Effect of Tetrasodium EDTA on Enzymatic Determinations of Urinary Oxalate

Jorgen Thode, Cheryl Amyx, Roland Valdes, Jr., and Gerald Kessler

We studied the effects of pretreating urine samples with tetrasodium EDTA (TEDTA) before measuring urinary oxalate with an enzymatic kit (Sigma). Mean analytical recovery of added oxalic acid was only 49% (SD ± 13%) when the assay was performed as recommended by the manufacturer, but treating samples with TEDTA improved recoveries (96 ± 10%). In 20 unselected 24-h urine samples assayed with and without TEDTA treatment, the mean oxalate concentrations were significantly (P < 0.001) different: 15.6 ± 8.7 and 12.2 ± 7.9 mg/L, respectively. TEDTA-treated urine samples stored for 14 days at −20 °C lost 20% of their oxalate concentration. Use of TEDTA simplifies sample preparation by eliminating the alkalinating step needed to dissolve EDTA or disodium EDTA.

Additional Keyphrases: sample preparation · calculous disease · oxalate oxidase

Measurement of urinary oxalate is becoming a standard procedure for evaluating renal stone formation (1, 2), but reliable measurement of oxalate in most clinical laboratories is difficult and subject to large errors (3–5). Recently, enzymatic methods have been developed for this assay (6–9). In response to increased requests for the measurement of this analyte, we evaluated the enzymatic procedure from Sigma (10). This assay is based on the oxidation of oxalate, catalyzed by the enzyme oxalate oxidase (EC 1.2.3.4), and quantification of the product, hydrogen peroxide. During our evaluation we observed low analytical recoveries of oxalate, consistent with results of other investigators who were using enzymatic methods for urine oxalate including the Sigma procedure (6–8). They corrected the problem of low recoveries by first alkalining the urine to permit solubilization of EDTA, which presumably acts to chelate oxalate-precipitating cations, before oxalate is measured (7, 9). The Sigma procedure (10) does not include an EDTA sample-preparation step. Here we demonstrate that low recoveries can also be corrected by treating urine with tetrasodium ethylenediaminetetraacetate (TEDTA). In addition, this approach simplifies the total analysis procedure by eliminating the need for a separate step to alkaline the urine specimen. We also measured values for TEDTA-treated 24-h urine specimens and evaluated the effect of long-term sample storage.

Materials and Methods

Materials. We measured urinary oxalate with the Sigma Oxalate kit (cat. no. 590-D; Sigma Diagnostics, St. Louis, MO 63178). Oxalate is first separated from interfering substances by adsorption onto aluminum hydroxide, then eluted with sodium hydroxide. The elute is carried through the enzymatic reaction steps, producing a colored indamine dye product, the absorbance of which is measured at 590 nm.

We used a Stasar II spectrophotometer (Gilford Instruments Labs., Oberlin, OH 44074). An oxalate standard is provided in the kit as a lyophilized urine preparation. The oxalic acid we used for the recovery experiments conformed to ACS specifications and was obtained from Sigma.

Specimen handling. Oxalate was determined in 24-h urine collections selected without conscious bias from those received during seven days from 20 different patients. Each specimen was well mixed and aliquoted into two 10-mL plastic tubes for measurement of oxalate. All aliquots were acidified to pH 3 or less with 10 mL of concentrated HCl per liter. To one of each pair of aliquots we added TEDTA (8 g/L), leaving the other portion untreated. After this addition, the pH was checked and adjusted to pH 3 or less if needed. We then measured oxalate in both aliquots on the day of collection. The samples were then stored at −20 °C and reassayed 14 days later.

Statistical analysis. The difference between means was determined with the Student's t-test. The correlation was determined by a least-squares analysis calculated from the least squares equation y = ax + b, substituting the definition of b = y − ax (where b is the intercept, and y and x are the means).

Results

Linearity and precision. We investigated the linearity of the standard curve by preparing three different concentrations of the lyophilized urine standard by adding various amounts of de-ionized water to give oxalate concentrations of 26, 52, and 104 mg/L. The standard curve was linear over the range investigated (0–104 mg/L). Precision was determined by using high (37 mg/L) and low (11 mg/L) urine oxalate controls provided in the kit. The within-run CVs for the high (37.3 ± 2.4 mg/L, mean ± SD, n = 10) and low (11.5 ± 0.5 mg/L, n = 10) controls were 6.5 and 4.6%, respectively.

Recovery with and without TEDTA pretreatment. Recovery of oxalate in samples not treated with TEDTA was determined for three different urines after addition of 10, 25, 50, and 100 mg of oxalic acid per liter. As listed in Table 1, low recoveries, ranging from 30% to 80% (mean 49.1%), were obtained. To 10 untreated urine specimens treated with TEDTA (8 g/L) we added 25 or 50 mg of oxalic acid per liter. On assay, the mean recovery was 96% (range 80–108%) (Table 1).

Urine sample comparisons with and without TEDTA pretreatment. Having noted improved recovery of oxalate by treatment with TEDTA, we investigated its effect on measured urinary oxalate before and after long-term sample storage. The relationship between urine oxalate concentration with and without TEDTA treatment in 20 unselected 24-h urine specimens is shown in Figure 1A. We noted a mean decrease of 21.8% for urine oxalate concentrations in samples without TEDTA pretreatment. The linear regression parameters are y = 12.2 = 0.853 (x − 15.6) and the correlation between the two measurements is significant (r = 0.94, P < 0.001) with a residual standard error, Sxy, of 2.74 mg/L.

The relationship between results for samples with TEDTA...
pretreatment measured within 24 h and then reassyayed after 14 days of storage at \(-20^\circ\text{C}\) is shown in Figure 1B. The mean urinary oxalate concentration decreased from 15.6 to 12.5 mg/L (18.7\%) during storage. The correlation between the two values measured was significant (r = 0.77, P < 0.001) with a residual variance of \(S_{r} = 4.56 \text{mg/L}\) and a regression of \(y = 12.5 - 0.618 (x - 15.6)\). In urine samples without TEDTA pretreatment the mean urinary oxalate concentration decreased by 7\% (from 12.2 to 11.4 mg/L) during the 14-day storage. The correlation between these two groups was significant (r = 0.95, P < 0.01) with a residual variance of \(S_{r} = 2.2 \text{mg/L}\) (not illustrated).

**Discussion**

This study confirms the observation that analytical recoveries of oxalate (mean 49.1\%) for acidified urine samples as measured with an enzymatic assay are low (10). Other studies have shown that treatment of urine samples with EDTA or disodium EDTA improves the recovery of oxalate (6-8). However, the use of these two forms of EDTA required alkalinization of the urine specimen, to dissolve EDTA or disodium EDTA. We demonstrate improved recoveries (mean of 96\%) when acidified specimens are treated with TEDTA without further specimen manipulation. We also observed increased values for patients' samples (mean increase of 20\%) as compared with untreated specimens. The mechanism leading to improved recoveries is not known, but addition of a strong chelating agent would preferentially bind cations, leaving oxalate available for reaction with the oxalate oxidase. We observed a disparity between improved analytical recovery of added oxalic acid (49\% nontreated, 96\% TEDTA-treated) and increased values (22\%) for TEDTA-treated vs nontreated urine specimens. The cause of the disparity is unknown. One explanation for the low analyti-
Automated Analysis for Taurine in Biological Fluids and Tissues

H. O. Goodman and Z. K. Shihabi

We have developed an automated method of analysis for taurine, based on incorporating an ion-exchange chromatography column into the continuous-flow AutoAnalyzer (Technicon). After removal of proteins and peptides by dialysis, taurine is selectively eluted from an ion-exchange column and reacted with o-phthaldialdehyde to yield a fluorescent compound. The advantages of this method are: full automation with no need for sample deproteinization or cleanup; sensitivity, detecting as little as 5 μmol/L; speed (20 samples per hour); and flexibility. It can be used for assaying taurine in urine, plasma, cerebrospinal fluid, and tissue homogenates. This method can be adapted for assays of other metabolites.

Additional Keyphrases: fluorometry · amino acids · chromatography · ion-exchange · continuous-flow analysis · urine · cerebrospinal fluid · platelets

The amino acid taurine is present in high concentration in most animal tissues, especially in excitable tissues such as brain and heart. Because it is not incorporated into proteins, however, its role is not clear, having been thought to act as a neurotransmitter, modulator, or both (1). Taurine also has a hypoglycemic effect (2) and protects against toxic materials in the cell (3). Deficiency of this amino acid causes retinal degeneration in the newborn kitten (4). In humans the mean output of taurine in urine is decreased in epilepsy (5) and in Down's syndrome (6) but increased in Friedreich's ataxia (7). Its concentration in cerebrospinal fluid is increased in bacterial meningitis (8).

Assay of this amino acid with an automated amino acid analyzer takes several hours and requires sample preparation, such as deproteinization and loading into another precolumn. More rapid assays of taurine have recently been developed with "high-performance" liquid chromatography (8), but this requires sample preparation.

Here we describe an automated method for the assay of taurine, in which we use a continuous-flow analyzer with ion-exchange chromatography.

Materials and Methods

The manifold. The instrument is constructed from AutoAnalyzer components (Technicon Co., Tarrytown, NY), with sampler II, and pump II. For detection we use a Gilson fluorometer (Gilson Medical Electronics, Middleton, WI), with o-phthaldialdehyde (OPA) filters and a recorder.

The manifold is diagrammed in Figure 1. Samples are placed in cups in the sampler. From the cups they are aspirated, diluted with acetic acid, segmented with air bubbles, and dialyzed (dialyzer 250-mm long for plasma and cerebrospinal fluid, 75 mm for urine), as in the traditional AutoAnalyzer, to remove proteins and peptides that might interfere with the taurine peak and also to further dilute the sample. However, in addition, we have incorporated into the manifold a 40 × 4.6 mm (i.d.) column packed with a mixture of two volumes of cation-exchanger AG 50W, ×8; 200–400 mesh, H+ form, and one volume of anion-exchanger Dowex 1, 8 ×, 200–400 mesh, Cl− (Bio-Rad, Richmond, CA) (Figures 1, 2). The column binds all the amino acids except taurine. We keep the column wet at all times and free from air bubbles.

The sampler is fitted with a cam, which enables the probe to dip for 26 s in the sample cup and 154 s in the wash (Figure 2). Thus the speed of the instrument is 20 samples per hour.

Reagents. We used acetic acid, 17 mmol/L, containing 200 μL of Brij-35 surfactant per liter. The water in the water line also contained 200 μL of Brij-35 per liter. To prepare the OPA reagent, dissolve 120 mg of OPA (Sigma Chemicals, St. Louis, MO) in 50 mL of ethanol, add 950 mL of sodium borate buffer (17 mmol/L, pH 9.0) and 600 μL of mercaptoethanolamine. This reagent is stable at room tem-

---

Debieve, Red Red (0.8) 100% air Line

Orange White (0.2) 100% air Line

ACETIC ACID

Water

Acetic acid

Air

Black Black (0.2)

Yellow (1.2)

Yellow (1.2)

Yellow (1.2)

Cell

WASTE

Dialyzer

Fig. 1. Schematics of AutoAnalyzer manifold for assay of taurine