Development and validation of ICP OES methodologies for the determination of metals in powder milk for in proficiency test applications

V Câmara¹,², T Araújo², R Caciano de Sena², A Osorio² and M Almeida²
¹ Postgraduate Programme in Biotechnology, INMETRO, BR
² Chemical and Thermal Metrology Division, National Institute of Metrology, Quality and Technology, INMETRO, BR
E-mail: vitoria.fontes.camara@gmail.com

Abstract. The main purpose of this work was the development and validation of methods for measuring the macro and microelements (Ca, Na, Mg, P, Fe, and Zn) in powder milk by inductively coupled plasma optical emission spectrometry (ICP OES). The validated methods were applied for measuring these elements in commercial samples in order to choose a suitable mix sample to prepare a reference material batch and use it for organizing a proficiency test. This comparison will be used to evaluate the performance of national laboratories for measuring the mass fraction of essential elements in powder milk. Samples were digested in a microwave reaction system and the elements were measured using the external calibration approach. The standard uncertainty for the mass fractions of Mg, P, Fe and Zn ranged from 1.2% to 2.8%. The method accuracy was evaluated using certified reference material (SRM 1849a) from the National Institute of Standards and Technology (NIST). The results obtained from the SRM 1849a and the measured values with the proposed methodologies was checked, and the results obtained for Mg, P, Fe, and Zn were considered consistent with the certified values, but not for Ca and Na. In this case, we need to perform additional studies in order to reevaluate these last two methods. After we checked the performance of methods, five commercial samples were chosen to determine the mass fraction of Mg, P, Fe, and Zn. Among these samples, in only one all elements were present at a suitable mass fraction for organizing a proficiency test.

Keywords. Measurement of essential inorganic elements, ICP OES, milk powder, method validation, proficiency test and ISO / IEC 17025.

1. Introduction

Milk is a product of animal origin, which is part of the Brazilians’ dietary intake and also present in the economic sector of Brazil. The country occupies the fourth place in a world ranking, as a producer of milk [1]. And in 2018, the Extended National Consumer Price Index (IPCA) of dairy products found that Brazilians have been consuming more milk powder than other dairy drinks over the years. [2]. This product has always had space in the nutrition
tables as part of the reconstructive foods group, and has recently gained more attention after the Brazilian Ministry of Health, through its Food Guide, recommended milk in three portions per day and considered it a food of high nutritional density [3], as it has a high concentration of nutrients in relation to its caloric content [4].

The inorganic macro and microelements present in this food perform several daily functions in our body, which are of interest in this work, Ca, Na, Mg, P, Fe and Zn, which act on bone and teeth formation, muscle contraction, in the constitution of the structure of body tissues, in the control of nerve impulses and in the activation or inhibition of food enzymes, among other functions [5]. These reasons underscore the importance of these elements for a healthy life, since the lack of these inorganic elements coming from fresh milk itself or coming from milk powder can cause serious damage to the body [5]. On the other hand, if ingested in excess, one mineral can cause the inhibition of the absorption of another one [5].

The use of the technique by Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) is intended to validate the methods and establish analytical performance for parameters such as accuracy, precision and detection limit [6], meeting ISO/ IEC 17025: 2017 requirements [7]. Another great advantage of the technique is that many elements can be easily determined in the same analytical run including non-metals such as phosphorus, increasing the flexibility of the analysis. Today more than 40 laboratories accredited by Inmetro [8] have access to this technique and their satisfactory performance in proficiency test activities is an indication of the laboratory’s competence to perform certain tests and calibrations in a specific matrix.

For these reasons, in order to provide tools for assessing milk quality, this work aims to validate measurement methods for Ca, Na, Mg, P, Fe and Zn elements in milk powder using ICP-OES. The developed methods will be used to assign the reference values of the aforementioned elements in a milk reference material (MR) mix batch. This MR will be used to organize and evaluate the performance of national laboratories through a proficiency test (CI) based on ISO / IEC 17025: 2017 [5].

2. Materials and Methods

2.1. Instrumentation

All experiments were performed using ICP OES, Ultima 2 (Jobin Yvon-Horiba) inductively coupled plasma optical emission spectrometer using argon 5.0. Sample digestion was performed in PFA vessels using a Microwave Reaction System (Multiwave PRO (Anton Paar)).

2.2. Reagents and standard solutions

All solutions were prepared with Grade 1 water (18 MΩ cm, Milli-Q®, Millipore). Hydrogen peroxide Suprapur® (Merck, Germany) and nitric acid sub-distilled in a Duo-PUR quartz distiller were used in the sample digestion procedure. Standard analytical solutions were prepared from 10,000 mg kg⁻¹ single-element certified calibration solutions of Ca (SRM 3109a, NIST), Na (SRM 3152a, NIST), Mg (SRM 3131a, NIST), P (SRM 3139a, NIST), Fe (SRM 3126a, NIST), Zn (SRM 3168a, NIST). For quality control, an infant/Adult Nutritional Formula I – Milk CRM, (SRM 1849a, NIST) was used.

2.3. Certified reference materials and sample preparation

Sample masses of 350 mg were weighed directly into PFA vessels, followed by the addition of 4 mL HNO₃ and 2 mL of H₂O₂. The digestion was using a power program consisting of seven steps as follows: (1) 500 W , for 10min on ramp; (2) 10 min retained at 500W; (3) 1,000W ramp for 5min; (4) 5 min kept at 1,000W; (5) ramp for 10 min to reach 1,300W; (6) 10 min at 1,300W; (7) cooling the system to the drop in these temperature and
pressure conditions (20 to 30 bar) the resulting solutions were transferred to polyethylene tubes and the volumes of up to 50.00 mL with grade water 1. Five commercial samples of milk powder were evaluated in this work. A similar procedure was applied to the certified reference material SRM 1849a.

2.4. Standards preparation and measuring procedure

The ICP OES quantification procedure was performed in a plasma radial view with 1350 W radiofrequency power, sample flow rate 2.5 L min⁻¹, plasma gas flow rate 20 L min⁻¹, auxiliary gas flow rate 0.0 L min⁻¹, nebulizer gas flow rate 0.02 L min⁻¹, integration time 20 s, stabilization time 15 if nebulization pressure 2.55 psi. Measured wavelength were: Calcium (317.933 nm), Sodium (589.599 nm), Magnesium (279.077 nm), Phosphorus (213.618 nm), Total iron (259.940 nm), Zinc (213.856 nm).

An intermediate solution (100 mg kg⁻¹) was gravimetrically prepared for each element by selecting the desired single-element calibration solution. HNO₃ (2%, v/v) was used as diluent. All solutions were stored protected from light and moisture. From this intermediate solution, all the calibration curves for each element were gravimetrically prepared to measure the elements present in the milk powder samples and in the CRM by ICP-OES.

Calibration curves used in this work ranged from: 3 to 13 mg kg⁻¹ for Ca, 0.5 to 9 mg kg⁻¹ for Na, 0.6 to 8.6 mg kg⁻¹ for Mg, 2 to 12 mg kg⁻¹ for P, 40 to 240 μg kg⁻¹ for Fe, 47 to 57 μg kg⁻¹ for Zn.

3. Results and discussion

3.1. Evaluation of the linearity of the calibration curves

For the adjustment of the calibration curve, the least squares model was used and based on the coefficients of determination (R²) obtained in the linear regression of the data set, it was possible to infer that the linear fit was a suitable model (R² ≥ 0.995).

The residual analysis was used also to check the adjustment of the calibration curves for the measurement of the mass fractions of Ca, Na, Mg, P, Fe, and Zn by ICP OES. The residual was appropriately arranged randomly and without an excess of trend, indicating that the variance remains homogeneous as a function of the mass fraction of each standard solution. As an example, in figure 1 we present a linear and residual plot for the determination of iron.
3.2. Limits of detection (LOD), limit of quantification (LOQ) and precision

Table 1 shows the detection limit (LOD) and the quantitation limit (LOQ) for the methods, and was calculated according to the equations 1 and 2, where \( DP \) is the standard deviation from blank intensities and \( fd \) is the mean dilution factor of the CRM obtained from a sample prepared in triplicate.

\[
LOD_{\text{method}} = \frac{3 \times DP_{\text{blank}}}{\text{inclusion}} \cdot f_d_{\text{samples}} \quad (1)
\]

\[
LOQ_{\text{method}} = \frac{10 \times DP_{\text{blank}}}{\text{inclusion}} \cdot f_d_{\text{samples}} \quad (2)
\]

Another important parameter for the validation of the method is the precision, which in this work was estimated to be the repeatability of the methods, calculated according to Equation 3, where \( DP \) is the standard derivation from the intensities which the samples analyzed in triplicate (n).

\[
% = \left( \frac{DP_{\text{intensit.}}}{\text{Median}_{\text{intensit.}}} \cdot \frac{1}{n} \right) \cdot 100 \quad (3)
\]

Table 1: Limits of detection (LOD), limits of quantification (LOQ) and precision for method of measurement of the mass fractions of macros and microelements.

| Analyte | LOD       | LOQ       | Precision |
|---------|-----------|-----------|-----------|
| Ca      | 18.08 mg kg\(^{-1}\) | 60.26 mg kg\(^{-1}\) | 0.25 – 0.45 % |
| Na      | 4.99 mg kg\(^{-1}\)   | 16.65 mg kg\(^{-1}\)   | 0.36 – 0.68% |
| Mg      | 0.43 mg kg\(^{-1}\)   | 1.45 mg kg\(^{-1}\)   | 0.42 - 0.48 % |
| P       | 4.49 mg kg\(^{-1}\)   | 14.97 mg kg\(^{-1}\)   | 0.24 - 0.82 % |
| Fe      | 2.18 mg kg\(^{-1}\)   | 7.29 mg kg\(^{-1}\)   | 0.45 - 1.0 % |
| Zn      | 2.53 mg kg\(^{-1}\)   | 8.43 mg kg\(^{-1}\)   | 0.80 - 1.3 % |

The accuracy of method was evaluated comparing the certified values from the SRM 1849a and the measured values. Table 2 shows the certified values of SRM 1849a and the measurement results for Ca, Na, Mg, P, Fe and Zn with the expanded uncertainties.

Table 2: Results of the analyses of the essentials elements by ICP OES.

| SRM     | Element | Certified value (mg kg\(^{-1}\)) | Measured value (mg kg\(^{-1}\)) |
|---------|---------|----------------------------------|---------------------------------|
| NIST 3109a | Ca      | 5253 ± 51                        | 4891.88 ±60.4                  |
| NIST 3152a | Na      | 4265 ± 83                        | 4498.09 ± 70.6                  |
| NIST 3131a | Mg      | 1648 ± 36                        | 1656.13 ± 20.9                  |
| NIST 3139a | P       | 3990 ± 140                       | 3926.45 ± 64.4                  |
| NIST 3126a | Fe      | 175.6 ± 2.9                      | 180.92 ± 3.5                    |
| NIST 3168a | Zn      | 151.0 ± 5.6                      | 151.69 ± 4.2                    |


The consistency between the results obtained through the proposed method and certified values from SRM 1849a was evaluated using equation 4 [9].

\[ |X_{\text{ref}} - X_{\text{med}}| \leq k \cdot \sqrt{u_{\text{ref}}^2 + u_{\text{med}}^2} \]  

(4)

The observed difference between the measured value and the declared property value in the certificate must be less than the standard uncertainty associated with the difference, under this condition the result obtained for the SRM shows agreement of the results obtained from the measurement procedure with the certified values. In accordance with equation 4 [9], the results obtained for the mass fraction of Mg, P, Fe and Zn were consistent with the certified values, but not for Ca and Na.

Based on these results, the methods for measuring the mass fraction for Mg, P, Fe, and Zn were considered adequate to perform the additional studies for preparing a batch of reference material for organizing a proficiency test. Data obtained for measuring Ca and Na show that it is necessary to perform additional studies.

3.3. Evaluation of commercial milk powder

Five commercial samples of whole milk powder from four different brands were collected, one of the brands sampled from two different lots to check if there was significant difference. Commercial samples were analyzed using the validated methods and the samples were codified as: AQ 228, AQ 257, AA 249, AG 01 and AI 01. The only sample that showed mass fractions of Mg, P, Fe and Zn larger than LOQ was AI 01 which is iron-fortified. The mass fraction for iron in the samples AQ 228, AQ 257, AA249, and AG 01 were below the detection limit. Table 3 shows the mass fraction for Mg, P, Fe and Zn in the sample AI 01.

Table 3: Results of the analyses of the essential elements in commercial sample AI 01 by ICP OES.

| Element | Determined value (mg kg\(^{-1}\)) |
|---------|---------------------------------|
| Mg      | 811.35 ± 17.6                   |
| P       | 7314.08 ± 178.7                 |
| Fe      | 94.55 ± 2.1                     |
| Zn      | 32.10 ± 0.62                    |

4. Conclusion

The methods for measuring Mg, P, Fe, and Zn in powder milk by the ICP OES technique were found to be satisfactory in accordance with the requirements of ISO / IEC 17025: 2017. Thus, the methods are suitable to assign the reference values of those metals in a powder milk RM for further proficiency test applications. Additional studies are necessary to improve the accuracy of methods for measuring the mass fraction of Ca and Na.

The developed methods were applied to the analysis of 5 commercial samples of powder milk. A commercial sample had quantifiable mass fractions of Mg, P, Fe and Zn, hence, it was identified as suitable for preparing a reference material mix batch. This reference material will be used to organize a proficiency test.
References

[1] BRAZILIAN COMPANY OF AGRICULTURAL RESEARCH - EMBRAPA 2016, Livestock Milk - Economic Importance.

[2] CEPEA (Center for Advanced Studies in Applied Economics) 2018- Broad National Consumer Price Index (IPCA) of dairy products and official inflation - Cumulative variation in 2017, organized by Embrapa Gado de Leite

[3] Brazil. Ministry of Health (MS) 2006. Health Care Secretariat. General Coordination of the Food and Nutrition Policy. Food Guide for the Brazilian population: promoting healthy eating. Brasilia: MS

[4] DREWNOWSKI A 2010. The nutrient-rich foods index helps to identify healthy, affordable foods. Am J Clin Nutr.; 91 [suppl]: 1095S-101S.

[5] Dietary reference intakes (DRI) 2018: Application of tables in nutritional studies. Preprint: http://www.guiadenutricao.com.br/minerais/2013-2018.

[6] MERMET J M 2005 “Is it still possible, necessary and beneficial to perform research in ICP-atomic emission spectrometry?”. J. Anal. At. Spectrom. 20 p 11.

[7] ABNT NBR ISO/IEC 17025:2017 – Norma para requisitos gerais para competência de laboratórios de ensaio e calibração. Rio de Janeiro.

[8] List of Laboratories in Chemical Testing in Food and Beverages, by ISO 17025:2017. Preprint: http://www.inmetro.gov.br/laboratorios/rble/lista_laboratorios.asp?sigLab=CRL&ordem=&titul oLab=&uf=&pais=&descr_escopo= milk & test_class = 006 & activity_activity = 8 & search_type = & page = 3. Accessed on: 25/07/2019

[9] ISO GUIDE 33 2015, Reference materials - Good practice in using reference materials