An Analysis for Blood Manganese Used to Assess Environmental Exposure

G. A. Hems and J. K. Fabri

In this graphite-furnace atomic-absorption spectrometric method for measuring manganese in whole blood, we use a pyrolytic platform to minimize interference by sample matrix. For optimal sample ashing we denature the sample within the furnace with nitric acid and use oxygen as the purge gas at low temperatures. The mean manganese concentration found in blood from 15 unexposed city dwellers was 215 (2 SD 135) nmol/L. By comparison, the range of manganese concentrations in blood sampled from a group of Australian aborigines living near a surface manganese ore deposit on Groote Eylandt, Northern Territory, was much higher (median 405 nmol/L, range 175 to 990 nmol/L).

Additional Keyphrases: graphite-furnace atomic-absorption spectrometry • reference values • environmental hazards • trace elements • toxicology • screening

Manganese is an essential trace element required for the maintenance of biological function in humans and other animals. Manganese neurotoxicity has been recognized after cases of exposure to dust derived from manganese ores. The symptoms of the neurotoxicity, which resemble Parkinson's disease, persist even when exposure is minimized and the body burden of manganese decreased. The nature of the neurological insult has not been determined, but possibly it is linked to the presence of neurotoxic dopamine oxidation products and hydrogen peroxide (1). A comprehensive review of manganese metabolism as related to occupational and environmental exposure has been published (2).

Several graphite-furnace atomic-absorption spectrometric (GFAAS) methods for manganese have been reported (3). Direct methods for blood samples are characterized by large nonatomic absorbances during atomization (4) or by accumulation of residue within the atomizer (5). One can correct for large nonatomic absorbance signals by using a Zeeman instrument, but this procedure fails to compensate for loss of manganese compounds that may be present in the gas phase during the atomization ramp. For GFAAS, nonatomic signals should be eliminated if possible, or at least minimized. Poor analysis precision owing to sample residue remaining after atomization must be addressed by modifying sample preparation and ashing. Wet digestion with various acids before sampling for furnace atomization is theoretically an answer, but this approach has associated contamination risks.

Hoening (6) recently reported the use of a combination of low-temperature oxygen ashing, nitric acid matrix modification, and furnace platform technique for the analysis for selenium in whole blood. Here we describe a modification of that technique for use in analysis for manganese.

Materials and Methods

Instrumentation

For the analysis we used a Model AA975 spectrometer equipped with a Model GTA95 graphite furnace with autosampler, all from Varian P.L., Mulgrave, Victoria, Australia. The solid pyrolytic graphite platforms were installed in pyrolytically coated graphite-furnace tubes.

In developing the analysis we used a remote computer running the Varian Tape Graphics program for the AA975. This enabled video display of both atomic and nonatomic (deuterium) signals during atomization. For routine analyses, atomic absorption signals corrected for background were displayed on the video screen of the GTA95.

Gases supplied to the furnace were medical-grade oxygen and "ultra high purity" argon (Commonwealth Industrial Gases, Surr Hills, N.S.W., Australia).

We stored polypropylene sampler cups (Varian no. 99-100282-00) in nitric acid before washing in metal-free water, drying, and using them as containers for the diluted blood samples.

Chemicals

A working manganese standard in dilute nitric acid (73.0 nmol of manganese per liter, in 7 g/kg HNO₃) was prepared by serially diluting a manganese atomic absorption standard solution (Sigma Chemical Co., St. Louis, MO).

Analytical-grade concentrated nitric acid (700 g/kg) was used for matrix modification and preparation of standards.

CLINICAL CHEMISTRY, Vol. 34, No. 6, 1988 1121
Water for analytical use (redistilled from a town water distillate and then de-ionized just before use) contained less than 2 nmol of manganese per liter. Sample diluent was prepared by adding one drop of Triton X-100 surfactant (BDH Chemicals Australia Pty. Ltd., Port Fairy, Victoria, Australia) to 40 mL of distilled de-ionized water. We checked the sample diluent for contamination before diluting any samples and used it only if no manganese absorption peaks were seen during atomization.

Polystyrene tubes containing lithium heparin (Mallinckrodt Pty. Ltd., Gladstone, N.S.W., Australia) were used to store the blood samples. These tubes did not add detectable manganese to normal whole blood.

Blood Collection

We collected 15 control blood samples from laboratory workers and 40 blood samples from the exposed population on Groote Eylandt, Northern Territory, Australia. Venous blood samples were collected with plastic syringes (Terumo Australia Pty. Ltd., Melbourne) fitted with 26-gauge stainless-steel hypodermic needles. The syringe-needle assembly was rinsed with the patient’s blood before we collected the sample to be used for manganese analysis.

Heparinized samples were stored at 4°C if the analysis was to be done within seven days; otherwise, they were frozen. Freezing and thawing blood samples several times did not affect the measured manganese, but it occasionally led to the formation of small clots, which caused sample-handling difficulties.

We diluted 100 μL of blood with 700 μL of sample diluent, using an air-displacement pipette (Finnpipette, Lab Supply, Marrickville, N.S.W., Australia) and performed the analysis within 60 min.

Analysis

The spectrometer was operated at 279.5 nm with a slit width of 0.2 nm. The lamp current was 5 mA. Table 1 lists the settings for the graphite furnace controller. The automatic pipette was programmed to deliver a total volume of 19 μL for each analysis (see Table 2).

The analysis was routinely calibrated with aqueous standards (range 0 to 700 nmol of manganese per liter of whole blood) after equivalence had been established between aqueous standard and dilute-blood-sample sensitivities. Occasionally, as the graphite furnace and platform aged, equivalence could not be shown. On these occasions the analysis was calibrated by the method of standard additions.

Diluted samples and standards were analyzed in duplicate, and the mean absorbance was used for calculation. Repeat analyses were made when the individual atomization readings deviated from the mean by >10%.

Results

Figure 1, which shows the atomic (hollow cathode minus deuterium) and the nonatomic deuterium signals during the atomization of a dilute blood sample, illustrates the low background intensity and the good separation of the atomization signals.

The coefficient of variation was 11.3% for analysis of a blood sample having a manganese concentration of 160 nmol/L. This precision estimate was based on 16 analytical runs on different days, with use of freshly prepared standards.

The atomic absorbance signal produced by this sample relative to the sample diluent was 0.04 peak-height unit. The absolute detection limit of the analysis (based on two standard deviations of the baseline noise) was 20 nmol/L. We considered the working detection limit to be 40 nmol/L.

---

**Table 1. Settings for Furnace Operation**

<table>
<thead>
<tr>
<th>Step no</th>
<th>Temp, °C</th>
<th>Time between steps, s</th>
<th>Gas flow, L/min</th>
<th>Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>150</td>
<td>5.0</td>
<td>3.0</td>
<td>Oxygen</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>15</td>
<td>3.0</td>
<td>Oxygen</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>30</td>
<td>3.0</td>
<td>Oxygen</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>20</td>
<td>3.0</td>
<td>Oxygen</td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>2.0</td>
<td>3.0</td>
<td>Argon</td>
</tr>
<tr>
<td>6</td>
<td>400</td>
<td>10</td>
<td>3.0</td>
<td>Argon</td>
</tr>
<tr>
<td>7</td>
<td>800</td>
<td>10</td>
<td>3.0</td>
<td>Argon</td>
</tr>
<tr>
<td>8</td>
<td>800</td>
<td>15</td>
<td>3.0</td>
<td>Argon</td>
</tr>
<tr>
<td>9</td>
<td>800</td>
<td>2.0</td>
<td>3.0</td>
<td>Argon</td>
</tr>
<tr>
<td>10</td>
<td>2600</td>
<td>1.0</td>
<td>0</td>
<td>Argon*</td>
</tr>
<tr>
<td>11</td>
<td>2600</td>
<td>1.0</td>
<td>0</td>
<td>Argon*</td>
</tr>
<tr>
<td>12</td>
<td>2600</td>
<td>8</td>
<td>0</td>
<td>Argon*</td>
</tr>
<tr>
<td>13</td>
<td>40</td>
<td>5.1</td>
<td>3.0</td>
<td>Argon</td>
</tr>
<tr>
<td>14</td>
<td>40</td>
<td>5.0</td>
<td>3.0</td>
<td>Argon</td>
</tr>
</tbody>
</table>

* Absorbance readings were taken over steps 10–12, inclusive. The injection temp was 150 °C.

---

**Table 2. Settings for Automatic Pipettor Operation**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Blank vol*</th>
<th>Modifier vol*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Std 1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Std 2</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Std 3</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Sample</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

* Standard manganese solution 73 nmol/L, see text.
  * Blank solution; nitric acid 700 g/L.
  * Modifier solution; de-ionized water containing a trace of Triton X-100.
The mean value for the laboratory workers was 215 (2 SD 135) nmol/L. The mean blood manganese concentration of the samples collected from the exposed population on Groote Eylandt was 490 nmol/L, and there was considerable asymmetry in the distribution of these data (range 175–990 nmol/L, median 405 nmol/L).

Discussion

This analysis has advantages over previously described techniques. The samples were analyzed after simply diluting them with water containing a trace of Triton X-100. Use of other reagents was minimized to minimize contamination risks. Low-temperature ashing in oxygen, combined with nitric acid matrix modification and use of the platform technique, resulted in an analysis characterized by low background signals. A particular advantage of the platform technique was the equivalence in analysis sensitivity observed for aqueous and dilute blood samples.

We find that routine analytical runs leave no significant residue on the platform if diluted blood is atomized alternately with sample diluent or aqueous standard. This approach allows continuous monitoring of baseline stability and analysis sensitivity in addition to assisting in cleaning the platform.

Reference intervals for blood manganese have been published (4, 7, 8) and are of the same order as found for this study's controls.

The increased blood manganese found in the Groote Eylandt aborigines is interesting. Groote Eylandt is characterized by the presence of a manganese ore body, which outcrops in the area surrounding the aboriginal settlement. Neutron activation analysis of soil and vegetation in the area show markedly elevated manganese concentrations (9).

In addition to the generally increased manganese concentrations in blood from the aboriginal population, there is also a subset of people with very high blood manganese concentrations, approaching 1000 nmol/L. There appears to be an association between very high concentrations of manganese in blood, low serum iron, and the occurrence of a group of neurological disorders that have a prevalence of about 2% (10, 11). The association has been reproducibly demonstrated, but at present it is not known whether these findings indicate a form of manganism affecting the community as a result of exposure to manganese in the environment.

The consistency of the increased values for blood manganese in the aboriginal community over a period of months indicates continued and uniform exposure to the metal. The group of aborigines showing symptoms of neurotoxicity and having very high values for blood manganese may have unusual or defective manganese regulatory processes.

We plan to apply the present analysis to detection of individuals at risk of accumulating a dangerous body burden of manganese and possibly suffering neurological insult.

We thank Professor J. Cawte (The Prince Henry Hospital, Sydney, N.S.W.) for inviting our contribution to his investigation of the Groote Eylandt condition, and Dr. C. Kilburn, on Groote Eylandt, for collecting the samples.

References