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# Method development and optimization for the determination of selenium in bean and soil samples using hydride generation electrothermal atomic absorption spectrometry

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ABSTRACT

The present investigation is the first part of an initiative to prepare a regional map of the natural abundance of selenium in various areas of Brazil, based on the analysis of bean and soil samples. Continuous-flow hydride generation electrothermal atomic absorption spectrometry (HG-ET AAS) with in situ trapping on an iridium-coated graphite tube has been chosen because of the high sensitivity and relative simplicity. The microwave-assisted acid digestion for bean and soil samples was tested for complete recovery of inorganic and organic selenium compounds (selenomethionine). The reduction of Se(VI) to Se(IV) was optimized in order to guarantee that there is no back-oxidation, which is of importance when digested samples are not analyzed immediately after the reduction step. The limits of detection and quantification of the method were 30 ng $L^{-1}$ Se and 101 ng $L^{-1}$ Se, respectively, corresponding to about 3 ng $g^{-1}$ and 10 ng $g^{-1}$, respectively, in the solid samples, considering a typical dilution factor of 100 for the digestion process. The results obtained for two certified food reference materials (CRM), soybean and rice, and for a soil and sediment CRM confirmed the validity of the investigated method. The selenium content found in a number of selected bean samples varied between $5.5 \pm 0.4$ ng $g^{-1}$ and $1726 \pm 55$ ng $g^{-1}$, and that in soil samples varied between $113 \pm 6.5$ ng $g^{-1}$ and $1692 \pm 21$ ng $g^{-1}$.



## 1. Introduction

Selenium is an essential micronutrient for humans and animals [1]. It has an important role as a substance with anti-cancer properties and its ability to prevent heart disease. Furthermore, selenium possesses antineoplasmatic properties since studies have demonstrated that dietary selenium supplementation can inhibit chemically induced tumors in rats [2]. The US Food and Nutrition Board established dietary reference intakes for selenium, calculated from the recommended dietary allowance (RDA) for certain groups with special physiological requirements [3], which are between 15 and 20 μg/day for infants and 70 μg/day for lactating mothers. An upper limit for safe intake was set at 400 μg/day. Nowadays, application of selenium-containing mineral supplements is very common for cattle breeding in the higher developed south and south east of Brazil.

Different types of food, such as biological materials and dairy products, are important sources of selenium in the human diet and its uptake depends on its chemical form. Various plants growing on selenium-rich soil absorb and accumulate this element [4]. Selenomethionine has been shown to be the predominant form of selenium in wheat, soybeans and selenium-enriched yeast [5].

Because of the importance of this element selenium maps are already available for many countries in the world (Selenium World Atlas) [6]; however, there is essentially nothing known about the natural abundance of selenium in areas of the Brazilian territory. In order to close this gap, a sampling campaign has already been completed to collect bean and soil samples from various states from the north to the utmost south of Brazil [7]. Bean samples have been chosen because they are grown all over Brazil, and they also represent a major part of the daily diet in this country. The purpose of the

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present work was to develop and validate a reliable routine method for the determination of selenium in a large number of bean and soil samples, taking into consideration all stages of the analytical procedure.

Because of the usually low concentration of selenium in biological and soil samples and the often complex matrix it is necessary to apply sensitive and selective analytical techniques for its determination. Electrothermal atomic absorption spectrometry (ET AAS) and hydride generation atomic absorption spectrometry (HG AAS) are frequently used for this purpose. While the response of HG AAS is strongly dependent on the selenium species present, ET AAS is adequate for the determination of both organic and inorganic selenium compounds [8]. Nevertheless, the accurate determination of selenium in biological materials and soil is still a major challenge [9–11]. In HG AAS the incomplete mineralization of refractory organic selenium compounds, such as selenomethionine, is one of the major challenges, whereas selenium losses by volatilization were often the cause for erroneous results in ET AAS. Furthermore, there are three nearby iron lines at 195.950 nm, 196.061 nm and 196.147 nm, which might cause spectral interference at the most sensitive selenium line at 196.026 nm when conventional line-source AAS is used [12]. This line is also in the range of strong molecular absorption due to PO and NO bands with pronounced fine structure, which might cause spectral interference due to under- or overcorrection in ET AAS [11,12].

In situ trapping of a hydride (arsine) in a pre-heated graphite tube was for the first time proposed by Drasch et al. [13] in 1980, and further refined by Sturgeon et al. [14]. Sturgeon and co-workers investigated carefully the sequestration of various hydride-forming elements, including selenium [15] in 'used' graphite tubes, which had a more active surface for trapping the hydrides at elevated temperature. In 1989 two papers were published almost simultaneously, in which a treatment of the graphite tubes with palladium was proposed in order to avoid the rather unpredictable behavior of a 'used' graphite tube [16,17]. This treatment was applied successfully by several authors to pre-concentrate and atomize selenium, and to combine the advantages of HG AAS and ET AAS [18–20]. The disadvantage of palladium was its low thermal stability in the atomization stage, so that the coating lasted only for one determination and had to be renewed before each measurement. In order to solve this problem, Shuttler et al. [21] proposed the use of iridium as a permanent modifier for the trapping of selenium and other hydride-forming elements in the graphite tube, a practice that was applied successfully by others later on [22–24]. Iridium applied as a permanent modifier was found to be a much more economic alternative; as such tubes could be used for several hundred measurements without any re-coating. The use of iridium coating also significantly improved the sensitivity of the method and the efficiency of hydride deposition in comparison with those obtained with other kind of modifiers [25].

Nevertheless, the first step of this combined hydride generation electrothermal atomic absorption spectrometric technique (HG-ET AAS) is the generation of the gaseous $SeH_2$, which requires that the selenium is present as inorganic Se(IV), i.e., selenite. This means that any organic selenium compounds have to be completely mineralized, which might require quite harsh conditions [26], and all Se(VI) has to be reduced to Se(IV) prior to hydride generation. Different methods have been suggested to convert the total organo-selenium in biological materials to inorganic selenium under varying oxidizing conditions. The digestion of human body fluids with only $HNO_3$ at 160 °C under pressure gave low selenium recoveries, but digestion with nitric, sulfuric and perchloric acids at a maximum temperature of 310 °C under reflux gave recoveries of 97–104% [26,27]. Comparison of several digestion methods for Se determination in biological samples has shown that only the $HNO_3 + HClO_4$ or $HNO_3 + H_2SO_4 + H_2O_2$ mixtures yielded complete recovery of Se [28]. Microwave-assisted wet digestion based on a mixture of $HNO_3$ and $H_2O_2$ followed by UV irradiation was successful to recover the total Se content in fish [29], but failed for shellfish samples. After digestion, boiling the solution with 5 or 6 mol $L^{-1}$ hydrochloric acid under reflux for 15–30 min was found to be sufficient to reduce all hexavalent to tetravalent selenium [30–32].

The goal of this work has been to develop a reliable routine procedure for the determination of selenium in a large number of bean and soil samples in order to establish the selenium status in as many states of Brazil as possible. Time and reagent consumption obviously played a major role, and all stages of the analytical procedure had to be optimized.

**Table 1**
Graphite furnace temperature program for the deposition of the iridium permanent modifier on the graphite platform; argon flow rate 1 L $min^{-1}$ in all stages.

| Step | Temperature (°C) | Ramp (°C $s^{-1}$) | Hold time (s) |
|---|---|---|---|
| 1 | 90 | 5 | 40 |
| 2 | 110 | 1 | 40 |
| 3 | 130 | 1 | 40 |
| 4 | 1200 | 300 | 25 |
| 5 | 2100 | 500 | 10 |
| 6 | 2100 | 0 | 5 |

## 2. Experimental

### 2.1. Instrumentation

All measurements were carried out using a Model AAS 5 EA atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with deuterium background correction, equipped with a transversely heated graphite tube atomizer and a Model HS1 hydride generation system (Analytik Jena). A selenium hollow cathode lamp (Analytik Jena) was used as the radiation source and operated with a current of 8.0 mA (wavelength 196.0 nm, spectral bandwidth 1.2 nm). The spectrometer was interfaced to an IBM PC/AT compatible computer. The hydride generation system was used in the continuous-flow mode and the gaseous hydrides were introduced into the pre-heated graphite tube using a Model MPE 50 furnace autosampler (Analytik Jena), equipped with a stainless steel capillary. A four-channel hose pump with snap-in hose cartridges was used for transporting the sample, the reducing agent and the acid to the reaction coil; the fourth channel was used to transport the waste from the gas–liquid separator into the receptacle. The gas–liquid separator was a modified 25-mL pointed flask with larger dead volume, which was half filled with 5-mm diameter glass beads. Pyrolytically coated graphite tubes without platform (Analytik Jena Part No. 407-A81.011) were used exclusively, and coated with 200 μg Ir, depositing five portions of 40 μL of a 1000 mg $L^{-1}$ Ir stock solution and executing the temperature program shown in Table 1 after each injection. The tube treated in this manner could be used for about 500 measurements without any re-coating. The graphite furnace temperature program for trapping the hydrogen selenide and atomization of selenium is shown in Table 2. In order to avoid loss of the permanent modifier at high temperatures, the

**Table 2**
Graphite furnace temperature programs for the sequestration of hydrogen selenide and atomization of selenium.

| Stage | Temperature (°C) | Ramp (°C $s^{-1}$) | Hold time (s) | Argon flow rate (mL $min^{-1}$) |
|---|---|---|---|---|
| Collection | 500 | 100 | 30 | 300 |
| Atomization | 2100 | 2000 | 5 | 0 |
| Cleaning | 2200 | 100 | 3 | 300 |

**Table 3**
Microwave digestion program for bean and soil samples.

| Parameters | Bean samples | | Soil samples |
|---|---|---|---|
| | 1 | 2 | |
| Temperature (°C) | 145 | 180 | 190 |
| Pressure (bar) | 40 | 40 | 40 |
| Power (%) | 70 | 80 | 80 |
| Ramp (min) | 10 | 5 | 1 |
| Hold time (min) | 5 | 10 | 25 |

cleaning temperature was set at 2200 °C, which was sufficient as no significant residues were expected in the graphite tube using this technique. Integrated absorbance (peak area) was used exclusively for signal evaluation and all values are the average of at least three successive measurements.

All sample digestions were carried out using a TOPwave IV laboratory microwave digestion system (Analytik Jena) with rotor CX 100 and independent, contact-free temperature and pressure control for each of the eight vessels. The system allows a maximum digestion temperature of 230 °C (short term 300 °C) and a maximum pressure of 100 bar. The microwave digestion programs used for bean and soil samples are shown in Table 3.

### 2.2. Reagents and samples

Deionized and further purified Milli-Q high purity water (Millipore, Bedford, MA, USA) was used throughout. The stock standard solution for inorganic Se, containing 1000 mg $L^{-1}$ Se was prepared from a Titrisol® concetrate (Merck, Darmstadt, Germany); an organic selenium standard solution containing 1000 mg $L^{-1}$ Se was prepared from selenomethionine, $C_5H_{11}NO_2Se$ (Fluka, Sigma–Aldrich, Belgium) by dissolving the reagent in 0.5 mol $L^{-1}$ HCl. A freshly prepared 1% (w/v) solution of sodium tetrahydroborate, (purum, Sigma–Aldrich, Steinheim, Germany) in 0.5% (w/v) NaOH (CAQ, Casa da Química, Brazil) was used as the reductant. An iridium standard solution containing 1000 mg $L^{-1}$ Ir (Merck) was used for coating the graphite tube. HCl (37% m/v, Merck) was further purified by sub-boiling distillation in a quartz still (Kuerner Analysentechnik, Rosenheim, Germany). Working standard solutions were prepared daily diluting the standards for HG-ET AAS measurements. All other reagents were of analytical reagent grade.

The certified reference materials (CRM) used in this work for the validation of the method for bean samples were GBW10013 soybean, with a proposed value of 22 ng $g^{-1}$ Se, and GBW10010 rice, with a certified value of $61 \pm 15$ ng $g^{-1}$ Se (Institute of Geophysical and Geochemical Exploration, Langfang, China). The CRM for the validation of the method for the soil samples were BCR-142, light sandy soil (European Community Bureau of Reference-BCR, Brussels, Belgium), with a proposed value of 530 ng $g^{-1}$ and Antarctic Marine Sediment (MURST-ISS-A1) from the Italian Programma Nazionale di Ricerche in Antartide, PNRA (Instituto Superiore di Sanitá, Rome, Italy) with a certified value of $2.2 \pm 0.1$ μg $g^{-1}$.

A number of arbitrarily selected soil and bean samples from the sampling campaign have been analyzed in order to show the applicability of the developed method for real samples. The sampling strategy and the mode of sample preparation were described in a previous paper of our group [33]. No attempt has been made to correlate the selenium concentration found in the samples with the location where they have been taken or to correlate selenium concentrations found in beans and soils, as this will be the topic of another, more extensive investigation.

### 2.3. Sample preparation

Before, each digestion program, the PTFE vessels were soaked overnight in diluted nitric acid (10% m/v $HNO_3$) at room temperature, followed by a cleaning program using reagent grade nitric acid (65% m/v) and the microwave cleaning program recommended by the manufacturer. For the digestion of the bean samples, 6 mL of concentrated nitric acid (65% m/v) and 6 mL of hydrogen peroxide (30% m/v) were added to about 800 mg of the bean sample in the PTFE digestion vessels of the microwave system. For the digestion of the soil samples, 2.5 mL of concentrated nitric acid (65% m/v) and 7.5 mL of hydrochloric acid (37% w/v) were added to about 1.0 g of soil sample in the PTFE digestion vessels. The vessels were closed and the content mixed for 5 min using an ultrasonic bath (Model 28X, DENTSPLY International, USA). Afterwards the closed vessels were removed from the ultrasonic bath and left at atmospheric pressure for 30 min. Finally, the vessels were introduced into the microwave oven and the digestion program given in Table 3 was used. In order to avoid foaming and splashing, the vessels were allowed to cool to room temperature for 30 min after the end of the digestion program, opened carefully and the volume was made up to 40 mL with water.

After cooling, 40 mL of hydrochloric acid (5% w/v) was added to 40 mL of the digested bean or soil samples in a round-bottom flask and the mixture heated for 15 min under reflux to reduce Se(VI) to Se(IV). During this process the flask was flushed with an argon stream of 0.5 L $min^{-1}$ in order to remove chlorine that might be forming during the reduction process. Hence, the dilution factor of bean and soil samples was 100 and 80, respectively.

## 3. Results and discussion

Whereas selenium in soils is usually present in the form of minerals, i.e., in inorganic form, selenium in biological materials, such as beans, is mostly present as complex organic compounds, such as selenomethionine, which require harsh conditions to be completely mineralized – a pre-condition for determinations using HG-ET AAS. Hence, the digestion procedure has to be investigated carefully to ensure complete mineralization of organo-selenium compounds. The oxidation state of selenium is of importance as well, as only Se(IV) forms a gaseous hydride, so that a reduction step is mandatory in case Se(VI) was formed during sample digestion. Finally, the different parameters in the HG-ET AAS procedure with in situ trapping in an Ir-coated graphite tube had to be optimized as well. These parameters are: trapping and atomization temperatures, argon carrier gas flow rate, concentration of carrier and reducing solutions, and the prevention of the back-oxidation of Se(IV) to Se(VI).

### 3.1. Trapping and atomization temperatures

The trapping temperature of the hydrogen selenide at the Ir-coated platform and the atomization temperature have been optimized using an aqueous standard solution of 10 μg $L^{-1}$ Se. The sensitivity for Se increased with increasing trapping temperature up to about 700–800 °C, as is shown in Fig. 1. However, an increasing memory effect has been observed for trapping temperatures higher than 500–600 °C, which was found to be due to decomposition of $H_2Se$ at these temperatures and the deposition of Se in the injection capillary. Hence, a compromise trapping temperature of 500 °C has been chosen for all future experiments. The fact that there is a steep increase in sensitivity between 500 °C and 700 °C trapping temperature already shows that trapping of $H_2Se$ at 500 °C is not quantitative. It has to be considered that this steep increase in sensitivity is in spite of an also increasing memory effect, i.e., an increasing decomposition of $H_2Se$ in the injection capillary. Hence, there are two competitive reactions going on above 500 °C, and it is not possible from this experiment to estimate the trapping efficiency of $H_2Se$ at this temperature. This topic will be discussed one more time in Section 3.6 in connection with the characteristic mass.

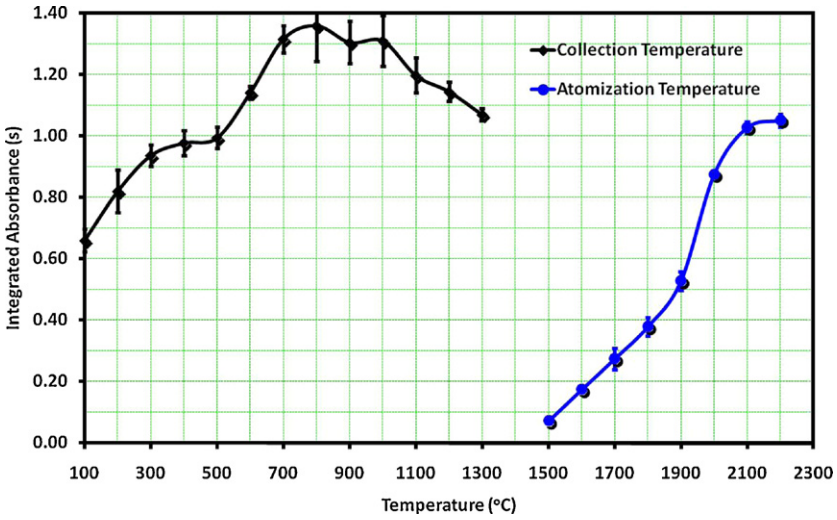


**Fig. 1.** Optimization of collection (trapping) temperature for hydrogen selenide in an Ir-coated graphite tube and atomization temperature for selenium; error bars are standard deviation of $n = 3$ repetitive collections and determinations of a 10 μg $L^{-1}$ Se solution.

The sensitivity for selenium increased up to an atomization temperature of 2100 °C, as is also shown in Fig. 1, above which temperature it started to decrease again; a temperature of 2100 °C has therefore been chosen for all future experiments. Table 2 shows the optimized graphite furnace temperature program used in the present work. The graphite tube was pre-heated to a temperature of 500 °C and a hold time of 30 s was used for trapping of the hydrogen selenide in the Ir-coated graphite tube (stage 1). Then, the trapped Se was atomized at 2100 °C and the integrated absorbance measured (stage 2). The graphite tube was cleaned with the purge gas flow on, using an only slightly higher temperature of 2200 °C (stage 3) in order not to vaporize the Ir permanent modifier. The optimized temperature values are somewhat different but comparable to trapping and atomization temperatures published in the literature [22,34–38].

### 3.2. Influence of argon gas flow rate

The influence of the argon gas flow rate used to transport the hydrogen selenide into the graphite tube on the integrated absorbance and the repeatability of the measurements is shown in Fig. 2. At argon flow rates lower than 300 mL $min^{-1}$, the integrated absorbance signal for Se was low and instable. This was probably due to incomplete and inconsistent liberation of the hydrogen selenide from the liquid phase, so that a significant part of the gas was still dissolved in the liquid and pumped to the waste. At argon flow rates between 300 and 600 mL $min^{-1}$, the sensitivity was approximately constant, with a slight decrease for higher flow rates. It is interesting to note that apparently the hydrogen that is developed in the reaction between the tetrahydroborate and the acid carrier is not capable of liberating the hydrogen selenide gas efficiently from the liquid phase. At argon flow rates higher than 600 mL $min^{-1}$ the total gas flow was apparently too high to ensure efficient trapping of the hydrogen selenide in the Ir-coated graphite tube. Therefore, an argon flow rate of 300 mL $min^{-1}$ was selected for analyte trapping in all future experiments.

### 3.3. Influence of acid and reducing agent concentration

The integrated absorbance signal for selenium increased rapidly with increasing hydrochloric acid concentration used as the carrier solution up to a concentration of 2% (w/v), and slightly more until a

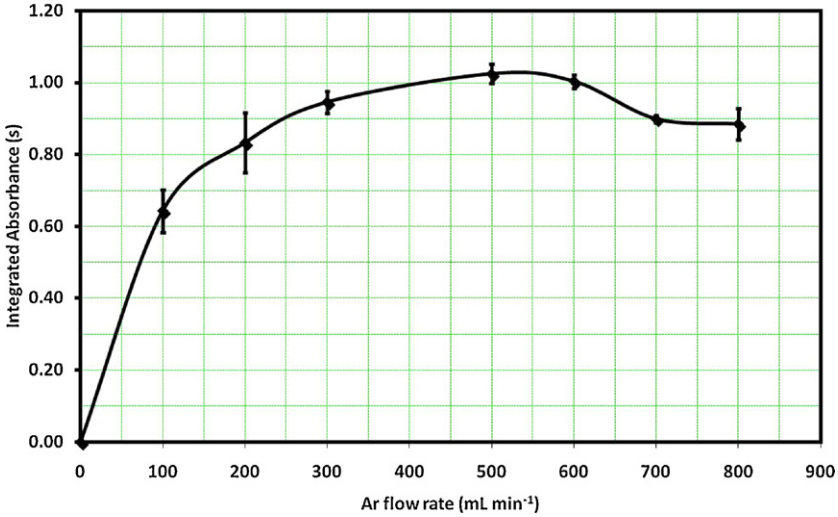


**Fig. 2.** Influence of the argon flow rate for the transport of the hydrogen selenide to the graphite tube atomizer on the integrated absorbance of selenium; error bars are standard deviation of $n = 3$ repetitive collections and determinations of a 10 μg $L^{-1}$ Se solution.

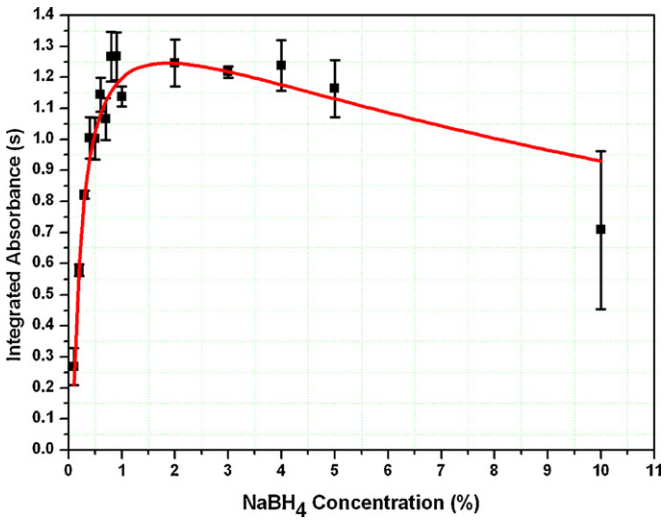


**Fig. 3.** Influence of the concentration of the reduction solution ($NaBH_4$) on the integrated absorbance of Se; error bars are standard deviation of $n = 3$ repetitive collections and determinations of a 10 μg $L^{-1}$ Se solution.

concentration of about 5% (w/v) HCl was reached. A further increase in the hydrochloric acid concentration did not have any significant influence on the Se signal except for an increasing imprecision. The pumping flow rate of hydrochloric acid as a carrier solution was fixed at 7 mL $min^{-1}$. A concentration of 5% (w/v) HCl was used for all further experiments.

The concentration of the reducing agent (sodium tetrahydroborate) was also optimized in the range between 0.1% and 10% (w/v), using a fixed concentration of 0.5% (w/v) sodium hydroxide for the stabilization of the solution. The pumping flow rate of the reduction solution was fixed at 3 mL $min^{-1}$. Fig. 3 shows that the selenium signal was increasing sharply with increasing concentration of the reduction solution up to about 1% (w/v) $NaBH_4$. Then the signal remained essentially constant within the concentration range from 1 to 5% (w/v), and decreased for further increasing $NaBH_4$ concentrations, which was accompanied by a significant decrease in precision, most likely due to the very violent reaction under these conditions. A sodium tetrahydroborate concentration of 1% (w/v), stabilized with 0.5% (w/v) sodium hydroxide was used for all future work.

### 3.4. Reduction of Se(VI) to Se(IV)

It is well documented in the literature that only ionic tetravalent selenium (selenite) is forming hydrogen selenide. This means that all selenium in calibration standards and sample solutions has to be in this from, and any hexavalent selenium (selenate) has to be reduced to Se(IV) prior to hydride generation. The reduction of Se(VI) to Se(IV) is usually carried out by boiling a 1 + 1 mixture of the digested sample solution + concentrated HCl under reflux for 30 min, a practice, which has become part of several national and international standard procedures. However, the recovery of selenium using HG AAS is also strongly dependent on the time that elapsed between the reduction of Se(VI) to Se(IV) and hydride generation [31,32]. Normally, complete recovery was only obtained if hydride generation was carried out immediately after the reduction, whereas lower and lower values were obtained for selenium with increasing time between reduction and determination. This problem was related to a back-oxidation of Se(IV) to Se(VI) by chlorine gas that was formed in the reduction process and was not removed from the solution [32]. The solution proposed in the same work to avoid this problem was to purge the solution with an inert gas during the reduction process in order to remove the chlorine gas from the solution. It was also mentioned that the time necessary for the reduction of Se(VI) could be reduced when the solution was purged during the reduction step [32].

The proposed purging of the solution during the reduction with HCl as well as the concentration of HCl necessary for reduction under these conditions has been investigated in this work. A model solution containing 10 μg $L^{-1}$ Se was digested with nitric acid and sodium peroxide, followed by reduction with HCl of different concentration for different periods of time, purging the solution during the reduction process with a flow of about 0.5 L $min^{-1}$ of argon. Complete recovery of Se was possible under these conditions for a 1 + 1 mixture of digested standard solution + 5% w/v HCl already after only 10 min of reduction time; the sensitivity for selenium did not change up to 40 min of reduction time. This means a quite significant reduction in the time required to convert all Se(VI) to Se(IV), and also in the concentration of HCl that is necessary for that process. Fig. 4 further shows the stability of the integrated absorbance values over 188 h (~8 days) obtained for the selenium standard treated this way. Besides the usual day-to-day variations of the sensitivity, the integrated absorbance signals did not show any tendency to decrease over time, indicating that there is no back-oxidation of Se(IV) to Se(VI). This stability is considered very important for a routine procedure, where the digested and reduced samples often cannot be analyzed immediately after their preparation.

### 3.5. Mineralization of organo-selenium compounds

It is well documented that organic selenium compounds have to be mineralized completely for their successful determination using HG AAS, and that very harsh conditions are necessary to completely mineralize organo-selenium compounds [26,27,31]. Nevertheless, relatively little has been published using modern microwave-assisted digestion techniques for that purpose; hence, we first investigated the procedure proposed by the manufacturer for the microwave-assisted digestion of food and agricultural products. In order to see if this digestion program is appropriate for the determination of selenium using HG AAS, two bean samples were selected arbiytarily. For each sample, nine digestions were carried out: (i) the bean sample without any addition, (ii) the bean sample with 10 μg $L^{-1}$ of inorganic Se(IV) standard solution added, and (iii) the bean sample with 10 μg $L^{-1}$ of organic selenium standard solution prepared from seleno-DL-methionine, $C_5H_{11}NO_2Se$; all digestions were carried out in triplicate. After microwave digestion, 20 mL of hydrochloric acid (5% w/v) was added to 20 mL of the digested bean samples in a round-bottom flask and heated for 20 min to boiling under reflux and flushing with argon. The integrated absorbance signal of selenium measured for all replicates by HG-ET AAS is shown in Fig. 5; the mean recovery of 100 ± 2% in the solution proves that all organo-selenium has been mineralized in the microwave-assisted acid digestion and no selenium has been lost in the sample preparation procedure. It is also worth mentioning that no perchloric or sulfuric acid had to be used in the microwave-assisted digestion. Similar experiments have also been carried out with soil samples with essentially identical results, which are not shown here.

### 3.6. Figures of merit

The characteristic mass, $m_0$, which is defined as the analyte mass that provides an integrated absorbance of 0.0044s, was found as 95 pg Se. This is significantly higher than values typically reported for ET AAS (between 25 and 30 pg Se), which indicates a non-quantitative trapping of selenium hydride in the electrothermal atomizer. A rough estimation indicates that only 25–30% of the $H_2Se$ can be retained in the graphite tube at 500 °C. This,

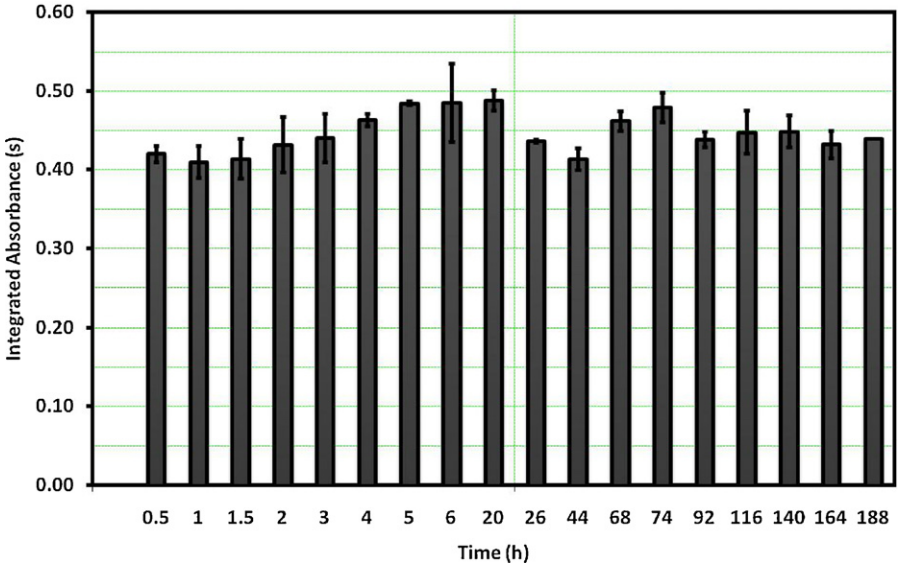


**Fig. 4.** Influence of the time between the reduction of Se(VI) to Se(IV) and the determination of Se by HG-ET AAS on the integrated absorbance signal; error bars are standard deviation of $n = 3$ repetitive collections and determinations of a 10 μg $L^{-1}$ Se solution.

however, is not a major problem as a low trapping efficiency can be compensated easily by a longer collection time if necessary. The limits of detection (LOD) and quantification (LOQ), defined as three times and ten times, respectively, the standard deviation of ten measurements of a blank, divided by the slope of the calibration curve, were 30 ng $L^{-1}$ Se and 101 ng $L^{-1}$ Se, respectively. The calibration curve for Se showed reasonable linearity up to an integrated absorbance ($A_{int}$) of about 0.5 s (5 μg $L^{-1}$ Se) with a linear regression equation of $A_{int} = 0.0973 m_{Se} + 0.0191$ and a correlation coefficient of $R^2 = 0.988$; for higher values of integrated absorbance, however, the curve exhibited pronounced non-linearity, necessitating dilution of the respective sample solutions.

### 3.7. Quantitative analysis of bean and soil samples

The results obtained for the bean samples and two related CRM are shown in Table 4. The result obtained for the rice CRM is well within the 95% confidence interval of the certified value, and the result found for the soy bean CRM is close enough to the recommended value to confirm that the proposed method is appropriate for the determination of selenium in the bean samples. Without going into details, the results show that the selenium content in the collected bean samples is varying by more than two orders of magnitude between 5.5 ng $g^{-1}$ and 1726 ng $g^{-1}$. The results also show that the proposed method has the capability and sensitivity to determine the selenium content in all the collected bean samples. The precision, which is typically between 5 and 10% RSD is quite acceptable for a routine procedure for the determination of a large number of samples.

The results obtained for the soil samples and two CRM are shown in Table 5. The values found for the two CRM are within the 95% confidence interval of the certified or recommended value, confirming the accuracy of the proposed procedure. The variation of the selenium content in the investigated soil samples, ranging between 113 μg $g^{-1}$ and 1692 μg $g^{-1}$, is much less pronounced than in the bean samples; however, no attempt will be made here to interpret these results. The precision for the determination of the soil samples was clearly better than that observed for the bean samples; this

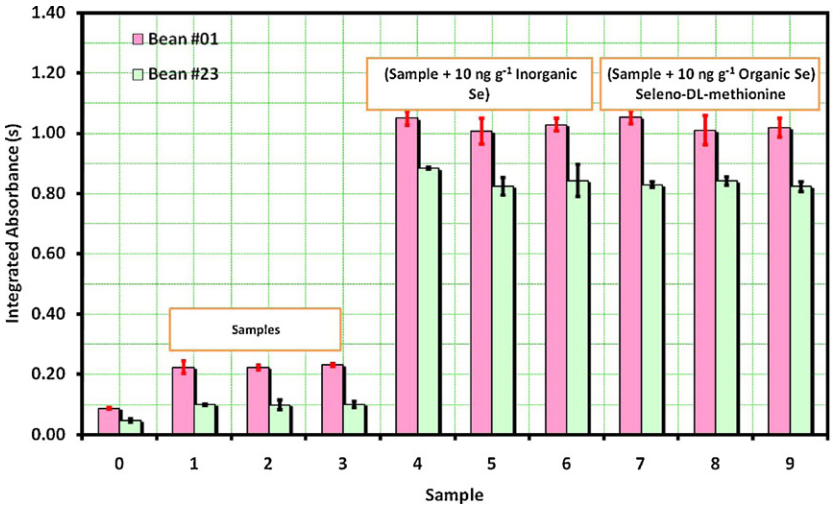


**Fig. 5.** Recovery of inorganic Se(IV) and organic selenium (seleno-DL-methionine) added to two bean samples before microwave-assisted digestion; all digestions were carried out in triplicate; error bars are standard deviation of $n = 3$ repetitive collections and determinations of Se in the digested solutions.

**Table 4**
Results obtained for Se in certified reference materials (CRM) and bean samples collected in various states of Brazil.

| Samples | Certified value ($ng\,g^{-1}$) | Found concentration ($ng\,g^{-1}$) | RSD (%) |
|---|---|---|---|
| Soy bean (CRM) | (22)[a] | 33 ± 2 | 5 |
| Rice (CRM) | 61 ± 15 | 68 ± 7 | 10 |
| B01 (FAM1) | | 94 ± 4 | 4 |
| B02 (FAM2) | | 209 ± 23 | 11 |
| B03 (FPA1) | | 345 ± 22 | 6 |
| B04 (PACE1) | | 1726 ± 55 | 3.2 |
| B05 (FPE1) | | 280 ± 23 | 8 |
| B06 (FPE2) | | 19 ± 1.3 | 7 |
| B07 (AL1) | | 191 ± 11 | 6 |
| B08 (FMS1) | | 178 ± 9 | 5 |
| B09 (GO) | | 5.5 ± 0.4 | 8 |
| B10 (FMO1) | | 107 ± 7 | 6.5 |
| B11 (FMO2) | | 101 ± 9 | 9 |
| B12 (FSP1) | | 35 ± 1.5 | 4 |
| B13 (FSP2) | | 7.4 ± 0.7 | 10 |
| B14 (FSP3) | | 18 ± 1 | 6 |
| B15 (FPR1) | | 17 ± 0.75 | 4.5 |
| B16 (FSC1) | | 8.8 ± 0.66 | 7.5 |
| B17 (FSC2) | | 35 ± 0.8 | 2.2 |
| B18 (FRS1) | | 17 ± 1.3 | 7.5 |
| B19 (FRS2) | | 13 ± 0.4 | 3.2 |

[a] Non-certified value.

**Table 5**
Results obtained for Se in certified reference materials (CRM) and soil samples collected in various states of Brazil.

| Sample | Certified value ($ng\,g^{-1}$) | Found concentration ($ng\,g^{-1}$) | RSD (%) |
|---|---|---|---|
| MURST-ISS-A1 (CRM) | 2200 ± 100 | 2076 ± 129 | 6.2 |
| BCR 142 (CRM) | (530)[a] | 518 ± 18 | 3.4 |
| S01 (F/IR/AM) | | 604 ± 15 | 2.5 |
| S02 (C/CG/MS) | | 113 ± 6.5 | 5.7 |
| S03 (F/TA/PA) | | 419 ± 18 | 4.2 |
| S04 (C/VA/RS) | | 248 ± 11 | 4.6 |
| S05 (C/LA/SC) | | 262 ± 20 | 7.7 |
| S06 (F/CA/MG) | | 1692 ± 21 | 1.2 |
| S07 (C/PA/CE) | | 599 ± 24 | 4.0 |
| S08 (F/FL/SC) | | 406 ± 19 | 4.6 |
| S09 (C/SH/GO) | | 215 ± 21 | 9.6 |
| S10 (C/CU/PR) | | 370 ± 12 | 3.3 |

[a] Non-certified value.

is most likely due to the greater heterogeneity of the bean samples and the significantly different hardness of its components, which might result in segregation effects [39].

## 4. Conclusion

The developed method for the determination of selenium in bean and soil samples using HG-ET AAS with in situ preconcentration in an Ir-coated graphite tube has been shown to be suitable for routine analysis of a large number of samples. The use of continuous-flow hydride generation with trapping of the hydride in an Ir-coated graphite tube offers better sensitivity than other AAS techniques. The limit of quantification of about 100 $ng\,L^{-1}$ was more than sufficient to determine selenium in all the investigated samples with good precision. The microwave-assisted digestion with nitric acid and hydrogen peroxide proved to be appropriate for the complete mineralization of organo-selenium compounds, avoiding the use of more hazardous reagents, such as sulfuric or perchloric acid. Bubbling argon through the solution during the reduction of Se(VI) to Se(IV) reduced the time necessary for boiling under reflux to 10–15 min. It also allowed reducing the hydrochloric acid concentration used for the reduction process to 5% (w/v), and the resulting sample solutions were stable for at least one week. All these parameters are of importance for routine analysis, and it might be expected that the developed procedure could be used equally for the determination of selenium in a variety of other types of samples.

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