Determination of Selenium in Biological Material using Automated Wet Digestion and an Automated Hydride Generation Atomic Absorption System

Application Note

Atomic Absorption

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Introduction

Selenium is both an essential and a toxic trace element. Therefore, analytical methods for accurate determination of selenium levels in biological material are important. Selenium deficiency diseases are well known among domestic animals and have also been described in man [1]. Selenium supplementation is therefore a common practice in livestock in many countries [2].

Several different methods are used to determine selenium in biological samples, such as neutron activation and graphite furnace atomic absorption. The fluorimetric method using diaminonaphthalene (DAN) as reagent has been used for many years [3-8]. Graphite furnace methods have gained increasing importance, especially when combined with Zeeman background correction. The hydride generation method is also commonly used. Although this method may have several advantages, it has proved difficult to automate until recently. However, hydride generation systems producing continuous absorption signals are now available. These systems can be easily automated.

It is essential to use perchloric acid in the digestion of biological samples when determining selenium by the hydride generation method. Intercalibration studies have shown that low recovery of selenium may result when perchloric acid is not used [9]. Therefore, an automated wet digestion system using nitric and perchloric acid has been adapted.

The present paper describes the combination of a wet digestion system and an automated hydride generation method for the determination of selenium in biological material, and summarizes the experience with this method.

Agilent Technologies

Materials and Methods

Equipment

A Tecator digestion system was used (Tecator AB, Höganäs, Sweden). The DS 40 aluminium block was temperature programmed with a 1012 Aotostep Controller, and 75 mL digestion tubes were used. The selenium concentrations were determined with an Agilent Vapor Generation Accessory (VGA 76), an Agilent atomic absorption spectrometer (AA-1475) using an Agilent hollow cathode lamp, an Agilent Programmable Sample Changer (PSC-55), and an Eaton 7000+ printer. The standard Agilent open quartz absorption cell with 5 mm i.d. heated by an air-acetylene flame was used. Nitrogen was used as inert gas. A schematic diagram of the hydride generation is given by Sturman [10].

Procedure

Up to 5 g wet weight or 1.5 g dry weight of biological material, containing a maximum of 1 g fat, or from 100 to 1000 ng of selenium standard, were placed in digestion tubes, and 17 mL of a 7 + 3 mixture of nitric and perchloric acid were added together with a few alundum granules to prevent bumping. In order to reduce foaming in some types of samples, the tubes were left overnight before digestion.

(Editor's note. Warning: Perchloric acid digests, — particularly of organic materials— are hazardous. Their use can cause a fire or explosion resulting in death or injury. ALWAYS evaporate digests in a suitable fume cupboard. NEVER evaporate to dryness.)

With the temperature program employed, digestion was complete within 20 hours. When the maximum temperature in the aluminium block is 225 °C, giving a temperature in the digest near the boiling point of perchloric acid, maximum oxidation power is reached [11]. The temperature program is described in Table 1. After cooling, the volume of perchloric acid was adjusted by visual estimate to 4 mL. Se (VI) was reduced to Se(IV) with hydrochloric acid. 10 mL of 2.4 M HCl was added to each of the tubes, which were then warmed in the aluminium block at 120 °C for 30 minutes [12]. After cooling, the solution was diluted to 25 mL.

The autosampler, hydride generator, and the AA spectrometer were operated under the conditions given in Table 2. Sodium borohydride was dissolved in sodium hydroxide to give a 0.6% w/v $NaBH_4$ in 0.5% w/v NaOH. The concentration of hydrochloric acid was 10 M, as recommended by the manufacturer.

Table 1. Temperature Program for the Digestion of Biological Material in a Mixture of Nitric and Perchloric Acid

Temperature (°C)	Ramp (h)	Time (h)
50		2.00
70		2.00
100		2.00
120		1.30
135		1.30
165	3.00	1.00
180	1.00	1.30
190		0.30
225		2-4

Table 2. Operating Conditions for Autosampler, Hydride Generator and Atomic Absorption Spectrometer

Autosampler	
Number of standards	3
Delay time	60 s
Rinse time	60 s
Number of replicates	3
Hydride generator	
Flow rate, sample	6.5 mL/minute
Flow rate $NaBH_4$	1.0 mL/minute
Flow rate HCl	1.0 mL/minute
Nitrogen pressure	3.5 kg/cm^2 (350 kPa)
AA spectrometer	
Wavelength	196.0 nm
Lamp current	10 mA
Slit	1.0 nm
Integration time	5.0 s
Background corrector	Off
Double beam mode	Yes

Control Analyses

Samples of National Bureau of Standards standard reference material 1577a bovine liver were analyzed to check the accuracy of the present method. In addition, 51 samples of blood were analyzed by the present method and by a fluorimetric method [8].

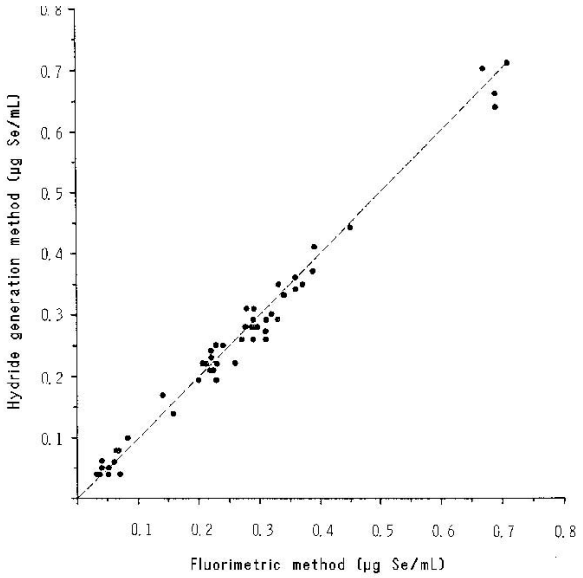

Figure 1. Selenium levels (mg Se/mL) in 51 whole blood samples determined by the fluorimetric method (x-axis) and the present hydride generation method (y-axis).
The dotted line is given by the equation y = x.
The regression line (r = 0.99) is given by the equation y = 0.97 x + 0.004.

Results and Discussion

Fifty-one blood samples were analyzed by the fluorimetric and the hydride generation method. The results are presented in Figure 1. All results are close to the 1:1 line. The regression line is given by the equation:

$y = 0.97x + 0.004$

where y is the result of the hydride generation method and x is the result of the fluorimetric method. The correlation coefficient was r = 0.99. Ten determinations of NBS bovine liver (1577a) gave an average concentration of 0.71 ± 0.03 µg Se/g, which is the same as the certified value.

The VGA-76 vapor generation accessory produces a continuous, integrable signal rather then a transient peak. Using an integration time of 5 s, the precision at an absorbance reading of 0.4 was < 1% with 27 replicates.

Up to 40 samples can be digested simultaneously in the Tecator block. In order to obtain complete digestion, it is necessary to use perchloric acid [13]. Perchloric acid must not be evaporated to dryness. (This will also prevent losses of selenium.) For some types of material, it may be possible to reduce the digestion time. However, the present temperature program is believed to be the safest. The Tecator digestion system has been used for many years without accidents.

The reduction of Se(VI) to Se(IV) with hydrochloric acid is dependent on the acidity and on time. Using 2.4 M hydrochloric acid, reduction is complete within 30 minutes at a block temperature of 120 °C. However, the amount of perchloric acid is critical, and 4 mL of this acid is required to complete the reduction [12].

The present method gives low blank readings. Twenty-seven blanks have an average absorbance of 0.003 ± 0.001, giving a relative standard deviation of about 30%. An absorbance reading equal to ten times the standard deviation corresponds to a selenium concentration of 0.67 ng/mL. Using a 1.0 g sample, this gives a quantification limit better than 0.02 µg Se/g of sample. The sensitivity was 0.3 ng/mL of the analyzed solution. In order to get good results, it is essential to have a stable zero reading on the AA instruments. A reslope calibration of the standard graph is carried out for every five to ten samples. No differences have been observed between digested and non-digested standards.

Several interferences have been detected in the hydride generation determination of selenium [14,15]. However, when analyzing animal or human tissues, only the interference from copper seems to be of practical importance. For copper levels above 7 µg/mL solution, it is recommended to dilute the sample, or use a standard addition method for the quantification of selenium.

The capacity of the present method is limited by the number of samples that can be digested. Under the conditions chosen, it takes about 2.5 minutes to run a sample. With two Tecator blocks, up to 80 samples can be digested during the night and run the next day.

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Printed in the USA
November 1, 2010
AA086

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