Selenium in serum measured by Zeeman graphite furnance atomic absorption spectroscopy

Application note

Clinical research

Authors

John Sanders

Agilent Technologies,
Melbourne, Australia

Introduction

Trace selenium levels of the order of 20 µg/L are encountered in some neonatal serum samples as a result of selenium deficiency in the mother's or infant's diet [9].

Numerous analytical methods [1,5,6,7,9] have been published for the determination of selenium in blood and serum by GFAAS. This study describes the use of new instrumentation and procedures which are important in obtaining the maximum accuracy and precision.

Selenium measurement suffers from a number of difficulties:

- The resonance wavelength of 196.0 nm is in the far UV region where both organic and inorganic compounds, likely to be present in the sample, will exhibit molecular absorption. This can be corrected using Zeeman background correction.

- The intensity of conventional hollow cathode lamps at this wavelength can cause limitations in the precision [6]. Boosted discharge lamps can increase the intensity of the light source, thus lowering noise levels. Selection of special photomultipliers can assist in providing superior response in this region of the spectrum.

- In this complex sample matrix, selenium may exist in any one of a range of chemical species which create atomization problems in the graphite furnace atomizer. This can be overcome by the use of chemical modifiers.

- Selenium is very volatile and can be lost during the ash stage. Suitable modifiers must be used to form compounds with the selenium and allow higher ash temperatures to remove as much of the matrix as possible before the analyte is atomized.

Experimental

Instrumentation

An Agilent SpectrAA-880 Zeeman graphite furnace atomic absorption spectrometer and programmable sample dispenser were used.

The instrument was fitted with an R166UH photomultiplier tube, the Se boosted discharge lamp was operated at 15 mA cathode current and 20 mA boost current.

Reagents Required

Antifoam B 10% Emulsion (Sigma Chemical). It is important this material be a smooth, non-separated emulsion that has a pure white milky appearance. Batches from some other suppliers have been found to separate; separated material is not satisfactory.

Triton X-100 (BDH)

Selenium standard	1000 mg/L Mallinckrodt AA standard
Seronorm standard	Nycomed Pharma AS Norway Batch # 010017
Serum control	UTAK Trace element control normal level, Lot # 7772
L-Ascorbic acid	BDH AnalaR Grade
Palladium chloride	1000 mg/L Mallinckrodt AA standard as $PdCl_2$

Reagent Preparation

Sample Diluent

0.056% wt/vol ascorbic acid
0.088% vol/vol Triton X-100
0.1% vol/vol Antifoam “B”

Prepare the sample diluent by dissolving 0.56 gram of ascorbic acid, 0.88 mL of Triton X-100 and 1 mL of Antifoam B in small portions of deionized water, add 0.1 mL of hydrochloric acid and dilute to 1.0 L.

The hydrochloric acid is added to lower the pH of the solution and ensure a clear solution is obtained when the diluent is mixed with serum samples. Although this solution has been found to be stable for up to 2 days, it is recommended that fresh solutions be prepared daily.

Autosampler Rinse

0.05% Triton X-100 , 0.1% Antifoam B

Pipette 0.5 mL of Triton X-100 and 1.0 mL of Antifoam B into a 1 L standar flask and dilute to 1.0 L with deionized water.

Modifier

500 mg/L palladium , 0.05% Triton X-100, 0.1% Antifoam B:

Pipette 10 mL of 1000 mg/L palladium standard and 2 mL of concentrated hydrochloric acid into a 20 mL standard flask. Add 100 µL of Triton X-100 and 20 µL of Antifoam B and dilute to 20 mL with deionized water.

Standards

Bulk standard 1.00 mg/L

Prepare a 10 mg/L standard in distilled water by diluting 10.00 mL of 1000 mg/L standard to 1 L with distilled deionized water, then prepare a 1.00 mg/L standard by diluting 10.00 mL of the 10 mg/L standard to 100 mL with sample diluent.

Working Standards

These are prepared by adding aliquots of standards prepared in the sample diluent to a portion of the seronorm standard, as per Table 1.

All solutions are diluted to a final volume of 10 mL in standard flasks.

In preparing the solutions, add the seronorm to dry 10 mL flasks and then add the 1 mg/L standard. Allow to stand for about one hour, then dilute to final volume with the sample diluent.

Table 1. Working Standard Dilution Scheme

Solution	Seronorm (mL)	Standard solution (µL)	Effective conc. (µg/L)
Blank	0	0	0
Addition 0	1.0	0	0
Addition 1	1.0	50	50.0
Addition 2	1.0	100	100.0
Addition 3	1.0	150	150.0

These standards have been found to keep for more than a week when stored in a refrigerator at 4 °C in stoppered glass standard flasks.

Autosampler Preparation

Fill the autosampler rinse bottle with the Triton X-100/ AntifoamB rinse liquid and purge the system as recommended in the operation manual.

The tip of the sample capillary must be kept scrupulously clean and should be rinsed at the start of each autorun with aqua regia. This is best accomplished by filling a sample vial with the acid, (**Caution** - extremely corrosive) and manually lowering the tip of the sampler probe into the acid. Then use the sampling syringe to draw the acid up into the capillary to ensure all residues and contamination are removed.

Allow the acid to contact the capillary for 2–3 mins using the syringe to rinse the acid in and out of the capillary.

Finally remove the acid and rinse the capillary two or three times using the Rinse command.

Sample Preparation

Caution: All samples should be treated as a potential biological hazard and full safety precautions in sample handling, required by your local health authorities, must be observed.

Dilute the samples 1:10 with sample diluent–this can be done on a microscale where sample volumes are limited, by diluting 10.0 µL with 90.0 µL of diluent and placing it directly into a sample cup.

In order to maintain the same levels of modifiers with additives in the samples and the standard addition set, samples should not be diluted with distilled water.

Analysis

Create a sample list using the SpectrAA-800 label file for sample identification. This can be directly imported from other data files such as a comma delimited file. The method illustrated uses premixed standard additions to achieve accuracy and consistency of reported results. The complex nature of the sample requires the use of premix standards.

Set up the operating conditions listed in Table 2 and start the sequence operation.

By using the QC Protocol incorporated in the SpectrAA-800 software, the system can be used to monitor the performance of the control samples, and instrument performance, with automatic recalibration or warning of suspect results.

Table 2. Instrument Settings Sampler Parameters

Method	Serum analysis
Element	Se
Instrument mode	Absorbance
Instrument type	Zeeman
Calibration mode	Standard additions
Calibration algorithm	Linear
Measurement mode	PROMT AREA
Sampling mode	Premix
Lamp position	1
Lamp current	15.0 mA
Slit width	1.0 nm
Slit height	Reduced
Wavelength	196.0 nm
EHT	394 V
Precision	3.0 %
Minimum reading	0.000
Replicates all	3
Background correction	On
Maximum absorbance	1.200
Standard 1 concentration	50.000 µg/L
Standard 2 concentration	100.000 µg/L
Standard 3 concentration	150.000 µg/L
Smoothing	9 Point
Expansion factor	1.0
QC Protocol	On

Furnace Parameters

Step no.	Temp (°C)	Time (sec)	Gas Flow (L/min)	Gas Type	Read
1	105	15.0	3.0	Normal	No
2	110	20.0	3.0	Normal	No
3	125	5.0	3.0	Normal	No
4	300	10.0	3.0	Normal	No
5	300	2.0	3.0	Normal	No
6	1200	10.0	3.0	Normal	No
7	1200	5.0	3.0	Normal	No
8	1200	1.0	0.0	Normal	No
9	2550	0.8	0.0	Normal	Yes
10	2550	1.0	0.0	Normal	Yes
11	2550	2.0	3.0	Normal	No
12	40	21.7	3.0	Normal	No

Sampler parameters

Sample volume (µL)	20
Total volume (µL)	20
Modifier 1 volume (µL)	25

Modifier injection parameters

Hot injection	Yes
Injection rate	1
Temperature °C	90
Custom injection	Yes

Mod no.	Pre inject	Co inject	Post inject
1	Yes	No	No
2	No	No	No
3	No	No	No
4	No	No	No
5	No	No	No
LDS	1	1	1
Count	1	1	1

Results

The following results highlight the accuracy and precision obtained using peak height mode from a series of analytical runs over different days.

Seronorm serum sample
Recommended value 96 µg/L
n = 45 Mean = 98.5 µg/L CV% 3.9
Max 104.1 Min 89.1

UTAK control sample
Recommended value 108 µg/L
n = 33 Mean = 108.9 CV% 4.4
Max 116.2 Min 99.2

Discussion

A 1:10 dilution was chosen as a compromise to allow accurate sample delivery in the range of 20-30 µL and to minimize syringe delivery errors. This also assists greatly in reducing ash residues in the graphite tube.

The calibration has been found to be essentially linear to above 0.3 absorbance, as shown in Figure 1. The regression formula for the calibration curve is:

ABS = 0.00082786*Conc + 0.00000003*Conc [2].

This enables confidence to be placed in the use of the standard additions technique and its application to a wide analytical range.

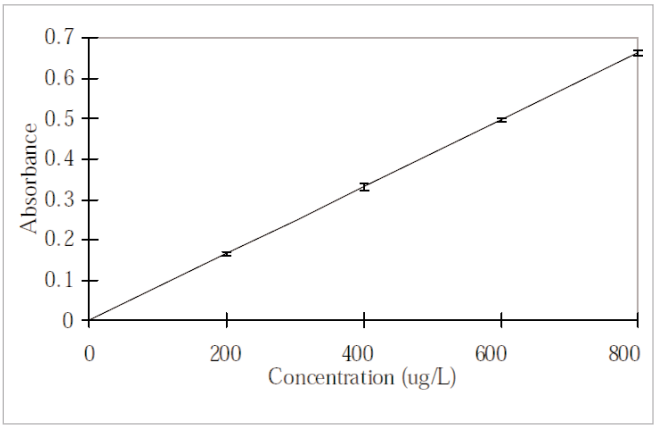

Figure 1. Selenium calibration graph.

The calibration graph in Figure 2 shows the difference in slope between pure aqueous selenium standard and that containing serum. This difference means that it is essential that calibration standards be prepared in serum using the standard addition method for initial calibration.

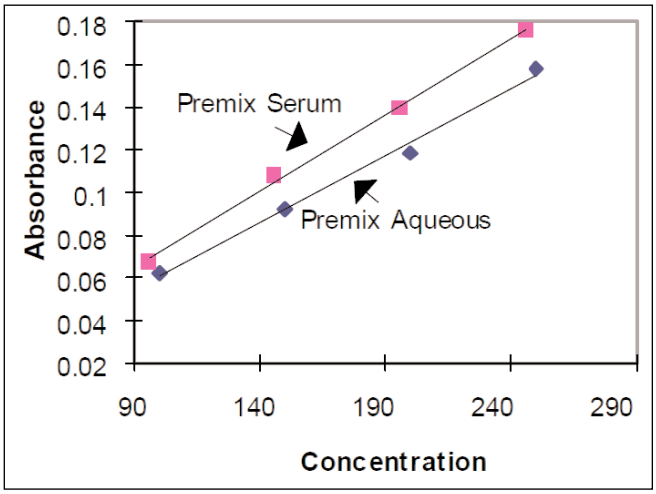

Figure 2. Serum standard addition calibrations.

Conditioning of the graphite tube is essential and this requires 6 to 9 firings with a sample or blank to form a coating on the interior of the tube. Once formed, this coating should not be disturbed. Avoid using a Tube Clean function to remove residue. This method has been found to permit analysis of 40–80 samples without interference from serum ash residue. Using Tube Clean will result in severe shortening of tube lifetime and deterioration in precision.

Palladium is used as a modifier for convenience and effectiveness [4,5,8]. Although other modifiers such as nickel, are recommended for use with Se, the use of nickel can create high nickel residues in the furnace. This will provide difficulties if the user later wishes to analyze for trace levels of nickel.

The instrument baseline noise level can limit the precision of the analysis. To minimize this noise source two avenues are available. Firstly, the use of boosted discharge lamps can increase the light output of the lamp.

Further gain can also be obtained by the use of special UV sensitive photomultiplier tubes, such as the Hamamatsu R166UH, which has approximately six times the radiant sensitivity in the low UV region compared to a normal R446. The R166UH photomultiplier has an added advantage in that it has very little response in the visible region and therefore is less subject to noise created from scattered furnace radiation when atomize temperatures above 2500 °C are used. Figures 3 and 4 compare the signals obtained using different sources and detectors. The improvement seen with the R166UH and boosted discharge lamp is clearly evident and reflects in the precision of the analysis. Detection limits are also lowered when using boosted discharge lamp with the R166UH photomultiplier tube.

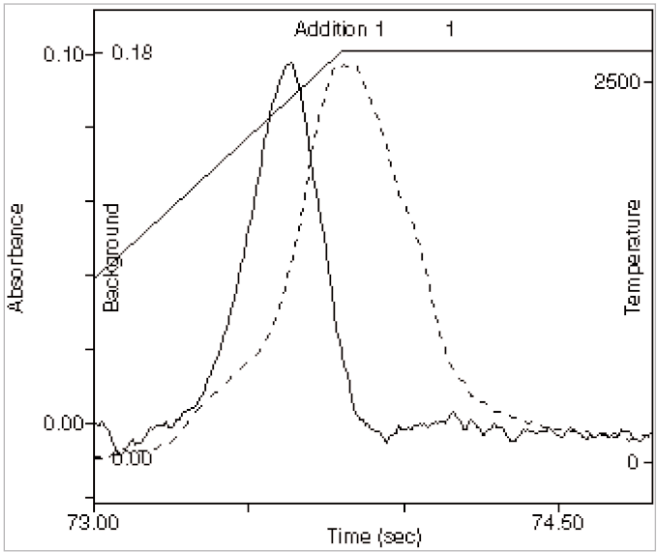

Figure 3. Typical graphic plot for selenium in serum with R166 PM tube and boosted discharge lamp.

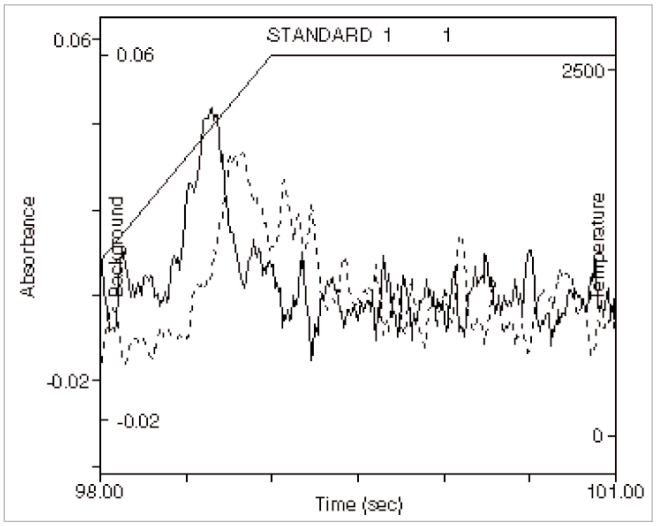

Figure 4. Typical graphic plot for selenium in serum with conventional R446 PM tube and normal hollow cathode lamp.

An added advantage of using a boosted discharge lamp for selenium is the increased sensitivity obtained due to the improved signal to background ratio at the 196.0 nm resonance line, when compared to a conventional lamp.

Speciation of selenium is a known problem in graphite furnace atomization, as discussed by Jacobson and Lockitch [9], and Johannessen et al. [4]. This creates some concern regarding the overall validity of the results obtained by the various techniques. In this context it may be advantageous to use peak area rather than peak height for this analysis. The SpectrAA-800 is particularly suitable for this task as sufficient numeric resolution is available when analyzing at low absorbance levels, for example, 4 decimal places, and it is possible to record both height and area results simultaneously. The ability to view the graphics plot of each firing allows those exhibiting spurious peaks or abnormal shape to be excluded when determining the final results. The data in Table 3 obtained over several days indicate the excellent results obtained by both peak area and peak height for UTAK control Lot # 7772.

Table 3. Summary of Results Over Several Days

	Peak area	Peak height
Mean µg/L	109.9	108.9
CV%	3.8	4.4
n3		
Min µg/L	99.1	99.2
Max µg/L	117.1	116.2
Recommended value	108 µg/L	

The speed of analysis may be improved by using the furnace PROMT measurement mode. In this mode the user defines the required precision and the maximum number of replicates. Once the desired precision has been achieved, the system automatically moves to the next sample. Coincident with this, the autosampler prepares the next solution ready for injection while the current sample is being atomized. This provides a saving of about 30 seconds per solution compared to conventional instrumentation, giving results to a known precision and significant time savings.

The complex nature of serum results in the accumulation of protein and fatty material in and around the sample probe. By using the Antifoam B, which contains an emulsified silicone oil, a thin coating of silicone oil is formed over the wetted surface of the probe, rendering it hydrophobic and ensuring a clean surface. This prevents carryover and improves reproducibility.

Antifoam B also causes the modifier and sample to spread uniformly in the graphite tube and minimizes foaming during the critical dry and ash stages.

Conclusion

The procedure described provides a reliable analytical method for the analysis of selenium in serum with coefficient of variation of better than 5% at the 100 µg/L level.

References

1. M. B. Knowles and K. G. Brodie, Determination of selenium in blood by Zeeman Graphite Furnace Atomic Spectrometry using a palladium-ascorbic -acid modifier. *J.Anal At.Spectrom* (1988),3, p 511.

2. Analytical methods for Graphite Tube Atomizers. Agilent Technologies. April 2014

3. B. Radzuik and Y. Thomassen, Chemical modification and spectral interference in selenium determination using Zeeman-effect Electrothermal Atomic Absorption Spectrometry. *J.Anal At.Spectrom* (1992), 7, p 397.

4. J. K. Johannessen, B. Gammelgaard, O. Jons, and S.H. Hansen, Comparison of chemical modifiers for similtaneous determination of different selenium compounds in serum and urine by Zeeman-effect Electrothermal Atomic Absorption Spectrometry. *J.Anal At.Spectrom* (1993), 8, p 999.

5. Chia-Ming Kao, Yunn-Jing Cherng, et al. Determination of total selenium in human urine and serum by Graphite Furnace Atomic Absorption Spectrophotmetry with palladium-nickel ammonium nitrates as a matrix modifier. *Journal Chinese Chemical Society.* (1993), 40, p 33.

6. B. Pohl, B. Horstkotte, U. Steeg, Determination of As and Se By AAS using high Intensity hollow cathode lamps. Agilent publication AA113. November 2010

7. B. Pohl, T. Schneider, Determination of normal levels of selenium in blood serum by GFAAS. Agilent Publication. AA103. November 2010

8. B. Welz, G. Schemmler, U. Voellkopf, Influence of the valency state on the determination of selenium in GFAAS. *Spectrochimica Acta.* (1984), 39B, p 501.

9. B. E. Jacobson, G. Lockitch, Direct determination of selenium in serum by GFAAS with Deuterium Background Correction and a reducing palladium modifier: Age-Specific Reference Ranges. *Clin Chem*, (1988), 34/4, p 709.

www.agilent.com/chem

For Research Use Only. Not for use in diagnostic procedures.

This information is subject to change without notice.

Published August 11 2015
Publication number: AA122

Agilent Technologies