Urinary Oxalate by Rate Analysis Compared with Gas-Chromatographic and Centrifugal Analyzer Methods

To the Editor:

We recently developed a method for measuring urinary oxalate by rate analysis with oxalate decarboxylase (ODC, EC 4.1.1.2; Sigma Chemical Co., St. Louis, MO) and formate dehydrogenase (FDH, EC 1.2.1.2; Boehringer Mannheim, Aust. Pty. Ltd.) in a Cobas Bio centrifugal analyzer. This method, which has been used successfully in our hospital laboratory for analyzing more than 150 patients’ specimens over the last nine months, is described below and its results are compared with those by gas chromatography (1) and by an end-point centrifugal analyzer method (2).

Collect 24-h urine into HCl (5 mol/L, 50 mL) and adjust a 1-mL aliquot to pH 3–5 with NaOH (0.1 mol/L), adding one drop of methyl red (0.2 g/L) pH indicator. Precipitate the urine oxalate with 14 mL of ethanol for 3 h or overnight and measure recovery with [U-14C]oxalate (1). Centrifuge (2000 rpm for 5 min) the precipitate, then dry (lyophilize, or use a rotary evaporator) and dissolve in 0.25 mL of H2SO4 (0.4 mol/L) and 0.75 mL of sodium citrate buffer (0.2 mol/L, pH 3.0). Incubate at 37°C for 60 min in Cobas Bio cups 0.1-mL aliquots of the urine extract and of potassium oxalate standards (0.5, 0.25, and 0.125 mmol/L) in the same citrate/H2SO4 buffer plus 20 μL of ODC solution (1 U/mL of sodium citrate buffer, 50 mmol/L, pH 4.5). Stop the ODC reaction by adding 50 μL of potassium phosphate buffer (2 mol/L, pH 7.80). Prepare freshly before use the enzyme reagent: 3 mg of FDH and 2 mg of NAD+ per milliliter of potassium phosphate buffer (0.5 mol/L, pH 7.8).

The Cobas Bio analyzer was run at the following settings: units, mmol/L; calculation factor, 0; standard 1, 0.125 mmol/L; standard 2, 0.25 mmol/L; standard 3, 0.5 mmol/L; limit, 0.5 mmol/L; temperature, 37°C; type of analysis, 4; wavelength, 340 nm (increasing absorbance reaction); sample volume, 25 μL; diluent volume, 65 μL; enzyme reagent volume, 250 μL; time of first reading, 15 s; time interval, 10 s; no. of readings, 6; blanking mode, 1.

We divided the oxalate result calculated by the analyzer for each urine by the percent recovery of oxalate to obtain the urinary concentration. In comparing the procedures we used the product moment correlation and Student’s paired t-test.

The within-day precision (CV) of the rate analysis for urine analyzed 15 times was 8.6% for an oxalate concentration of 0.23 mmol/L. The same urine supplemented with 0.2 mmol of oxalate per liter gave a CV of 7.0% and an analytical recovery of 0.21 (SD 0.02) mmol/L (105 ± 9.5%, mean ± SD). Analysis of these same urines 16 times over 30 days gave results (mean ± SD) of 0.21 ± 0.02 (CV 9.5%) and 0.40 ± 0.03 (CV 7.5%) mmol/L with an average analytical recovery of 95.0 ± 10.5% (0.19 ± 0.02 mmol/L, added oxalate).

To test for specificity, we treated the urine with ODC before precipitation (1). The result for a urine containing 0.33 mmol of oxalate per liter indicated a specificity >94%. The lower limit of detection obtained by serial twofold dilutions was 0.02 mmol/L.

The 95% reference range was 0.10 to 0.50 mmol/day and a mean of 0.23 mmol/day for 16 men and 12 women; there was no significant sex-related difference in oxalate excretion. This reference range compares favorably with that obtained by the more specific FDH/ODC (3) and gas-chromatographic methods (1). The mean value is lower than by the oxalate oxidase (4, 5), colorimetric (6), isotope dilution (7), and ion-chromatographic (8) methods.

Figure 1 shows a comparison of our method with the gas-chromatographic method (1) and the recently published end-point centrifugal analyzer method of Urdal (2). There was a significant (p <0.01) agreement between these methods: mean rate method 0.32 (SD 0.17) mmol/L, mean gas-chromatographic method 0.31 (SD 0.17) mmol/L (r = 0.91, n = 24); and mean rate method 0.29 (SD 0.11) mmol/L, mean Urdal’s method 0.23 (SD 0.11) mmol/L (r = 0.99, n = 10).

Our rate method is more complex and time consuming than the end-point method of Urdal (2), but has the advantages of (a) consuming only about half as much FDH, ODC, and NAD+ (b) taking less time on the Cobas Bio analyzer (one full rotor of 24 urines by our method takes about 5 min of analysis time vs about 40 min for 12 urines by Urdal’s method), and (c) being less error prone because one does not have to subtract one large value (oxalate + formate) from another (formate). In our method the blanks (sample plus reagent minus ODC) were consistently low (0.0011 SD 0.0022) ΔA/min, equivalent to <0.005 mmol/L (n = 24) and therefore could be ignored. Finally, this rate method involves only a single addition of reagent, whereas the end-point method requires addition of a second reagent. Our method therefore can be used in many centrifugal analyzers that lack the facility of second reagent addition. In fact, before purchase of the Cobas Bio, our initial analyses were done satisfactorily with a Centrifichem 400 (Union Carbide Pty., Ltd.) with the following settings: sample 25 μL; sample plus wash 90 μL; reagent 250 μL; time delay 3 s; ΔA/min measured every 15 s for four readings; abnormal absorbance 1.0; blank automatic; concentration factor 0; temperature 37°C; filter 340 nm. Oxalate concentrations were calculated manually from the average ΔA/min for standards and samples.

The most disturbing aspect of Urdal’s method is the high value reported for the upper limit of the reference range: 0.67 mmol/day. Most reputable methods have an upper limit of the reference range of about 0.5 mmol/day (1, 3–5). We feel therefore that it is important to elucidate why the upper limit of this reference range is so high.

We thank the John P. Kelly Mater Research Foundation for a grant supporting this project. The gas-chromatographic analyses were kindly performed by Ms. Beryl Mazzacli, Clinical Biochemistry Unit, Finders Medical Centre, Adelaide.
Astra Creatinine Reagent Stable

To the Editor:

In a Letter, Alan Posner (1) refers to the paper by Bromberg et al. (2), who state that the Beckman Astra creatinine reagent is stable for one week and can be used on the RA-1000, and he questions their statement on stability.

We use the Astra creatinine reagent on our RA-1000 and find that it is stable for at least a month. We used the same bottle of creatinine reagent from May 1 to May 31. The tabulation below shows our creatinine QC data for the month of May.

During the time this reagent was being used on the RA-1000, the creatinine was recalibrated three times. On May 8 the calibration factor was 54.11, on May 9 the calibration factor was 54.17, and on May 24 the calibration factor was 54.11.

The fact that our QC’s showed no shifts or drifts and the standard deviation remained relatively constant, along with the data showing that the concentration factor was not drifting verifies that the Astra creatinine reagent is stable for at least a month.

References


John W. Koenig

Barnes Hospital
St. Louis, MO 63110

<table>
<thead>
<tr>
<th></th>
<th>Low QC</th>
<th></th>
<th>High QC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>CV, %</td>
</tr>
<tr>
<td>Week 1</td>
<td>13</td>
<td>0.58</td>
<td>0.06</td>
<td>10.38</td>
</tr>
<tr>
<td>Week 2</td>
<td>24</td>
<td>0.58</td>
<td>0.04</td>
<td>7.16</td>
</tr>
<tr>
<td>Week 3</td>
<td>10</td>
<td>0.59</td>
<td>0.03</td>
<td>5.36</td>
</tr>
<tr>
<td>Week 4</td>
<td>13</td>
<td>0.59</td>
<td>0.05</td>
<td>8.33</td>
</tr>
<tr>
<td>Week 5</td>
<td>15</td>
<td>0.60</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>