm 21	254 \pm 5	922 \pm 15	175 \pm 3	7.5 \pm 0.2	1210 \pm 12	1833 \pm 11	107 \pm 6
A2	167 \pm 7	109 \pm 5	232 \pm 8	23 \pm 2	1.16 \pm 0.05	87.5 \pm 0.4	376 \pm 17	52 \pm 2
A3	41 \pm 2	208 \pm 3	1134 \pm 79	238 \pm 7	9.8 \pm 0.2	84 \pm 2	1228 \pm 35	115.5 \pm 0.2
A4	2492 \pm 36	130 \pm 2	5414 \pm 153	425 \pm 7	73 \pm 0.07	854 \pm 20	2677 \pm 36	36 \pm 1
A5	4559 \pm 118	56 \pm 1	7534 \pm 219	343 \pm 12	0.94 \pm 0.02	1147 \pm 41	2713 \pm 82	46 \pm 2
A6	2424 \pm 55	118 \pm 4	5915 \pm 18	522 \pm 9	10.3 \pm 0.4	808 \pm 11	3004 \pm 28	40 \pm 1
A7	3663 \pm 81	302 \pm 15	1610 \pm 25	265 \pm 10	11.0 \pm 0.2	2112 \pm 29	3015 \pm 74	141 \pm 6
A8	2635 \pm 79	268 \pm 14	997 \pm 30	60 \pm 2	1.49 \pm 0.07	1681 \pm 62	1820 \pm 57	129 \pm 29
A9	5771 \pm 107	73 \pm 1	11882 \pm 474	624 \pm 8	0.31 \pm 0.02	1862 \pm 13	3923 \pm 62	49.6 \pm 0.4
A10	2456 \pm 33	344 \pm 11	1539 \pm 21	220 \pm 3	5.6 \pm 0.1	1211 \pm 16	1927 \pm 16	179 \pm 38
A11	2909 \pm 80	187 \pm 9	3244 \pm 62	575 \pm 37	22 \pm 2	991 \pm 34	2511 \pm 89	108 \pm 9
A12	6000 \pm 156	77 \pm 2	10252 \pm 234	584 \pm 19	0.40 \pm 0.01	1593 \pm 44	3653 \pm 86	52 \pm 1
A13	8726 \pm 184	3.6 \pm 0.4	19164 \pm 246	771 \pm 19	0.080 \pm 0.009	2772 \pm 77	7699 \pm 164	29 \pm 1
A14	13475 \pm 212	36 \pm 11	18818 \pm 548	784 \pm 21	1.0 \pm 0.2	2680 \pm 63	8073 \pm 181	94 \pm 3
A15	2854 \pm 180	42 \pm 2	7767 \pm 638	527 \pm 31	0.69 \pm 0.05	1128 \pm 56	1769 \pm 108	46 \pm 3

Higher concentrations of As were observed for rice-based food samples (A1, A3 and A7), which can be correlated with the known accumulation of As in rice. Unlike other cereals, rice is generally grown in flooded soils (anaerobic conditions with excess water) that provide mobilization of As and, consequently, a high accumulation of this element in the plant [28]. On the other hand, high Sn concentrations were observed for samples A5, A9, A12 and A15, all classified as infant formula. This behavior can be correlated with the metallic packaging of these formulations, known as one of the main sources of contamination of inorganic Sn [7].

For determination of macro and microelements (Table VIII), the concentration ranges determined in infant foods and supplements samples were 41 to 13475 mg kg⁻¹ for Ca; 3.6 to 344 mg kg⁻¹ for Fe; 232 to 19164 mg kg⁻¹ for K; 23 to 784 mg kg⁻¹ for Mg; 0.080 to 11.0 mg kg⁻¹ for Mn; 84 to 2772 mg kg⁻¹ for Na; 376 to 8073 mg kg⁻¹ for P, and 29 to 179 mg kg⁻¹ for Zn.

According to the recommended daily intakes (RDI) from RDC no. 269 [12] and the average concentrations of Ca, Fe, K, Mg, Mn, P and Zn in samples, it is possible to assign a minimum mass value of food that meets the recommendations of the Brazilian resolution for different population groups. To do so, the average concentrations of the analytes determined in the samples were grouped according to children's food indication and then a mean value was calculated for each element. Table IX shows the recommended daily intake (RDI), minimum mass of food that meets the RDI for each analyte according to the population group (infants and children) considering the mean concentrations calculated based on indication. It was observed that the intervals of minimum mass of food for infants from 0 to 6 months, infants from 7 to 11 months, children from 1 to 3 years old and children from 4 to 6 years old, are respectively from: 4 to 83 g, 38 to 161 g, 9 to 559 g, and 37 to 609 g, respectively. Considering a 5 g portion of sample, it was possible to calculate an average percentage of recommended daily intake (%RDI) for the foods indicated for infants from 0 to 6 months of 6% for Ca; 91% for Fe; 10% for K; 6% for Mg; 136% for Mn; 11% for P; and 8% for Zn. For a 30 g portion of sample, it was also possible to calculate an average %RDI for the foods indicated for infants from 7 to 11 months, children from 1 to 3 years old and children from 4 to 6 years old ranged from 21 to 28% for Ca; 72 to 89% for Fe; 5 to 19% for K; 17 to 20% for Mg; 22 to 35% for Mn; 19 to 29% for P; and 51 to 80% for Zn. These results evidence the high nutritional potential of these foods.

Table IX. Mean concentration, recommended daily intake (RDI), minimum mass of food (g) and average percentage of recommended daily intake for infants (0-6 months and 7-11 months) and children (0-3 years and 4-6 years) according to the chemical element

Element	Mean concentration (mg kg ⁻¹)				RDI ^a (mg day ⁻¹)			
	0-6 months	7-11 months	1-3 years	4-6 years	0-6 months	7-11 months	1-3 years	4-6 years
Ca	3707	3716	3779	4103	300	400	500	600
Fe	49	215	178	162	0,27	9	6	6
K	7651	4349	5363	6240	400	700	3000	3800
Mg	435	358	369	405	36	53	60	73
Mn	1	7	13	11	0.003	0.6	1.2	1.5
Na	1138	1523	1317	1380	N/A	N/A	N/A	N/A
P	2241	2669	3106	3211	100	275	460	500
Zn	46	109	94	87	2.8	4.1	4.1	5.1

Element	Minimum mass of food (g)				%RDI			
	0-6 months	7-11 months	1-3 years	4-6 years	0-6 months	7-11 months	1-3 years	4-6 years
Ca	81	108	132	146	6	28	23	21
Fe	6	42	34	37	90	72	89	81
K	52	161	559	609	10	19	5	5
Mg	83	148	163	180	6	20	18	17
Mn	4	87	9	136	135	34	33	22
Na	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P	45	103	148	156	11	29	20	19
Zn	61	38	44	59	9	80	69	51

^aResolution no. 269, of September 22, 2005 [12].

Dairy products, such as milk, yogurt and cheese, are the most Ca-rich foods in Western diets. Although grains are not particularly rich in calcium, the use of calcium-containing additives in these foods accounts for a substantial proportion of the calcium ingested by people who consume large amounts of grains [29]. In the analyzed samples, Ca occurred in higher quantities in samples A13 and A14: 8726 and 13475 mg/kg, respectively.

Manganese presents the lowest concentrations determined for all samples. Manganese (Mn) is an essential element, but it can also be toxic when in high concentrations. The essentiality of Mn is reflected in national and international policies on formulas and foods for children, which stipulate minimum concentrations of Mn. Infant formula regulations have not yet been adjusted to reflect this growing body of research on neurotoxicity of Mn; while the maximum content of Mn is regulated for baby formulas in some jurisdictions, others do not establish a maximum content for Mn in formulas for children and babies [30].

Samples A1, A7, A8, A10 and A11 had the highest concentrations of Fe. As bovine milk is a poor source of bioavailable Fe, its use is not recommended for babies under 1 year. Inadequate early intake of cow's milk is associated with an increased risk of Fe deficiency anemia. Pediatric societies have concluded that babies who are not or are only partially fed human milk should receive an Fe-enriched formula. Supplementation is also recommended for premature babies, as their Fe stores are low [30].

Comparison with other methods

Due to the importance of monitoring elemental concentrations in infant foods, procedures for determination of macro, micro and toxic elements in children's food have been developed [31-33] Table X shows a comparison of different procedures for those determinations, highlighting a similar type of sample, cereal (porridge) rice-based, and studied elements among the cited papers [31-33].

Table X. Some procedures for determination of macro, micro and toxic elements in infant food samples

Type of children's food	Method	Element	Calibration strategy	Sample preparation	Concentration range in cereal rice-based (mg kg ⁻¹)	Reference
Infant food rice-based	ICP-MS	As, Ca, Cd, Fe, K, Mg, Mn, Na, Pb, Sn, Zn	No calibration strategy evaluated	0.3-0.5 g sample mass. Microwave digestion (concentrated HNO ₃) and dilution with distilled-deionized water	As: 0.08 ± 0.02	[31]
					Ca: 4454 ± 1797	
					Cd: 0.007 ± 0.005	
					Fe: 65 ± 32	
					K: 4024 ± 809	
					Mg: 535 ± 104	
					Mn: 0.9 ± 0.1	
					Na: 2756 ± 758	
					Pb: 0.18 ± 0.02	
					Sn: 0.05 ± 0.04	
					Zn: 35 ± 9	

Table X. Some procedures for determination of macro, micro and toxic elements in infant food samples (Continuation)

Type of children's food	Method	Element	Calibration strategy	Sample preparation	Concentration range in cereal rice-based (mg kg ⁻¹)	Reference
Infant formula and infant food	ICP-MS	As, Ca, Cd, Fe, Mg, Mn, Pb and Zn	No calibration strategy evaluated	1 g sample mass. Microwave-assisted digestion (2 mL 65% HNO ₃ + 3 mL deionized water) and dilution with 20% HNO ₃	As ^a : 33.0 ± 0.6 Ca: 38.0 ± 0.6 Cd ^a : 1.70 ± 0.04 Fe: 1.2 ± 0.6 Mg: 127 ± 1 Mn: 3.16 ± 0.01 Pb ^a : 1.2 ± 0.1 Zn: 1.50 ± 0.01	[32]
Infant food and other rice-based products	ICP-MS	As, Cd, Fe, Mn, Pb and Zn	Use of IS (In, Y, Ge, Sc) but without prior analysis	0.15 g sample mass. Digestion block (1.5 mL of 65% HNO ₃ + 1 mL of H ₂ O ₂ 30%) and dilution with distilled-deionized water	As: 0.10 ± 0.04 Cd: 0.012 ± 0.007 Fe: 20 ± 94 Mn: 11 ± 4 Pb: 0.03 ± 0.02 Zn: 85 ± 46	[33]
Infant food, infant formula and children's supplements	ICP-MS and ICP OES	As, Cd, Sn, Pb, Ca, Fe, K, Mg, Mn, Na, P and Zn	Use of IS (Y, Ge, Pd) with prior analysis	0.5 g sample mass. Microwave-assisted digestion (5 mL of 7 mol L ⁻¹ HNO ₃) and dilution with distilled-deionized water	As: 0.09 ± 0.01 Cd: 0.009 ± 0.001 Pb: <0.031 Sn: < 0.025 Ca: 3663 ± 81 Fe: 302 ± 15 K: 1610 ± 25 Mg: 265 ± 10 Mn: 11.0 ± 0.2 Na: 2112 ± 29 P: 3015 ± 74 Zn: 141 ± 6	This study

^aConcentration in (µg kg⁻¹).

Similarly to the proposed procedure in reference [32], the procedure here developed also is based on a less concentrated nitric acid solution. According to these studies, concentrations of toxic elements (As, Cd and Pb) in cereal-based samples do not pose a risk to this consumer group, despite highlighting the importance of monitoring these elements to prevent health problems. This conclusion is also true to the samples here analyzed.

CONCLUSIONS

In this study, a tailored microwave-assisted sample preparation for supplements and infant foods using only 7 mol L⁻¹ nitric acid solution was developed. Precise and accurate determinations of As, Cd, Sn and Pb were performed using ICP-MS and Ca, Fe, K, Mg, Mn, Na, P and Zn using ICP OES. ICP-MS presents wide range of concentrations, however, thinking about laboratories that do not have the option of performing

analyzes by ICP-MS, since it is a more expensive instrumental method, we also evaluated the ICP OES performance. ICP OES can be considered a suitable analytical method for applying the Resolution no. 269 since it is less expensive and contamination issues during sample preparation are less critical when compared to ICP-MS. For ICP-MS, the combination of instrumental strategies based on aerosol dilution and CCT as well as the use of the calibration strategy with internal standardization, allowed minimum dilution of digests and the introduction of digests with TDS of up to 0.5% m/v and residual acidity of up to 1% v/v. Considering the limits for As, Cd, Sn and Pb recommended by RDC no. 193 and the RDI for Ca, Fe, K, Mg, Mn, P and Zn recommended by RDC no. 269, the developed analytical procedure using ICP-MS and ICP OES presented sufficient detectability to meet both Brazilian resolutions.

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Conflicts of interest

All authors declared that they have no conflict of interest.

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