

Atomic Absorption Full Method Al in Serum

Key Words

- Aluminium
- Serum
- Background Correction
- Graphite Furnace
- Atomic Absorption
- Extended Lifetime Cuvettes

Introduction

The determination of aluminium in biological materials by Graphite Furnace Atomic Absorption Spectrometry has been the subject of many publications. Most attention has been given to the analysis of blood serum, since serum aluminium levels can be used for the diagnosis and monitoring of dialysis patients at risk of aluminium intoxication. In patients with renal failure treated by means of dialysis it is well established that, besides the ingestion of aluminium containing phosphate binders, aluminium contaminated water and dialysis fluids can cause aluminium toxicity.

There are indications that aluminium may have certain essential functions, but in excess it can also induce toxic manifestations. High concentrations have been shown to be toxic to the nervous system. The concentrations of aluminium in blood serum reported as normal are low, at less than 2.0 µg/L. Serum aluminium levels above 50 µg/L can indicate increased absorption leading to monitoring and clinical symptoms at the 100 µg/L and 200 µg/L levels respectively.

Aluminium is normally measured direct by Atomic Absorption Spectrometry using a graphite furnace, although it has been shown due to the nature of the serum matrix that dilution of the serum sample is necessary. Contamination has been shown to be the most important problem.

Analytical Range

A method for the determination of aluminium in human blood serum samples is presented. The 3 sigma method detection limit is approximately 1 µg/L.

Principle

Aluminium is determined directly in blood serum by direct calibration against aqueous standards using Graphite Furnace Atomic Absorption Spectrometry. Background correction may be used but there is little background signal from the serum matrix. The use of a furnace vision system, such as Thermo Scientific GFTV, is a distinct advantage when performing this analysis.

Method

Reagents:

Nitric acid (Spectrosol grade).

Aluminium master standard (1000 mg/L Spectrosol or equivalent).

Triton X-100 (High purity grade or equivalent).

Silicone anti-foam (High purity grade or equivalent).

Sample Collection

Serum samples were prepared in acid washed autosampler cups immediately before analysis. 100 µL portions of blood serum were mixed with 200 µL of diluent containing 0.1 % m/v Triton X-100, 0.5 % v/v nitric acid and 0.05 % m/v antifoaming agent.

Working standards were prepared daily by serial dilution of the master standard with the diluent mixture.

Method Development

It is necessary to dilute the serum samples before analysis, so that they can be measured and transferred accurately by the Furnace Autosampler. For this work, a dilution factor of 3 (1 volume of serum mixed with 2 volumes of diluent) was used. Serum samples are viscous and sticky, so to further improve their handling qualities, a surfactant (Triton X-100) was added to the diluent. The optimum concentration was found to be 0.1 % m/v.

Serum contains large quantities of chloride ion, which can cause some of the aluminium to be lost as volatile aluminium chloride during the Ash phase of the furnace program. This can be prevented by adding an excess of nitric acid to the sample, so that the chloride ion is lost as hydrogen chloride during the dry phase. It is convenient to add the nitric acid to the sample in the diluent solution, and a concentration of 0.5 % v/v was found to be sufficient to overcome the interference without causing the proteins in the serum sample to precipitate.

Serum samples diluted with a suitable nitric acid / Triton mixture can be analysed successfully. However, there is a strong tendency for the mixture to form foam and bubbles, particularly during the later stages of the Dry phase, as can be clearly seen on the GFTV Figure 1.



Figure 1: Serum + Triton sample bubbling in the Dry phase

All reagent examples available from:

Fisher Scientific
Bishop Meadow Rd
Loughborough,
LE11 5RG
UK.

Reference blood samples were obtained from:

Nycomed Pharma AS, PO Box 4284, Oslo N-0401, Norway

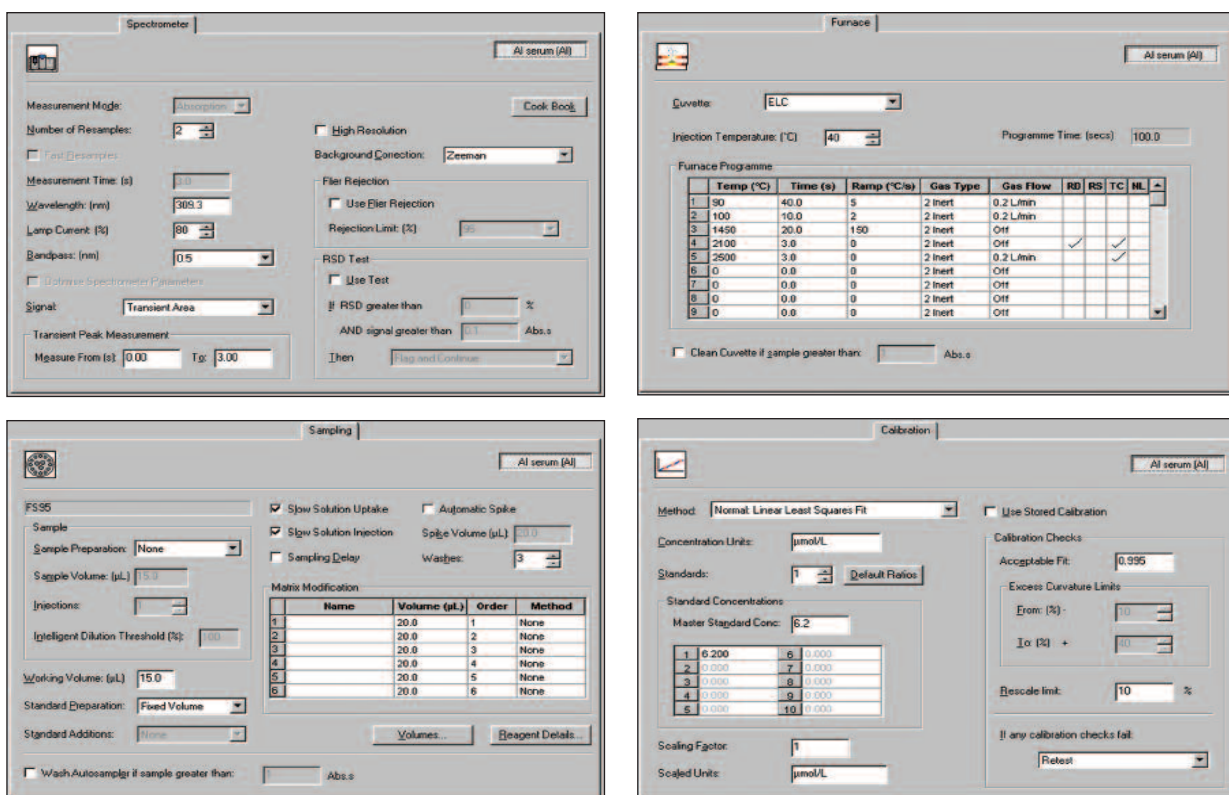


Figure 2: Analysis parameters

This degrades the measurement precision and can, to some extent, be overcome by using a long, slow drying phase. Better results and a shorter program cycle were obtained, however, when an anti-foaming agent was added to the diluent. Silicone antifoaming agent described as 'an aqueous emulsion containing 30 % m/m of silicone' was used, added to the diluent at a concentration of 0.05 % m/v of the supplied emulsion. This completely prevented any foaming and bubbling and also improved the injection, as it prevented the sample from running back up the outside of the capillary tip.

Residue build-up in the cuvette is a common problem when analysing serum samples. The residues are believed to be carbonaceous materials from the thermal degradation of the fats and proteins in the serum. They cannot be removed by a conventional cuvette clean phase. The formation of these residues can be reduced by including an oxidising Ash phase in the furnace program, typically using air or oxygen as the inner gas for 30 seconds at 600 - 650 °C. This should be followed by a phase at the same temperature with the inert gas flowing for around 20 seconds to desorb oxygen trapped in the graphite and avoid undue shortening of the cuvette lifetime. This procedure adds nearly a minute to the furnace cycle. It was also observed that the aluminium signal from both standards and samples was reduced by 5-10 % when an oxygen ash phase was used, presumably because some of the metal forms refractory Al_2O_3 which is not fully atomised.

During the development of this method, two factors were identified that influenced the rate at which residues built up in the cuvette. It was found that if the sample decomposition in the Ash phase could be accomplished quickly, by rapidly increasing the temperature to a high

value, much of the material was volatilised as a 'puff' of smoke and little residue was produced. With a slower rate of temperature increase, the decomposition took place more slowly, no 'puff' of smoke was observed and there was a much stronger tendency for the residue to form. Furthermore, as the Dry phase was optimised, and especially as the bubbling and foaming were eliminated, the amount of residue formed was also reduced.

Sample injection

Serum samples diluted as described were deposited in the cuvette by the autosampler in the form of a droplet. This persisted throughout the Dry phase, resulting in a solid 'lump' of material in the centre of the cuvette at the start of the Ash phase. It then decomposed to leave a similar 'lump' of residue (see Figure 3a).

However, if the cuvette surface was 'pre-wetted' by the injection of a suitable volume of the diluent alone, before the injection of the diluted serum sample, the serum spread to form a pool in the base of the cuvette. This not only could be dried more rapidly and with better precision than the droplet, but also did not form a 'lump' of residue. Under these conditions, the decomposition of the serum will be more efficient. As the material is spread over a relatively large area, any residue will form a film, rather than a 'lump', which does not affect subsequent measurements (see Figure 3b).

Using this technique, it was possible to analyse a full autosampler tray of serum samples (60 samples) without an oxygen ash phase, and without observable residue build up.

Furnace Program

The Ash and the Atomise temperatures were optimised using the automatic Ash Atomise facility provided in the SOLAAR software package. The Ash Atomise graph is shown in Figure 4. The optimum Ash temperature was found to be 1800 °C, and atomisation at 2300 °C gave clean, well shaped signals. A 12 second ash phase was found to be sufficient to remove all the matrix material, and a 2 second atomise phase included all of the signal.



Figure 3a: Dry injection



Figure 3b: Pre-wetted injection

It was found that a 10 µL volume of diluted serum could be smoothly dried in 45 seconds at 110 - 115 °C if the diluent was the nitric acid/Triton/ silicone antifoam mixture described above. The cuvette pre-wetting was achieved by using a 'dummy' Dry phase of 1 second at zero temperature, whose only purpose was to allow a Return to Standby command to be inserted in the program. 10 µL of the 'pre-wetting' solution, which was the pure diluent, was then added as a matrix modifier using the Dry mixing method. The effect of this procedure is that 10 µL of the diluent is added to the cuvette, and the system then performs the first (dummy) Dry phase.

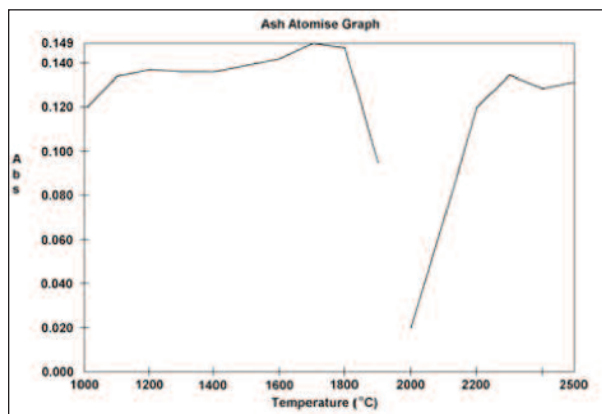


Figure 4: Ash/Atomise plot for Al in the diluent mixture

The Return to Standby command then causes the normal sample injection to take place into the wet cuvette, after which the normal furnace program is executed.

Both pyro-coated and Extended Lifetime cuvettes were evaluated and no significant difference in performance was seen, although full lifetime tests were not performed. It would be expected that an ELC cuvette would last for 5 - 10 times more measurements than a pyro-coated one.

Spectrometer parameters

With the optimised furnace program described above, clean signals with no measurable backgrounds were produced. A typical signal is shown in Figure 5.

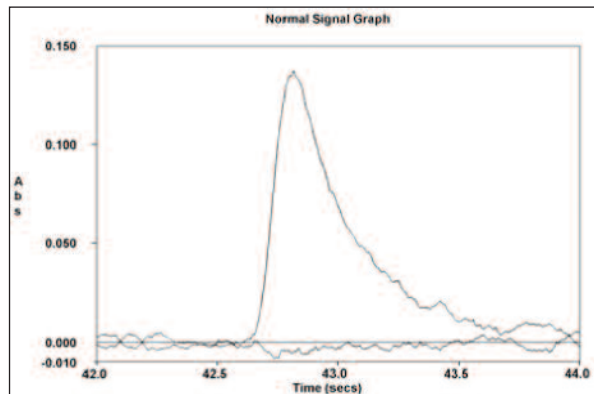


Figure 5: Signal display for Al, background correction off

There was no consistent difference between results obtained with, and without, the Quadline background correction system selected. All the results reported below were measured with no background correction.

The default wavelength for aluminium determination is 309.3 nm. Under normal conditions, use of this wavelength results in a somewhat curved calibration graph. The alternative wavelength at 396.2 nm is only about 20 % less sensitive, and gives a much more linear calibration, resulting in a greater dynamic range for the analysis. This wavelength was used for this work and the lamp current was increased to 100 % from the default value of 80 %, improving the baseline noise levels without compromising the calibration linearity.

Calibration

Two volumes of the diluent solution were mixed with one volume of deionised water. This solution was used directly as the blank for the analysis and a standard containing 50.0 µg/L of aluminium in the same mixture was prepared by serial dilution of a 1000 mg/L master solution.

Standard addition experiments were performed using several different serum samples and the slopes of the calibration lines were compared with the slope measured for a simple aqueous solution. When the optimised diluent, sample injection and furnace program were used, the slopes were identical within the experimental precision, indicating that the Normal calibration method could be used.

The calibration line at the 396.2 nm line was linear up to at least 50 µg/L of aluminium. The Furnace Autosampler automatic standard preparation facilities were used to prepare a three point calibration and the linear least squares fit algorithm was used.

Method Validation

The method was tested by first analysing a standard reference serum, Seronorm batch 311089, produced by Nycomed Pharma AS, Oslo, Norway. The certified aluminium level in this sample is 61 µg/L; this method gave a result of 61.4 µg/L with a relative standard deviation (n = 3) of 1.3 %.

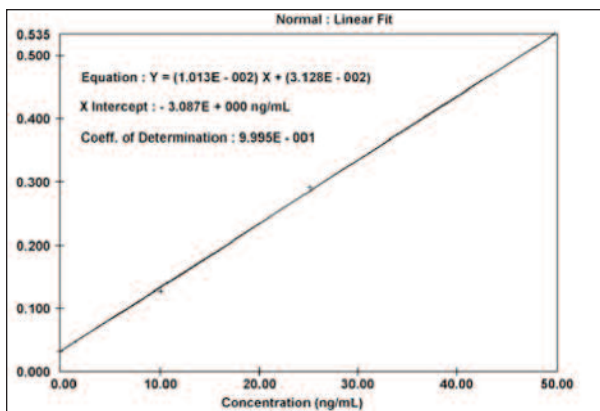


Figure 6: Calibration graph, Al in diluent mixture

A number of serum samples with known aluminium levels, kindly supplied by the Trace Metals Unit at the Robens Institute, were analysed by this method. The results are given in Table 1.

Robens Samples

SAMPLE NO.	FOUND (µg/L)	REF. VALUE (µg/L)
402	12.6	12.9
403	43.4	43.4
409	28.7	29.9
411	21.6	19.2
416	13.7	14.3
417	36.5	34.5
419	21.9	22.1

Table 1: Validation experiments, Al in reference sera

Finally, a run with a full autosampler tray of serum samples was set up. A Robens sample was designated as a QC Check and analysed after every five samples. A blank solution, prepared as described above, was designated as a QC Blank and also analysed after every five samples. Calibration Rescale actions were programmed after every

ten sample measurements, particularly to compensate for evaporation of the solutions. This was significant as the experiments were performed on an instrument operated under a laminar flow of air filtered to Class 100.

Results

The results obtained for both the certified reference serum and the samples obtained from the Robens Institute agree well with the expected values, showing that the method is accurate. The QC results from the 50 sample run show that the method is stable. It is necessary, under the environmental conditions used for this experiment, to compensate for sample evaporation by using the Rescale facility of the SOLAAR software.

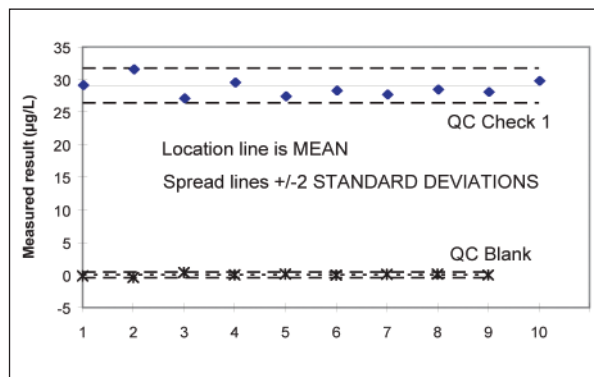
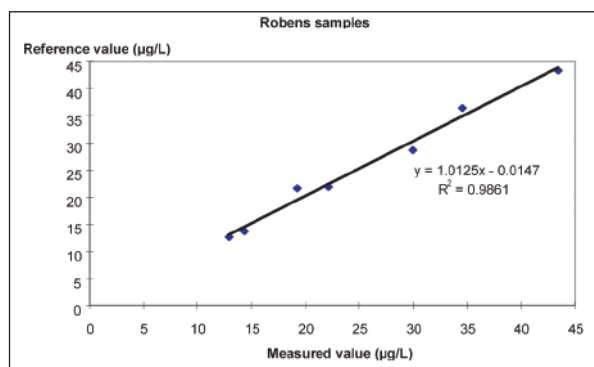


Figure 7: QC results, Al in reference serum

Conclusion

A simple method suitable for the routine determination of aluminium in serum samples from patients on dialysis is presented. The method has been shown to be both accurate and repeatable.

The method of sample treatment described in this publication should be performed only by a competent chemist or technician trained in the use of safe techniques in analytical chemistry. Users should acquaint themselves with particular hazards which may be incurred when toxic materials are being analysed and handled in the instruments, and the instrument must be used in accordance with the operating and safety instructions given in the Operators manual.

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