

# Determination of Oxalate in Urine by Ion Chromatography

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## **INTRODUCTION**

Urinary oxalate was successfully determined by ion chromatography with suppressed conductivity detection. Ion chromatography using an IonPac® AS4A anion chromatographic column is a method recognized by the U.S. Pharmacopoeia (USP) for determining oxalate in calcium gluconate.<sup>1</sup> This method can be performed in less than 10 minutes. Since the analysis is performed at pH 9.2, the decomposition of urinary ascorbate to oxalate is minimized. The analysis is selective, rapid, sensitive, and requires no sample pretreatment other than dilution.

The measurement of oxalate in urine is important in the diagnosis of hereditary and enteric hyperoxaluria and in the evaluation and management of patients with urinary calculi ("stones"). Calculi are deposited chemicals in compact form, and these concretions are frequently found in the urinary tract.

Seven factors may contribute to the formation of urinary calculi: 1) metabolic disturbances, such as cystinuria and gout; 2) endocrinopathies, such as hyperparathyroidism; 3) urinary obstruction; 4) infections; 5) mucosal metaplasia, which occurs in vitamin A deficiency; 6) extrinsic conditions, such as dehydration, dietary excess, drug excess, or chemotherapy; and 7) isohydruria, which refers to the loss of the normal acid-alkaline tides ("fixations of pH"). The presence of two or more of these conditions is usually associated with calculi formation, and, among the seven listed factors, isohydruria is found most often.<sup>2</sup>

The composition of a calculus depends to some degree on its location. Adult bladder stones frequently are composed of uric acid, whereas kidney stones generally

contain calcium oxalate, apatite, and phosphate. Apatite is a mineral composed of complex calcium phosphate that sometimes contains carbonate. Calcium is the chief cation found in calculi and is generally associated with oxalate, phosphate, or carbonate. Apatite occurs frequently with calcium oxalate and uric acid in acidic urine, and with magnesium ammonium phosphate in alkaline urine, which suggests that apatite may precipitate over the entire physiological urinary pH range.<sup>2</sup> Calcium oxalate is the chief constituent of most urinary calculi.

Calcium oxalate is relatively insoluble in urine. Increases in urinary oxalate concentration can result in the formation of calculi. The understanding of calcium oxalate calculi formation has been hampered by the lack of an adequate analytical method. This application note presents a specific, sensitive, rapid, and reproducible method for determining oxalate levels in urine.

## **EQUIPMENT**

Any Dionex chromatograph consisting of:

High-performance pump

Conductivity detector

AI-450 Chromatography Workstation or computing  
integrator

## **REAGENTS AND STANDARDS**

Sodium Tetraborate, decahydrate (ACS Grade)

Boric Acid (ACS Grade)

Oxalic Acid, dihydrate (ACS Grade)

## CONDITIONS

Columns: IonPac AG4A-SC (4 mm) guard & IonPac AS4A-SC (4 mm) analytical

Eluent: 22 mM Sodium tetraborate/  
22 mM boric acid

Flow Rate: 2.0 mL/min

Injection Volume: 50  $\mu$ L

Suppressor: ASRS™ Anion Self-Regenerating Suppressor, power level 3

Detection: Suppressed conductivity, AutoSuppression™ Mode

## PREPARATION OF SOLUTIONS AND REAGENTS

### 22 mM Sodium Tetraborate / 22 mM Boric Acid:

Prepare eluent in a 1.0-liter volumetric flask. Fill flask half full with deionized water and dissolve 8.39 g of sodium tetraborate, decahydrate, by swirling flask in an ultrasonic bath. Add 1.36 g of boric acid to the flask and dissolve. Add deionized water to a total volume of 1.0 liter and vacuum filter the eluent through a 0.45- $\mu$ m filter.

### 1000 mg/L Oxalate Standard:

To prepare 1.0 liter of 1000 mg/L oxalate, add 1.43 g of oxalic acid, dihydrate to a 1.0-liter volumetric flask. Dissolve oxalic acid in 500 mL of deionized water and add deionized water to a final volume of 1.0 liter.

## SAMPLE PREPARATION

In most cases, the only sample preparation required is dilution and filtration of the urine sample. The maximum concentration of sulfate that can be injected without interfering with oxalate is approximately 300 mg/L. Generally, a 1:10 dilution of urine in deionized water is appropriate. Filter the diluted sample through a 0.45- $\mu$ m or smaller filter before injection.

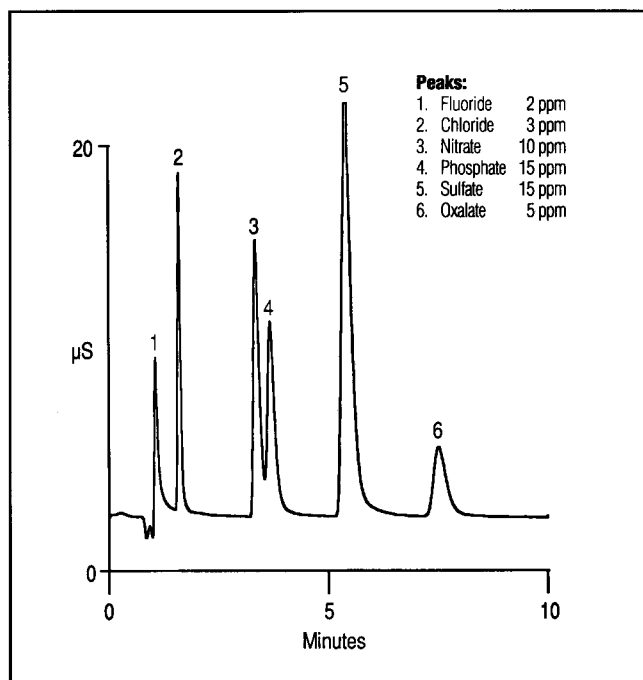


Figure 1 Common ions and oxalate

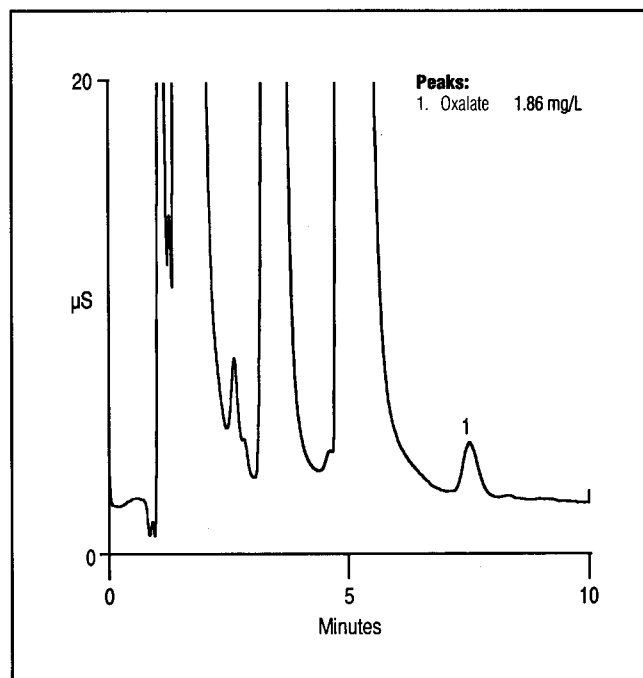


Figure 2 Oxalate in diluted urine

## RESULTS AND DISCUSSION

The analysis of oxalate in urine by ion chromatography is straightforward. Oxalate is rapidly determined and well resolved from sulfate and other inorganic constituents in urine. The use of a borate-buffered eluent at pH 9.2 minimizes decomposition of urinary ascorbate to oxalate, which can occur at higher pH levels.<sup>3</sup> A chromatogram of oxalate and moderate levels of other inorganic anions is shown in Figure 1.

The average concentration of oxalate in urine is 20 to 40 mg/L and of sulfate 1700 to 2700 mg/L.<sup>4</sup> The method described here applies to sulfate:oxalate ratios of 500:1, making the method suitable for the analysis of oxalate in urine. The maximum concentration of sulfate that should be injected is 300 mg/L, which requires about a 10-fold dilution of urine prior to analysis. The detection limit for oxalate using the method described is 0.2 mg/L. Assuming the use of a 1:10 dilution, this corresponds to a detection limit of 2.0 mg/L oxalate in a urine matrix. The method is linear ( $r^2=0.9996$ ) for oxalate from the detection limit (0.2 mg/L) to approximately 25 mg/L.

Figure 2 shows the analysis of a sample of commercially available lyophilized male human urine. The sample was diluted 10-fold with deionized water and filtered prior to analysis. The concentration of oxalate in the urine was  $18.9 \pm 0.4$  mg/L ( $\pm 1.8\%$ ).

## PRECAUTIONS


Low level oxalate calibration standards should be prepared on a daily basis to prevent errors arising from oxalate decomposition. Stock solutions of 1000 mg/L oxalate are stable for several days. Samples should also be analyzed as soon as possible.

Some urine samples may contain organic material capable of fouling ion exchange columns; therefore, the use of a guard column is strongly recommended for this analysis. Generally, fouling of the ion exchange resin is evidenced by a gradual decrease in retention times of the analytes. If fouling occurs, the IonPac AG4A-SC can be cleaned with a combination of solvent and salt. Refer to the IonPac AG4A-SC column manual for a detailed description of the column clean-up procedure.

## REFERENCES

1. United States Pharmacopoeia XXII and National Formulary XVII, Supplement 2, U.S. Pharmacopoeial Convention: Rockville, Maryland, 1990.
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