RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

# Determination of micro and macro elements in iron supplements used for treatment of anemia and evaluation employing chemometric analysis tools

Uenderson Araujo Barbosa[a,b,c], Ivanice Ferreira dos Santos[a,b], Sergio Luis Costa Ferreira[a,b], Ana Maria Pinto dos Santos[a,b]

[a]Universidade Federal da Bahia, Instituto de Química, CEP 40170-270, Salvador-Bahia, Brazil.

[b]Instituto Nacional de Ciência e Tecnologia, INCT, de Energia e Ambiente, Universidade Federal da Bahia, 40170-290 Salvador-BA, Brazil.

[a]Universidade Federal da Bahia, Faculdade de Medicina, CEP 40110-100, Salvador-Bahia, Brazil.

Corresponding author's

Ana Maria Pinto dos Santos

E-mail: amps@ufba.br

Uenderson Araujo Barbosa

E-mail: uendersonaraujo@hotmail.com

Tel/Fax: 55-71-32355166

## Abstract

This paper propose a method using inductively coupled plasma optical emission spectrometry (ICP OES) for the determination of Ca, K, Mg, Mn, Na, P and Zn in iron supplement used for the treatment of anemia and evaluation of the results by chemometric analysis tools, principal component analysis (PCA) and hierarchical cluster analysis (HCA). Sample preparation was performed by acid digestion using 3.0 mL of $HNO_3$ and 1.0 mL of $H_2O_2$ (30% v/v). Limits of quantification (mg $L^{-1}$) were 0.52 for Ca, 0.14 for K, 0.03 for Mg, 0.07 for Mn, 0.40 for Na, 0.36 for P and 0.24 for Zn, showing that the method is sensitive for the determination of elements. There is no certified reference material of ferrous supplement for evaluation of the accuracy. Thus, addition/recovery tests were performed to evaluate the accuracy of the method. The recovery values achieved varied from 89.75 to 114.97%, confirming the applicability of this method for quantification of the mentioned elements in these iron supplements. The method proposed was applied for determination of Ca, K, Mg, Mn, Na, P and Zn in seventeen iron supplement samples with different chemical composition. All results were evaluated by multivariate analysis tools, which have the ability to characterize the samples by chemical composition and the analyte content. Some samples showed higher values for some metals, indicating the importance of specific legislation also for these metals.

**Keyword:** Macro and micro element; Iron supplement; Anemia; Multivariate analysis; ICP OES; PCA and HCA.

## 1. Introduction

Iron deficiency is the principal cause of nutritional anemia in humans, quite common in children and pregnant women. When it is severe, it causes anemia microcytic characteristic and hypochromic, secondary to reduction in hemoglobin synthesis. [1] Iron is a component of hemoglobin, and its deficiency may affect the metabolism of the muscles, the activity of mitochondrial enzymes, gas exchange and has shown an association with behavioral and learning problems in children.[2] The content of chemical elements in pharmaceutical products is a global concern. In general, during development and manufacturing of pharmaceuticals, the raw materials used in manufacturing these products, residues of catalysts derived from manufacturing processes may cause contamination with elemental impurities.[3] Other causes of contamination can be the use of improper production processes or unclean laboratories. Considering this, many papers have been developed involving the determination of toxic chemical elements in different pharmaceuticals. Rao & Talluri published a review paper reporting applications of inductively coupled plasma-mass spectrometry (ICP-MS) in determination of inorganic impurities in drugs and pharmaceuticals.[4] Recently, Lewen presented a comprehensive discussion about the use of atomic spectroscopy in the pharmaceutical industry for the determination of trace elements in pharmaceuticals. Advantages and drawback of this technique for this purpose were presented.[5] Støving *et al.* proposed a method for determination of elemental impurities in tablets pharmaceuticals by inductively coupled plasma optical emission spectrometry (ICP OES). Eighteen elements were determinated.[6] Barbosa *et al.* proposed a method using slurry sampling for determination of mercury in iron supplements. The mercury content varied from 3.17 to 34.86 ng $g^{-1}$.[7] Tanase *et al.* proposed a simple and fast method for routine determination of nickel in magnesium stearate using electrothermal atomic absorption spectrometry (ETAAS), after simple sample dissolution in acidified ethanol without mineralization.[8] Antes *et al.*, proposed the development of a method using ICP-MS for determination of impurities in compound utilized for parenteral nutrition.[9] Carvalho *et al.* evaluated the performance of the laser induced breakdown spectrometry for the determination of macro and micronutrients in pharmaceutical tablets.[10] Portugal *et al.* determined lead in aluminum and magnesium antacids using ETAAS.[11]

The determination of macro and microelements using ICP OES has been applied in several matrices. Dos Santos *et al.* determined the mineral composition of raw and cooked okra.[12] Anderson *et al.* evaluated the chemical profiling of coffee samples to differentiate their geographic growing origins.[13] The use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for the result analysis is an good strategy used as routine in the evaluated of chemical results. Barbosa *et al.* proposed the evaluation of metals and metalloids content in the Chapeu-de-Couro (*Echinodorus macrophyllus* (Kunth) Micheli) employing chemometric analysis tools.[14] Froes *et al.* proposed the use of exploratory analysis for evaluate of microelements in fruit juice determination by ICP OES. Other papers describe the application of these statistic tools for evaluation of chemical dates. [15] [16] [17]

The intake of macro and micro elements is associated to main foods used by anemic patients in daily diet and the intake of drugs are by a long periods of time, even after the recovered values of erythrocytes in the blood.[18] As drugs for treatment of anemia are potential sources of high level of chemical elements and because of long term use, the ingestion of large quantities may be harmful to health. These elements even macronutrient and non-toxic elements can have harmful effects for health if consumed at high levels.[22-25]

Therefore, this paper proposes a method using ICPOES for the determination of Ca, K, Mg, Mn, Na, P and Zn in iron supplement used for the treatment of anemia and evaluation of the results using the multivariate analysis techniques, PCA and HCA.

## 2. Experimental

### 2.1. Instrumentation

A Varian model Vista PRO inductively coupled plasma optical emission spectrometer (Mulgrave, Australia) with axial viewing and a charge coupled device detector was used for multi-element determination. A Sturman-Master chamber and a V-Groove nebulizer were also used. The instrument conditions and analytical lines for each element are given in Table 1. For sample preparation a digester block Model TE-040/25, TECNAL (São Paulo, Brazil) with closed vessels and electrical heating was used.

**Table 1**

### 2.2. Reagents and solutions

All chemical reagents used were of analytical grade and obtained from Merck (Darmstadt, Germany). Ultrapure water with resistivity of 18 MΩ cm was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). The nitric acid solutions were prepared using the reagent from Merck (Germany) that double distilled in a MILESTONE quartz distillation system. All containers and glassware were maintained in a 10 % v/v nitric acid solution for at least 12 h for decontamination before use. Calibration solutions for all elements were prepared daily by the serial dilution of stock solution (1000 mg $L^{-1}$ or 4000 mg $L^{-1}$) from Merck (Germany) with a 0.05% (v/v) nitric acid solution.

## 2.3. Sample collection and Preparation

Seventeen commercial samples of the iron supplements used in the treatment of iron deficiency anemia were purchased from pharmacies located in different cities of Brazil. Samples from different laboratories, have been maintained in their original containers, only were opened when they were used in the experiments. The mass 0.1 g of solid sample or 500 µL of liquid sample (approximately 0.5 g) were directly introduced in the digester tube. Then, 3 mL of concentrated bidistilled nitric acid and 1 mL of hydrogen peroxide were added. The system was closed with cold finger and heated to 150 ºC for 4 hours using heating block. Later, the contents were transferred to centrifuge tubes and topped up with ultrapure water to the 25 mL mark.

# 3. Results and discussions

## 3.1. Validation study according to the ICH Guideline specificity[19]

In the validation step, some analytical characteristics of the method used for metals quantification were evaluated. The linearity of any analytical methodology used for impurities quantification should be evaluated from the limit of quantification to the maximmum point of the calibration curve. The limits of detection (LD) and quantification (LQ) were determined based on ICH recommendations and IUPAC[20], calculated as 3δ/s and 10δ/s, respectively, where δ is the standard deviation of the blank solution and s the slope of the analytical curve employed. The precision (expressed as relative standard deviation RSD) was evaluated by intraday and intermediate precision (different days) with experiments using the same iron supplement samples used in the recovery studies. For evaluate the accuracy of the method, two additions were performed, more details and results are presented in Table 2. The robustness was evaluated by an Mg II/Mg I emission intensity ratio equal to or greater than 8, point to a robust plasma condition, wherein the ICP system able to accommodate alterations in the concentrations of major elements, acids, and other components, without any significant variation in the intensities of the analyte lines[21]. In this study, the intensity ratio calculated (to calibration curve and samples) was greater than 8. The results demonstrate that the proposed method is robust, sensitive and accurate for determination of the analytes.

**Table 2**

### 3.2 Determination of micro and macro elements in iron supplements

Seventeen samples from various pharmacies located in Brazil were prepared and analyzed. Inductively coupled plasma optical emission spectrometry was used to determine calcium, potassium, magnesium, manganese, sodium, phosphorus and zinc. The results are shown in Table 3 with concentrations expressed as mg of analyte per kg of sample.

**Table 3**

### 3.3. Principal component analysis

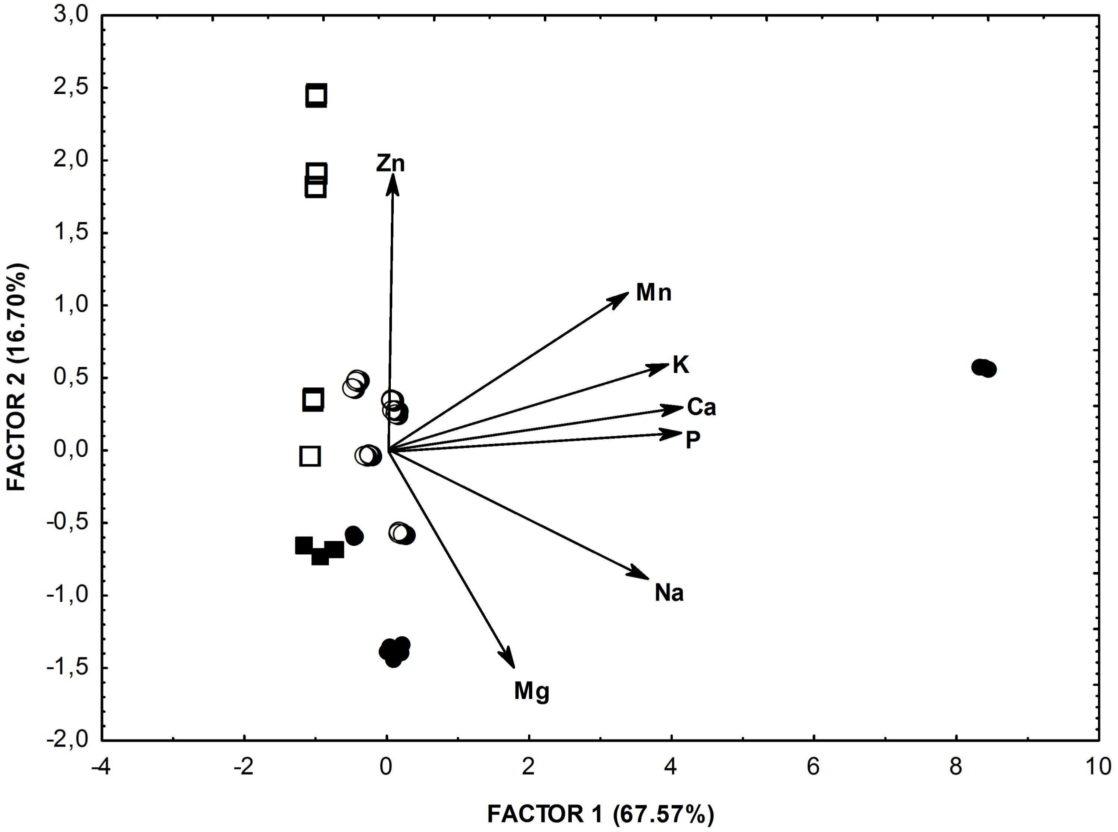
The results of the determination of the elements in the seventeen iron supplement samples analyzed in triplicate were evaluated using principal component analysis (PCA). The evaluation was performed on auto-scaled data because of different orders of magnitude in element concentrations. PCA was performed using cross-validation as a validation method, and after a normalized Varimax rotation, that maximizes the loading of the variables in a factor and minimizes it in all the others, 84.27% of the total variance was explained by two principal components (PCs), which show eigenvalues of >1.0 (4.72 and 1.16 for PC1 and PC2, respectively).

**Table 4**

From loadings of variables along the first two PCs listed in Table 4, Ca, K, Na and P concentrations are the dominating features in PC1, and which PC1 can explain 67.57% of total variance. These four elements contribute to the major variability presented in the iron supplement samples. The biplot graph of the first two components is shown in Figure 1.

**Figure 1 -** Biplot graph for PC1 and PC2 for samples. (*Iron supplement - organic tablets (●), inorganic tablets (❍), organic liquids (■), inorganic liquids*

(□).



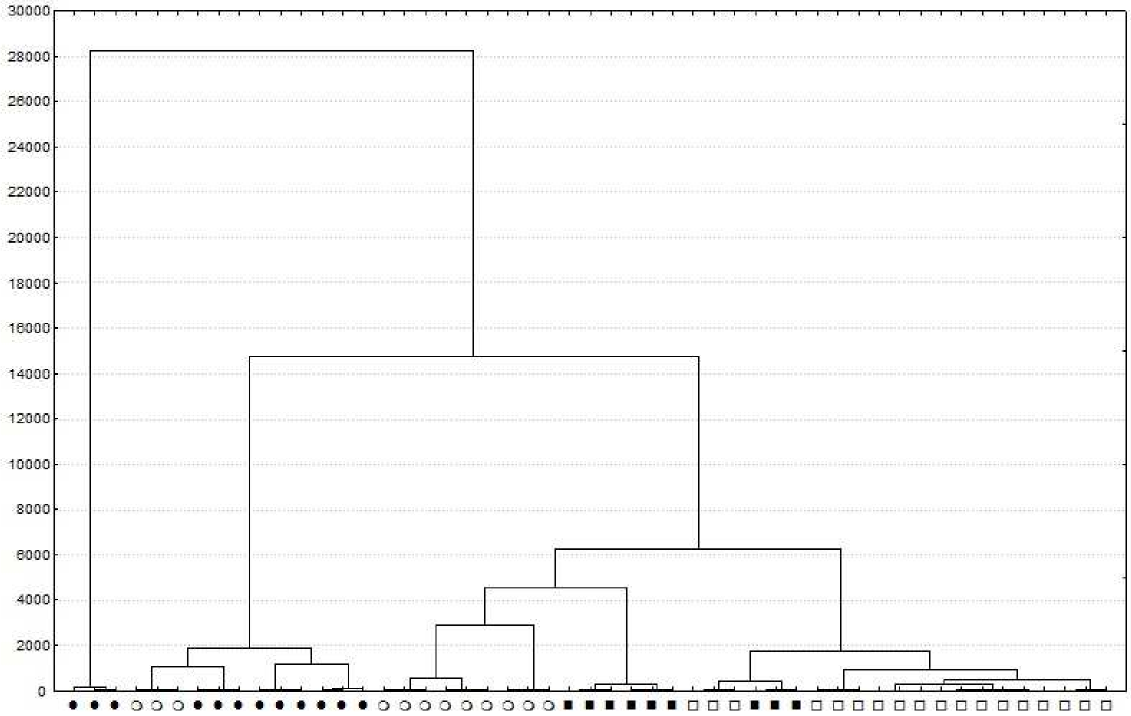
The triplicates of one sample (iron supplement organic table) with high concentrations for these four elements have high scores on PC1 and other samples have low concentrations for these four elements on PC1. The second PC offers the highest weights for Zn and Mg contents (Table 5), explains 16.70% of the total variance of the data set and was able to characterize the samples by chemical composition and magnesium showed negative loading. Thus, samples with high Mg concentrations have low Zn content. The inorganic samples (tablets and liquids) have major content of the zinc. In general, inorganic tablets have higher concentrations than the liquid inorganic samples. The inorganic tablet samples have higher content of magnesium and form a group separated from organic tablets and organic liquids.

Indeed, the tablets are produced with magnesium stearate to reduce friction between the solid particules.[22]

### 3.4. Hierarchical cluster analysis

HCA was applied to the scaled dates using Ward's method with Euclidean distances to calculate the sample interpoint distances, where all points are rotated to orthogonal axes with no change to preserve all of the information in the original data matrix. The dendrogram obtained is shown in Fig. 2 and show a non-complete separation of the groups. One sample of iron supplement organic table with high concentrations for all elements is separate of all other samples to a distance of approximately 28000. The other samples are separated into three clusters to a distance of approximately 6000, tablets samples, liquid samples and a cluster of organic liquid samples that resemble to inorganic solid samples. This last cluster is separated to a distance of 4000, showing that there are differences in the compositions of the samples.

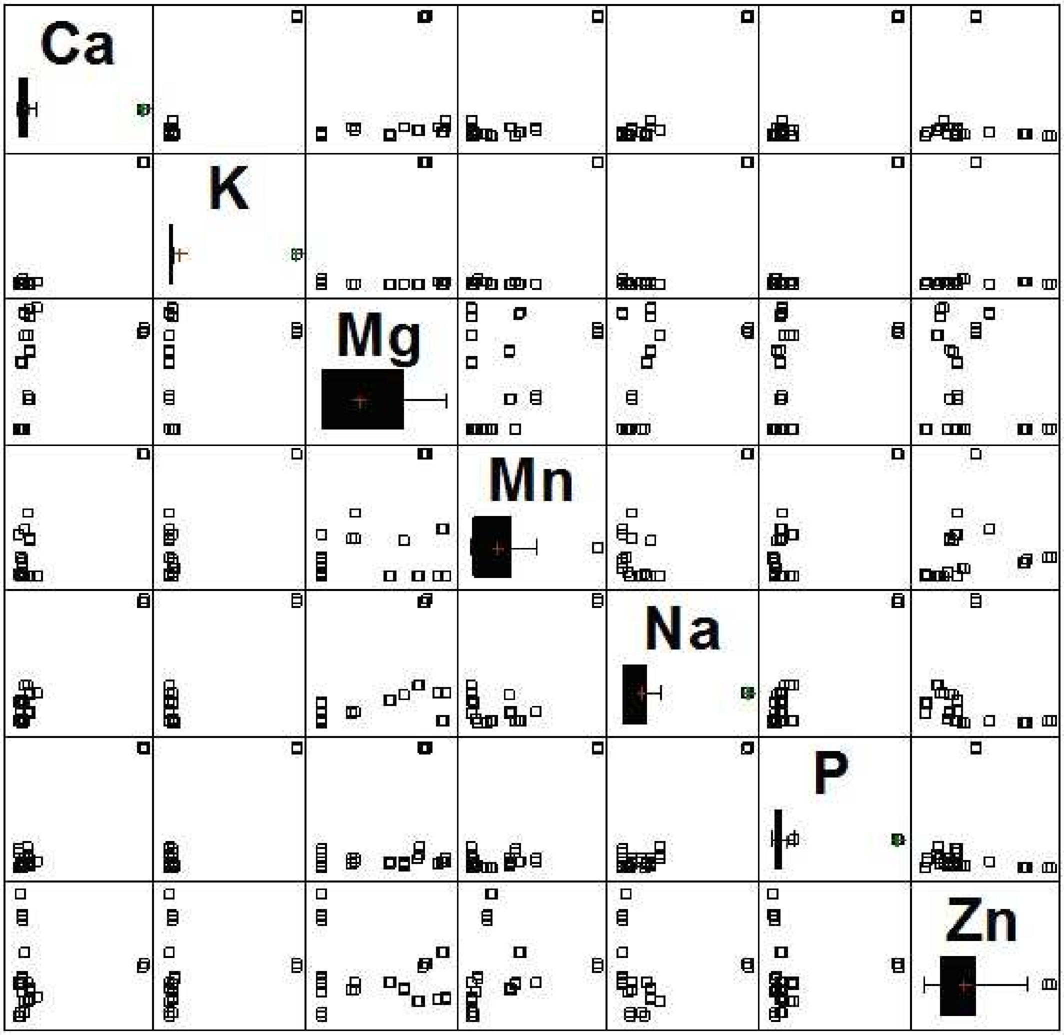
**Figure 2 -** Dendrogram for iron supplement samples showing Ward's method with Euclidean distances



### 3.5. Graph of matrix plot

The graph of matrix plot was used for evaluate the samples comportment for the all analytes determination. A matrix plot is a graph that you can use to assess the relationship among several pairs of variables at the same time. A matrix plot is a set of individual scatterplots. A matrix of plots is useful when you have many variables and you would like to examine relationships between all pairs of variables. An each Y versus each X plot displays a plot for each possible Y-X combination, when specify the y variables and x variables. The matrix 7x7 has 21 different combinations for the all analytes. The graph of matrix obtained showed in Figure 3.

**Figure 3 -** Graph of matrix to samples comportment for the all analytes determinated



These plots are also known as draftsman plots or casement displays. Each axes X and Y represents the concentration for the analytes. In this graph, plots between Mg, Mn, Na and Zn show that the concentrations of these analytes increase together. The other plots show a possible weaker positive relationship between the variables. This results show the analytes are the main responsible by analytes variations in the samples and these combinations is shown in the graph so as similar the PCA.

### 3.6. Evaluation of the micro and macro elements content in iron supplements

The content of micro and macro elements of iron supplements was determined by the analysis of samples collected from the brazilian pharmacies. The average and concentration ranges (expressed as mg of analyte per kg of sample) for calcium, potassium, magnesium, manganese, sodium, phosphorus and zinc are shown in Table 5.

**Table 5**

The Pharmacopoeias (Brazilian, US and European) does not establish maximum allowable limits for these elements in medicines. The pharmacopoeias establish maximum values for daily intake under mass of element per day. The pharmacopoeias not establish maximum limits by not consider these elements as potentially dangerous. However, as can be seen in Table 5, organic medicines should be administered with precaution in patients with hypertension (with cardiovascular risk associated)[23] and renal insufficiency as showing greater potassium and sodium values that can generate a hyperkalemia[24] and metabolic acidosis[25]. The tablets of iron supplements show high values for magnesium that may cause acute renal failure.[26]

## 4. Conclusions

The results confirm that the tablets iron supplements have highest concentrations of zinc and magnesium, principally associated with the production process.

Some samples demonstred to have higher concentrations of all analytes and organic iron supplements higher concentrations of sodium. This way, the use of iron supplements in patients with altered physiology should be made with precaution, as the literature describes the negative effects of the ingestion of high quantities of sodium and magnesium.

The existing Pharmacopoeias should include maximum values for each analyte in the regulamentation.

## Acknowledgements

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnalógico (CNPq) and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing grants and fellowships and for financial support.

256 **References**

[1] L. de Falco, M. Sanchez, L. Silvestri, C. Kannengiesser, M.U. Muckenthaler, A. Iolascon, L. Gouya, C. Camaschella, C. Beaumont, *Haematologica*, 2013, **98**, 845.

[2] M. Andro, P. Le Squere, S. Estivin, A. Gentric, *Eur. J. Neurol.*, 2013, **20**, 1234.

[3] D.R. Abernethy, A.J. De Stefano, T.L. Cecil, K. Zaidi, R.L. Williams, *Pharm. Res.-DORDR*, 2010, **27**, 750.

[4] R.N. Rao, M.V.N.K. Talluri, *J. Pharmaceut. Biomed. Anal.*, 2007, **43**, 1.

[5] N. Lewen, *J. Pharmaceut. Biomed. Anal.*, 2011, **55**, 653.

[6] C. Støving, H. Jensen, B. Gammelgaard, Stefan Stürup, *J. Pharm. Biomed. Anal.*, 2013, **84**, 209.

[7] U.A. Barbosa, L.O.B. Silva, I. F. dos Santos, S.L.C. Ferreira, A.M.P. dos Santos, *Curr. Anal. Chem.*, 2014, **11**, 44

[8] A. Tanase, A. Miu, *Rev. Roum. Chim.*, 2013, **57**, 971.

[9] F.G. Antes, M.F. Mesko, J.S. Barin, C.M. Moreira, E.M.M. Flores, V.L. Dressler, *Microchem. J.*, 2011, **98**, 144.

[10] G.G.A. de Carvalho, L.C. Nunes, P.F. de Souza, F.J. Krug, T.C. Alegre, D. Santos Jr., *J. Anal. At. Spectrom.*,2010, **25**, 803.

[11] L.A. Portugal, G.D. Matos, D.C. Lima, G.B. Brito, A.P. Fernandes, S.L.C. Ferreira, *Microchem. J.*, 2011, **98**, 29.

[12] I.F. dos Santos, A.M.P dos Santos, U.A. Barbosa, J.S. Lima, D.C. dos Santos, G.D. Matos, *Microchem. J.*, 2013, **110**, 439.

---

[13] K.A. Anderson, B.W. Smith, *J. Agric. Food Chem.*, 2002, **50**, 2068.

[14] U.A. Barbosa, I.F. dos Santos, A.M.P dos Santos, D.C. dos Santos, G.M. da Costa, *Biol. Trace Elem. Res.*, 2013, **154**, 412.

[15] J. dos Santos, M.L.P. dos Santos, M.M. Contia, *J. Braz. Chem. Soc.*, 2010, **21,** 1468.

[16] A. Kolasani, H. Xu, M. Millikan, *Food Chem.*, 2011, **127**, 1465–1471.

[17] L. Tormen, D.P. Torres, I.M. Dittert, R.G.O. Araújo, V.L.A. Frescura, A.J. Curtius, *J. Food Compos. Anal.*, 2011, **24**, 95-102.

[18] L.S. Goodman, A.G. Gilman, L.L Brunton, elenveth ed. Rio de Janeiro, Rio de Janeiro, 2006.

[19] ICH Validation of Analytical Procedures: Text and Methodology Q2(R1), 2006.

[20] IUPAC Analytical Chemistry Division, *Spectrochim. Acta B*, 1978, **33**, 242.

[21] J.M. Mermet , *Anal. Chim. Acta*, 1991, **250**, 85.

[22] K. Sugisawa, T. Kaneko, T. Sago, T. Sato, *J. Pharm. Biomed. Anal.*, 2009, **49**, 858.

[23] L. Wei, I.S. Mackenzie, T.M. MacDonald, J. George, *Expert Opin. Drug Saf.*, 2014, **13**, 1515.

[24] M. Tazoe, M. Narita, R. Sakuta, T. Nagai, N. Narita, *Brain Dev.-JPN*, 2007, **29**, 369.

[25] D. Street, J.J. Nielsen, J. Bangsbo, C. Juel, *J. Physiol.-LONDON*, 2005, **566**, 481.

[26] W.H., Huang, J.L. Lin, *J. Toxicol.-Clin. Toxic.*, 2004, **42**, 305.