

Dynamic Reaction Cell-ICP-MS as a Powerful Tool for Quality Control of a Se-Enriched Dietary Supplement

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Abstract The aim of this work was to evaluate different approaches employing a reaction cell to circumvent spectral interferences over $^{80}\text{Se}^+$ to increase the sensitivity in the determination of Se by inductively coupled plasma mass spectrometry (ICP-MS). Different gases (NH_3 , CH_4 , and O_2) were employed, and the operating conditions were optimized by a central composite design, evaluating the effect of the reaction gas flow rate and the rejection parameter q (Rpq) on the limit of detection (LOD) of the method. All reaction gases studied reduced the interference by $^{40}\text{Ar}_2^+$ over $^{80}\text{Se}^+$, reducing the LOD when compared to the LOD obtained with ICP-MS operating in the standard mode (LOD = $0.2 \mu\text{g kg}^{-1}$). A better LOD ($0.01 \mu\text{g kg}^{-1}$) was obtained with CH_4 , while $0.1 \mu\text{g kg}^{-1}$ was the LOD with NH_3 . The use of O_2 as a reaction gas enabled the determination of Se at m/z 96 ($^{80}\text{Se}^{16}\text{O}^+$) with adequate analytical performance (LOD = $0.03 \mu\text{g kg}^{-1}$). The accuracy of the method was evaluated by analyzing the certified reference material (CRM) Selm-1, and the results obtained ranged from 99 to 104% of the certified value. In addition, Se dietary supplements of four different brands and commercial yeast were analyzed, and the major sources of uncertainty were studied. The dietary supplements followed the criteria adopted by Brazilian legislation, although the uncertainty study highlighted the great

variation in the Se content of each brand. Clearly, the manufacturing process of dietary supplements needs more rigorous quality control, considering that this product can affect the health of consumers.

Keywords Selenium · ICP-MS · Reaction cell · Dietary supplement · Yeast sample

Introduction

Selenium is an essential element in the antioxidant function of some enzymes. Selenium-deficient diets are known to be responsible for some health disorders such as Keshan disease (a cardiomyopathy that affects women of childbearing age) and Kaschin-Beck disease (an articular disease that results in bone damage) (Chen et al. 1980; Ge and Yang 1993). To minimize the impact of a Se-deficient diet, Se-enriched dietary supplements are commonly used. In this case, it is important to know the Se chemical form, since the organic Se is more bioavailable than inorganic Se in biochemical processes. Commercial Se supplements are widely used and generally are obtained from *Saccharomyces cerevisiae* enriched in organic Se, which has selenomethionine as the main species.

Although Se is considered an important element in biological processes, the limit between its essentiality and its toxicity is narrow, and it is very important to control the ingestion of Se in a diet (Reid et al. 2004). The occurrence of Se intoxication is rare, but a critical poisoning of 201 people by ingestion of a Se dietary supplement occurred in 2001 in different American states. The US Food and Drug Administration (FDA) laboratories demonstrated that the Se content in the commercialized supplement was approximately 200 times higher than indicated by the labeled value (MacFarquhar et al. 2010). Veatch and co-workers (Veatch et al. 2005)

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quantified Se in different brands of dietary supplements and reported that the concentrations in micrograms per tablet were higher than the labeled value in 14 of the 15 products analyzed. In this study, measured values differed from -7 to $+39\%$ relative to stated values.

The easy access to dietary supplements makes their uncontrolled use common, which can be potentially dangerous, highlighting the necessity of rigorous quality control in manufacturing and commercialization. For example, in Brazil, in 2014, problems related to misformulated dietary supplements, considering the content of protein and carbohydrate (R7 2014), the origin of the protein (vegetal or animal), and the presence of non-declared substances (INMETRO 2014), considered most samples inadequate for commercialization.

The biological importance of Se made it the focus of many scientific works developing analytical methods to enable the accurate and precise determination of Se in complex matrices (Stivanin de Almeida et al. 2009; Sucharová 2011; De Albuquerque et al. 2012; da Silva and Arruda 2013). Inductively coupled plasma mass spectrometry (ICP-MS) is the most powerful technique for fast elemental determination due to the multi-elemental capacity and the wide linear range. This technique is also useful for Se, which is not totally ionized in the plasma and is subject to spectral interferences in all isotopes. The most abundant isotope ($^{80}\text{Se}^+$, 49.61%) encounters interference mainly from the argon dimer ($^{40}\text{Ar}^{40}\text{Ar}^+$) and the less abundant isotopes ($^{76}\text{Se}^+$ 9.37%, $^{77}\text{Se}^+$ 7.63%, $^{78}\text{Se}^+$ 23.77%, $^{82}\text{Se}^+$ 8.73%) used to be employed for quantification, but all of these isotopes are interfered with at some level by polyatomic ions generated mainly with Ar, by recombination in the plasma with other common elements ($^{40}\text{Ar}^{36}\text{Ar}^+$, $^{40}\text{Ar}^{37}\text{Cl}^+$, $^{40}\text{Ar}^{38}\text{Ar}^+$, $^{40}\text{Ar}^{40}\text{Ca}^+$, $^{40}\text{Ar}^{42}\text{Ca}^+$), which are also called argide ions (Lum and Leung 2016). In addition, interference by doubly charged ions from rare earth elements (REEs) can be of concern for the analysis of specific samples (Bishop et al. 2015). As mentioned by D'Ilio et al. (2011), to solve the problems related to spectral interferences over $^{80}\text{Se}^+$, it is necessary to use a high-resolution ICP-MS operating at a resolution ($m/\Delta m$) of 9521.

The dynamic reaction cell (DRC) was developed to solve problems related to spectral overlap for low-resolution ICP-MS spectrometers. The DRC is a multipole, operating as a mass filter, similar to the quadrupole, by applying electric and radio frequency fields to each pair of poles (Tanner et al. 2002). The DRC is inside a low-pressure compartment, in which a reaction gas (H_2 , NH_3 , CH_4 , O_2 , etc.) is added to react with interfering species, changing their m/z ratio, to eliminate them before the ion beam reaches the quadrupole. Therefore, the reaction gas should be chosen considering the expected interfering species and the analyte of interest. Naturally, there are many possible mechanisms that can occur inside the reaction cell, such as charge transfer, proton transfer, hydrogen transfer, and atom transfer (Iglesias et al. 2002). Knowing the mechanisms and

thermodynamic parameters can help to choose the reaction gas and the best operating conditions for each situation (Lum and Leung 2016). In the case of charge transfer reactions, the potential of ionization (PI) can be used to estimate the thermodynamic probability of the reaction between the reaction gas and the interfering species or the analyte.

Concerning Se determination, the spectral interferences caused by argide ions or by doubly charged REEs over every Se isotope can be eliminated by many different approaches. Tanner et al. (2002) demonstrated that the same reaction gases used to solve the spectral overlap of Ar, ArH, ArO, etc., can be employed for argide ions. In the case of interference by doubly charged ions from REEs, according to Jackson and co-workers (Jackson et al. 2015), the PI of these reactions is between 11 and 12 eV, making the reaction with ammonia (PI ~ 10 eV) thermodynamically allowed. However, the same is not true for methane, for which the ionization potential is 12.6 eV. Hydrogen was used as the reaction gas to circumvent the problems related to doubly charged REEs over Se isotopes. The employment of oxygen as the reaction gas was an option for eliminating interference over selenium, by a mass shift reaction, and monitoring Se at m/z 96. Sucharová (2011) evaluated the employment of ammonia and methane for selenium determination in plants, and ammonia was the reaction gas of choice considering the best performance in solving the spectral interference by a doubly charged REEs. "The use of methane as the reaction gas was impaired by the presence of interfering species such as Br, which in the plasma forms the interfering species $^{79}\text{Br}^1\text{H}^+$ over $^{80}\text{Se}^+$ at high concentrations in the sample matrix. In another paper, Bishop et al. (2015) evaluated the efficiency of He, H_2 , and O_2 as collision/reaction gases for the determination of selenium in serum samples in the presence of gadolinium using ICP-MS/MS. The authors concluded that He as the collision gas and H_2 as the reaction gas at low flow rates were not able to eliminate the interference of doubly charged Gd. However, at higher flow rates, hydrogen considerably reduced the incidence of $^{156}\text{Gd}^{2+}$. The authors finally concluded that the employment of oxygen in the mass shift reaction was the most effective in solving the problems related to the spectral interference of doubly charged gadolinium over the selenium isotopes. Hu and co-workers (Hu et al. 2016) developed a method to perform an accurate quantification of As and Se in food plant samples by ICP-MS/MS, studying H_2 , O_2 , and He as reaction/collision gases. All gases could significantly reduce the spectral interferences over selenium isotopes in a matrix containing 100 mg L^{-1} of Ca and $1 \text{ } \mu\text{g L}^{-1}$ Se. However, H_2 and O_2 showed better performance with all isotopes studied ($^{77}\text{Se}^+$, $^{78}\text{Se}^+$, $^{80}\text{Se}^+$, and $^{82}\text{Se}^+$). The same behavior was not observed when the authors simulated a sample matrix containing $1 \text{ } \mu\text{g L}^{-1}$ of Se and $1 \text{ } \mu\text{g L}^{-1}$ of REEs. In this case, only the reaction gases could solve the interferences from doubly charged REEs. In another attempt to solve the spectral

interferences over selenium isotopes, Bolea-Fernandez et al. (2015) evaluated CH_3F as a reaction gas. This approach is interesting once CH_3F reacts with selenium to generate $^{80}\text{SeCH}_2^+$ ($m/z = 94$), a species free of known interferences. Considering the importance of selenium to the diet, Kubachka et al. (2017) developed a method to investigate the selenium composition in a dietary supplement for quality control purposes in a routine analysis using hydrogen as the reaction gas. The authors report that this method can be used for the quality control of dietary supplements, which is becoming increasingly important for sub-populations that need to control selenium intake.

The goal of this work was to evaluate different reaction gases (CH_4 , NH_3 , and O_2) and different conditions to resolve the spectral interferences over Se determination by ICP-MS, applying this analytical method for Se determination in dietary supplements and in its main raw material (yeast enriched in selenium). These approaches intend to provide reliable results, thus assuring food safety and helping the manufacturer of dietary supplements to pay attention to the food safety criteria of the product and, consequently, the safety of customers.

Materials and Methods

Reagents and Samples

Hydrogen peroxide and nitric acid (both from Merck, Darmstadt, Germany) were used for sample decomposition. The nitric acid was purified by sub-boiling distillation in a quartz still (Duo-PUR, Milestone, USA) and also used to prepare internal standards and calibration solutions. Distilled water was deionized in a Milli-Q system (Millipore, Bedford, MA, USA) to a minimum resistivity of 18 $\text{M}\Omega$ cm.

Argon (Ar, 99.9996%) was used as the plasma gas. Methane (CH_4 , 99.995%), ammonia (NH_3 , 99.999%), and oxygen (O_2 , 99.995%) were used as reaction gases. All gases were supplied by White Martins Ltda. (Rio de Janeiro, RJ, Brazil). Other gases not available in the laboratory were not studied.

A solution containing 1 $\mu\text{g kg}^{-1}$ of Mg, In, U, Ce, and Ba (PerkinElmer, Brazil) was employed for the daily performance optimization of the ICP-MS spectrometers.

A 0.2- $\mu\text{g kg}^{-1}$ Ga solution (SRM NIST 3119a, USA) was used as the internal standard.

The calibration solutions were prepared by gravimetric dilution in HNO_3 (2%, v/v) from a Se standard reference material (SRM) NIST 3149 (10,042 mg kg^{-1} Se solution, from the National Institute of Standards and Technology, NIST, USA).

The Se-enriched yeast certified reference material (CRM) SELM-1 (2059 \pm 64 mg kg^{-1} for total Se, from the National Research Council Canada [NRCC], Canada) was employed for method optimization and accuracy checking.

A commercial yeast sample with approximately 2000 mg kg^{-1} of Se was kindly supplied by Biorigin (São Paulo, Brazil). The yeast sample had a calcium concentration of the same order of magnitude as Se, approximately 1000 $\mu\text{g kg}^{-1}$ in the sample, which corresponds to approximately 15 $\mu\text{g kg}^{-1}$ in the optimization solution.

Samples of Se dietary supplements of four different brands were purchased from a local market and codified as follows: A (27 $\mu\text{g Se/12 tablets}$), B (Se content not listed), C (34 $\mu\text{g Se/tablet}$), and D (34 $\mu\text{g Se/tablet}$).

Equipment

A high-pressure asher (HPA-S), from Anton Paar (Graz, Austria), was used for sample decomposition, and an analytical balance with resolution of 0.01 mg (model ME 235S), from Sartorius (Göttingen, Germany), was used for samples and calibration solution preparation.

Two ICP-MS spectrometers (DRC II and NexIon 300X, both from PerkinElmer, USA) were employed for Se determination in the dietary supplements and yeast samples. The optimization of plasma conditions was carried out by the daily performance procedure, recommended by PerkinElmer, to assure a compromise between maximum sensitivity in the entire mass spectrum ($^{24}\text{Mg}^+$, $^{115}\text{In}^+$, $^{208}\text{U}^+$) and minimum levels of oxides (CeO^+) and doubly charged (Ba^{++}) ions. The internal standard was introduced in line via a t-connector with all solutions (blanks, calibration standards, and samples) to correct instrumental drift and transport effects. Different approaches were evaluated to minimize the spectral interferences over $^{80}\text{Se}^+$. The DRC II model equipment employed NH_3 and the NexIon 300X employed CH_4 and O_2 .

An inductively coupled plasma optical emission spectrometry (ICP OES) spectrometer (Ultima 2, Horiba-Jobin Yvon, Longjumeau, France) was used as an independent technique to compare to the results obtained by ICP-MS. The methodology employed was adapted from Silva et al. (2013).

All spectrometers were equipped with introduction systems composed of a Meinhard nebulizer and a cyclonic spray chamber (Glass Expansion, USA). The ICP-MS and ICP OES instrumental conditions are summarized in Tables 1 and 2, respectively.

Sample Preparation

The Se-enriched yeast and CRM SELM-1 samples had the humidity gravimetrically determined for correction of the results. This procedure was not applied to dietary supplements because the value stated in the label was not expressed as dry mass.

The dietary supplement samples were macerated and homogenized, and 0.25 g was weighed and decomposed in the quartz flasks of the HPA-S using 3 mL of a 50% (v/v) HNO_3 /

Table 1 ICP-MS operational conditions

Parameter	DRC II	NexIon
RF power (W)	1350	
Plasma gas (L min ⁻¹)	15	
Auxiliary gas (L min ⁻¹)	1.1	
Nebulizer gas (L min ⁻¹)	0.92–0.96	1.0–1.04
Cones	Pt	
Scan mode	Peak-hopping	
Sweeps/reading	5	
Readings/replicate	1	
Replicates	7	
Dwell time (ms)	50	
Detector operation	Dual	
CH ₄ gas flow rate (mL min ⁻¹)	–	0.9
R _{pq}	–	0.45
R _{pa}	–	0
NH ₃ gas flow rate (mL min ⁻¹)	1	–
R _{pq}	0.40	–
R _{pa}	0	–
O ₂ gas flow rate (mL min ⁻¹)	–	1.6
R _{pq}	–	0.65
R _{pa}	–	0

H₂O₂ solution. This equipment operates with a nitrogen atmosphere to assure better control of the digestion process. The sample decomposition was carried out using the program recommended by the software of the HPA-S system: The program started with the maximum heating power to achieve 70 °C, and then the heating rate was lowered to achieve 120 °C over 20 min; the power was increased again to the maximum to achieve 240 °C, and the temperature was kept at this value for 60 min.

After decomposition, the flasks were cooled to room temperature (21 ± 2 °C), and the digested samples were quantitatively transferred to a polypropylene flask.

Table 2 ICP OES operational conditions (Silva et al. 2013)

Parameter	Condition
RF power (W)	1400
Plasma gas (L min ⁻¹)	15
Auxiliary gas (L min ⁻¹)	0.4
Nebulizer gas (L min ⁻¹)	0.8
Acquisition mode	Peak height
Integration points to measure	5
Integration points for calculation	3
Replicates	7
Integration time (s)	1
Spectral line, λ (nm)	Se(I) 196.026

Results and Discussion

Optimization of Dynamic Reaction Cell

Although univariate studies for optimization purposes are widely used, this practice could lead to errors in the determination of the best operating conditions, mainly by failing to consider the possible interaction effects between the variables. To check if the interactions between the parameters of the reaction cell, the reaction gas flow rate and the rejection parameter q , R_{pq} , are statistically significant, a central composite design was carried out by measuring the signal intensity obtained for Se in the blank and in a yeast sample solution with 25 μg kg⁻¹ of Se and approximately 15 μg kg⁻¹ of calcium. These optimizations were carried out with the commercial yeast sample, given by Biorigin, to set the best conditions for the analysis of commercial food supplements. The criterion for reaction cell optimization was the detection limit (DRC_{LOD}), evaluated in a composite central design, using the modified Eq. (1) described by Tanner et al. (2002). DRC_{LOD} does not express the detection limit of the analytical method but is only a tool used for the optimization purposes.

$$\text{DRC}_{\text{LOD}} = \frac{3 \times \sqrt{I_{\text{Blank}}}}{I_{\text{Sample Solution}} - I_{\text{Blank}}} \times C_{\text{Sample Solution}} \quad (1)$$

Preliminary studies with the different reaction gases, not shown here, were carried out by monitoring the behavior of ⁷⁸Se⁺ and ⁸⁰Se⁺, and due to the higher sensitivity, ⁸⁰Se⁺ was chosen for the following steps of the optimization protocol, described below.

Ammonia as Reaction Gas

Before the multivariate study and to define minimum and maximum values for the ammonia flow rate, a univariate optimization was performed (Fig. 1), keeping the rejection parameter q fixed at 0.4 for a blank solution (HNO₃ 2%) and for a Se-enriched yeast sample solution (25 μg kg⁻¹).

The increasing NH₃ flow rate reduced the signal intensity obtained for m/z 80 in the blank and in the sample solution, when compared to signals obtained in the standard mode (NH₃ flow rate = 0 mL min⁻¹, Se intensities of approximately 5,000,000 cps in both solutions). For higher NH₃ flow rates (up to 1.2 mL min⁻¹), the intensities for the sample solution continued to decrease while remaining constant for the blank solution. This behavior can be attributed to the thermodynamically allowed reaction between NH₃ and Se, as mentioned in the literature (Bouchoux et al. 2012).

Bouchoux et al. (2012) carried out a theoretical investigation of selenium interferences in ICP-MS, evaluating different reaction gases. The authors reported that the formation of

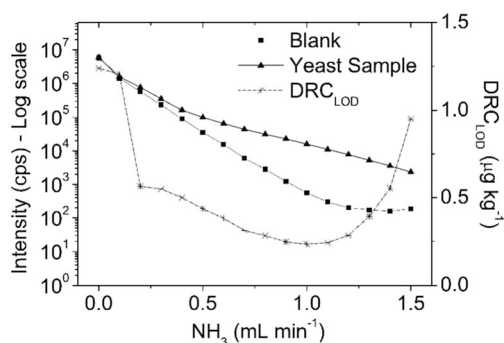


Fig. 1 Effect of NH_3 flow rate on signal obtained at m/z 80, with Rpq of 0.4

SeNH_3^+ (or HSeNH_2^+) and NH_4^+ corresponds to exothermic processes with $\Delta H = -240$ and -50 kJ mol^{-1} , respectively, suggesting the two-step mechanism for this reaction, as shown as follows



These reactions are thermodynamically allowed to occur inside the dynamic reaction cell and are in agreement with the experimental behavior reported by Sucharová (2011). According to these authors, ammonia is a good clustering ligand that is able to generate species of the general form $\text{M}(\text{NH}_3)_n^+$ or ionic condensation products such as $\text{M}(\text{NH})(\text{NH}_3)_n^+$ or $\text{M}(\text{NH}_2)(\text{NH}_3)_n^+$.

Although the formation of these species is undesirable in ICP-MS employing DRC technology, in this case, the reaction between the reaction gas (NH_3) and the interfering species (mainly $^{40}\text{Ar}^{40}\text{Ar}^+$) is faster than the reaction with the analyte ($^{80}\text{Se}^+$), as confirmed by the behavior observed in Fig. 1, allowing its use for analytical purposes. The reaction between $^{40}\text{Ar}^{40}\text{Ar}^+$ and NH_3 is proposed to occur by charge transfer, as shown in the following equation, since the ionization potential of $^{40}\text{Ar}^{40}\text{Ar}$ (14.456 eV) is higher than the ionization potential of NH_3 (10.16 eV) (D'Ilio et al. 2011; Bouchoux et al. 2012).



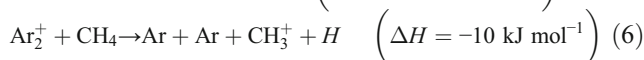
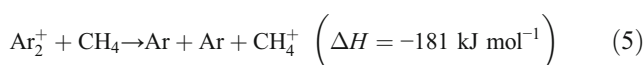
Defined by the limits for the ammonia flow rate, the multivariate optimization by central composite design of the ammonia flow rate and Rpq was carried out, and the resulting Pareto chart and response surface are shown in Fig. 2.

Figure 2a shows that the Rpq demonstrates statistical significance at 95% of the confidence level on the DRC_{LOD} , while the reaction gas flow rate did not affect it significantly. This behavior was expected, since the minimum (0.5 mL min^{-1}) and the maximum (1.5 mL min^{-1}) NH_3 flow rate values employed in the experimental design were selected considering the values obtained in the univariate study. In this experiment, the

intensity for m/z 80 reached a minimum constant value for the blank, indicating that the influence of the $^{40}\text{Ar}^{40}\text{Ar}^+$ species could be disregarded.

Methane as the Reaction Gas

According to Tanner et al. (2002), methane can be used as the reaction gas leading to reactions with polyatomic species of argon. D'Ilio et al. (2011) mentioned that the reaction between CH_4 and $^{40}\text{Ar}^{40}\text{Ar}^+$ occurred by a charge exchange mechanism, since the ionization potential of CH_4 is 12.6 eV, lower than the ionization potential of $^{40}\text{Ar}^{40}\text{Ar}$ (approximately 14.456 eV) and confirmed by the enthalpy data for the possible reactions



Then, methane is expected to be adequate for eliminating the interfering species, mainly argon dimer, over selenium isotopes.

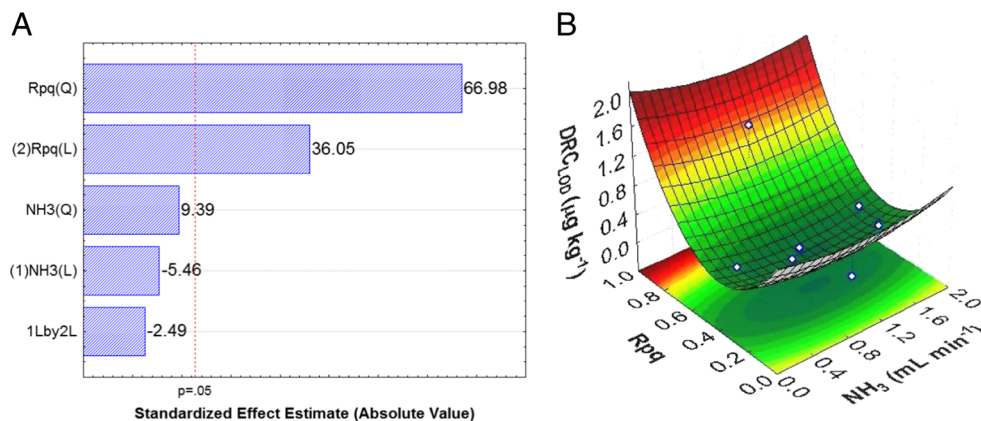
The same optimization protocol used for NH_3 was employed to evaluate the ability of methane to eliminate interfering species over $^{80}\text{Se}^+$ and once again, the interaction factor between reaction gas flow rate (CH_4) and Rpq was not statistically significant (results not shown here).

Figure 3 shows the behavior of methane as the reaction gas for m/z 80, using a blank solution of HNO_3 (2%) and a yeast sample solution of $25 \mu\text{g kg}^{-1}$ of Se.

As expected, when the equipment was operated in the standard mode, the intensities obtained for the blank and the sample solution were of the same order of magnitude due to the high incidence of spectral overlap by interfering species. Figure 3 shows that the intensity for both solutions was reduced until reaching a complete discrimination between the blank and the sample solution at approximately 0.5 mL min^{-1} CH_4 flow rate. For the sample solution, the signal was reasonably constant up to 1 mL min^{-1} CH_4 flow rate, and then the signal decreased slightly.

Some considerations can be done based on the experimental data obtained in this work. The reaction between CH_4 and $^{40}\text{Ar}^{40}\text{Ar}^+$ is thermodynamically allowed, occurring by means of a charge transfer mechanism. However, the signal intensities decreased for a CH_4 flow rate higher than 1.0 mL min^{-1} , probably due to collisional processes inside the dynamic reaction cell that can cause Se to defocus from the ion beam, considering that the incidence of non-effective collisions is proportional to the quantity of molecules inside the DRC. In this way, the optimization of reaction gas flow rate must be a compromise between reaction efficiency and reduction of analytical signal by collisional processes.

Fig. 2 **a** Pareto chart and **b** response surface obtained by central composite design of Rpq and NH₃ flow rate



Oxygen as Reaction Gas

An interesting approach in DRC technology is the possibility of employing a reaction gas to shift the *m/z* of the analyte by a transfer reaction, and oxygen can be used for this purpose (Stürup et al. 2006; da Silva and Arruda 2013). The reaction between selenium and oxygen is thermodynamically allowed, enabling the analysis of this element by measuring the *m/z* 96, without the undesirable interference of ⁴⁰Ar⁴⁰Ar⁺. In fact, Se reacts with O₂ following Eq. (7)

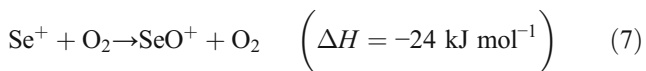


Figure 4a, b shows the univariate experiment carried out to check the feasibility of this approach for the determination of Se in yeast samples.

Figure 4a shows the behavior observed when O₂ was applied as the reaction gas to convert ⁸⁰Se⁺ into the ⁸⁰Se¹⁶O⁺ species by monitoring the *m/z* 96. The signal intensity obtained for the sample solution increased slightly with the increase in the O₂ flow rate up to 0.6 mL min⁻¹. However, when the signal-to-background ratio (not shown here) was evaluated, the maximum was obtained at a 1.6 mL min⁻¹ O₂ flow rate.

The reaction between Se and O₂ inside the dynamic reaction cell is not complete because only a small portion of the

total Se converted into the oxidized form (SeO⁺). The efficiency of this reaction was estimated using Eq. (8), at approximately 10%. This statement is in agreement with a previous study described in the literature (Stürup et al. 2006).

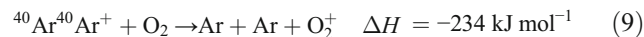
$$\% \text{SeO} = \frac{I_{\text{SeO}}}{I_{\text{SeO}} + I_{\text{Se}}} \times 100 \quad (8)$$

where

*I*_{SeO} = net intensity at *m/z* 96

*I*_{Se} = net intensity at *m/z* 80

Figure 4b shows the behavior of O₂ as the reaction gas to solve the interference of ⁴⁰Ar⁴⁰Ar⁺ over ⁸⁰Se⁺. Hattendorf and Günther (2003) mentioned that O₂ reacts with ⁴⁰Ar⁴⁰Ar⁺ by a charge transfer mechanism, according to Eq. 9.



Therefore, as previously demonstrated in that work, the reaction of O₂ with Se also occurred, but to a lesser extent, with the Δ*H* of -24 kJ mol⁻¹ (Hattendorf and Günther 2003; Bandura et al. 2006), which makes possible its use for analytical purposes, as shown in Fig. 4. Therefore, the determination of Se in both *m/z* ratios (96 and 80) was studied with further experiments.

Figures of Merit

The limit of detection was obtained employing the 3σ of the blank concentration criteria. Table 3 shows the instrumental LODs obtained with the different approaches (different gases and optimized conditions) studied in this work.

The lowest LOD was obtained with CH₄ as the reaction gas, mainly because this gas reacts specifically with the ⁴⁰Ar⁴⁰Ar⁺ interfering species, keeping the Se signal constant. This behavior was not observed when NH₃ and O₂ were employed as reaction gases, although both significantly reduced the incidence of interfering species. Figures 1 and 4

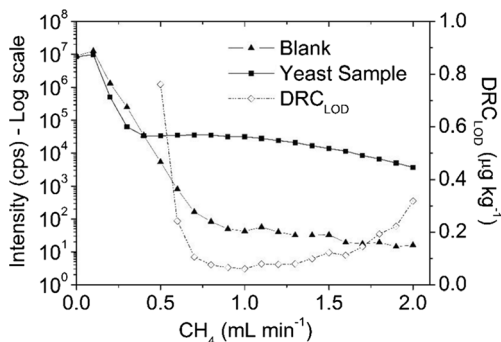


Fig. 3 Effect of CH₄ as reaction gas on signal obtained for *m/z* 80 with Rpq of 0.45

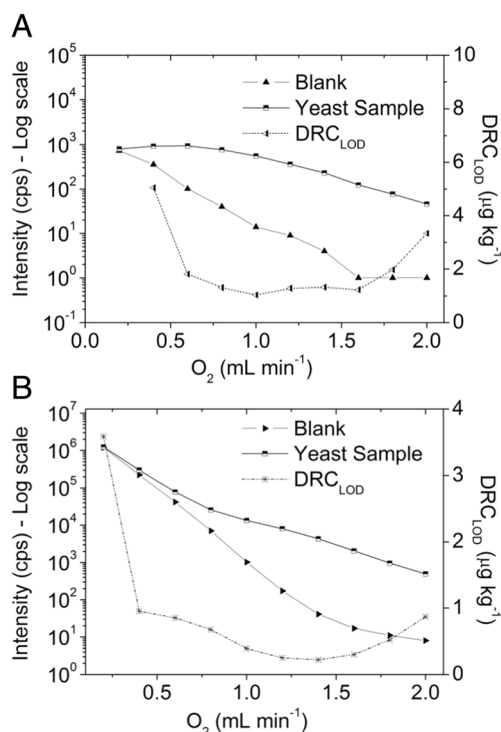


Fig. 4 Effect of O_2 as reaction gas on signal obtained for **a** m/z 96 ($^{80}\text{Se}^{16}\text{O}$) and **b** m/z 80 (^{80}Se) with R_{pq} of 0.65

show that NH_3 and O_2 also react with Se, decreasing its signal intensity and consequently raising the limits of detection. Although the approach employing O_2 and monitoring the m/z 96 has presented the lowest sensitivity (approximately 10% of ^{80}Se is converted into $^{80}\text{Se}^{16}\text{O}^+$), this approach showed a lower LOD than the approach using NH_3 and O_2 and monitoring the $^{80}\text{Se}^+$.

Analytical Application

Analysis of Se-Enriched Yeast and Dietary Supplement Samples Table 4 shows the concentration of selenium in commercial yeast obtained employing the different reaction gases studied in this work and the result obtained by an independent technique—ICP OES.

The results obtained by ICP-MS employing different reaction gases were in good agreement with the certified value,

Table 3 DRC conditions selected for the determination of Se in yeast samples and obtained limits of detection

Approach	Gas flow rate (mL min ⁻¹)	R _{pq}	LOD (µg kg ⁻¹)
Methane (^{80}Se)	0.9	0.45	0.01
Oxygen ($^{80}\text{Se}^{16}\text{O}$)	1.6	0.65	0.03
Oxygen (^{80}Se)	1.6	0.65	0.06
Ammonia (^{80}Se)	1.0	0.40	0.1
Standard mode (^{82}Se)	–	–	0.2

Table 4 Concentration of selenium in yeast samples

Approach (measured isotope)	Yeast sample (mg kg ⁻¹)	CRM SELM-1 (2059 ± 64 mg kg ⁻¹) (% of the certified concentration)
Methane (^{80}Se)	2136 ± 111	2141 ± 139 (104%)
Ammonia (^{80}Se)	2187 ± 209	2048 ± 105 (99%)
Oxygen (^{80}Se)	2298 ± 176	2137 ± 181 (104%)
Oxygen ($^{80}\text{Se}^{16}\text{O}$)	2286 ± 173	2122 ± 204 (103%)
Standard mode (^{82}Se)	2171 ± 131	–
ICP OES (196 nm)	2169 ± 107	2048 ± 69 (99%)

Results expressed as $\bar{x} \pm U(n = 5; p = 0.05)$

and the degree of accuracy was in the range of 99 to 104% for the CRM SELM-1. Comparing the results obtained by ICP OES and the approaches employed in ICP-MS, all results were statistically similar, considering the uncertainty ranges. These results demonstrate the efficiency of the reaction gases studied in this work in circumventing the spectral interferences over $^{80}\text{Se}^+$, not only those Ar-based species but also Ca species, once the yeast sample had a calcium concentration of approximately 1000 mg kg⁻¹ (determined by semiquantitative analysis).

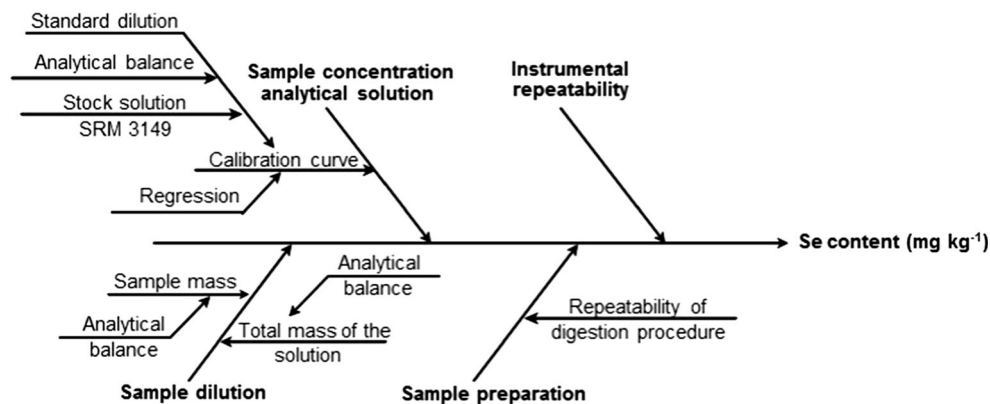
The measurement uncertainty was obtained employing the concept of error propagation according to ISO GUM (JCGM 101: 2008). The uncertainty sources considered in this work were as follows: (i) Se concentration in analytical solution— u_{Co} , (ii) instrumental repeatability— u_{rep} , (iii) standard dilution— u_{std} , (iv) sample dilution— u_{dil} , and (v) sample preparation— u_{prep} . These sources of uncertainty are shown in the Ishikawa diagram (Fig. 5).

The methodology developed in this work was applied to an evaluation of some commercial brands of dietary supplement obtained in the local market. Since all gases had similar behavior in the interference correction and due to technical facilities, only NH_3 was used as the reaction gas in this analysis. Table 5 shows the selenium content measured in these samples.

In 2003, the Brazilian Health Surveillance Agency (Agência Nacional de Vigilância Sanitária (ANVISA)) has published a resolution (RDC no. 360) establishing technical requirements for the labels of packaged food, as well as of dietary supplements. This document (RDC no. 360) presents aspects concerned with nutritional information, including the adoption of a tolerance of +20% of the discrepancy between the real content of the nutritional parameter (for example, Se mg per tablet) and the value declared on the label (ANVISA 2003).

According to the criteria adopted by Brazilian Legislation (ANVISA 2003), the brands C and D can be considered adequate for consumption considering the uncertainty of the

Fig. 5 Ishikawa diagram of uncertainty sources



measurement. Although the results found for Brand A correspond only to 71% of the labeled value for Se content, this sample was not considered inappropriate for consumption according to the legislation because RDC no. 360 does not address differences lower than labeled.

In Brazil, the recommended dietary allowance for Se is 34 µg for adults, according to ANVISA (2005), but this limit might be different according to country or authority (WHO 2004; Baghurst 2005; Høybråten 2012). Therefore, the dietary supplement samples analyzed in this work do not represent a real risk for consumers.

To perform a critical evaluation of the results found for selenium content in dietary supplements, an uncertainty study was carried out (Fig. 6).

In this study, the overall uncertainty is fragmented as to its individual sources, making possible the evaluation of the contribution of each source to the final result. As seen in Fig. 6, the variability between tablets of each dietary supplement represents the major uncertainty source, contributing almost 50 and 80%, in relation to the combined uncertainty, for brands C and D, respectively.

In general, the contribution of the uncertainty sources arising from the analytical method did not contribute significantly to the final result, except for brand B, in which the uncertainty of Se concentration in analytical solution— u_{C_0} —contributed almost 80%, mainly because the selenium content was much lower in this sample than in the others. Despite the results

being in agreement with the legislation, the great variability inside each bottle, near 20%, highlights the need of a more robust quality control in the manufacturing process of dietary supplements. This evidence can be confirmed by the uncertainty study shown in Fig. 6.

Other recent studies dealing with method development and quality control for dietary supplements have found similar results, confirming that misformulated products are commonly available in the market (MacFarquhar et al. 2010; Niedzielski et al. 2016). In 2002, Valiente et al. (2002) published a comparative study of 15 samples of commercial dietary supplements, and the authors found Se content ranging from -50 to +224% related to the labeled value. In another study, Niedzielski et al. (2016) evaluated different selenium-enriched dietary supplements available in the Polish market, where the real selenium content ranged from 12 to 280% in agreement with the declared selenium content. Although these studies had found disagreement between measured and labeled Se content, the dietary supplements also did not represent a health risk for the consumers. We also found in the literature a study in which the results measured in dietary supplements were in reasonable agreement with the manufacturer-declared value (Tyburska and Jankowski 2013).

All these studies and ours corroborate the necessity of a more rigorous quality control of supplement contents, considering that the label claims might be inaccurate (B'Hymer and Caruso 2000; Valiente et al. 2002).

Table 5 Selenium content in commercial dietary supplement

Samples	Se content (µg Se tablet ⁻¹)	Variability inside the bottle (%)	Lower (µg Se tablet ⁻¹)	Upper (µg Se tablet ⁻¹)	Discrepancy with labeled value (%)
Brand A (2.25 µg) ^a	1.6 ± 0.1	6	1.5	1.7	-29
Brand B (NI)	0.25 ± 0.02	8	0.23	0.27	-
Brand C (34 µg)	42 ± 8	19	34	50	+23
Brand D (34 µg)	39 ± 6	15	33	45	+15

Results expressed as microgram per tablet; $\bar{x} \pm U$ ($k = 2; n = 5$)

NI not informed

^a Value labeled 27 µg 12 tablets⁻¹

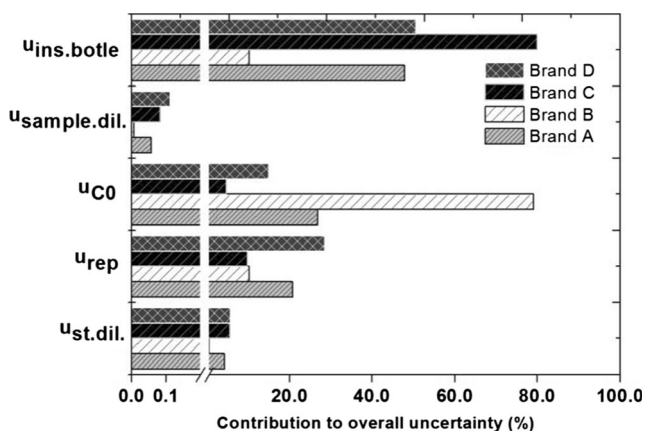


Fig. 6 Contribution of individual sources to overall uncertainty. $u_{ins.botte}$ the variation between tablets of the same brand, $u_{sample.dil.}$ sample dilution, u_{CO} Se concentration in analytical solution, u_{rep} instrumental repeatability, $u_{st.dil.}$ standard dilution

Conclusions

The employment of different reaction gases in DRC-ICP-MS is an interesting alternative to solve the problems related to spectral overlap of argon polyatomic species over $^{80}\text{Se}^+$. Methane was the most adequate reaction gas, which reacted specifically with $^{40}\text{Ar}^{40}\text{Ar}^+$ dimer, resulting in a lower limit of detection than the limit of detection obtained with NH_3 and O_2 . Although CH_4 has shown better performance, NH_3 and O_2 were also able to satisfactorily solve the spectral interference over $^{80}\text{Se}^+$. Nevertheless, NH_3 and O_2 also react with Se^+ , but their reaction with interfering species is faster than with the analyte, enabling its use for analytical purposes.

The dynamic reaction cell technology is demonstrated to be a powerful tool to circumvent problems related to spectral interferences over the most abundant isotope of selenium in yeast samples. The method allowed the analysis of dietary supplements with good precision and accuracy. The results found for these samples showed a great variation between the tablets from the same brand and a considerable discrepancy of selenium content with respect to labeled value. In addition to these discrepancies, the dietary supplements were considered adequate for consumption, according to Brazilian legislation requirements. Supported by the results obtained in this work, we can conclude that there is a need for a most rigorous quality control of dietary supplement in Brazil.

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