Improved Rapid Assay of Uric Acid in Serum by Liquid Chromatography

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This improved method for rapid determination of uric acid in serum is based on high-performance liquid-gel-permeation chromatography, with hydrophilic and highly porous vinyl alcohol copolymer as packing material. It has the following advantages: no need for sample deproteinization or use of a precolumn, more than 500 serum samples can be analyzed without having to regenerate or recondition the analytical apparatus, and the analysis for uric acid is a one-step procedure. Correlation coefficients between this method and other methods are very good ($r = 0.998, 0.999$).

Uric acid in serum is widely determined enzymatically with uricase-peroxidase. Unfortunately, numerous substances such as ascorbic acid or bilirubin interfere strongly in the method (1-4). Recent improvements to high-performance liquid chromatographic (HPLC) methods avoid such interferences by using different packing materials: an anion-exchange resin (5) or a reversed-phase (6-9). However, neither of these methods is really convenient, because serum samples must be pretreated with a deproteinizing agent.

To improve and simplify the determination of uric acid in sera, we have recently developed a new HPLC gel-permeation technique, with a hydrophilic porous polymer gel as a packing material. Here we report our findings.

Materials and Methods

Reagents

The uric acid Standard Reference Material was from U.S. National Bureau of Standards (SRM 913). Lithium carbonate, special grade, was from Wako Chemicals Inc., Tokyo, Japan. All other reagents were from Merck Japan Ltd., Tokyo, Japan. The mobile-phase solution, phosphate buffer (50 mmol/L, pH 7.0) is filtered through a 0.45-μm pore-size filter (Millipore Corp., Bedford, MA) before use.

To prepare the stock standard solution of uric acid (1 g/L, 5.95 mmol/L), dissolve 500 mg of uric acid and 375 mg of lithium carbonate in 500 mL of warm distilled water. Keep at room temperature when the solution cools.

Prepare the working solution by diluting 1 mL of the stock standard solution fivefold with distilled water. To make a calibration curve, further dilute the standard with the mobile-phase solution to give the following final concentrations: 200, 100, 75, 50, 25, 10, and 5 mg/L (1190-29.75 μmol/L).

Apparatus

The sample injector (Model 7125; Reodyne Inc., Cotati, CA) with syringe, is equipped with a rotary valve. The volume of the sample was controlled at 20 μL with a loop system.

For the enzymatic determination, we used an automated analyzer (Model 105 auto system; Hitachi Co., Ltd., Tokyo, Japan) and the uricase-catalase method (10).

We used a newly developed column (10 cm × 7.6 mm, i.d., Asahipak GS-320; Asahi Chemical Co., Ltd., Tokyo, Japan) containing hydrophilic polymer gel, a rigid and highly porous vinyl alcohol copolymer (particle size 9 ± 0.5 μm). We also used a pre-column filter (pore size 2.0 μm; Gasukuro-kogyo Inc., Tokyo, Japan). To measure absorbance at 290 nm for determination of uric acid, we used a Model 655A ultraviolet detector (Hitachi; chart speed, 5 mm/min) and an SIC Chromatocorder 11 integrator (System Corporation, Tokyo, Japan).

Chromatographic Procedure

Dilute 20 μL of each standard solution or serum sample with 400 μL of mobile-phase solution and inject 20 μL of this directly into the chromatograph. The retention time of uric acid on the column is ~5.8 to 6 min under the following chromatographic conditions: flow rate 0.8 mL/min, measurement wavelength 290 nm, and sensitivity 0.04 A full-scale.

Using a calibration curve obtained with the series of uric acid standards, calculate the concentration of uric acid in the samples, by the peak-area technique.

No pretreatment of the column, such as washing or reconditioning, is necessary before injecting the next sample.

Results

Figure 1 shows chromatograms of a standard solution and a pooled serum. Under the described conditions uric acid has a retention time of 5.8 min, which is the same as that for the standard solution or free uric acid. Treatment of the pooled serum with uricase (EC 1.7.3.3) eliminated this peak.

Analytical Variables

Sensitivity. The limit of detection for the assay was <2.5 mg/L. The signal/noise ratio was >5 at 2.5 mg of uric acid per liter.

Precision. Repeated analysis of pooled serum specimens at three different concentrations—19.2, 30.8, and 78.3 mg/L—gave within-day CVs ranging from 0.3% to 1.3% (n = 20). Day-to-day CVs for concentrations of 8.4, 32.4, and 82.4 mg/L ranged from 1.1% to 3.7% (n = 5).

Analytical recovery. Analytical recoveries for uric acid ranged from 99% to 100.3% for pooled serum to which extra amounts of uric acid (10, 20, 30, 40, 70, and 100 mg/L) were added; the pooled serum contained 14.6 mg of uric acid per liter originally.

Standard curves and linearity. Standard curves exhibited a correlation coefficient of 0.999 for peak area (y) vs uric acid concentration (x): $y = 1.197x - 0.19$. By this new assay method the concentration of uric acid is linearly measurable up to 200 mg/L.

Interference. We further studied analytical interference in this assay by using aqueous solutions and samples of serum containing ascorbic acid or bilirubin, which are known to interfere strongly in the enzymatic method (1-4). These substances did not interfere in this method because of their complete separation from uric acid (Figure 2).

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Discussion

Clearly, this newly improved method is much simpler and more rapid than any other HPLC-based method being used. Instead of requiring only a single dilution of serum, previous methods require time-consuming and (or) discontinuous steps for the pretreatment of serum before use, i.e., either centrifugation (6, 7, 11) or a precolumn (12). Beside those disadvantages, the method involving anion-exchange HPLC requires regeneration and conditioning steps after running a set of samples or after each day's run. The present method obviates these troublesome procedures, resulting in the ability to determine uric acid in serum at the rate of 10 samples per hour, 500 samples per column. The internal standard addition is not needed, because this method is simple and yields good analytical results. Furthermore, the separation column is easily regenerated by washing with 6 mol/L urea after 500 serum samples have been run.

We consider this improved method the most suitable method for uric acid in serum because of its great rapidity, simplicity, and accuracy.

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References