Use of Chemometric Tools for HG-AAS Instrumental Optimization in the Determination of Se in Nuts Grown in Brazil

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INTRODUCTION

Selenium (Se) and its related metalloid species are essential for humans and animals due to their medicinal properties (1-3), powerful antioxidant characteristics, and protectors against heavy metal toxicity (4). Due to the health-related benefits, the U.S. Food and Nutrition Board recommends 55 µg/day for adults and 70 µg/day for lactating mothers as a dietary reference intake (DRI) of selenium (5, 6). The tolerable upper intake for adults is set at 400 µg/day (6, 7).

Food is considered the most important source of Se, and poultry, beef, eggs, seafood, wheat flour, oat grains, and brown rice are the main dietary staples (8-11). In addition, species of the genus *Allium* (onion, garlic, leek, etc.) and *Brassica* (cabbage, cauliflower, broccoli, mustard, brussels, turnip, radishes, etc.) also contain small amounts of selenium (12). However, among these numerous foods that easily furnish the recommended daily intake of selenium (13), the Brazil nut (*Bertholletia excelsa*) is worldwide known and has a high content of selenium (9, 12, 14, 15). Due to the environmental and climatic diversity in Brazil, there is an extensive variety of nuts grown. These species also deserve dedicated attention and are discussed below.

ABSTRACT

The determination of total selenium (Se) is described in nuts such as bacuri (*Platonia insignis* Mart.), baru (*Dipteryx alata* Vog.), bociuiva (*Acrocomia aculeata* (Jacq.) Lodd), and cashew (*Anacardium occidentale* L.) (other than the well-known Brazilian nut) using hydride generation coupled to a flame atomic absorption spectrometer (HG-AAS). The best experimental conditions to generate the volatile hydride (H₂Se) were established by multivariate statistical analysis. The conditions of the pre-reduction step to generate the Se hydride were optimized using the Pareto diagram and Doehlert design. Best results were achieved when 0.6 mol L⁻¹ HCl, 1.0% (m/v) NaBH₄ in 0.5% (m/v) NaOH, and a 00-cm reaction coil were used. For Se determination by HG-AAS, analytical curves were built in the 0.50 - 25.0 µg L⁻¹ range resulting in a linear coefficient correlation better than 0.9970. The accuracy of the sample preparation procedure and HG-AAS determination were evaluated by addition and recovery tests, and the recoveries were found in the 90.3-95.8% range with a relative standard deviation (%RSD) of < 4.7%. Using the proposed methodology, the limit of detection obtained was 0.1315 µg L⁻¹ with a %RSD up to 1.9%. The Se concentrations in the samples were in the 0.515±0.017-0.657±0.029 µg g⁻¹ Se range. The multivariate analysis applied to the experimental results showed to be a useful tool because it allows reducing the number of experiments (from 60 to 8) with satisfactory accuracy.

The determination of Se can be carried out through different spectrometric techniques including electrothermal atomic absorption spectrometry (ETAAS) (16-18), inductively coupled plasma mass spectrometry (ICP-MS) (19-21), inductively coupled plasma optical emission spectrometry (ICP-OES) (22, 23), hydride generation atomic absorption spectrometry (HG-AAS) (19, 24), hydride generation atomic fluorescence spectrometry (HG-FAAS) (25, 26), instrumental neutron activation analysis (8), and the hyphenated technique of HPLC-ICP-MS (27). With regard to Se speciation, hyphenated techniques are usually preferred and applied for analytical purposes (28, 29). However, these techniques result in high laboratory costs. Hydride generation coupled to an AAS is an alternative and can also produce high sensitivity and be used as an optional and satisfactory technique to determine low Se concentrations in different matrices (31, 32). In addition, the experimental parameters of HG-AAS can be successfully established by multivariate analysis, allowing ideal conditions and requiring a reduced number of experiments for the determination of hydride elements (32, 33).

Due to the lack of information about the content of Se in native nuts from midwestern Brazil, this paper reports the development of a novel method for the determination of selenium in bacuri (*Platonia insignis* Mart.), baru (*Dipteryx alata* Vog.), bociuiva (*Acrocomia*

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aculeata (Jacq.) Lodd), and cashew (*Anacardium occidentale* L.) nuts by hydride generation coupled to atomic absorption spectrometry, and using multivariate statistical analysis to establish the best conditions for the generation of H₂Se.

EXPERIMENTAL

Instrumentation

A Varian 240FS flame atomic absorption spectrometer (Agilent Technologies®, Mulgrave, Victoria, Australia), equipped with a deuterium background corrector, was used throughout this work and the instrumental operating parameters are listed in Table I. A VGA 77 Vapor Generation (Agilent Technologies) with a thermal quartz flow cell atomizer was used to generate and atomize the Se hydride. It was controlled by a peristaltic pump, and measurement of the solutions was carried out in four replicates, and the peak height (absorbance) of the blank, analytical solutions, and samples were obtained under optimum instrumental conditions (Table I). Atomization of the SeH₂ was performed using an oxidizing air-acetylene flame. High-purity acetylene (99.7%) and argon (99.999%) (White Martins®, Dourados, MS, Brazil) were used as fuel and carrier gas, respectively. A 515 Orion (Fanem®, São Paulo, SP, Brazil)

forced air oven and a TE-361 (Tecnal®, Piracicaba, SP, Brazil) stainless steel mill were used to dry and powder the nuts, respectively. The appropriate amount of samples was digested in a MARS 6 microwave oven (CEM Corporation, Matthews, NC, USA).

Standard Solutions and Reagents

High purity deionized water obtained from a Milli-Q® Plus reverse osmosis system (resistivity 18.2 MΩ-cm, Millipore Corporation, Bedford, MA, USA) was used to prepare all solutions. Digestion of the nuts was carried out using hydrochloric acid [37% (v/v)] and nitric acid [65% (v/v)] (Sigma-Aldrich, St. Louis, MO, USA), plus sulfuric acid [98% (m/v)] and hydrogen peroxide [30% (m/v)] (Vetec®, Duque de Caxias, RJ, Brazil).

Aqueous analytical solutions containing 0.5–25.0 µg L⁻¹ Se were prepared daily using appropriate dilutions of mono-element 1000-mg L⁻¹ standard stock solutions (SpecSol®, SRM-682, USA) of Se(IV) in 1.0% (v/v) nitric acid. All solutions were stored in high-density polypropylene bottles (Nalgene®, Rochester, NY, USA).

A 100 mL 1.0% (m/v) NaBH₄ solution [97% purity (m/m), Vetec®, Duque de Caxias, RJ, Brazil] was prepared daily in 0.5% (m/v) sodium hydroxide [99% purity (m/m), Vetec] medium and used as a reducing agent in the HG system. A 0.60 mol L⁻¹ HCl solution was prepared and used as the carrier solution and during the generation of the Se hydride. To convert Se(VI) to Se(IV), a pre-reduction step was carried out using 10.0% (m/v) KI [99% purity (m/m) (Vetec), in 2.4 mol L⁻¹ HCl medium.

The best experimental conditions (reaction coil, concentration of HCl carrier solution, and % (m/v) of the reducing agent) to generate the H₂Se were established by multivari-

ate statistical analysis using working solutions containing 25.0 µg L⁻¹ Se.

Plastic bottles and glassware were separated and cleaned by soaking in 10% (v/v) HNO₃ for at least 24 hours, followed by thoroughly rinsing with deionized water before use.

Sample Preparation

Samples of native bacuri, baru, bociuiva, and cashew nuts were collected from the trees during 2015 from Bonito, Campo Grande, Dourados and Rochedo Cities (Mato Grosso do Sul, Brazil), respectively. A 1000-g amount of each nut was randomly collected, stored in individually labelled paper bags, and then transported to the laboratory. The nuts were separately and thoroughly washed with running tap water as well as with deionized water. A 5.0-g subsample of each species was dried at 50 °C for 30 hours in a forced air oven, ground in a stainless steel mill, and then sieved using a No. 20 sieve (0.84 mm opening size). All samples were individually stored in identified plastic bags and kept under refrigeration until sample preparation.

The native nuts were mineralized using acid digestion in a closed vessel microwave oven. For this purpose, a 0.5000-g portion of pre-treated bacuri, baru, bociuiva, and cashew samples was accurately weighed (± 0.0001 g) and transferred into a 100-mL microwave flask, followed by the addition of 6.0 mL of HNO₃ and 2.0 mL of HCl. The optimized heating program used is listed in Table II. After digestion and cooling, the resulting solutions were transferred to 20-mL volumetric flasks, and the volume was completed with deionized water. Three sample and blank replicates were used for the acid decompositions.

Since the determination of Se requires only the Se(IV) oxidation

TABLE I
HG-AAS Instrumental
Operating Conditions
for the Determination of Se

Instrumental Conditions	Se
Working range	2.0–25.0 mg L ⁻¹
Wavelength	196.0 nm
Lamp current	10 mA
Burner head	100 mm
Air flow rate	13.0 L min ⁻¹
Acetylene flow rate	2.0 L min ⁻¹
Argon flow rate	0.1 L min ⁻¹
Slit width	1.0 nm

state to produce SeH_2 , a pre-reduction step was performed to reduce all Se(VI) to Se(IV). Thus, after the sample preparations, 6.0-mL aliquots of the digested sample were transferred to 15-mL polyethylene bottles, followed by addition of 1.0 mL of 10% (m/v) KI and 3.0 mL HCl [37% (v/v)]. The solutions were mixed with a vortex stirrer for 10 seconds to complete homogeneity and kept at rest for 30 seconds. The procedure resulted in homogeneous and colorless solutions. All solutions were prepared in triplicate.

Optimization of HG-AAS Experimental Conditions

In order to find the optimum HG-AAS experimental conditions for SeH_2 generation in the determination of Se in the native Brazilian nuts, a 2^3 full factorial design was

employed. The influence of HCl concentration, NaBH_4 concentration, and reaction coil length were evaluated and the maximum (+) and minimum (-) levels studied (see Table III). The experiments were randomly chosen and carried out in duplicate to avoid possible systematic errors. The significance of each factor was evaluated from the analysis of variance (ANOVA) performed at 95% confidence interval and graphically represented by a Pareto chart. For final optimization of the HCl and NaBH_4 concentrations, a Doehlert matrix for two variables and a response surface methodology were used. All results obtained from the multivariate analysis were processed using Statistica® software (Version 10) (TIBCO Software Inc., Palo Alto, CA, USA) at a 95% confidence level.

TABLE II
Microwave-assisted Digestion Heating Program for Nuts

Step	t_{ramp} (min)	t_{hold} (min)	Power (W)	T (°C)
1	5	5	1000	85
2	7	5	1000	120
3	11	13	1000	200
3	0	20	0	30 (Ventilation)

TABLE III
Factors Studied in 2^3 Full Factorial Design, Levels, and Analytical Responses

	Factor			Level	
				Low (-)	High (+)
	HCl (mol L ⁻¹)			1.0	5.0
	NaBH_4 (%)			0.15	1.5
	Reaction Coil Length (cm)			30	100
Experiment	HCl	NaBH_4	Coil Length	Absorbance Sb(IV) (Peak Height)	
1	-	-	-	0.253/0.256	
2	+	-	-	0.311/0.317	
3	-	+	-	0.427/0.418	
4	+	+	-	0.502/0.505	
5	-	-	+	0.285/0.273	
6	+	-	+	0.387/0.387	
7	-	+	+	0.508/0.506	
8	+	+	+	0.549/0.534	

Analytical Measurements

Standards, samples (bacuri, baru, bocaiuva, and cashew), carrier (HCl), and a reduction (NaBH_4) solution were pumped into the HG gas/liquid separator system using three Tygon® tubes with a 1.00-mm i.d. For SeH_2 generation, the aspiration rates of the sample/standards, carrier and reduction solutions were 8.0, 1.0, and 1.0 mL min⁻¹, respectively. A 0.6 mol L⁻¹ HCl and a 1.0% (m/v) NaBH_4 in 0.5% (m/v) NaOH solution were used for Se(IV) determination. The reagents were mixed with the standard solutions or samples by dispersion in the 100-cm reaction coil at 1.0 mm i.d. to generate SeH_2 . The hydrogen selenide was conducted into a 17-cm cell T-type quartz insert on the AAS burner at a 100-mL min⁻¹ argon flow rate (99.999%, White Martins®, Dourados, MS, Brazil). The measurements were performed at the main atomic line for Se (196.0 nm) using an oxidizing air-acetylene flame (see Table I) to atomize Se, and its determination was carried out within 2.0–25.0 µg L⁻¹ intervals of the calibration curves.

The accuracy was evaluated by recovery tests using all samples spiked with 3.00 and 6.00 µg L⁻¹ Se(IV), which corresponds to 0.300 and 0.600 µg g⁻¹ Se, respectively. The precision of the method was checked by means of calculating the relative standard deviation (%RSD) of the sample measurements. The limits of detection (LOD) and quantification (LOQ) for Se were calculated according to the IUPAC recommendations (34).

RESULTS AND DISCUSSION

HG-AAS Instrumental Optimization Using Multivariate Analysis

The influence of the most important factors for the selenium generation step, which involved hydrochloric acid concentration as a carrier solution, concentration of the

reducing agent (sodium tetrahydroborate - NaBH_4) and a reaction coil length using a $25 \mu\text{g L}^{-1}$ Se(IV) standard solution, was studied by means of a 2^3 full factorial design. Table III shows the analytical responses for each assay and the results were analyzed through a Pareto chart (Figure 1) where all studied variables and one of the interactions were statistically significant at the 95% confidence interval. Among the studied factors, the NaBH_4 concentration proved to be a most significant positive effect (65.664). This demonstrates clearly that an increase on the NaBH_4 concentration provides improvements on the hydride generation due to a reduction of Se(IV) to H_2Se in a strongly acidic medium. The NaOH concentration in the reduction solution [0.5%, (m/v)] does not have to be optimized since NaOH is only added to the reducing agent in order to stabilize the NaBH_4 in the solution (35). It is important to emphasize that the working solutions of NaBH_4 in the alkaline medium were prepared daily and minutes before starting analysis due to the degradation of the NaBH_4 caused by an increase in the evolution of the hydrogen from the hydrolysis of BH_4^- (36). The pump

flow rate of the reduction solution was fixed at 1.0 mL min^{-1} .

The hydrochloric acid concentration, which is the second most important factor in Se determination, demonstrated a strong influence on the hydride generation with a positive effect (25,112) since the presence of protons in the reaction is essential to form the Se(IV) hydride (35, 37). In addition, in samples containing high OH^- concentration, the HCl concentration is necessary to neutralize the sample and to react with the reducing agent and the analytes. The pump flow rate of the hydrochloric acid as a carrier solution was fixed at 1.0 mL min^{-1} . Although the factors of NaBH_4 and HCl concentrations have presented a positive effect when analyzed individually, its interaction of 1by2 (HCl by NaBH_4) showed a negative effect, indicating an antagonistic effect of both of these factors on the Se(IV) determination. This implies that better results in the Se(IV) determination are obtained when one of the factors is at its highest level while the other is at its lowest, as studied within the experimental domain.

Using 1.0 mL min^{-1} for the carrier solution and reducing agent,

and 8.0 mL min^{-1} for the Se(IV) standard flow rates, the 100 cm reaction coil length was the third most significant factor. The positive effect on the Se(IV) hydride determination due to the higher reaction time with the carrier gas, the reducing agent, and the Se(IV) species before reaching the optical pathway was observed. Thus, a reaction coil of 100 cm was chosen for all studies. Although this factor proved to be a significant factor when analyzed independently (see Figure 1), their interactions with other factors, such as reducing agent (NaBH_4) and carrier gas (HCl), were not significant at the 95% confidence interval.

Therefore, according to the achieved results and considering that during the assays with the NaBH_4 concentration at its highest level, a decrease in precision of the results (%RSD) was observed most likely due to the very violent reaction under these conditions. For this reason, a Doehlert matrix (Table IV) with triplicate at the central point was used for obtaining the optimum values for the reducing agent (NaBH_4) and carrier solution (HCl). From the Doehlert design, the quadratic model, which establishes the relationship between the two factors and the analytical response (absorbance as peak height), was obtained. The model was described according to the following equation:

$$\text{Abs} = -0.505 (\pm 0.016) + 0.522\text{RA} (\pm 0.018) - 0.284\text{RA}^2 (\pm 0.007) + 3.014\text{CS} (\pm 0.044) - 2.574\text{CS}^2 (\pm 0.038) + 0.077\text{RA} \times \text{CS} (\pm 0.021).$$

According to ANOVA, the obtained $F_{\text{calculated}}$ (6.737) was lower than $F_{\text{tabulated}}$ (18.51) at the 95% confidence interval, which confirms the absence of lack of fit and demonstrates that the regression model is considered to be an accurate representation of the experimental data. In addition, the high determination coefficient ($R^2 = 0.99904$) also points out that the quadratic model fits

Fig. 1. Pareto's Chart obtained from the 2^3 full factorial design for the determination of Se by HG-AAS.

very well to the experimental results. Using the quadratic model, the response surface was constructed, showing a maximum point at the NaBH_4 concentration of 1.00% and HCl concentration of 0.60 mol L^{-1} (Figure 2). These values were further used as the best conditions for determining the analytical features of the developed method. Validation of the model also has been performed by replacing RA (reducing agent) and CS (carrier solution) with the optimum values obtained. The predicted values by the qua-

dratic model are very close to that obtained experimentally, which means that the model can be used to predict the response in all experimental domains (Table IV).

Analytical Features of HG-AAS for the Determination of Se

The only way to generate the SeH_2 species is from ionic tetravalent selenium (selenite) (35, 38). Since Se has other oxidation forms [e.g., Se(VI)], the reduction step must be performed to produce the desired species of Se. The reduc-

tion procedure is commonly carried out by adding a reducing agent and concentrated HCl into the selected digested sample solution volume (6). Moreover, the efficiency of the Se determination by HG-AAS depends on the time between the reduction step and hydride generation (35, 38). According to literature (6), satisfactory recoveries are obtained if Se hydride generation is carried out immediately after the reduction. It should be noted that by increasing the time between reducing step and analysis, problems related to a back-oxidation of Se(IV) to Se(VI) by chlorine gas formed in the reduction process have been reported (32). In this work, the pre-reduction step of Se(VI) to Se(IV) was evaluated using 6.0 mL of digested sample, 1.0 mL of 10% (m/v) KI, 3.0 mL HCl [37% (v/v)], and an aliquot of 150 μL of 1000 $\mu\text{g L}^{-1}$ Se(VI) standard solution in order to contain 10.0 $\mu\text{g L}^{-1}$ Se. The solution was completed to 15 mL with high purity deionized water and vortex-stirred for 10 seconds. Homogeneity of the solutions was obtained after 30 seconds; in other words, it was satisfactory and is enough time to convert the Se(VI) to Se(IV) . Recoveries of $94.0 \pm 2.0\%$ indicates that this pre-reduction step, followed by HG-AAS analysis, is satisfactory to reduce Se(VI) to Se(IV) . Besides, this pre-reduction step was evaluated day-to-day and no variations were observed between them at the 95% confidence level (Student's *t*-test).

Under the optimum conditions established for the pre-reduction step and by multivariate analysis for Se hydride generation, analytical curves were built in the 0.50–25.00 $\mu\text{g L}^{-1}$ interval for Se(IV) . Typical linear correlation coefficients better than 0.9970 were constantly obtained and the limits of detection (LOD) and quantification (LOQ), defined as three times and ten times, respectively, of the standard

Fig. 2. Response surface for NaBH_4 [% (m/v)] and HCl (mol L^{-1}) concentrations.

TABLE IV
Doehlert Matrix Design Used for Optimizing Reducing Agent (NaBH_4) and Carrier Solution (HCl) in the Determination of Se for HG-AAS

Experiment	Reducing Agent (%, m/v)	Carrier Solution (mol L^{-1})	Absorbance (Peak Area)	Predicted Values by Quadratic Model
1	1 (0)	0.5 (0)	0.640	0.635
2	1 (0)	0.5 (0)	0.636	0.635
3	1 (0)	0.5 (0)	0.630	0.635
4	1.8 (1)	0.5 (0)	0.442	0.447
5	1.4 (0.5)	0.8 (0.866)	0.525	0.520
6	0.2 (-1)	0.5 (0)	0.465	0.460
7	0.6 (-0.5)	0.2 (-0.866)	0.210	0.215
8	1.4 (0.5)	0.2 (-0.866)	0.196	0.191
9	0.6 (-0.5)	0.8 (0.866)	0.502	0.507

The number between parentheses represents the Doehlert matrix coded values.

deviation of 12 measurements of a blank, divided by the slope of the calibration curve, were 0.13 and 0.44 $\mu\text{g L}^{-1}$, respectively. However, since the results for the determination of Se in native nuts are expressed as $\mu\text{g g}^{-1}$, the limits of detection and quantification were converted to this order and the values are expressed as 0.013 and 0.044 $\mu\text{g g}^{-1}$ Se, respectively. The precisions, which are expressed as the relative standard deviation (%RSD) for $n=4$, were 1.9% using the HG-AAS atomization system.

Determination of Se in Different Native Brazilian Nuts by HG-AAS

The Se concentration in nuts, as well in the most varied types of environmental samples, is in the $\mu\text{g g}^{-1}$ range (12). Since the limit of detection obtained with the proposed methodology was (0.013 $\mu\text{g g}^{-1}$), it allows below $\mu\text{g g}^{-1}$ determination in the native Brazilian nuts.

The accuracy of the sample preparation procedure and the determination method was evaluated using the addition/recovery tests for all samples. Different volumes of a 1.00-mg L^{-1} Se(IV) standard solution were added in order to contain 3.00 and 6.00 $\mu\text{g L}^{-1}$ in the sample before starting the sample preparation procedure, representing close to 50% (0.300 $\mu\text{g g}^{-1}$) and 100% (0.600 $\mu\text{g g}^{-1}$) of natural Se concentration in the samples analyzed. The recovery values were in the 90.3–95.8% range, with the RSD in the 0.4–3.9% range (see Table V).

According to the results of the present study, the relative standard deviations (%RSD) obtained varied from 1.6–4.7%. The recovery tests show that the established methodology is suitable for the determination of Se in the selected nut samples (see Table V).

According to the U.S. Food and Nutrition Board, an amount of 55 $\mu\text{g/d}$ of Se can be considered the dietary reference intake (DRI) for

adults. Since the Se concentration in the selected nuts varied from 0.515 to 0.657 $\mu\text{g g}^{-1}$, the DRI can be reached by daily ingestion of 83, 106, 91, and 96 g of bacuri, baru, bociúva, and cashew nuts, respectively. According to the Brazilian National Health Surveillance Agency or ANVISA (Agência Nacional de Vigilância Sanitária), any food can be considered a nutritional source when 100 g provides 15% of the DRI and is considered rich in Se when it provides about 30%. It can, therefore, be stated that all nuts evaluated in this study are rich sources of Se.

CONCLUSION

The use of the multivariate analysis has proven to be an outstanding tool in developing an analytical methodology for Se determination by using diluted solutions of HCl and NaBH_4 with HG-AAS as a detection technique. The proposed fast and robust procedure reduces Se(VI) to Se(IV) and shows good recoveries (90.3 – 95.8%). In addition, the HG-AAS technique presented limits of detection (LOD) close to 0.013 $\mu\text{g g}^{-1}$ and provided an RSD better than 4.7%.

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TABLE V
Results (Mean $\pm$ Standard Deviation) for the HG-AAS Determination ($n=3$) of Se ($\mu\text{g g}^{-1}$) in Native Brazilian Nuts

Sample	Se Added ($\mu\text{g g}^{-1}$)	Se Measured ($\mu\text{g g}^{-1}$)	Recovery (%)	RSD (%)
<i>Platonia insignis</i> Mart.	0.000	0.657 $\pm$ 0.029	-	4.7
	0.300	0.933 $\pm$ 0.005	92.0	2.2
	0.600	1.206 $\pm$ 0.008	91.5	1.5
<i>Dipteryx alata</i> Vog.	0.000	0.515 $\pm$ 0.017	-	4.3
	0.300	0.794 $\pm$ 0.015	93.1	1.1
	0.600	1.057 $\pm$ 0.022	90.3	0.4
<i>Acrocomia aculeata</i> (Jacq.) Lodd	0.000	0.602 $\pm$ 0.009	-	1.6
	0.300	0.879 $\pm$ 0.011	92.4	2.6
	0.600	1.177 $\pm$ 0.019	95.8	0.9
<i>Anacardium occidentale</i> , L.	0.000	0.568 $\pm$ 0.026	-	3.1
	0.300	0.852 $\pm$ 0.021	94.7	3.9
	0.600	1.131 $\pm$ 0.029	93.9	2.4

REFERENCES

1. B.D. Wake, A.R. Bowie, E.C. Butler and P.R. Haddad, *Trac-Trend. Anal. Chem.* 23, 491 (2004).
2. C.B. Hymer and, J.A. Caruso, *J. Chromatogr.* 1114, 1 (2006).
3. Y. Wang and L. Fu, *Biol. Trace. Elem. Res.* 149, 110 (2012).
4. C.W. Nogueira, G. Zeni and J.B.T. Rocha, *Chem. Rev.* 104, 6255 (2004).
5. P. Trumbo, A.A. Yates, S. Schlicker, and M. Poos, *J. Am. Diet. Assoc.* 101, 294 (2001).
6. A.S. Abdallah, I.N.B. Castilho, B. Welz, E. Carasek, I.B.G. Martens, A. Martens and S.M.F. Cozzolino, *Talanta* 85 1350 (2011).
7. G.F. Combs Jr., L.C. Clark and B.W. Turnbull, *Bio. Factors.* 14, 153 (2001).
8. P.P. Parekh, A.R. Khan, M.A. Torres and M.E. Kitto, *J. Food. Comp. Anal.* 21, 332 (2008).
9. M.P. Rayman, *Br. J. Nutr.* 100, 254 (2008).
10. B.M. Thomson, R.W. Vannoort and R.M. Haslemore, *Br. J. Nutr.* 99, 614 (2008).
11. EPA, <http://www.epa.gov/ttnatw01/hlthef/selenium.html>, Last visited: 28/12/2016.
12. C.A. Prauchner, A importância do Selênio para a agropecuária e saúde humana, Ed. da UFSM, Santa Maria, Brazil (2014).
13. M. Tuzen, K.O. Saygi and M. Soylak, *Talanta* 71, 424 (2007).
14. E. Dumont, F. Vanhaecke and R. Cornelis, *Anal. Bioanal. Chem.* 385, 1304 (2006).
15. M. Lemire, M. Fillion, F. Barbosa Jr., J.R.D. Guimarães and D. Mergler, *Sci. Total. Environ.* 408, 4076 (2010).
16. R. Sabe, R. Rubio, and L. Garcia-Beltran, *Anal. Chim. Acta* 419, 121 (2000).
17. A.P. Oliveira, J.A.G. Neto, J.A. Nóbrega, P.R.M. Correia and P.V. Oliveira, *Food. Chem.* 93, 355 (2005).
18. A. Bidari, E.Z. Jahromi, Y. Assadi and M.R.M.H. Hosseini, *Microchem. J.* 87, 6 (2007).
19. W.T. Buckley, J.J. Budac and V. David, *Anal. Chem.* 64, 724 (1992).
20. S.J. Santos, H. Mokudai and S. Tanaka, *J. Anal. At. Spectrom.* 12, 409 (1997).
21. A.S. Ribeiro, M.A. Viera and A.J. Curtius, *Spectrochim. Acta. B.* 59, 243 (2004).
22. P.C. Hernandez, J.F. Tyson and C. Peter, *J. Anal. At. Spectrom.* 22, 298 (2006).
23. P. Qiu, C. Ai, L. Lin, J. Wu and F. Y., *Microchem. J.* 87, 1 (2007).
24. K. Agrawal, K.S. Patel, and K. Shrivastava, *J. Hazard. Mater.* 161, 1245 (2009).
25. P. Smrkolj and V. Stibilj, *Anal. Chim. Acta.* 512, 11 (2004).
26. J.B. García, M. Krachler, B. Chen and W. Shotyk, *Anal. Chim. Acta* 534, 255 (2005).
27. H.F. Li, E. Lombi, J.L. Stroud, S.P. McGrath and F.J. Zhao, *J. Agr. Food. Chem.* 58, 11837 (2010).
28. J.L. Capelo, C. Fernandez, B. Pedras, P. Santos, P. Gonzalez and C. Vaz, *Talanta* 68, 1442 (2006).
29. P. Cuderman, I. Kreft, M. Germ, M. Kovacevic and V. Stibilj, *J. Agr. Food. Chem.* 56, 9114 (2008).
30. M. Navarro, H. Lopez, M.L. Ruiz, S. Gonzalez, V. Perez and M.C. Lopez, *Sci. Total. Environ.* 175, 245 (1995).
31. P.C. Aleixo, J.D.A. Nóbrega, D. Santos Júnior and R.C.S. Müller, *Quim. Nova* 23, 310 (2000).
32. R.F. Teófilo and M.M. Ferreira, *Quim. Nova* 29, 338 (2006).
33. S.L.C. Ferreira, M.D.G.A. Korn, H.S. Ferreira, E.G.P. da Silva, R.G.O. Araújo, A.S. Souza, S.M. Macedo, D.D.C. Lima, R.M. de Jesus, F.A.C. Amorim and J.M. Bosque Sendra, *Appl. Spectrosc. Rev.* 42, 475 (2007).
34. L.A. Currie, *Anal. Chim. Acta.* 391, 105 (1999).
35. J. Dedina and D.L. Tsalev, *Hydride Generation Atomic Absorption Spectrometry*, John Wiley & Sons Ltd., Chichester, UK (1995).
36. N. Erta, Z. Arslan and J.F. Tyson, *J. Anal. At. Spectrom.* 23, 223 (2008).
37. I. Takase, H.B. Pereira, A.S. Luna, P. Grinberg and R.C. de Campos, *Quím. Nova* 25, 1132 (2002).
38. B. Welz and M. Sperling, *Atomic Absorption Spectrometry*, 3th ed., WILEY VCH Verlag GmbH, Weinheim, Germany (1999).