
Four Methods for Determining Uric Acid Compared with a Candidate Reference Method

Ronald J. Elin, Ernestine Johnson, and Ruth Chesler

Uric acid as measured in serum by three different uricase (EC 1.7.3.3) methods (aca, Ektachem, and SMAC) and by the SMAC method with phosphotungstic acid was compared with a candidate Reference Method for uric acid. Serum specimens from 83 patients (uric acid concentrations, 19 to 141 mg/L) were analyzed by all five methods. Results were compared by using linear regression analysis, and the mean difference between results by the candidate Reference Method and the four other methods was calculated. Compared with the candidate Reference Method, the aca method gave the smallest deviation from zero for the intercept and the smallest mean difference, and the SMAC phosphotungstic acid method showed a slope closest to unity. The SMAC uricase method had the largest intercept and greatest deviation of the slope from unity.

Additional Keyphrases: enzymic methods · discrete analysis · continuous-flow analysis · multilayer film analysis

Two widely accepted methods are used to determine uric acid in serum. In the older method uric acid reduces colorless phosphotungstic acid (PTA)\(^1\) to tungsten blue, which usually is measured by its absorbance at 700 nm. The newer method involves enzymic conversion of uric acid and oxygen to allantoin and hydrogen peroxide by the enzyme uricase (urate oxidase, EC 1.7.3.3; urate:oxygen oxidoreductase). Several uricase methods are available, differing in their indicator systems. Because common biological reducing substances such as glutathione, ascorbic acid, and several antibiotics cause a positive interference in the PTA determination of uric acid, it is generally accepted that there is a constant bias between the two methods, with the uricase result being 1 to 10 mg/L lower than the PTA result (1–3). The initial uric acid method available for the Sequential Multiple Analyzer Computerized (SMAC; Technicon Instruments Corp., Tarrytown, NY 10591) was the PTA method. Recently, Technicon introduced a uricase method for determination of uric acid with the SMAC. Our laboratory also can determine uric acid concentration with the Automatic Clinical Analyzer (aca; Du Pont Instruments, Wilmington, DE 19898) and the Ektachem (Eastman Kodak Co., Rochester, NY 14650), in both of which a uricase method is used. To select the optimum method for the SMAC and to determine comparability among the four methods mentioned, we compared results by all four methods with those by a candidate Reference Method—a uricase method involving equilibrium kinetics and measurement of ultraviolet absorption (4, 5).

Materials and Methods

Samples. Blood specimens from patients in the Clinical Center, submitted to the Clinical Chemistry Service for uric acid measurement, are determined with the SMAC PTA method. We selected specimens for this study by reviewing the uric acid results so obtained, selecting specimens that contained 3 mL or more of serum and that were distributed uniformly over the range of concentration for uric acid. Thus, we tried to obtain an equal number of specimens below the lower limit of the reference interval, within the reference interval, and above the reference interval. The serum specimens, stored at \(-20^\circ C\), were thawed the same day that the analyses were performed by the five methods. A human serum pool obtained from normal volunteers and stored at \(-70^\circ C\) was also assayed each day of the study by the five methods.

Apparatus. The spectrophotometer used with the candidate Reference Method was equipped with a 37 °C temperature control and ultraviolet source (Model 25; Beckman Instruments, Inc., Carlsbad, CA 92008). The SMAC, aca, and Ektachem were calibrated and used according to the manufacturers’ instructions.

Candidate Reference Method. The candidate Reference Method was used as described (4, 5). Based on the ultraviolet uricase equilibrium method of Remp (6), this method requires both a test and a blank: Incubate 0.5 mL of specimen with 2.5

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\(^1\) Nonstandard abbreviations: PTA, phosphotungstic acid; TCA, trichloroacetic acid; NBS, U.S. National Bureau of Standards; and SRM, Standard Reference Material.

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mL of Tris (0.1 mol/L, pH 8.5) and 1.0 mL of a 0.1 kU/L solution of microbial uricase for 60 min at 37 °C, add 2.0 mL of a 100 g/L solution of trichloroacetic acid (TCA), centrifuge (1200 x g), and measure the absorbance of the supernate at 283 nm.

We used the following chemicals and reagents with this method:

Tris(hydroxymethyl)aminomethane (Tris), SRM 922; Tris hydrochloride, SRM 923; uric acid, SRM 913; and lithium carbonate, SRM 924, obtained from the National Bureau of Standards (NBS); uricase from Candida utilis (lot no. M003543) from Beckman Instruments, Inc., Microbes Operations, Carlsbad, CA 92008; TCA from J. T. Baker Chemical Co., Phillipsburg, NJ 08865; and crystallized bovine plasma albumin from Reheis Chemical Co., Div. of Armour Pharmaceutical Co., Carlsbad, CA 92028.

We calibrated the instrument with materials supplied by the manufacturer (calibrator 1, Kodak no. 0517001; calibrator 2, no. 0518002; and calibrator 3, no. 0505003).

SMAC method. For the SMAC, we used two different methods. In the older method, protein is separated from serum by a dialysis membrane, and uric acid in the dialysate reduces a phosphotungstate complex to a phosphotungstic acid, the absorbance of which is determined at 660 nm. In the SMAC uricase method, uric acid is converted to allantoin and hydrogen peroxide. The latter is determined by oxidative coupling of 3-methyl-2-benzothiazolinone hydrazide hydrogen chloride and anthranilic acid in the presence of peroxidase, forming a colored complex (indamine dye) that absorbs at 570 nm. We assumed a carryover factor of 5% with each method. Technicon Instruments Corp. provided us with a tape that enabled us to use both methods simultaneously on one instrument. We calibrated the SMAC with Technicon Reference I (lot no. B9A043) and Reference II (lot no. B9A016). The uric acid set points for Reference I were 76 mg/L for the PTA method, 72 mg/L for the uricase method.

Data analysis. To compare the results by the candidate Reference Method (absissa) with those by the other four methods (ordinate), we used linear regression. Debiascd linear regression (7) was used to compare the two SMAC methods. We also calculated the mean difference between results by the candidate Reference Method and by the four other methods. For all the above analyses, we used the Statistical Analysis System (SAS Users' Guide, SAS Institute Inc., Raleigh, NC, 1979, pp 391–396).

Results

Table 1 shows the results of this study. The deviation from zero for the intercept and mean difference were smallest when aca results were compared with the candidate Reference Method. The SMAC phosphotungstate results showed the slope closest to unity but the largest mean difference. The SMAC uricase results showed the largest deviation of the slope from unity and the largest intercept among the four methods compared with the candidate Reference Method. The aca method had the smallest standard deviation of the regression (S_YX) and the SMAC PTA method the largest. Results (mg/L) with the control materials showed all assays (n = 6 each) to be in control: Reference Method, 52 ± 1.3; aca, 56 ± 0.8; Ektachem, 49 ± 1.2; SMAC-UA, 53 ± 0.8; SMAC-PTA, 54 ± 2.4 (means ± SD).

The two methods for determination of uric acid with SMAC were inter-compared with conventional linear regression and debiased linear regression (Table 2). A similar comparison was done by Technicon Instruments Corp., and the data appear in the SMAC uric acid method (no. SG4-0054F9J) (8). The regression equations for our studies and those by Technicon are in good agreement.

Discussion

We found good agreement for two of the three uric acid methods when compared with the candidate Reference Method. The method used in the aca involves the same indicator system as the candidate Reference Method, with which it agreed well. The Ektachem uric acid method involves a different indicator system.

The two uric acid methods used in the SMAC showed the largest mean difference from the candidate Reference Method. The SMAC phosphotungstate method had the slope closest to unity and thus the smallest proportional bias. However, this method showed an intercept of 3.88 mg/L, indicating a systematic bias. Musser and Ortigosa (9) reported a mean of 3 mg/L for non-urate chromogens in pooled sera studied by the phosphotungstate method. The results of this study are consistent with this systematic bias for the SMAC phosphotungstate method.

The SMAC uricase method has a systematic (intercept of 4.07 mg/L) and a proportional (slope of 0.964 or 3.6%) bias. This regression line crosses the 45° line at a uric acid concentration of 113 mg/L. Thus, uric acid concentrations below this value would tend to be falsely high and above this value, falsely depressed.

A comparison of the two SMAC uric acid methods in this

Table 1. Four Methods for Determination of Uric Acid Compared with a Candidate Reference Method

<table>
<thead>
<tr>
<th>Method</th>
<th>Slope</th>
<th>Intercept</th>
<th>S_YX</th>
<th>Mean difference, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>aca</td>
<td>0.990</td>
<td>1.00</td>
<td>2.98</td>
<td>0.42</td>
</tr>
<tr>
<td>Ektachem</td>
<td>0.991</td>
<td>-1.23</td>
<td>3.10</td>
<td>-1.75</td>
</tr>
<tr>
<td>SMAC-uricase</td>
<td>0.964</td>
<td>4.07</td>
<td>4.11</td>
<td>2.02</td>
</tr>
<tr>
<td>SMAC-PTA</td>
<td>0.996</td>
<td>3.88</td>
<td>5.82</td>
<td>3.68</td>
</tr>
</tbody>
</table>

Table 2. SMAC Phosphotungstate and Uricase Methods Compared for Determination of Uric Acid

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Slope</th>
<th>Intercept</th>
<th>S_YX</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technicon</td>
<td>597</td>
<td>0.951</td>
<td>3</td>
<td>2.0</td>
<td>0.996</td>
</tr>
<tr>
<td>NIH</td>
<td>83</td>
<td>0.953</td>
<td>1</td>
<td>2.7</td>
<td>0.995</td>
</tr>
<tr>
<td>NIH, de-biased</td>
<td>83</td>
<td>0.964</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The phosphotungstate method is the independent variable (absissa).
study and one by Technicon showed similar results. In the Technicon study, the regression line crosses the 45° line at a uric acid concentration of 61 mg/L (Table 2), which is within the reference interval. Thus, the SMAC uricase value will exceed the phosphotungstate value when the uric acid concentration is <61 mg/L. The results from our study show a similar pattern but the regression line crosses the 45° line at a uric acid concentration of 21 mg/L. A previous study of the SMAC PTA method for determination of uric acid showed a positive bias when compared with a uricase continuous-flow method (10). In this study paired uric acid analyses were done on 1185 unselected specimens. The difference between the means of values obtained by each of the two methods was 4.6 mg/L, the higher value being obtained with the SMAC PTA method. The equation of the regression line was $y = 6.2 + 0.967x$, where $y$ was the SMAC result and $x$ the uricase result. Here the SMAC PTA method is plotted on the ordinate, while in our study it is plotted on the abscissa. The regression line crosses the 45° line at a uric acid concentration of 187 mg/L. Thus, the results for this study show that the PTA method gave higher results than the uric acid method over the entire physiologic range. As was mentioned previously, there is a documented positive systematic bias for the PTA method compared with the uricase method for uric acid of approximately 3 mg/L (9). Because our study and that of Technicon show that the SMAC uricase method produces higher results than the PTA method at concentrations within the reference interval and below, we conclude that the SMAC uricase method needs additional study to resolve this discrepancy.

References
4. Duncan, P. H., Gochman, N., Cooper, T., et al., Development and evaluation of a candidate Reference Method for uric acid in serum. U.S. Dept. of HEW, Public Health Service, 1980. (Copies may be obtained from the Clinical Chemistry Division, Centers for Disease Control, Bldg. 17, Rm. 302, Atlanta, GA 30333.)

Determination of Fluoxetine and Norfluoxetine in Plasma by Gas Chromatography with Electron-Capture Detection

J. F. Nash, R. J. Bopp, R. H. Carmichael, K. Z. Farid, and L. Lemberger

This gas-chromatographic method for assay of fluoxetine and norfluoxetine in human plasma involves extraction of the drugs and use of a 63Ni electron-capture detector. The linear range of detection is 25 to 800 µg/L for each drug. Overall precision (CV) in the concentration range of 10 to 100 µg/L for both drugs was approximately 10%. Accuracy (relative error) in the same concentration range was approximately +10%. None of the commonly prescribed antidepressants or tranquilizers that we tested interfere with the assay.

Additional Keyphrases: drug assay · antidepressants

Fluoxetine hydrochloride, or dl-N-methyl-3-phenyl-3-[α,α,α-trifluoro-p-tolyloxy]propylamine hydrochloride, a specific neuronal inhibitor of serotonin reuptake, is currently undergoing clinical trials as an antidepressant drug (1). After oral dosing, the drug is well absorbed and rapidly metabolized to its desmethyl metabolite, norfluoxetine, which has selectivity similar to and pharmacological activity equivalent to fluoxetine (2). There is a therapeutic effect when 60 mg of fluoxetine hydrochloride is administered daily as a single dose. With this regimen, steady-state concentrations in plasma range from 103 to 282 µg of fluoxetine and 47 to 181 µg of norfluoxetine per liter. Lemberger et al. (1) previously reported minimal anticholinergic or other side effects at a dose as large as 90 mg.

We describe a sensitive, specific, and precise procedure for determining fluoxetine and norfluoxetine in plasma, in which we use a gas chromatograph with an electron-capture detector.

Materials and Methods

Instrumentation. We used a gas chromatograph equipped with a 63Ni electron-capture detector (Model 5710A; Hewlett-Packard, Avondale, PA 19311) and an automatic sample injector (Hewlett-Packard, Model 7671A). The siliconized looped glass column, 1.2 m by 3 mm (i.d.), was packed with 3% SP 2100 on Supelcoport, 80/100 mesh (Supelco, Inc., Bellefonte, PA 16823).