Dry- and Wet-Ashing Techniques Compared in Analyses for Zinc, Copper, Manganese, and Iron in Hair

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Preparation of hair specimens for trace-metal analyses is routinely done by wet- or dry-ashing. Wet-ashing is more time consuming than dry-ashing and can be dangerous. We wished to determine if dry-ashing was a suitable alternative to wet-ashing with HClO₄:HNO₃ or HNO₃ alone in preparing hair for measurement of zinc, copper, iron, and manganese by atomic absorption spectroscopy. Concentrations of Zn, Cu, and Mn were not differently affected in hair that was dry- or wet-ashed. Analytical recovery of these elements added to hair samples ranged from 102 to 108%; day-to-day CVs were <5%. Fe was lost during dry-ashing of hair, and wet-ashing with HNO₃ produced results for Fe comparable with those obtained with HClO₄:HNO₃. Therefore we recommend dry-ashing of hair to be analyzed for Zn, Cu, and Mn, but wet-ashing with HNO₃ for assays of Fe.

Additional Keyphrases: trace elements • atomic absorption spectroscopy • sample preparation • variation • source of neural tube defect

Dry- and wet-ashing have been compared as digestion techniques in the analyses of tissues from various sources, including fish and plants (1–4). Dry (5–8) and wet (9–11) digestion are routinely used in preparing hair for analyses of trace elements by various techniques. To our knowledge, no study as yet has compared the effects of wet- and dry-ashing on analyses for zinc, copper, manganese, and iron in hair. This is an important consideration in preparing hair for trace-metal analysis, given previous comparisons of wet- and dry-ashing of different tissues that indicated that results may be tissue dependent (4). Therefore we wished to assess whether dry-ashing is as suitable as wet-ashing for preparing hair samples for determinations of zinc, copper, manganese, and iron by atomic absorption spectroscopy.

Materials and Methods

Apparatus

The entire spectrometric system was from Perkin-Elmer Corp., Norwalk, CT 06856. For flame analyses we used the Model 2380 atomic absorption spectrophotometer with deuterium background correction. Flameless analyses were performed with the graphite furnace (Model HGA 300). Atomization signals were recorded from the digital readout. The light sources were zinc, copper, manganese, and iron hollow-cathode lamps. For the graphite furnace we used pyrolytically coated tubes (no. 0290-1821).

Reagents and Materials

De-ionized water with a specific resistance >18 MΩ/cm was obtained from the Barnstead purification system (Barnstead Co., Boston, MA 02132). We washed the hair samples with a 10 mL/L solution of a non-ionic detergent ("Acutionox"; American Scientific Products, IL 60085) in de-ionized water. For dry-ashing we used "Suprapur"-grade HNO₃ (E. Merck, Damstadt, F.R.G.); for wet-ashing we used reagent-grade HNO₃ and HClO₄ (Fisher Scientific Co., Fair Lawn, NJ 07410).

To avoid contamination, we soaked all glassware in dilute (50 mL/L) HNO₃ for two days, and then rinsed with de-ionized water. For all injections into the graphite furnace we used trace-element-free disposable polypropylene pipette tips (Fisher Scientific Co., no. 22 34 190-1).

Standards. Zn, Cu, Fe, and Mn standards were prepared by diluting 1 g/L stock solutions (Fisher Scientific Co.) with de-ionized water. Working standards at two concentrations for both Zn and Cu were 1 and 4 μg/mL, for Fe 0.5 and 5 μg/mL, and for Mn 5 and 15 ng/mL.

Procedures

Spectrophotometer setting. For flame procedures, we used the glass impact bead in the assays of Zn, Cu, and Fe. The Zn hollow-cathode lamp current was 15 mA; wavelength, 213.9 nm; slit width, 0.7 nm. The Cu hollow-cathode lamp current was 15 mA; wavelength, 324.8 nm; slit width, 0.7 nm. The Fe hollow-cathode lamp (multi-element lamp) current was 30 mA; wavelength, 248.3 nm; slit width, 0.2 nm. For all flame analyses the integration time was 2 s; results were calculated from the average of three readings. Flameless analyses for Mn were performed with deuterium arc background correction; the Mn hollow-cathode lamp current was 15 mA, wavelength, 279.5 nm; slit width, 0.2 nm.

Graphite furnace program. The basic settings used for the graphite furnace were: Dry: 130 °C, ramp 20 s, hold 60 s; Char: 1070 °C, range 30 s, hold 30 s; Atomize: 2400 °C, ramp 0 s, hold 5 s; Clean: 2700 °C, ramp 1 s, hold 2 s. The argon gas flow was 300 mL/min during the entire procedure except for 4 s during atomization, when it was 0 mL/min.

Collection of hair samples. For the control study, we collected hair from various areas of the head of one of us (J. K. F.), cut it with stainless-steel scissors, and stored it in polyethylene tubes. The entire fibers (not segments) were included in the samples.

Using standardized procedures (12), we also collected hair samples from 20 women who had recently given birth to infants with neural tube defects or to normal, healthy full-term infants. For both groups of women, hair was cut above the nape of the neck, as close to the scalp as possible. Only the 1–2 cm of hair closest to the scalp was retained for analysis.

Preparation of hair samples. We placed the hair sample for the control study in a 500-mL acid-washed Erlenmeyer flask and added 200 mL of the Acutionox solution. The flask was covered with Parafilm and agitated at room temperature for 60 min with a mechanical shaker. We then filtered...
the hair through an acid-washed Büchner funnel connected to a vacuum aspirator and rinsed the sample several times with a total volume of 2 L of de-ionized water before covering it and leaving it to dry overnight at 55 °C in a drying oven. Fourteen portions of this control sample of hair were transferred to acid-washed 15-mL porcelain crucibles, and 28 portions were placed in acid-washed 250-mL Pyrex beakers. All samples weighed between 100 and 200 mg.

Dry-ashing. Ten of the control hair samples were ashed in a muffle furnace (Fisher "Isotemp" muffle furnace, Model 184) for 12 h at 450 °C, after oven-drying at 250 °C for 2 h. After they had cooled for 1 h, we removed the samples from the furnace and added five drops of "Suprapur" HNO₃. One hour later, we returned the samples to the muffle furnace, where they were heated at 250 °C for 2 h, then ashed at 450 °C for an additional 8 h (11). We again let the samples cool for 1 h, then added 1 mL of Suprapur HNO₃, in 4 mL of water, to each beaker. After 30 min, we decanted the samples into 25-mL volumetric flasks, washed the beakers with de-ionized water, and used the combined washings to bring the samples to volume.

Wet-ashing. We placed 10 control samples of hair in 250-mL wide-mouthed Pyrex beakers, and added 10 mL of reagent-grade HNO₃ to each beaker and let these sit overnight. Ten other control samples of hair were placed in similar beakers and left to digest in 5 mL of HNO₃ overnight. The following morning we added 10 mL of HClO₄:HNO₃ (1:4 by vol) (4) to the latter 10 beakers. All samples were covered with watch glasses and refluxed at 120–140 °C for about 4 h until the digests were clear and 2–3 mL of acid remained. After cooling for 1 h we decanted the digested samples into 25-mL volumetric flasks and brought to volume with added rinsings of de-ionized water.

Assessing accuracy and precision. Because no certified hair standard is currently available, we used U.S. National Bureau of Standards certified oyster tissue (Standard Reference Material no. 1566) containing known amounts of Zn, Cu, Fe, and Mn to assess the accuracy of the methods. For analytical-recovery experiments, we added a known amount of each metal to four portions of control hair for each of the dry-ashing, HNO₃-ashing, and HClO₄:HNO₃-ashing procedures. We also added known amounts of each metal to pure solutions, which were analyzed without any intervening ashing steps.

Measuring metals in hair samples. For assay of Zn, Cu, and Fe, we aspirated wet- and dry-digested samples directly into the flame. For Mn determinations, we injected samples manually into the graphite tube with an Eppendorf pipette. Sample size was 20 μL, and each sample was analyzed in triplicate. We repeated the assays of 10 samples on different days to assess between-run variation. We also repeated the assay of one sample 10 times during the same day to assess within-run variation.

Statistical analysis. Differences between hair concentrations in each group were assessed by one-way analysis of variance (13).

Results

Table 1. Concentrations of Zinc, Copper, Manganese, and Iron in Control Hair Treated by Dry- and Wet-Ashings

<table>
<thead>
<tr>
<th></th>
<th>Zn</th>
<th>Cu</th>
<th>Mn</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>216 (4)</td>
<td>44 (1)</td>
<td>0.74 (0.06)</td>
<td>27 (9)*</td>
</tr>
<tr>
<td>HNO₃</td>
<td>216 (28)</td>
<td>42 (5)</td>
<td>0.82 (0.11)</td>
<td>38 (6)*</td>
</tr>
<tr>
<td>HNO₃:HClO₄</td>
<td>227 (31)</td>
<td>44 (6)</td>
<td>0.77 (0.12)</td>
<td>41 (12)*</td>
</tr>
</tbody>
</table>

* Values with different superscripts are significantly different from each other at p < 0.05. n = 10.

Table 2. Analytical Recovery of Zinc, Copper, Manganese, and Iron Added to Four Samples of Hair

<table>
<thead>
<tr>
<th></th>
<th>Zn</th>
<th>Cu</th>
<th>Mn</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>102 (7)</td>
<td>100 (13)</td>
<td>100 (5)</td>
<td>110 (9)</td>
</tr>
<tr>
<td>HNO₃</td>
<td>102 (2)</td>
<td>97 (3)</td>
<td>103 (1)</td>
<td>114 (14)</td>
</tr>
<tr>
<td>HNO₃:HClO₄</td>
<td>102 (3)</td>
<td>103 (3)</td>
<td>120 (10)</td>
<td>100 (11)</td>
</tr>
</tbody>
</table>

* Amount (μg) added to 100 ± 10 mg of control hair samples: Zn, 20; Cu, 4; Mn, 0.2; Fe, 2.

Discussion

Hair is analyzed to evaluate both toxicological and nutritional status in the human population (12, 14–16). Many different washing procedures (11) and analytical techniques—including atomic absorption spectroscopy (6, 6), neutron activation analysis (12, 13), x-ray fluorescence (17), and PIXE (18)—have been used in hair analyses. Neutron activation analysis requires only simple preparation (12), but relatively few laboratories have access to irradiation facilities. PIXE and x-ray fluorescence require isotopic analytical equipment, also not commonly available in a clinical laboratory. Atomic absorption spectroscopy offers a convenient method for analysis of metals in biological tissues and is one of the most commonly used techniques (19). Preparation of samples for atomic absorption is often done with perchloric acid, which can be dangerous. We were interested

Table 3. Zinc, Copper, Manganese, and Iron Measured in Four Samples of NBS Oyster Tissue (SRM no. 1566), and Certified Values

<table>
<thead>
<tr>
<th></th>
<th>Zn</th>
<th>Cu</th>
<th>Mn</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certified value</td>
<td>852 (14)</td>
<td>63 (3.5)</td>
<td>17.5 (1.2)</td>
<td>195 (34)</td>
</tr>
<tr>
<td>Dry</td>
<td>859 (1)</td>
<td>65 (0.4)</td>
<td>18 (0.8)</td>
<td>202 (7)</td>
</tr>
<tr>
<td>HNO₃</td>
<td>864 (2)</td>
<td>72 (3)</td>
<td>20 (1.6)</td>
<td>192 (4)</td>
</tr>
<tr>
<td>HNO₃:HClO₄</td>
<td>835 (100)</td>
<td>58 (8)</td>
<td>15.9 (0.8)</td>
<td>218 (9)</td>
</tr>
</tbody>
</table>
in determining if dry ashing of hair samples provides a satisfactory alternative to wet-ashing with either HClO₄:HNO₃ or HNO₃ alone.

Zinc. Concentrations of Zn in hair treated by three different ashing procedures did not differ (Table 1), suggesting that dry ashing is a suitable preparation for analysis for Zn in hair. Volatilization losses during ashing have not been reported at the ashing temperature we used, but some Zn may be retained on crucible walls (20). We did not find any losses of Zn after dry-ashing, because we evaporated all samples with HNO₃, a method known to improve recovery (4). Recovery of added Zn (102 ± 7%, Table 2) and accuracy of oyster tissue analysis (Table 3) supports the suitability of the dry-ashing treatment prior to analysis of Zn in hair.

Copper. Concentrations of Cu in control hair samples did not differ among the three treatments (Table 1). Recovery (108 ± 13%) and accuracy of Cu determinations after dry-ashing were comparable to those results obtained by wet-digestion (Tables 2 and 3). Contamination with Cu from porcelain crucibles has been reported previously (11) and may explain the slightly higher analytical recovery for dry-ashed samples than was found for wet-ashed samples. Cu is not volatile at the ashing temperature we used (20); however, there have been reports (4) of variable and increased losses of Cu as the temperature of dry-ashing increases above 400 °C. Dry-ashing of hair samples is a reliable treatment in preparation for Cu analysis.

Manganese. Concentrations of Mn in hair treated by dry-ashing did not differ significantly from those results obtained after wet-ashing (Table 1). Mn volatilization during dry ashing is unlikely below 550–600 °C, although some retention of Mn on crucible walls has been reported (21). Recovery of Mn is improved with HNO₃ (22), and the recovery from porcelain crucibles during dry-ashing in this experiment was acceptable (108 ± 5%, Table 2). Recovery of Mn after wet-digestion by HNO₃:HCIO₄ was high (120 ± 10%, Table 2). This may be attributed to pipetting error or contamination, which is a risk because of the very low concentrations of Mn in hair as compared with those of other metals (Table 4). We do not think that the slightly lower values for Mn obtained during dry-ashing are ascribable to either volatilization or retention of manganese on crucible walls. Other workers (4) have used ashing aids for manganese analyses in tissues, but this extra step would not be useful, considering the low concentrations of manganese in hair and the correspondingly increased probability of contamination. Recently, Guillard et al. (23) reported on manganese analysis in hair after wet-digestion in Teflon bombs. The recovery within-run CV (4% vs 8%) and recovery (104 ± 9.2% vs 108 ± 5%) of Mn were lower than ours. The slightly lower findings (23) may not justify the expense of the mini-autoclave (current cost of the Perkin-Elmer no. 074846, $140.00 U.S.) in comparison with the cost of porcelain or silica crucibles ($6.00 U.S., Fisher Scientific Co.).

Iron. Measured concentrations of Fe in hair were significantly lower in samples treated by dry-ashing as compared with wet-ashing (Table 2). Fe concentrations of oyster tissue analyzed after preparation by the dry-ashing technique (Table 3) were within the expected range (202 ± 7 μg/g vs 195 ± 34 μg/g, certified value). Matrix differences may have accounted for lower measured Fe concentration in hair as compared with the measured Fe concentration in oyster tissue. Some Fe may volatilize during dry-ashing, but previous researchers have reported no Fe losses at 500 or 600 °C (24). Retention losses for Fe have also been reported during dry-ashing (27), although our analytical recovery by the dry-ashing technique (110 ± 9%) was comparable to that of the HNO₃ digestion method (114 ± 14%) and higher than the recovery by the HClO₄:HNO₃ digestion technique (100 ± 11%, Table 2). Recovery of Fe after dry-ashing was higher than that found for Zn, Cu, or Mn. Previous researchers have also reported higher recovery for Fe as compared with that found for Zn and Cu in liver (19) and hair (5). Dry-ashing may not be suitable prior to determining Fe in hair. Dry-ashing for Fe has previously been recommended only for large and (or) fatty samples (20). We found that treatment with HNO₃ gave results comparable with those obtained by HClO₄:HNO₃ digestion (Table 1). Treatment with HNO₃ has previously been reported to be suitable for Fe analysis in hair (25). It should be noted that, if temperatures exceed 150 °C during wet-ashing, some Fe may be lost (4).

Watting and Wordale (4) reported that concentrations of metals in fish tissue after wet or dry-ashing were comparable. They suggested that contamination during wet oxidation may result from the addition of reagents to samples, the contamination frequently exceeding the amounts in the sample itself. We used Suprapur-grade HNO₃ only during dry-ashing, because each sample required only 1 mL Suprapur HClO₄ is not available and the large amount of acid required for wet digestion (10 mL per sample) precludes the use of Suprapur HNO₃ because of the expense. Therefore blank values obtained with reagent-grade acid during wet-ashing were higher than Suprapur blank values obtained during dry-ashing. Blank values from dry-ashing of 18 blanks, calculated in micrograms per gram of dry hair, were low for Zn (0.04), Cu (0.04), and Mn (0.02), but were high for Fe (0.85)—about 5–8% of the usual Fe concentration in hair.

A further difficulty during wet oxidation is the preparation of acid-matched standards, because the amount of acid that is evaporated differs for each sample.

We suggest that dry-ashing is a suitable treatment for hair before analyses for Zn, Cu, and Mn. Wet-digestion with HNO₃ rather than HClO₄:HNO₃ is acceptable before the analysis of Fe in hair. Zn, Cu, and Mn concentrations in hair analysed after dry-ashing are presented in Table 4 for mothers who gave birth to infants with neural tube defect and mothers of normal healthy children. The results suggest a difference in trace-metal status of these two groups. A larger study of these two groups is currently in progress.

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Table 4. Concentrations of Zinc, Copper, and Manganese in the Hair of Mothers of Infants with Neural Tube Defect or Normal Healthy Children

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<thead>
<tr>
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<th>Mean (SD), μg/g dry wt</th>
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<tbody>
<tr>
<td></td>
<td>Zn</td>
</tr>
<tr>
<td>Neural tube defect (n = 10)</td>
<td>177 (26)</td>
</tr>
<tr>
<td>Normal (n = 10)</td>
<td>193 (30)</td>
</tr>
</tbody>
</table>

References


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