Simultaneous Determination of Oxalate, Glycolate, Citrate, and Sulfate from Dried Urine Filter Paper Spots in a Pediatric Population, Nenad Blau,1* Ana Matasovic,1 Anna Lukasiewicz-Weliechowicz,1 Claus W. Heizmann,1 and Ernst Leumann2 (Divisions of 1Clinical Chemistry and Biochemistry and 2Nephrology, University Children’s Hospital, Steinwiesstrasse 75, 8032 Zürich, Switzerland; * address for correspondence: University Children’s Hospital, Division of Clinical Chemistry and Biochemistry, Steinwiesstrasse 75, CH-8032 Zürich, Switzerland; fax 411 266 7169, e-mail blau@kispi.unizh.ch)

Measurement of oxalate in urine is important for the diagnosis of primary hyperoxaluria (McKusick 259900) and the secondary forms produced by excessive intake or abnormal intestinal absorption of oxalate (1). Determination of glycolic acid is essential for the diagnosis of primary hyperoxaluria type 1. Finally, to estimate the risk of stone formation in calcium oxalate urolithiasis and nephrocalcinosis, simultaneous determination not only of calcium but also of citrate (a potent inhibitor of calcium oxalate and calcium phosphate crystallization) and other constituents (electrolytes, phosphate and sulfate) is required to calculate urinary calcium saturation (2).

Ion-chromatography HPLC (3, 4) and specific enzymatic assays (5, 6) are available only in specialized laboratories. In addition, preservation and storage of liquid samples may influence the stability of oxalate and glycolate (7). Use of urinary filter spots is a practical alternative for the collection and safe transport of samples to be analyzed for many metabolic disorders.

To evaluate the age-related changes of oxalate, glycolate, citrate, and sulfate in a pediatric population, we developed an automated ion-chromatography system for the simultaneous measurement of these anions in urine of a pediatric patient with primary hyperoxaluria type 1. Concentrations of urinary oxalate on the standard mixture, nondiseased urine, and urine from a patient with primary hyperoxaluria type 1 and other hyperoxalurias. Stability of oxalate and glycolate is one of the most important factors and may be affected by ascorbate, pH, and time of storage. We investigated the recovery of liquid samples compared with the dried urine on filter paper stored for 1 week and 2 weeks at room temperature in two different urine pools (controls and patient with primary hyperoxaluria type 1). Concentrations of urinary oxalate in the filter spot were higher (P < 0.005) when stored for 1 week and almost identical after 2 weeks compared with values in the control liquid sample measured immediately after collection (Table 1). No significant differences were found between the liquid sample, the dried spot after 1 week, and the dried spot after 2 weeks for glycolate, citrate, and sulfate. In the sample from a patient with the primary hyperoxaluria type 1, values for oxalate and glycolate were much higher than the controls measured in the liquid sample, the dried spot after 1 week, and the dried spot after 2 weeks (Table 1). The values of oxalate were unaffected by storage, whereas glycolate decreased after 1 week (P < 0.001) and 2 weeks (P < 0.001). Collection of urine on filter paper was more critical for glycolate than for oxalate when present in higher concentrations. The values for citrate were similar to those in the control urine and were only slightly lower after 2 weeks (P < 0.05; Table 2).
1). Sulfate was higher in pathological urine than in controls, and collection on filter paper produced lower values than in liquid urine ($P < 0.005$). There was no difference in values between filter papers stored at room temperature or at 4 °C (data not shown).

Recovery experiments were performed with control urine. One aliquot with the four ions added was spotted on filter paper, stored for 1 week at room temperature, and analyzed in triplicate. Mean recoveries were 98% ± 8.1% for oxalate, 87% ± 12.3% for glycolate, 83% ± 2.3%
for citrate, and 96% ± 7.0% for sulfate. These findings suggest that urine dried on filter paper can be stored for more than 1 week without diagnostically significant changes in concentrations of oxalate, glycolate, citrate, and sulfate.

The molar ratios of urinary oxalate, glycolate, and citrate vs creatinine were found to be age-dependent. This is in agreement with data published previously by other methods (8–11). The ratios were highest in infants, particularly below the age of 2 years (Table 1). For oxalate and citrate, the highest values were observed in infants below 6 months of age. In children over 2 years of age, values for oxalate, glycolate, citrate, and sulfate decreased. The Friedman test showed an age-dependent distribution of values for all four analytes.

In conclusion, our improved method allows the simultaneous determination of oxalate, glycolate, citrate, and sulfate in liquid urine and urine filter paper spots, enabling differential diagnosis of oxaluria. Urine filter papers can be stored up to 1 week without diagnostically significant changes in concentrations of the above compounds. The greatest advantage of this new method is that the samples can be mailed in envelopes, speeding delivery and reducing shipping costs.

References