Direct Determination of Selenium in Blood and Urine Samples by Platform Furnace Atomic Absorption Spectrometry

Application Note

Atomic Absorption

Introduction

Earlier reports on the furnace determination of selenium have been often contradictory and confusing — probably more so than for any other metal. Some of the problems result from the background effects encountered with all the volatile metals in the presence of high concentrations of alkaline and alkaline earth elements.

Selenium has proved to be particularly difficult to determine in urine and blood samples. Some authors claim that the pre-atomization losses of the analyte may be responsible [1]. The interference from iron [2] or phosphorus [3] adds to the problems.

Normal selenium level in blood ranges between 50 and 100 µg L⁻¹ [4]. Similar concentrations may be found in urine samples [5].

The problems encountered in platform AAS determinations of selenium in biological fluids were recently reported by Carnrick et al. [1]. The presence of phosphates produces a spectral interference for the selenium determination at 196.0 nm, avoided by using Zeeman background correction. On the other hand, Hoenig and Van Hoeyweghen show that spectral interference of phosphates in animal tissues may be circumvented by using the nickel nitrate matrix modifier and measuring the selenium signal in the peak height mode [6].

This study shows the possibilities of selenium determination in blood and urine using a modern graphite furnace device, including platform and modifier approaches, in conjunction with deuterium arc background correction.
Experimental

All work was performed on an Agilent Series AA-1275 BD Spectrometer equipped with Agilent GTA-95 graphite furnace and programmable sample dispenser.

Pyrolytically coated tubes and solid pyrolytic graphite platforms were used.

Details of furnace programs are given in Tables 1 and 2. A Hewlett-Packard 82905 A printer was used for plotting absorbance-time profiles.

A selenium hollow cathode lamp operated at 8 mA current, and a spectral slit width of 1.0 nm were used for all experiments.

The blood samples were diluted (1 + 4) with Triton X-100, 0.2% solution (proanalysis MERCK). The 1% nickel matrix modifier was prepared by dissolving the NiO (Speccure, Johnson and Matthey) in concentrated nitric acid (Suprapure, MERCK). The urine samples were diluted (1 + 9) with demineralized, distilled water. The selenium standards were prepared in 2% nitric acid from commercial standard solution (Titrisol, Merck).

Results

Diluted blood and urine samples are deposited on the preheated platform as specified previously [7]. In this case, the programmable injection temperature facility of the Agilent GTA-95 is used. The droplet is dispensed at 120–150 °C, and the ashing ramp starts directly after the dispensing. Under this condition overflowing the platform is avoided.

For blood, the ashing step is performed with oxygen as the alternative gas up to 500 °C (steps 1–2 in Table 1). Quantitative removal of the organic matter is then achieved without periodical scraping of the carbon residues on the platform which are observed if air ashing is used. After the oxygen ashing and residual oxygen blow-out with argon at 500 °C (step 3), the pyrolysis continues up to 1200 °C (steps 4–5) followed by atomization at 2700 °C (steps 6–7) and cooldown to deposition temperature (step 8). No pre-atomization selenium losses are observed with the applied sample pretreatment in the presence of 20 µg Ni as matrix modifier (2 µL of 1% Ni) and 2 µL nitric acid (1 + 3) as ashing aid, for a 5 µL diluted-blood sample (1 + 4). If necessary, the sensitivity may be enhanced by programming the multiple injection facility after the fourth step.

Determination of selenium in urine samples is performed with a similar graphite furnace program (Table 2). However, no improvement is observed with the oxygen ashing and in the case of urine analysis it is unnecessary.

Urine is diluted with demineralized water (1 + 9) and a 10 µL sample is dispensed on the preheated platform together with 20 µg Ni (2 µL of 1% Ni) and 2 µL nitric acid (1 + 1).

Introduction of samples larger than 10 µL leads to loss of reproducibility.

<table>
<thead>
<tr>
<th>Step no.</th>
<th>Temperature (°C)</th>
<th>Time (sec)</th>
<th>Gas flow (L/min)</th>
<th>Gas type</th>
<th>Read command</th>
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<td>6</td>
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Last dry phase step for multiple injections is step 6. Maximum heating rate was used in step 6.
Discussion

When determining cadmium in sea water, Pruszkowska et al. [8] have shown that background absorbance from sodium chloride may be reduced by using nitric acid as a matrix modifier in much the same way as using ammonium nitrate. In our case also, addition of nitric acid to urine samples reduced the background signal considerably.

In the case of both urine and blood samples, large amounts of calcium and magnesium phosphates lead to negative signals, uncorrectable by the deuterium arc device. In an earlier work ([9] we described a procedure for the determination of selenium in animal tissues which also contain high levels of phosphates in their mineral matrix. The addition of an adequate amount of nickel prevents the formation of phosphorus molecular species which produce a structured background. This allows the analyte absorbance signal to develop completely. With addition of nickel, the negative signal produced by the phosphate is much smaller and is shifted towards higher temperatures.

A similar situation is observed with urine and blood samples (Figures 1 and 2). Peak area is strongly altered by the baseline drift, but the peak height is unaffected since the baseline drift follows the peak value. The selenium analysis is then a typical example for the preferential choice of peak height signal processing.

We also checked the use of palladium as a matrix modifier. Unfortunately, contact of palladium nitrate with blood causes immediate coagulation. With nickel nitrate no coagulation of blood occurs.

No significant matrix effect is observed during the determination of selenium in blood and urine samples. The calibration curve in a simple nitric acid medium achieved with the same modifiers is very close to the standard additions curves obtained for blood and urine samples. Direct comparison with nitric acid standards is then possible. This was checked with blood and urine samples spiked with selenium.

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Maximum heating rate was used in step 5.

Table 2: Furnace Operating Parameters for Selenium Determinations in Urine. Injection Temperature 120 °C

Figure 1. Selenium absorbance-time profiles for blood samples (pyrolysis 1200 °C, atomization 2700 °C).

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Performances

Under the described conditions, the characteristic mass for selenium is about 15 pg for 0.0044 absorbance units. It is easily possible to dispense 10 µL volumes of diluted blood onto the platform. However, using the multiple injection facility, dispensing of two 5 – µL volumes provided better reproducibility of results. In this case, we consider that real selenium concentrations ≥ µ30 µg L⁻¹ in undiluted blood can be measured with sufficient accuracy.

A similar determination limit may be attained for the urine samples despite the smaller dispensed volume. This is a reasonable limit, considering normal levels of selenium in blood and urine.

References


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