Determination of Selenium in Human Blood Serum by Electrothermal Atomic Absorption Spectrometry

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Abstract

A method for direct selenium determination in human blood serum by electrothermal atomic absorption spectrometry (ETAAS) was developed. Total selenium was measured by ETAAS employing 10 µg of palladium as matrix modifier in a graphite atomizer with pyrolytically coated tubes and Zeeman background correction. Blood serum was diluted 1+2 with 0.1 % v/v nitric acid and 0.1 % Triton X-100. Pyrolysis and atomization temperatures for palladium modifier are 1100 ºC and 2500 ºC, respectively. The measurements were confirmed by the analyses of standard reference material and by the method of standard additions. Reference serum material Seronorm, Trace elements, Serum level 1 (lot JL 4409) was analyzed and the results are in agreement with the certified value. The limit of detection of the direct ETAAS based on 3σ of the blank signal is 0.60 µg L⁻¹ Se in blood serum samples. The precision of the method ranges from 2.06 % to 5.95 %. The obtained data from the selenium concentrations in serum samples from 83 patients show that the content of selenium is relatively low, ranging from 43.91 ± 4.80 µg L⁻¹ for female to 45.21 ± 5.60 µg L⁻¹ for male.

Keywords: Selenium; serum; determination; ETAAS.

1. Introduction

Selenium is an essential micronutrient at low concentration but toxic at high concentration with a relatively small difference between these levels. Human body uses selenium to produce glutathione peroxidase [1], which works with vitamin E [2] to protect cell membranes from damage caused by dangerous, naturally occurring substances known as free radicals produced by oxidative metabolism [3]. Selenium is taking center stage as a potential anticancer agent [4, 5] by promoting formation of white blood cells which destroys the cancer cells and is an essential component of more than 10 selenoproteins with multiple biochemical functions. Moreover, it boosts the immune system [6] by increasing the activity and number of white blood cells and prevents premature aging, degenerative diseases, cardiovascular diseases, inflammatory diseases, stroke, cataracts, and rheumatoid arthritis. It is also necessary for normal thyroid functions [7] and protection of heavy metal toxicity. Deficiency of the element can cause Keshan disease, characterized by an enlarged heart and poor heart function [8] or to be a factor for essential hypertension [9]. This disorder is endemic in some of most selenium-poor soils in the world. High blood levels of selenium can result in selenosis, which is associated with gastrointestinal upsets, hair loss, white blotchy nails and mild nerve damage [10]. The optimum daily dietary intake of selenium is 55 µg/day for women and 70 µg/day for men [11].

The trace elements concentration in blood, serum, urine and tissue is used as an indicator of the trace elements status of the human body. Namely, determination of metals in vivo in organs is not possible; therefore indirect determination of metals in accessible tissue can point at the presence of toxic elements. Because of that, it is very important to introduce accurate and precise methods for trace metal determination (and their chemical forms, as well) in different biological fluids and tissue. There are a number of methods for selenium determination in different biological materials, mainly by atomic absorption spectrometry (AAS), both hydride generation AAS (HG-AAS) [12, 13] and electrothermal AAS (ETAAS) [14-17]. Atomic absorption spectrometry is one of the most used techniques for trace element determination.

Direct electrothermal atomic absorption spectrometry (ETAAS) shows sensitivity and accuracy adequate for selenium determination in biological fluids and requires little sample preparation, but is very sensitive to matrix interference and reliable methods have been validated only for blood plasma and serum [18, 19]. In direct ETAAS assay on diluted biological fluids, the main concern is reliability of background correction and
efficiency of thermal stabilization for all forms of endogenous selenium. Zeeman is the most powerful current tool.

The purpose of this study was to define optimal instrumental parameters, suitable modifiers and calibration procedure for direct ETAAS determination of selenium in serum. Analytical quality assurance was carried out by analyzing standard reference materials and by the method of standard additions. The proposed method was applied to the determination of selenium in the serum of normal presumable healthy individual.

2. Methods

2.1. Instrumentation

A Varian SpectrAA 640Z Zeeman electrothermal atomic absorption spectrometer equipped with a GTA-100 graphite furnace (Varian, USA) and PSD-100 autosampler (Varian, USA) was used. Pyrolytically coated tubes were used as atomizers. A Varian selenium hollow cathode lamp was used and the measurements were performed at 196.0 nm. Argon was applied as protective gas and 10 µL samples were injected into the graphite furnace (GF). The graphite furnace operating parameters are presented in Table 1. Only integrated absorbance values (peak height) were used for quantification.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>196.0 nm</td>
</tr>
<tr>
<td>Slit</td>
<td>1.0 nm</td>
</tr>
<tr>
<td>Lamp current</td>
<td>10.0 mA</td>
</tr>
<tr>
<td>Calibration mode</td>
<td>Absorbance, peak height</td>
</tr>
<tr>
<td>Background correction</td>
<td>Zeeman</td>
</tr>
<tr>
<td>Drying</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>85; 95; 120 °C</td>
</tr>
<tr>
<td>Time</td>
<td>5; 40; 10 s</td>
</tr>
<tr>
<td>Pyrolysis</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>1100 °C</td>
</tr>
<tr>
<td>Ramp time</td>
<td>5 s</td>
</tr>
<tr>
<td>Hold time</td>
<td>32 s</td>
</tr>
<tr>
<td>Atomization</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>2500 °C</td>
</tr>
<tr>
<td>Ramp time</td>
<td>1 s</td>
</tr>
<tr>
<td>Hold time</td>
<td>3 s</td>
</tr>
<tr>
<td>Cleaning</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>2500 °C</td>
</tr>
<tr>
<td>Hold time</td>
<td>2 s</td>
</tr>
<tr>
<td>Gas</td>
<td>Argon</td>
</tr>
</tbody>
</table>

2.2. Reagents and samples

All reagents and standards were of analytical grade. Stock standard solutions for selenium were 1000 µg mL⁻¹ Solution Plus Inc. (USA). The working standard solutions were prepared weekly by appropriate dilution and kept refrigerated at 4 °C. The palladium matrix modifier solution was prepared by the dilution (10 g L⁻¹) Pd(NO₃)₂ (Merck, Darmstadt, Germany), and iridium AA standard solution, 1000 g mL⁻¹ in 20% HCl (Alfa Johnson Mathey), 0.1 % V/V nitric acid prepared by dilution trace pure 65 % nitric acid (Merck, Darmstadt, Germany), and 0.1 % Triton X-100 (Merck, Darmstadt, Germany) were used. Doubly distilled water was used in all operations.
The following certified reference materials were used for the validation purposes: Seronorm, Trace elements, Serum level 1 (lot JL 4409). All disposable devices were rigorously clean before use by brief immersion in hot concentrated nitric acid and rinsing twice with distilled water.

Serum samples were obtained from 83 presumable healthy volunteers. All patients signed agreement for selenium testing of their serum during systematic medical checkup. Clinical experiments were performed according to the Ethics Committee provisions of the Institute of Preventive Medical Care and Toxicology at the Clinical Health Institution Center (Skopje, Macedonia). The serum samples were collected with plastic IV cannula No. 24 (TIK, Slovenia) with an injection valve. The samples were kept frozen (-18°C) until analysis.

2.3. Procedures

Blood serum samples (500 µL) were diluted 1+2 with 0.1% V/V nitric acid and 0.1% Triton X-100 and 10 µL were introduced into graphite furnace with appropriate volume of palladium modifier (1.2 µg; 2 µg; 2.4 µg; 5 µg and 10 µg) and iridium modifier (0.1 µg; 0.5 µg and 1 µg). The calibration curves (4–60 µg mL⁻¹ Se) were prepared using human serum in nitric acid-Triton X-100 mixture spiked with known amount of selenium standard solution and palladium or iridium solution as matrix modifier.

3. Results and Discussion

3.1. Selection of chemical modifier

Several preliminary experiments were carried out to select an efficient chemical modifier for the measurement of selenium in serum. The modifiers tested are: palladium (10 g L⁻¹) and iridium (1 g L⁻¹) modifier. Typically, relatively high pyrolysis temperatures could be used for selenium determination, so the action of the modifier is to assist and ensure complete matrix mineralization and removal during the ashing step. The matrix modifiers used in this work were Pd and Ir with optimal concentration of 500 µg mL⁻¹ for Pd modifier and 100 µg mL⁻¹ for Ir modifier. Different masses of modifiers were tested, for palladium (1.2 µg; 2 µg; 2.4 µg; 5 µg and 10 µg) and for iridium (0.1 µg; 0.5 µg and 1 µg). Best results yielded 10 µg or palladium and 1 µg of iridium. From this point of view, palladium is very useful to its high efficiency at relatively low masses and good performance in presence of organic matter. It was observed that in presence of palladium the nonspecific absorbance signals were remarkably lower than iridium; therefore this modifier was selected for all further investigations.

3.2. GFAAS program optimization

Optimization of temperature program for Se determination by electrothermal atomic absorption spectrometry in human blood serum samples was performed. For the optimization of pretreatment and atomization temperatures, pyrolysis-atomization curves were constructed from serum sample previously spiked with 10 µg L⁻¹ Se in the presence of palladium (10 µg) and iridium (1 µg) as most widely used modifiers for thermal stabilization of selenium in ETAAS. The modifiers were applied through the autosampler directly into the graphite furnace with a volume of 10 µL for 10 µL serum sample. Parameters of the drying step were carefully optimized so as to ensure complete matrix decomposition and removal during this step. Ashing temperatures (from 800 to 1200 °C) and atomization temperatures (from 2000 °C to 2700 °C) were assayed by using wall atomization (pyrolytically graphite coated graphite tubes). Effects of pyrolysis and atomization temperatures and times on integrated absorbance for serum samples with Pd modifier are given in Fig. 1 and with iridium modifier are given in Fig. 2. As it can be seen, the optimal pyrolysis temperature with Pd and Ir modifiers was found to be 1100 °C, while the optimal atomization temperature was 2500 °C using Pd modifier and 2600 °C with Ir modifier. The optimal pyrolysis ramp time was established to be 5 s and hold time was 32 s (the last 2 s without argon flow). The optimal atomization ramp time was 1 s and hold time 3 s. The behavior of Pd and Ir for thermal stabilization of selenium proved identical. Both modifiers ensured loss-free ashing up to 1100 °C is serum samples diluted with 0.1% V/V nitric acid and 0.1% Triton X-100. Lower atomization temperatures are preferable in this case because of lower background absorption signals, as well as better shaped absorbance-time profiles for selenium. Therefore palladium is recommended as efficient modifier for ETAAS determination of total Se in serum samples [20-23].
Figure 1: Effect of pyrolysis (a) and atomization (b) temperature on the integrated absorbance signal for Se in serum (▲) and in serum with 50 µL Se (■) using Pd modifier 10 µg. Pyrolysis and atomization temperature - 1100 °C and 2500 °C.

Figure 2: Effect of pyrolysis (a) and atomization (b) temperature on the integrated absorbance signal for Se in serum (▲) and in serum with 50 µL Se (■) using Ir modifier 1 µg. Pyrolysis and atomization temperature - 1100 °C and 2600 °C.

3.3. Calibration

In order to evaluate the degree of matrix depression on atomization of selenium, several serum samples were spiked with Se in the range 4–60 µg L⁻¹, and 10 µL of these samples were introduced into the graphite furnace. Spiking of serum samples was also performed by using the capabilities of the autosampler, introducing into graphite furnace 10 µL serums and 10 µL aqueous standards of Se. Matrix interferences were evaluated by the ratio of the slopes of calibration curves obtained in the presence of serum and in presence of aqueous standard solutions. Results obtained are shown in Table 2 and clearly illustrate strong matrix depression. In the presence of a matrix modifier there is a slight improvement and a decrease in matrix interferences. It is worth mention that the matrix depression effect is much more pronounced for previously spiked samples than for samples spiked directly into the graphite furnace. It might be assumed that some chemical reaction between Se and sample components take place and only previously spiked serum samples...
represent real standard addition. Evidently, the method of standard addition should be used for calibration and preliminary standard addition should be prepared by adding a Se standard to the serum samples. The autosampler facilities to prepare standard additions should not be used for standard addition preparation. It is known that matrix components contents in serum of different persons slightly varied and it might be expected a diverse degree of depression on Se determination would be observed for different serum samples. However, experiments performed with around 80 samples of serum have shown that in all these cases, statistically equal slopes of standard addition calibration curves were obtained, which means that standard additions prepared to at least one of the serum samples is enough to prepare a regression equation valid for Se quantification in all serum samples.

Table 2: Slopes of the best fit linear regression models for calibration curves.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Slopes of calibration curves</th>
<th>Mean value</th>
<th>RSD, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous standard solutions</td>
<td>500.3</td>
<td>3.65</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>543.4</td>
<td>10.14</td>
<td></td>
</tr>
</tbody>
</table>

*n = 5; RSD – Relative Standard Deviation

3.4. Accuracy and precision

The accuracy of the proposed procedure for direct ETAAS determination of selenium in serum samples was checked by spike recovery experiments. Serum samples were spiked with Se in the concentration range 4–60 µg L⁻¹ Se. Recovery experiments performed with human serum samples in nitric acid-Triton X-100 mixtures spiked with known amount of selenium standard solution showed 94.6–100.4 % yield for palladium modifier, 93.2–104.8 % yields for iridium modifier. The results of recovery are shown in Table 3. The accuracy of the analytical method developed was also checked by analyzing certified reference material Seronorm, Trace elements, Serum level 1 (lot JL 4409) for serum. The result 74.4 ± 2.4 µg L⁻¹ is in very good agreement with certified values (range 72.8–82.4 µg L⁻¹), thus confirming the validity and versatility of the analytical procedure.

The limit of detection (LOD) and limit of quantification (LOQ) were evaluated on the basis of repeated analysis of blanks. LOD and LOQ were calculated as the average Se level in blank plus 3 times and 10 times the standard deviation of the blank, respectively. For serum samples the LOD was 0.60 µg L⁻¹ and the LOQ was 1.99 µg L⁻¹. The linearity range was 0.6–80 µg L⁻¹ Se. The precision of the method ranges from 2.06 % to 5.95 %. Between batches precision (calculated as the standard deviation for results obtained for parallel samples analyzed during different days) is 10–17%.

Table 3: Recovery results of serum samples after ETAAS with Pd and Ir modifier.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Addition, µg L⁻¹</th>
<th>Pd modifier</th>
<th>Ir modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Found, µg L⁻¹</td>
<td>Recovery, %</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>33.88</td>
<td>66.15</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>52.11</td>
<td>96.7</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>58.92</td>
<td>24.49</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>109.32</td>
<td>100.4</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>57.04</td>
<td>60.64</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>101.22</td>
<td>94.6</td>
</tr>
</tbody>
</table>

3.5. Analytical application

The method was applied to the determination of Se in serum samples of 83 presumably healthy volunteers (47 female and 36 male) with average age of 46 years. Serum samples were collected from patients of Clinical centre "Bit Pazar" in Skopje during systematic medical checkup. The results obtained revealed that the selenium content varied in the range of 12.25 to 93.02 µg L⁻¹. No significant difference has been revealed
between women 43.91 ± 4.80 (16.88-89.93) µg L\(^{-1}\) and men 45.21 ± 5.60 (12.25-93.02) µg L\(^{-1}\) [9]. Our data reveal that serum selenium levels of healthy people in Macedonia are among the lowest in Europe. These values are comparable with those in serum of adults in Bulgaria (66.5±15.5 µg L\(^{-1}\)) [14], in Czech Republic (46±14 µg L\(^{-1}\)) [24], in Montenegro (51±26 µg L\(^{-1}\)) [25] in Greece (63±14 µg L\(^{-1}\)) [26], in Hungary (50±11 µg L\(^{-1}\)) [27], in Poland (57±8 µg L\(^{-1}\)) [28], in Bosnia-Hercegovina (64±19 µg L\(^{-1}\)) [25], in Croatia (69±17 µg L\(^{-1}\)) [29] and in Serbia (41±20 µg L\(^{-1}\)) [25].

Table 4: Content of Se in blood serum samples for total, male and female.

<table>
<thead>
<tr>
<th>N</th>
<th>Sex</th>
<th>Average age (from-to)</th>
<th>Range/µg L(^{-1})</th>
<th>Median/µg L(^{-1})</th>
<th>(Mean ± CL*)/µg L(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>f</td>
<td>46 (15-84)</td>
<td>16.88 - 89.93</td>
<td>42.35</td>
<td>43.91 ± 4.80</td>
</tr>
<tr>
<td>36</td>
<td>m</td>
<td>46 (18-75)</td>
<td>12.25 - 93.02</td>
<td>52.63</td>
<td>45.21 ± 5.60</td>
</tr>
<tr>
<td>83</td>
<td>f + m</td>
<td>45 (15-84)</td>
<td>12.25 - 93.02</td>
<td>42.90</td>
<td>44.47 ± 5.13</td>
</tr>
</tbody>
</table>

N – Number of patients; f – female; m – male; *CL - Confidence level

4. Conclusion

The method for total selenium determination in human blood serum by ETAAS was optimized. It was found that Pd modifier should be applied with the optimal pyrolysis temperature of 1100 °C and optimal atomizing temperature of 2500 °C. It was also established that the serum samples should be diluted 1+2 with a mixture of 0.1% v/v nitric acid and 0.1% Triton X-100. This method was applied for selenium determination in 83 samples of human serum collected during systematic medical checkup from patients of Clinical Centre "Bit Pazar" in Skopje. The obtained data show that the content of selenium is relatively low, ranging from 16.88 to 89.93 µg L\(^{-1}\) for female and from 12.25 to 93.02 µg L\(^{-1}\) for male, which indicate that dietary intake of Se in the Republic of Macedonia is low [19, 20]. The highest value for Se was found to be 93.02±1.52 µg L\(^{-1}\) and the lowest value was 12.25±0.57 µg L\(^{-1}\). These levels are in agreement with previous reports for this biogeochemical region by Maksimović [25]: 35±7 µg L\(^{-1}\) for selenium in blood serum. In comparison to other European countries these selenium levels are among the lowest.

Competing Interests

The authors declare that they have no competing interests.

Authors’ Contributions

TS supervised the experimental work, preparation of manuscript and communicated to the journal; FČ did the actual work and prepared the manuscript.

References


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