DETERMINATION OF SELENIUM IN BIOLOGICAL TISSUES AND FOODS
BY HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROSCOPY

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Abstract: The use of dry ashing with magnesium nitrate, as an ashing aid, is evaluated for the reliable
determination of selenium in biological tissues and foods by hydride generation atomic absorption
spectroscopy (HG-AAS). Results obtained by the dry ashing method are comparable to those obtained
by wet digestion with HNO₃-HClO₄ and HNO₃-H₂SO₄ mixtures. However, the percentage recovery and
reproducibility obtained with the dry ashing method were superior to those obtained with wet digestion
methods. The dry ashing method was successfully used for reliable determination of selenium in
bovine liver, horse kidney, prawn and wheat samples by HG-AAS.

Keywords: Selenium, HG-AAS, liver, kidney, prawn, wheat, dry ashing, wet, digestion

Introduction

Hydride generation atomic absorption spectroscopy (HG-AAS) is the most widely used technique
for the determination of selenium because of its high sensitivity, low detection limit and minimum
interference from most other elements in biological materials (Clinton, 1971; Vorinden, Deelsra &
Adriaenssen, 1981). Several studies have indicated that the sensitivity of HG-AAS for selenium is
influenced by the nature and concentration of decomposition reagents, especially by the concentration
of residual acids (Clinton, 1971; Vijan, & Leung, 1980; Welz, 1985). For example, it has been
suggested that the presence of large amount of nitric acid hinders the reduction of Se (VI) to Se (IV)
and, hence, lowers the sensitivity of the technique (Clinton, 1971; Vijan, & Leung, 1980). Also, it has
been shown that the presence of excessive amounts of sulphuric and hydrochloric acids in sample
digest can decrease the HG-AAS response for selenium (Welz, 1985). Yet, the accurate determination
of selenium in solid biological materials often requires complete decomposition of the organic matrixes
and subsequent conversion of different forms of selenium to selenium (IV) with hydrochloric acid.

Several studies have demonstrated that dry ashing with magnesium nitrate as ashing aid,
and wet decomposition with acid mixtures such as nitric-perchloric acid and nitric-sulphuric acid are
suitable for the decomposition of biological materials (Bajo, Suter & Aeschliman, 1983; Bye, 1986;
Hansson, Pettersson & Olin, 1989; Lloyd, Hold, & Delves, 1982; May, & Kane, 1984; Neve, Hanoqc,
Mollee, & Lefebvre, 1982; Reamer, & Veillon, 1981; Vijan, & Leung, 1980; Welz, 1985). In contrast,
other studies have claimed that wet digestion with these acid mixtures are inadequate for either complete
decomposition of a wide range of biological materials or can result in loss of selenium during digestion
(Beal, 1975; Hansson, Pettersson & Olin, 1989; Mulford, 1966; Neve, Hanoqc, Mollee, & Lefebvre,
1982). It has also been claimed that the use of dry ashing can result in loss of selenium (Bajo, Suter
& Aeschliman, 1983; Hansson, Pettersson & Olin, 1989; Neve, Hanoqc, Mollee, & Lefebvre, 1982;
Vijan, & Leung, 1980; Welz, 1985). In view of my interest in the HG-AAS determination of selenium
in biological tissues and foods, it was necessary to carefully evaluate a chosen digestion method by
comparison with a number of the more acceptable decomposition methods. The dry ashing method
with magnesium nitrate, as ashing aid, was chosen because of its simplicity in terms of chemical requirements and required attention (Adelroja, 1989; Adelroja, Bond, & Hughes, 1983). While most wet digestion methods could be carried out much faster, they unfortunately require more chemicals and a great deal more attention than dry ashing. Nevertheless, the concern expressed above about possible losses by dry ashing warrants further investigation to ensure its use for reliable HG-AAS determination of selenium in biological tissues and foods.

In this study the effectiveness of dry ashing with magnesium nitrate, as an ashing aid, is evaluated for the reliable HG-AAS determination of selenium in biological tissues and foods. As part of our evaluation, comparison is made between the dry ashing method and a number of the common wet digestion methods. The influence of the state of the magnesium nitrate added as ashing aid, in the form of solid or solution, was also investigated to ascertain possible factors that may be responsible for previously reported losses of selenium by this dry ashing method (Bajo, Suter & Aeschliman, 1983; Hanson, Pettersson & Olin, 1989; Neve, Hanocq, Mollee, & Lefebvre, 1982; Vijn, & Leang, 1980; Welz, 1985). The application of the dry ashing method to reliable determination of selenium in liver, kidney, prawn and wheat by HG-AAS was also investigated.

Experimental Materials and Methods

Reagents and Standards

All reagents used in this study were of analytical grade and the solutions were prepared with Milli-Q water. Selenium stock solution (1 g/l) was prepared by dissolving 0.1g of selenium powder in 3ml of HNO3, and then diluting to 100ml. Working standards were prepared daily by diluting the stock solution. Sulphuric acid (98%, BDH), Hydrochloric acid (32.33%, BDH), nitric acid (70%, Rhone-Poulenc) and perchloric acid (70%, BDH) were used.

The standard reference materials were obtained as follows: bovine liver (1577b) from NIST, Gaithersburg, U.S.A.; horse kidney (H-8) from IAEA, Vienna, Austria; and prawn tissue (AGAL 3) from Australian Government Analytical Laboratories, Pymble, NSW, Australia.

Instrumentation, Glassware and Procedure

**Instrumentation.** All HG-AAS measurements were performed on a Varian Spectra AA-20+ atomic absorption spectrometer operated with deuterium background correction. The conditions employed for the measurements were wavelength: 192.6nm, slit width: 1.0nm, lamp current: 10mA; and electrically heated quartz cell.

**Glassware.** All glassware and plastic containers were soaked in nitric acid (2M) for at least 24 hours and rinsed 8-10 times with water prior to use.

**Procedure for HG-AAS measurement.** A 25ml glass tube with ground neck containing 3ml sample or standard solution was connected to the electrically heated quartz atomising cell and a source of purge gas through a draschal head. A mixture of argon and 1% oxygen was constantly bubbled through the solution. 0.4ml of 4% (w/v) sodium borohydride was injected through the bubbler with the aid of a built-in capillary, and the resulting peak height was measured.

**Decomposition methods**

Dry ashing with Mg(NO3), as ashing aid [M1]. 0.3g of bovine liver (0.25g of prawn tissue or 0.1g of horse kidney or 0.3g of wheat flour) was accurately weighed into a dry crucible followed with addition of 2.5ml of concentrated nitric acid and 2ml of 80% w/v (or 1.6g) magnesium nitrate. The partially covered crucible was heated slowly to 120°C and this temperature was maintained overnight. The temperature was then raised to enable complete dryness, before transferring the crucible into a muffle furnace at 500°C for one hour. The resulting white ash was dissolved in 8ml of 50% HCl by heating for 20 minutes at 100°C, and then the solution was diluted with 4M HCl to 25ml.

Wet decomposition with nitric-perchloric acid [M2]. 0.3g of bovine liver (0.1g of horse kidney or 0.25g of prawn tissue) was accurately weighed into a 100ml Erlenmeyer flask followed by the addition of 20ml of concentrated HNO3 and 3ml of HClO4. The flask covered with a glass funnel was heated at 100°C on a hot plate until all the solid was dissolved and vigorous reaction stopped. The temperature
was raised to 150°C and the flask was allowed to reflux until HClO₃ fumes were visible. The flask was heated overnight to obtain a clear solution. Additional nitric acid (1.00ml) was added where necessary and the heating was repeated until a clear colourless solution is obtained. The flask was then cooled and 8ml of 50% (v/v) HCl was added. The resulting solution was heated at 100°C for 20 minutes and the solution was made up to 25ml with HCl. A special perchloric acid fumehood was used for this method.

Wet decomposition with nitric-sulphuric acid [M3]. This digestion method was carried out as for M2, but with the addition of 20ml concentrated HNO₃ and 1ml of concentrated sulphuric acid. The flask was covered with a glass funnel and heated overnight at 150°C on a hot plate. Then, the temperature was increased to 230°C and heating continued until brown fumes ceased to evolve. After allowing the flask to cool, 8ml of 50% (v/v) HCl was added and the flask was heated at 100°C for 20 minutes. The resulting solution was diluted to 25ml with 4M HCl.

Wet decomposition with reverse aqua-regia [M4]. Required sample was weighed accurately (as for M1-M3) into 50ml teflon tube with screw cap (Oak Ride-Centrifuge tubes, NALG 3114). Then 6ml of concentrated HNO₃ and 2ml of concentrated HCl were added. The tube was screw-capped tightly and the contents were heated overnight on a water bath. After cooling the tube and releasing its pressure, 8ml of 50% (v/v) HCl was added before heating further at 100°C for 20 minutes. The resulting solution was diluted to 25ml with 4M HCl.

Wet decomposition with aqua-regia [M5]. This digestion method was carried out as for M4, except that 6ml of concentrated HCl and 2ml of concentrated HNO₃ were used.

Table 1: Influence of digestion mixture on the detection limit and sensitivity of HG-AAS for selenium

<table>
<thead>
<tr>
<th>Digestion method</th>
<th>Detection Limit (ng)</th>
<th>Sensitivity (ng/0.0044 Abs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>M2</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>M3</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>M4</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>M5</td>
<td>0.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Based on three times standard deviation; n = 10

Results and Discussion

Table 1 shows the influence of the reagents used in the dry ashing and wet digestion methods on the detection limit and sensitivity of HG-AAS response for selenium. The detection limits obtained with wet digestion based on the use of reverse aqua-regia and aqua-regia (M4 and M5) were lower than those of dry ashing (M1) and other wet digestion methods (M2 and M3). It can also be noted that the detection limits and sensitivities obtained with the use of aqua-regia were comparable. Likewise, the detection limits obtained with reagents for dry ashing (M1) was significantly higher than those of M2 and M3. The order of preference, on the basis of detection limit of selenium determination, can therefore be summarised as: M4 = M5 > M1 > M3 = M2. However, this trend may be significantly altered when the mixtures are employed for the decomposition of biological materials. Under this condition, the result will depend upon the effectiveness of the method in breaking down the organic matrix and releasing the selenium in its inorganic Se (IV) form.
Table 2: Recovery of spiked selenium in bovine liver and prawn tissue

<table>
<thead>
<tr>
<th>Digestion method</th>
<th>Bovine liver (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Prawn (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>94.5 ± 1.4</td>
<td>95.6 ± 1.3</td>
</tr>
<tr>
<td>M2</td>
<td>96.7 ± 2.7</td>
<td>95.8 ± 2.2</td>
</tr>
<tr>
<td>M3</td>
<td>92.3 ± 6.5</td>
<td>94.6 ± 7.7</td>
</tr>
<tr>
<td>M4</td>
<td>68.5 ± 5.3</td>
<td>71.2 ± 5.6</td>
</tr>
<tr>
<td>M5</td>
<td>79.0 ± 5.1</td>
<td>87.9 ± 4.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on three independent digestions and three measurements on each digestion; error is mean deviation.  
<sup>b</sup> Spiked concentration is 0.5mg/kg of bovine liver.  
<sup>c</sup> Spiked concentration is 2.0mg/kg of prawn tissue.

The effectiveness of the digestion methods in recovering inorganic selenium spikes in reference materials is given in Table 2. The selenium recoveries obtained with the use of reverse aqua-regia and aqua-regia (M4 and M5) were significantly lower than those obtained with the dry ashing (M1) and other wet digestion methods (M2 and M3). The low recoveries obtained with M4 and M5 may be due to incomplete digestion which resulted in considerable foaming during the hydride generation step. In contrast, the use of dry ashing and other wet digestion methods did not result in excessive foaming during the hydride and their spiked recoveries were comparable. These results therefore suggest that dry ashing (M1) and two of the wet digestion methods (M2 and M3) are superior to methods M4 and M5 in the decomposition of liver and prawn for the HG-AAS determination of selenium.

Table 3: Selenium concentrations (mg/kg) in bovine liver and prawn tissue

<table>
<thead>
<tr>
<th>Digestion method</th>
<th>Bovine liver (mg/kg)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RSD (%)</th>
<th>Prawn (mg/kg)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.75 ± 0.03</td>
<td>4.0</td>
<td>2.76 ± 0.04</td>
<td>1.5</td>
</tr>
<tr>
<td>M2</td>
<td>0.71 ± 0.04</td>
<td>5.6</td>
<td>2.71 ± 0.09</td>
<td>3.3</td>
</tr>
<tr>
<td>M3</td>
<td>0.69 ± 0.07</td>
<td>10.2</td>
<td>2.50 ± 0.30</td>
<td>12.0</td>
</tr>
<tr>
<td>M4</td>
<td>0.31 ± 0.03</td>
<td>9.6</td>
<td>1.17 ± 0.06</td>
<td>5.1</td>
</tr>
<tr>
<td>M5</td>
<td>0.21 ± 0.04</td>
<td>18.5</td>
<td>0.57 ± 0.05</td>
<td>8.7</td>
</tr>
<tr>
<td>Certified value</td>
<td>0.73 ± 0.06</td>
<td>2.74 ± 0.37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on three independent digestions and three measurements on each digestion; error is mean deviation.

Table 3 also shows the concentrations of selenium found in bovine liver and prawn samples with the various methods. The selenium concentrations found with methods M4 and M5 were significantly lower (<50%) than those obtained by the other methods. The selenium concentrations obtained with dry ashing (M1) and two of the wet digestion methods (M2 and M3) for the bovine liver and prawn samples agreed favourably with the certified values. Evidently, wet digestion with aqua regia and reverse aqua-regia at 100°C was inadequate for the decomposition of these biological materials for HG-AAS determination of selenium. Again, the incompleteness of the decomposition with methods M4 and M5 was manifested by excessive foaming during hydride generation. The presence of excessive amounts of nitric acid in the digests obtained with methods M4 and M5 may also interfere with the reduction of Se (VI) to Se (IV), as previously reported (Bajo, Suter & Aeschliman, 1983; Hansson, Pettersson & Olin, 1989).
Based on recovery efficiency, concentration found in real samples and relative standard deviation, digestion of liver and prawn by dry ashing (M1) appeared to be superior to the wet digestion methods for the HG-AAS determination of selenium. The percent relative standard deviation (% RSD) obtained with method M3 was significantly higher than those of dry ashing (M1) and wet digestion with nitric-perchloric acid (M2). This may be due to varying amounts of residual acid present in the final sample digest.

In general, there was no advantage in using any of these digestion methods, based on the required digestion time as overnight digestion at low temperature was required for all methods. Attempts were made to accomplish rapid digestion with nitric-sulphuric acid mixture (M3) as a basis for reduction of digestion time, but this resulted in loss of selenium and low recoveries of spiked selenium in bovine liver and prawn samples. The loss of selenium during digestion of biological materials with nitric-sulphuric acid is well known in cases where oxidising conditions are not maintained and charring of samples occurs (Grant, 1963). Furthermore, it may result in exothermic and violent reactions when large amounts of sample are digested. This method (M3) was most difficult to optimise for HG-AAS measurement of selenium in biological materials.

Nitric-perchloric acid digestion mixture (M2) was very effective, but necessary safety considerations must be made when employing perchloric acid as a digestion reagent. Moreover, it cannot be used without a special fume hood for perchloric acid. In contrast, there is no special requirement for dry ashing method (M1), as overnight heating at low temperature is required. Overall consideration of all the above factors revealed that the use of dry ashing with Mg(NO₃)₂ as ashing aid provides the best conditions in terms of safety and effectiveness for reliable and accurate determination of selenium in biological materials by HG-AAS.

Table 4: Effect of solid and soluble Mg(NO₃)₂ on selenium concentration (mg/kg) found in some standard reference materials

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mg(NO₃)₂ Solid</th>
<th>Mg(NO₃)₂ Solution</th>
<th>Certified Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine Liver</td>
<td>0.72 ± 0.01</td>
<td>0.75 ± 0.03</td>
<td>0.73 ± 0.06</td>
</tr>
<tr>
<td>Prawn (Agal 3)</td>
<td>2.71 ± 0.06</td>
<td>2.76 ± 0.04</td>
<td>2.73 ± 0.37</td>
</tr>
<tr>
<td>Horse Kidney</td>
<td>4.69 ± 0.29</td>
<td>4.73 ± 0.23</td>
<td>4.67 ± 0.30</td>
</tr>
</tbody>
</table>

* Based on three independent digestions and three measurements on each digestion: units mg/kg; error is mean deviation

Table 4 shows that there was no significant difference in using either soluble or solid form of Mg(NO₃)₂ as ashing aid for the HG-AAS determination of selenium in biological materials. However, the use of solid salt as ashing aid avoids the dilution of nitric acid which usually occurs with the addition of Mg(NO₃)₂ solution and, thus, provides an instantaneous oxidising conditions for the pre-decomposition of biological materials.

Recovery studies of selenium in dry-ashed wheat samples, with solid Mg(NO₃)₂ as ashing aid revealed that 95-99% of the element was recovered by this method. The typical concentrations of selenium obtained by HG-AAS for different wheat samples after dry ashing with solid Mg(NO₃)₂ as ashing aid, is given in Table 5. The mean concentration of selenium in these wheat samples varied from 0.623 to 1.019 mg/kg.
Table 5: Selenium concentrations found in some wheat samples by HG-AAS after dry ashing with magnesium nitrate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Selenium* (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.623 ± 0.006</td>
</tr>
<tr>
<td>S2</td>
<td>0.644 ± 0.005</td>
</tr>
<tr>
<td>S3</td>
<td>0.683 ± 0.007</td>
</tr>
<tr>
<td>S4</td>
<td>0.756 ± 0.005</td>
</tr>
<tr>
<td>S5</td>
<td>0.869 ± 0.004</td>
</tr>
<tr>
<td>S6</td>
<td>0.916 ± 0.005</td>
</tr>
<tr>
<td>S7</td>
<td>1.004 ± 0.012</td>
</tr>
<tr>
<td>S8</td>
<td>1.019 ± 0.009</td>
</tr>
</tbody>
</table>

* n = 6; error is mean deviation.

Conclusion

The accurate determination of selenium in biological materials by HG-AAS requires careful consideration of the choice of decomposition method. Dry ashing with magnesium nitrate as ashing aid was found to be effective for the complete recovery of spiked selenium in biological tissues and food samples. The decomposition method proved to be most satisfactory for the reliable HG-AAS determination of selenium in bovine liver, horse kidney, prawn and wheat samples.

References