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Short communication

A simple and fast ultrasound-assisted extraction procedure for Fe and Zn determination in milk-based infant formulas using flame atomic absorption spectrometry (FAAS)

Ignacio Machado, Gabriela Bergmann, Mariela Pistón *

Analytical Chemistry, Faculty of Chemistry, Universidad de la República (UdelaR), Av. Gral. Flores 2124, P.O. Box 1157, 11800 Montevideo, Uruguay

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ABSTRACT

A simple and fast ultrasound-assisted procedure for the determination of iron and zinc in infant formulas is presented. The analytical determinations were carried out by flame atomic absorption spectrometry. Multivariate experiments were performed for optimization; in addition, a comparative study was carried out using two ultrasonic devices. A method using an ultrasonic bath was selected because several samples can be prepared simultaneously, and there is less contamination risk. Analytical precision (sr(%)) was 3.3% and 4.1% for iron and zinc, respectively. Trueness was assessed using a reference material and by comparison of the results obtained analyzing commercial samples using a reference method. The results were statistically equivalent to the certified values and in good agreement with those obtained using the reference method. The proposed method can be easily implemented in laboratories for routine analysis with the advantage of being rapid and in agreement with green chemistry.

1. Introduction

Milk-based infant formulas are manufactured food that can be used as a substitute for breast milk in infants. Ideally, infants should be feed with breast milk for at least the first 6 months of life (World Health Organization, 2001a,b), but this is not always possible. Thus, manufactured food is important to meet infanťs minimum nutritional requirements in certain populations because of poverty and malnutrition.

Iron (Fe) and zinc (Zn) are essentials trace elements for infant nutrition, and milk-based infant formulas are supplemented by the addition of salts with acceptable bioavailability (MacLean et al., 2010).

Iron is an essential element for the development of vital functions. The World Health Organization considers iron deficiency the most common and widespread nutritional disorder in the world, with high prevalence in infants (World Health Organization, 2001a,b).

Zinc is also an essential trace element, and low Zn intakes may cause growth stoppage and damages in the immune system (Roohani, Hurrell, Kelishadi, & Schulin, 2013).

The potential interaction between Fe and Zn has been a cause of concern and must be taken into account in these formulations. High doses of inorganic Fe decreases Zn uptake (Roohani et al., 2013).

Salts providing readily absorbable Fe and Zn, such as sulphate, gluconate or acetate, are recommended for infant formula since they are water-soluble (Allen, de Benoist, Dary, & Hurrell, 2006).

Analytical methods for infant formulas have been discussed previously (MacLean et al., 2010). The classical AOAC standard methods require concentrated acids and high temperature for matrix digestion, which is not in agreement with the principles of green chemistry. The analytical determination of Fe and Zn in milk and infant formulas is often performed by flame atomic absorption spectrometry (FAAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES) (International Dairy Federation, 1986, 2000; AOAC, 1996a,b).

Development or selection of appropriate analytical methodologies must consider the trueness and precision of measurements, available facilities and equipment, simplicity of procedure and rapidity of determination. To accomplish this, drastic treatment of the matrix is not necessarily required and extraction under mild conditions may be adequate.

The use of ultrasound energy in liquid and solid media has been widely used in food-processing applications and trace elements determinations. Ultrasound-assisted methods for sample preparation are recognized as an efficient technique that significantly

* Corresponding author.
E-mail address: mpiston@fq.edu.uy (M. Pistón).

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reduces working times and acid consumption (Korn et al., 2008; Pistón, Silva, Pérez-Zambra, & Knochen, 2009; Seidi & Yamini, 2012).

Microwave-assisted extraction is another strategy for sample preparation that has been shown to be very efficient for Fe and Zn determination in milk and infant formulas. This methodology has the advantage of working with closed vessels, which reduces the risk of contamination (Nóbrega, Trevizan, Araújo, & Nogueira, 2002; Saracoglua, Saygib, Uluozlub, Tuzenb, & Soylakc, 2007), but is expensive, consumes more energy and is not as safe and fast as the ultrasound-assisted procedures.

The goal of this work was the optimization and validation of a very simple and rapid ultrasound-assisted method, using an ultrasonic bath, for Fe and Zn determination simultaneously in milk-based infant formulas. In addition, a comparative study was performed by applying the proposed method using two different ultrasonic devices (probe and bath). To the best of our knowledge, a method as simple and rapid for quality control as the proposed approach (without external heat and using diluted acid) for the determination of Fe and Zn in infant formulas has not been reported before.

2. Materials and methods

2.1. Reagents

All reagents were made up or diluted with ultrapure water (18.2 MΩcm resistivity) obtained from a Millipore Simplicity 185 purifier (São Paulo, Brazil).

A 1000 mg L^{-1} Zn standard solution was prepared from Zn metal (Aldrich, 99.99%) dissolved in hydrochloric acid 1 + 1 (v + v). A 1000 mg L^{-1} Fe standard solution was prepared from Mohr's salt (ferrous ammonium sulphate hexahydrate, Baker).

All other reagents were of analytical reagent grade.

All glassware was soaked overnight in 10% (v/v) nitric acid and then rinsed with ultrapure water.

2.2. Instruments

Ultrasound devices: Cole-Parmer 8893 bath (Ultrasonic Cleaners) 47-kHz; 230 VAC and Ultrasonic homogenizer (Sonics vibracell) 750-Watt; 20-kHz; 230 VAC equipped with a 13-mm titanium alloy probe.

A PerkinElmer AAnalyst 200 (Norwalk, CT, USA) atomic absorption spectrometer (air-acetylene flame) operated at the 248.33 nm (Fe) and 213.86 nm (Zn) analytical lines was used for the analytical determinations.

2.3. Samples

Commercial infant formulas products available in local markets (Montevideo, Uruguay) were purchased for analysis. A Standard Reference Material (SRM) – NIST 1846 Infant Formula – was used for optimization and to assess the trueness and precision of the analytical methods. The certified concentration values were: 63.1 ± 4.0 mg kg^{-1} (Fe) and 60.0 ± 3.2 mg kg^{-1} (Zn) (dry basis).

2.4. Sample preparation

2.4.1. Acid digestion (reference method)

Samples were prepared as follows: 0.5 g of the sample were accurately weighed in a glass vessel, and then 15 mL of concentrated nitric acid and 2 mL of 30% (w/w) hydrogen peroxide were added. The vessel was placed in a hot plate at 100 °C for 15 minutes, and then it was left to cool at room temperature. Afterwards, the content of the vessel was transferred quantitatively to a 25-mL volumetric flask and made up to volume with purified water. The resulting suspension was filtered using a 25-mm diameter, 0.45-μm PTFE membrane filter and the analytical determinations were carried out directly with the filtrate. Reagent blanks were also run.

2.4.2. Ultrasound-assisted method using a probe (method A)

For the optimized method 0.5 g of the sample were accurately weighed in a glass vessel and then 25.0 mL of nitric acid 15% (w/w) were added. Then the ultrasound probe was immersed in this suspension for 5 min (35% sonication amplitude). The analytical determinations were carried out directly using the slurry obtained. Reagent blanks were also run.

2.4.3. Ultrasound-assisted method using an ultrasound bath (method B)

For the optimized method 0.5 g of the sample were accurately weighed in a glass vessel and then 25.0 mL of nitric acid 15% (w/w) were added. The vessel was put into the ultrasonic bath for 5 min. The analytical determinations were carried out directly on the slurry. Reagent blanks were also run. Up to eight samples were processed simultaneously in the bath.

2.5. Optimization and validation

The influence of three variables (concentration of nitric acid, sonication amplitude and time) was studied for the method with the ultrasonic probe (A), by means of a three-level central composite design (Massart et al., 1997). In the case of the method using the ultrasonic bath (B), as the amplitude is fixed in the instrument, the design was performed varying the acid concentration and the sonication time.

Precision and trueness of the selected method was evaluated by means of perform the selected method using the reference material ($n = 5$) and comparing the results obtained for commercial samples performing an acid digestion (reference method).

3. Results and discussion

3.1. Optimization

Considering the concentration of Fe and Zn commonly found in the commercial product, 0.5 g of sample and a volume of 25 mL were adequate for the proposed methods.

The calibration curves presented a linear fit through zero up to 3.0 mg L^{-1} and 1.0 mg L^{-1} for Fe and Zn respectively. Depending on the metal content of the commercial product, some required further dilution with ultrapure water.

The conditions tested by means of a three-level central composite design to achieve the optimal experimental conditions are described in Tables 1 and 2.

The response to select the optimal conditions was the recovery percentage estimated as R (%) = obtained concentration (mg k^{-1}) × 100/certified concentration (mg k^{-1}) for each experiment. The most simple and rapid experiment with an R (%) statistically equal to 100% (for both elements) was selected as optimal for subsequent validation.

In order to verify if the reference material was within specifications for Fe and Zn, content and as comparative method for the analysis of commercial samples, an acid digestion was carried out as detailed in Section 2.4.1. This procedure was validated previously (Machado, Pistón, & Bühl, 2013) and the performance shown to be statistically equivalent to the standard AOAC method 985.35 but quicker.

Table 1
Central composite design for method A.

Experiment	HNO_3 (% w/w)	Sonication time (min)	Sonication amplitude (%)
1	5	5	20
2	5	5	35
3	5	10	10
4	5	10	35
5	10	8	25
6	15	5	20
7	15	5	35
8	15	10	20
9	15	10	35

Table 2
Central composite design for method B.

Experiment	HNO_3 (% w/w)	Sonication time (min)
1	5	5
2	5	10
3	10	8
4	15	5
5	15	8

Table 3
Results corresponding to the optimization experiments for method A.

Experiment	% *R* (Fe) (mean%; *n* = 3)	% *R* (Zn) (mean%; *n* = 3)
1	<60	<90
2	<60	<90
3	<60	<90
4	<60	<90
5	85.2	94.6
6	87.5	94.5
7	**95.9**	**98.0**
8	95.4	98.2
9	96.4	97.6

Table 4
Results corresponding to the optimization experiments for method B.

Experiment	% *R* (Fe) (mean%; *n* = 3)	% *R* (Zn) (mean%; *n* = 3)
1	<60	<90
2	<60	<90
3	83.2	91.4
4	**101.2**	**99.7**
5	85.2	94.6

The results obtained for methods A and B are summarized in Tables 3 and 4, respectively.

For method A, the experiments 1–4 were not adequate since the recoveries were low. This shows that the use of nitric acid 5% (w/w) was not enough for a quantitative extraction. Experiment 7 had the best performance for both elements in the minimum time.

In the case of method B, optimum conditions occurred in experiment 4 where an acid concentration of 15% (w/w) and 5 minutes sonication were enough for a quantitative extraction of both elements.

The efficiency of the treatment using dilute acids was in good agreement with the fact that common Fe and Zn salts added to these products have good solubility in water. Milk-based infant formulas have a proportion of powdered cow's milk, meaning ultrasound plus dilute acid were required for the extraction of Fe and Zn, but drastic treatments are not justified.

Table 6
Fe and Zn contents in commercial infant formula (dry basis). LC: Label claim; AD: Acid digestion; PM: proposed method; s: standard deviation (*n* = 3).

Sample	Fe (mg kg^{-1})			Zn (mg kg^{-1})		
	LC	AD	PM	LC	AD	PM
A	91	94.4 ± 1.3	95.4 ± 1.1	38	37.3 ± 1.0	39.4 ± 0.9
B	30	27.9 ± 0.6	28.2 ± 0.4	40	39.3 ± 0.7	38.0 ± 1.1
C	89	86.8 ± 1.5	88.0 ± 0.8	46	46.7 ± 0.6	45.7 ± 0.4
D	95	92.0 ± 1.9	93.7 ± 0.6	39	41.8 ± 0.9	40.2 ± 0.5
E	83	84.1 ± 2.0	84.1 ± 1.1	39	38.1 ± 1.5	37.6 ± 1.2

3.2. Method comparison and validation

To establish whether there was a difference between using an ultrasonic probe or an ultrasonic bath, method A and B where compared under the optimal conditions (experiment 7 for method A and 4 for method B).

The results presented in Table 5 show that the proposed methods performed well in terms of trueness and precision. All the experimental *t* values were below the theoretical *t* (0.05, 4) 2.78. Thus, at the 95% significance, concentrations obtained using either method A or B did not differ significantly from the certified value, and the trueness of these methods is ensured.

Some advantages for method B can be highlighted. When an ultrasound probe is employed, only one sample at a time can be prepared, and the contamination risk is high because the probe is immersed into the solution. On the other hand, when an ultrasonic bath is used, several samples can be prepared at the same time. Moreover, the proposed method can be performed at room temperature with instruments commonly found in laboratories.

For the reasons stated above, method B was the most advantageous.

Analytical precision for method B (sr(%)) was 3.3% for Fe and 4.1% for Zn (*n* = 5).

Trueness for method B was evaluated using a *t* test and analyzing the reference material, as described above and by analyzing commercial samples using a reference method (Table 6).

These results were also compared with the label claim for quality control purposes.

Five commercial brands of milk-based infant formulas representative of the Uruguayan marker were purchased locally, three of them for infants from 0 to 6 months or for infants from 6 to 12 months and two generic ones.

The results presented in Table 6 show that the concentration levels of Fe and Zn obtained using the proposed method are equivalent to those obtained by means of a wet acid digestion (reference method) and they agreed within 10% with the label claims.

Table 5
Metal contents found in reference material and comparison with reference value by Student's *t*-test; s: standard deviation; *t* (0.05, 4) = 2.78 (Miller & Miller, 1993).

	Certified value	Method A (mean ± s; *n* = 5)	Method B (mean ± s; *n* = 5)	*t*-Experimental
Fe (mg kg^{-1})	63.1 ± 4.0	69.1 ± 4.6	63.8 ± 2.1	2.45 (A)/0.23 (B)
Zn (mg kg^{-1})	60.0 ± 3.2	60.6 ± 1.9	59.8 ± 0.8	1.56 (A)/0.80 (B)

4. Conclusions

A simple and rapid ultrasound-assisted method for the determination of Fe and Zn in infant formulas was optimized and validated. It is in good agreement with the principles of green chemistry because the method uses only dilute acid and an ultrasonic bath. It can be postulated as an alternative to standard quality control procedures for assuring nutrient content of infant formulas.

Compliance with ethics requirements

The authors declare no conflicts of interest.

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