Determination of selenium in some food matrices by electrothermal atomic absorption spectrometry after preconcentration with diethyldithiophosphate

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Selenium preconcentration for its electrothermal atomic absorption spectrometric determination in biological tissues, such as food samples, is described. The method is applicable to matrices containing 0.05–1 mg kg⁻¹ Se, like fish, meat, and flour. After a closed nitric acid digestion procedure, an aliquot of the sample is diluted with 0.2% v/v HNO₃. In order to minimize diethyldithiophosphate interference, a chemical modifier is used, chosen aiming at this purpose, but detection limits are not always satisfactory and matrix effects may still persist, even after comparison among Rh, Pd, Ir and Ni, and Rh proved to be the best. The furnace program can include a pyrolysis step at 1000 °C or, alternatively, omit it, skipping from a drying step to the atomization. A typical analytical curve goes up to 4 ng mL⁻¹ Se. An enrichment factor of 65 is possible, taking 6 min for each preconcentration step. Good results were obtained for several certified reference materials. The entire procedure, including the digestion, can proceed rapidly, because there is no need for a pre-reduction step, coprecipitation or a lengthy solvent extraction.

1. Introduction

Like some other nutrients, selenium illustrates the dual aspect of essentiality and toxicity.¹ Reports concerning both naturally occurring human selenium deficiency and toxicity are available. Selenium was considered a toxic element for many decades before it was recognized as an essential nutrient in the 1950s. Its toxic effects were identified long ago in farm animals, in areas where there are relatively high levels of the element in the soil.² The major biological functions of selenium can be attributed to its antioxidative properties, its role in the regulation of thyroid hormone metabolism, cell growth and eicosanoid biosynthesis.³ Both organic and inorganic forms of selenium may be used by the body, but the selenoamino acids have the greatest bioavailability.³

There are significant differences in selenium concentration in foods and in dietary intake over the world, mainly due to the level of the element in agricultural soils, in each country.⁴ Official methods for the determination of selenium in food are time consuming and need a great deal of sample manipulation and reagents.⁵ Electrothermal atomic absorption spectrometry (ETAAS) is a mature and ubiquitous technique that can be chosen aiming at this purpose, but detection limits are not always satisfactory and matrix effects may still persist, even when Zeeman background correction is used.⁶

In order to overcome these limitations, a preconcentration step may be utilized. Solid phase extraction has been largely used for this purpose and SiO₂–C₁₈ is one of the most common sorbents employed.⁷ Considering the good results that have been published, using diethyldithiophosphate (DDTP) as a complexing agent for the quantification of many elements, including selenium(v), by using other techniques,⁸–¹⁰ this reagent was evaluated for solid phase separation and preconcentration of selenium in food samples, followed by ETAAS determination. The DDTP–selenium complex was retained on a minicolumn filled with SiO₂–C₁₈ and was eluted with ethanol for analysis. There is evidence from Hocquellet and Candillier¹¹ that, in practice, complete nitric acid digestion of food samples converts the organically bound selenium into the inorganic selenium(vi). Relying in this premise, and keeping in mind that DDTP reacts only with selenium(v), a straightforward method for the determination of selenium in some food matrices is proposed.

2. Experimental

2.1. Instrumentation

A PE 4100 ZL graphite furnace atomic absorption spectrometer (PerkinElmer, Norwalk, CT, USA), equipped with Zeeman effect background correction, an AS-70 autosampler and a hollow cathode lamp (Intensitron, Perkin Elmer), were applied for selenium determination (wavelength 196.0 nm; bandpass 2.0 nm; lamp current 12 mA; 20 µl sample volume injected). Two temperature-time programs for the furnace were investigated and are given in Tables 1(a) and 1(b).

2.2. Reagents

All solutions were prepared using ultrapure water (Milli-Q Water Purification System, Millipore, Bedford, MA, USA). Nitric acid and magnesium nitrate were analytical grade reagents (Suprapur, Merck).

The 1000 mg l⁻¹ stock solution of selenium was prepared from metallic Se in nitric acid (> 99.5%, Merck). A solution of Rh (1 g l⁻¹) was prepared from RhCl₃·3H₂O (for synthesis, Merck 38% Rh), dissolved in water. Alternatively, a 1 g l⁻¹ solution of Rh was prepared, replacing the chloride ions by...
SiO2–C18. For all determinations by ETAAS, dilutions were filtered and purified by passing through a column filled with (95%, Aldrich), diluted in water. These DDTP solutions were prepared weekly, from diethyldithiophosphate ammonium salt Phase was purchased from Fluka. The DDTP solutions were water (final concentration in nitric acid: 1.5% v/v). The 1 g l−2 was obtained from 10 g l−2 IrCl3 solution of Ir, in 1.2% v/v nitric acid, was made from 1.5H2O (Sigma, 62.2% Ir). Silica gel 100 C18-Reversed, 200 ppm Ni, in 1% v/v nitric acid, was made by dilution of Tirtisol (NiCl2, Merck). The 1 g l−1 solution of Pd was obtained from 10 g l−1 Pd(Pd(NO3)2, Merck) diluted with water (final concentration in nitric acid: 1.5% v/v). The 1 g l−1 solution of Ir, in 1.2% v/v nitric acid, was made from IrCl3·1.5H2O (Sigma, 62.2% Ir). Silica gel 100 C18-Reversed Phase was purchased from Fluka. The DDTP solutions were made with 0.2% v/v nitric acid, using the autosampler. Other reagents were of analytical grade.

2.3. Sample preparation

Reference certified materials (Fish Tissue IAEA MA-B-3-TM, Mussel Tissue CRM 278, Animal Blood IAEA A-13, Wholemeal Flour BCR 189, Bovine Liver BCR 185) were digested by means of two independent procedures. A closed microwave digestion system, MDS-2000, from CEM Corporation (Matthews, NC, USA) and an oxygen bomb calorimeter, Parr Instrument Company (Moline, IL, USA) were utilized. For the microwave procedure, a program was developed avoiding too severe conditions in terms of acidity and power (four steps; power 441 W; pressure 30, 50, 100 and 120 psi; time 10, 10, 10 and 5 min; time at pressure 10, 10, 10 and 5 min). Aliquots of 0.2–0.3 g of the sample were reacted with 1 ml of water + 2 ml of concentrated nitric acid. The digests were filtered and diluted to 25 ml with water in volumetric flasks. The oxygen bomb procedure can accommodate up to 1 g of sample, depending on its density. The sample aliquots were submitted to instantaneous combustion, under an oxygen pressure of 25 atm. The volatile components formed by burning the sample were quantitatively trapped in 10 ml of 10% v/v nitric acid and transferred to 25 or 50 ml volumetric flasks, with filtration, completing the volume with water.

2.4. Preconcentration procedure

A manual procedure was utilized, using a peristaltic pump with a 5 ml min−1 flow-rate and a 1.5 cm minicolumn filled with 30 mg of SiO2–C18. Following the nitric acid digestion procedure, an aliquot (usually 0.5–2 ml) of the sample solution was diluted to 25 ml (samples expected to contain 0.05–1 mg kg−1 selenium) with 0.2% v/v nitric acid, in order to dilute the matrix, which was found to affect the analysis. Then, 1 ml of DDTP solution was added (the optimised concentration of the DDTP was 0.5–1% v/v), the mixture homogenized, and the complex formed loaded onto the minicolumn. The entire nitrate through successive evaporation and dissolution of the residue in nitric acid (final concentration in nitric acid: 1% v/v). The 1 g l−1 solution of Ni, in 1% v/v nitric acid, was made by dilution of Tirtisol (NiCl2, Merck). The 1 g l−1 solution of Pd was obtained from 10 g l−1 Pd(Pd(NO3)2, Merck) diluted with water (final concentration in nitric acid: 1.5% v/v). The 1 g l−1 solution of Ir, in 1.2% v/v nitric acid, was made from IrCl3·1.5H2O (Sigma, 62.2% Ir). Silica gel 100 C18-Reversed Phase was purchased from Fluka. The DDTP solutions were made with 0.2% v/v nitric acid, using the autosampler. Other reagents were of analytical grade.

Table 1 Temperature–time program used for selenium determination

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature/°C</th>
<th>Ramp/s</th>
<th>Hold/s</th>
<th>Ar flow/ml min−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drying I</td>
<td>110</td>
<td>1</td>
<td>30</td>
<td>250</td>
</tr>
<tr>
<td>Drying II</td>
<td>130</td>
<td>5</td>
<td>50</td>
<td>250</td>
</tr>
<tr>
<td>Pyrolysis</td>
<td>See text</td>
<td>10</td>
<td>20</td>
<td>250</td>
</tr>
<tr>
<td>Atomization</td>
<td>1900</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Cleaning</td>
<td>2400</td>
<td>1</td>
<td>2</td>
<td>250</td>
</tr>
</tbody>
</table>

3. Results and discussion

3.1. Determination of Se in food matrices without analyte separation/preconcentration

Theoretically, the detection limits achieved in ETAAS guarantee good results in the determination of selenium in foods containing at least 1–2 mg kg−1 of the element, without preconcentration. To verify this possibility, microwave and oxygen bomb digestion procedures were applied to CRM 186 Pig Kidney Reference Material (10.3 ± 0.5 µg g−1 selenium). The resulting solutions were analyzed by ETAAS, using the temperature–time program in Table I(a). Considering that the results12,13 have shown that Rh is an efficient chemical modifier for the ETAAS determination of selenium, it was used in this work. Using 5 µg of Rh the pyrolysis temperature was optimized at 1300 °C. The experiment was also performed using Pd (5 µg)–Mg(NO3)2 (3 µg) and a pyrolysis temperature equal to 1300 °C, as recommended by the manufacturer of the spectrometer.

Considering the high level of selenium in the Pig Kidney digests, it was necessary to dilute the resulting solutions in order to interpolate the integrated absorbance into the analytical curve, which went up to 40 ng ml−1. It is necessary to emphasize that the concentration of selenium in all the dilutions made was within the linear range. In spite of this, only at high dilutions was the correct value for the certified material obtained. This illustrates the matrix effect, which would cause negative error if such a dilution was not possible, as in samples with selenium concentrations lower than 1 mg kg−1. The same strategies were used by Hanna et al., for selenium in nutritional supplements.

Similar results were obtained following the oxygen bomb procedure applied to CRM 278 Mussel Tissue (1.66 ± 0.04 µg g−1 selenium) and IAEA MA-B-3/TM Fish Tissue (1.35–1.70 µg g−1 selenium) certified reference materials.

3.2. Evaluation of the DDTP effect on selenium absorbance—comparison among chemical modifiers

It was noticed that the presence of DDTP could cause depression of selenium absorbance. To minimize such an effect an experiment was developed to compare Rh, Ni, Ir and Pd as the chemical modifier, transferring to the platform 5 µg of each metal (from a 1 g l−1 solution), 0.8 ng of selenium (from a 200 ng ml−1 solution) and 40 ng of DDTP (from a 2% w/v solution), through the autosampler. The program described in Table I(a) was applied, varying the pyrolysis temperature. For better comparison, curves with selenium only, and selenium + DDTP, without metallic modifier, were included. Fig. 1 shows...
the results. Clearly, the addition of Rh proved to be the best choice, either the chloride or the nitrate form, whereas Pd depressed selenium absorbance still more. No memory effects were detected from any of the metallic modifiers experimented.

3.3. Preconcentration results

In order to evaluate the use of DDTP for selenium preconcentration from food matrices, different quantities of SiO$_2$–C$_{18}$ for minicolumn filling, were tested. Best results were achieved by using 30 mg of the sorbent material. To get reasonable precision in the procedure (relative standard deviation equal to 5%, for a 0.25 ng ml$^{-1}$ selenium standard, $n = 3$), not less than 200 µl of ethanol could be used for elution of the DDTP–selenium complex, adsorbed on the minicolumn. After optimization, the DDTP concentration was fixed at 0.1% w/v for the 5 mL procedure (foods with more than 1 mg g$^{-1}$ selenium) and 1% w/v for the 25 ml procedure (selenium $\leq$ 1 mg kg$^{-1}$). The aliquot of sample digest to be diluted to 5 or 25 ml needed to be determined, through successive dilutions or recovery tests.

Considering that Rh modifier can compensate for the depressing effect on the absorbance of selenium caused by the presence of DDTP, 5 µg of the metal was used for determination of the analyte in the eluate.

The temperature–time program for the furnace is in Table 1(a), with the pyrolysis temperature equal to 1000 $^\circ$C or less. An attempt was made to make it equal to 1300 $^\circ$C, but the results showed a decrease in sensitivity. On the other hand, the possibility of omitting the pyrolysis step was studied, with good results. Table 1(b) shows the temperature–time program used.

The best enrichment factors (EF) are achieved using the program without a pyrolysis step. A typical analytical curve for the 25 ml procedure may be represented by the equation $y = 0.0733x - 0.000271$, $r = 0.9995$. The EF$^{14}$ was calculated to be 65, and the limit of detection$^{12}$ in solution was 0.14 ng ml$^{-1}$. For the determination of EF, an analytical curve without preconcentration was considered, obtained by using the same program depicted in Table 1(b), with the addition of the same chemical modifier. The resulting equation was $y = 0.001125x + 0.003931$ and the limit of detection 5 ng ml$^{-1}$, a number much higher than the corresponding figure obtained with preconcentration.

It was observed that the complexation and adsorption of the DDTP–selenium complex can be performed in 0.2–2% v/v nitric acid or even more concentrated, but it is convenient to keep acid concentration low (like 0.2% v/v), in order to preserve the column.

The optimized preconcentration conditions were applied to certified reference materials, using solutions prepared as described before. Some matrix effects are clearly present also during the preconcentration process with DDTP–SiO$_2$–C$_{18}$, but they are overcome when appropriate dilution of the sample digest is provided. Results are shown in Table 2 and can be seen the good agreement with the certified values.

The method was also applied to a blind food sample (no data about matrix and Se concentration interval) from a national intercomparison exercise organized by TACO—Tabela Brasileira de Composição de Alimentos—project, NEPA–UNICAMP. Participants were asked to perform four replicates and were not allowed to extend this number, and the Se concentration was determined as being 0.09 ± 0.02 µg g$^{-1}$. Afterwards, the blind sample was identified as corresponding to NIST SRM 1846 material (infant formula), whose informed value for Se is 0.08 µg g$^{-1}$.

4. Conclusion

Solid state extraction with DDTP–SiO$_2$–C$_{18}$ performed well, especially when an adequate chemical modifier, like Rh, was used in the ETAAS determination of selenium in the eluate. Moreover, in the furnace temperature–time program, it is possible to omit the pyrolysis step, saving time and probably graphite parts as well. The entire procedure, including the nitric acid digestion, can be completed rapidly, because a pre-reduction step to eliminate oxidant residues (such as in hydride generation), coprecipitation or a lengthy solvent extraction are not necessary. The application of the method to certified reference materials, representing some food matrices, led to good results. A few reagents are necessary and the method generates low volumes of residues.

Acknowledgements

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References


Table 2 Final results for certified reference materials, obtained by preconcentration with DDTP–SiO$_2$–C$_{18}$ (means of three replicates at least, $P = 0.05$)

<table>
<thead>
<tr>
<th>Certified reference material</th>
<th>Se found/µg g$^{-1}$</th>
<th>Se found/ng g$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mussel Tissue CRM 278/1.66</td>
<td>1.63 ± 0.20</td>
<td>—</td>
</tr>
<tr>
<td>Fish Tissue IAEA MA-B-3/TM/</td>
<td>1.54 ± 0.21</td>
<td>—</td>
</tr>
<tr>
<td>Animal Blood IAEA A-13/</td>
<td>0.24 ± 0.03</td>
<td>—</td>
</tr>
<tr>
<td>Bovine Liver BCR 185/446</td>
<td>436 ± 68</td>
<td>—</td>
</tr>
<tr>
<td>Wholemeal Flour BCR 189/</td>
<td>135 ± 15</td>
<td>—</td>
</tr>
<tr>
<td>132 (122–142) ng g$^{-1}$ Se</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
13 V. M. C. Dias, S. Cadore and N. Bacca, *Comparação entre vários modificadores para a determinação de selênio por ETAAS*, Encontro Nacional de Química Analítica, Santa Maria, Brazil, 10th edn., 1999, p. EA-50.