Electrothermal Atomic Absorption Spectrometric Determination of Aluminum: Elimination of Serum Matrix Effects

Hillel J. Gltelman and Frances R. Alderman

An improved electrothermal atomic absorption spectrometric method for determination of aluminum in serum is described. An ammonium hydroxide/sulfuric acid diluent is used to control matrix effects from serum constituents. Because serum produces no apparent matrix effects with this diluent, one can accurately calibrate the procedure with aqueous standards.

We have previously described a technique for determining aluminum in serum by use of electrothermal atomic absorption spectrometry (1). Our inability to adapt this procedure to a newer instrument, as well as our difficulties with other existing atomic absorption spectrometric procedures (2,3), prompted us to re-evaluate the electrothermal measurement of aluminum in serum. This study led to the development of a modified sample diluent that, in combination with slightly altered instrumental settings, permits us to measure aluminum in serum without apparent matrix interferences.

Materials and Methods

**Materials**

*Instrumentation:* Aluminum was measured in a Zeeman/3030 instrument, equipped with an HGA-600 graphite furnace, an AS-60 auto sampler, and a PR-100 printer. We used pyrolytically coated graphite tubes (0091-504) with solid pyrolytic graphite platforms (B0121-091), all from Perkin-Elmer Corp., Norwalk, CT. Table 1 lists the instrumental settings for the furnace program.

*Standards:* We used 1000 ppm (1000 mg/L) certified atomic absorption aluminum reference solution (Fisherscientific, Pittsburgh, PA 15219) to prepare standards. Aliquots of this stock were diluted with dilute nitric acid (0.1 mL/L, "Ultrex" grade; J.T. Baker Co., Phillipsburg, NJ 08865) to yield working standards containing 100 and 600 μg of aluminum per liter. To avoid contamination, we found it necessary to rinse the Parafilm squares used for capping and mixing the dilutions.

*Samples:* Blood was collected in red-top Vacutainer Tubes (Becton Dickinson Co., Rutherford, NJ 07070). After the specimens had cotted, but within an hour, the tubes were centrifuged, and the serum was removed with disposable polyethylene pipettes and stored in disposable polystyrene or polypropylene tubes (all determined to be aluminum free).

* Diluent: This solution contains, per liter, 8.35 mL of 200 mL/L ammonium hydroxide (Ultrex, J.T. Baker Co.), 1.0 mL of sulfuric acid (Ultrex, J.T. Baker Co.), 5 mmol of disodium EDTA (Fisher), and 1.5 mL of Triton X-100 surfactant (scintillation grade; Mallinckrodt, Inc., St. Louis, MO 63147). This diluent solution is stable indefinitely when stored in a polyethylene bottle.

*Cleaning solution:* This solution contains, per liter, 10 mmol of sodium carbonate, 1.0 mmol of disodium EDTA, 2.0 mmol of sodium citrate, and 0.15 mL of Triton X-100.

**Procedure**

Dispense 200 μL of serum, distilled water, or appropriate standards by means of a 1-mL Selectapette (Clay Adams, Becton Dickinson) with a disposable polypropylene tip into 800 μL of diluent in a 12-mL polycarbonate or polypropylene tube. Vortex-mix.

As reported (4), the standard curve for sample concentrations of aluminum >100 μg/L is curvilinear. For calibration we used Al standards of 100 and 600 μg/L and a two-coefficient calibration procedure.

To minimize contamination, we performed all operations under dust covers. The pipette tips used for dispensing serum were rinsed in dilute (30 mL/L) nitric acid and distilled water immediately before use. Sample dilutions were made in tubes that had been soaked in cleaning solution for at least 4 h, then extensively rinsed with distilled water immediately before use. After vortex mixing the diluted specimens, we poured them into disposable autosampler cups that had been rinsed with distilled water and shaken dry just before use. For most purposes we ran duplicate dilutions for each sample. Results were considered reproducible when peak absorptions for the pair agreed within 10%.

**Results and Discussion**

*Preliminary studies:* Our initial studies examined the
analytical recovery of aluminum from distilled water, isotonic saline, pooled serum from normal individuals, and serum from patients receiving hemodialysis therapy. At the outset we used the magnesium nitrate diluent procedure recommended by Slavin (3), except we did not use O₂ during the ashing step at 600 °C. Figure 1 displays these recovery results along with results from samples diluted with the proposed new diluent. Aluminum recoveries from aqueous and serum-containing samples prepared with the magnesium nitrate diluent differed markedly and thus precluded the use of this methodology with our instrument. Because we thought the incomplete recoveries could be indicating loss of analyte at the recommended char temperature of 1700 °C, we repeated these studies with the char temperature at 1500 °C. This improved the absorption values from aqueous samples, but the recoveries of aluminum from serum samples were incomplete.

Incomplete recoveries were also observed when we used an ammonium hydroxide/sulfuric acid diluent that we had previously recommended for the older Model HGA 2000 graphite furnace (1). This diluent had been designed to eliminate chloride interference by removing chloride during the early heating phases of the graphite furnace protocol. The subsequent development of a reliable methodology for the new equipment was made possible when we recognized that chloride interference could be controlled in the new instrument with approximately one-tenth the concentration of matrix modifiers previously used. In retrospect, we think it likely that the markedly smaller volume of the newer graphite tube and the restricted surface area of the L'vov platform account for this discrepancy.

Furnace conditions: We designed the furnace settings to proceed as rapidly as possible through the heating cycle while maintaining full recovery of aluminum. The temperature chosen for the initial drying stage produces rapid drying of samples on the L'vov platform without boiling or spattering. Because these settings may vary between instruments, the setting for the drying temperature must be established for a given instrument. In the subsequent step we found the rate of temperature increase to 500 °C to be critical for complete recovery of aluminum in serum samples. This accounts for the 20-s ramp and 10-s hold at 500 °C. A char temperature of 1450 °C is the hottest temperature we could use in our instrument without loss of analyte signal from aqueous samples. Once again, because the actual temperature achieved in the HGA 600 furnace is not measured directly, the maximal thermal pretreatment temperature must be established for a given instrument. We found 2600 °C optimal for atomization and followed this with a short period at 2700 °C to avoid sample carryover.

Method assessment: We evaluated the proposed methodology with analytical-recovery experiments and serial-dilution studies. When we used the ammonium hydroxide/sulfuric acid diluent, the peak areas observed for aluminum recoveries from saline and from pooled normal and pooled hemodialysis serum were virtually identical to the peak areas obtained with aluminum in water (Figure 1). Figure 2 shows a serial dilution study of a pooled serum sample from hemodialysis patients. The linearity of this dilution curve is consistent with the conclusion that the proposed procedure is substantially free of matrix interferences.

Individual recovery data from normal serum, hemodialysis serum, and normal urine specimens are presented in Table 2. In all instances the mean recoveries and range of recoveries are consistent with complete recovery of added aluminum.

To evaluate the precision of the procedure, we used pooled serum as reference material. We found the precision satisfactory for clinical use (Table 3). Because there is no effective way to exclude the possible contamination of an individually diluted sample, we routinely measure duplicate dilutions and accept the result if results for the dilutions agree within 10%.

We found that 12 pg of aluminum produced an absorption of 0.0044 absorbance seconds (A-s). This corresponds to an absorption of approximately 0.125 A-s for a 100 μg/L aluminum standard prepared by fivefold dilution of the sample with the diluting fluid as outlined above. A typical blank measurement was 0.004 A-s, with a standard deviation of 0.0009 A-s. Consequently, the detection limit (2 SD) for our assay is approximately 5 pg of aluminum. After correction for sample dilution, this corresponds to a value of 1.5 μg/L for a single determination, or ~1 μg/L for the duplicate dilutions we routinely use.

The mean aluminum value in serum of 10 normal individuals was 3.1 (SD 1.1) μg/L, not significantly different from the normal values we have previously observed in this laboratory (1).

Advantages and disadvantages of the method: Although it is generally agreed that electrothermal atomic absorption spectrometry is the method of choice for the determination of
Table 2. Analytical Recovery Data

<table>
<thead>
<tr>
<th>Sample</th>
<th>Range in sample</th>
<th>Added</th>
<th>Mean (SD) recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal serum</td>
<td>0-6</td>
<td>95</td>
<td>96 (3.6)</td>
</tr>
<tr>
<td>Hemodialysis serum</td>
<td>18-61</td>
<td>83</td>
<td>85 (2.6)</td>
</tr>
<tr>
<td>Normal urine</td>
<td>4.5-10</td>
<td>86</td>
<td>87 (2.4)</td>
</tr>
<tr>
<td>n = 5 each.</td>
<td></td>
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</tr>
</tbody>
</table>

Table 3. Precision Data for Serum Samples

<table>
<thead>
<tr>
<th>N</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within run</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4-6</td>
<td>5</td>
<td>0.6</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>95-109</td>
<td>102</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Between run</td>
<td>15*</td>
<td>43-57</td>
<td>50</td>
<td>4</td>
</tr>
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<td></td>
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* Measured over a 10-week period.

aluminum in serum, there is no general agreement regarding optimal control of matrix effects with this technique (5). Several studies performed with the stabilized temperature platform have strongly recommended the utilization of magnesium nitrate diluent (2, 3). The studies we report were prompted by our inability to completely eliminate the matrix effects of serum with this diluent (see above). Others using a magnesium nitrate diluent have also reported matrix effects with serum and have recommended standardization with protein-containing solutions (2) or a protein precipitation technique (6). The basis for our new procedure is the finding that these matrix effects can be eliminated with a diluent of ammonium hydroxide/sulfuric acid. The fact that the new procedure can be standardized with aqueous standards makes this methodology distinctly advantageous.

The accuracy and precision of the procedure and the values observed in normal individuals are similar to results obtained by us previously (1) and by others (2, 3).

Nonetheless, the proposed methodology has several disadvantages worth noting. The heating cycle is relatively long, which slows the rate at which samples can be run. Thus far, our efforts to significantly decrease the duration of the heating cycle have been unsuccessful. The need to use prediluted samples is another disadvantage. It would obviously be much more convenient to assay serum samples directly, but our attempts to use undiluted serum have not been successful, presumably because the matrix modifier does not mix easily with undiluted serum.

Finally, we recognize that our method yields only a small absorbance value for serum containing physiological (<10 μg/L) amounts of aluminum. In this instance we have found that scrupulous care must be taken to avoid contamination because contamination is the major limiting factor. In addition, we recommend the routine use of triplicate dilutions in this concentration range to improve the absolute precision of the assay.

References


CLIN. CHEM. 35/7, 1519-1523 (1989)

Influence of Blood Pressure, Heart Rate, Age, and Sex on Concentrations of Atrial Natriuretic Factor and Cyclic GMP in 124 Volunteers

Marion Wencker,¹ Susanne Hauftlorenz,¹ Willi Moll,² and Bernd Puschendorf¹,³

The significance of increased atrial natriuretic factor (ANF) in relation to blood pressure and age is still controversial. We investigated the influence of blood pressure, age, and some other variables on ANF and its putative second messenger, cGMP. Samples for ANF and cGMP detection were taken from 124 ostensibly healthy individuals who were donating blood. Samples were also collected from 27 volunteers before and after blood donation, to study the influence of bleeding. During blood donation, ANF increased from 78.9 to 87.4 ng/L (P = 0.0035), whereas cGMP remained unchanged. ANF concentrations in 124 healthy individuals, corrected for the influence of bleeding, were 61.5 (SD 26.1) ng/L, with a 95% confidence interval of 10.0 to 112.1 ng/L. Mean cGMP concentrations in plasma were 2.9 (SD 1.45) nmol/L, with a 95% confidence interval of 0.4 to 5.75 nmol/L. Multivariate analysis revealed no significant influence of blood pressure, age, heart rate, or sex on concentrations of either ANF or cGMP in plasma.

Additional Keyphrases: variation, source of...