

Application Note

► Determination of Citrate and Oxalate in Urine with IC



Category	Clinical
Matrix	Urine
Method	IC
Keywords	Ion Chromatography (IC), organic anions, Citrate, Oxalate, Urine
Analytes	Citrate, Oxalate
ID	VCL1, December 2007

Summary

A simple and fast analysis of Oxalate and Citrate in the presence of sulfate ions can be carried out applying anion exchange chromatography.

Introduction

In the last few years, Ion Chromatography (IC) has achieved a high level of acceptance. One of the most important applications of IC in the clinical area is the analysis of organic and anorganic ions in physiological fluids, such as Urine, Plasma, and Serum. On average, 3 g of assorted acids including Oxalic Acid, Citric Acid, and free Amino Acids are excreted in the Urine daily. Inborn metabolic diseases such as primary hyperoxaluria and inflammatory intestinal illnesses such as Morbus Crohn's disease are associated with an increase in the Oxalate concentration in Urine and thereby promoted buildup of Calcium Oxalate stones. An increase in the Oxalate concentration in Urine can be an indicator of future urinary stones. Citrate, on the other hand, acts as an important inhibitor of Calcium Oxalate and Calcium Phosphate crystal formation. This is the reason why alkaline Citrates are so often effective in current therapies. The chief mechanism of action in the treatment of urinary stones is to raise the Urine pH value and to produce a concomitant increase in renal Citrate excretion. The Citrate concentration in Urine is due not only to that amount which is produced in the body but also to that which is contributed by certain foods. High consumption of animal proteins as well as a calcium deficiency can lead to decreased Citrate excretion. A decreased excretion of Citrate (< 2.5 mmol in 24 h) is detectable in 50% of patients with Calcium Oxalate stones. In order to understand and supervise a treatment for the overdevelopment of Calcium Oxalate containing urinary stones, a specific and sensitive method for the determination of Citrate and Oxalate is necessary.

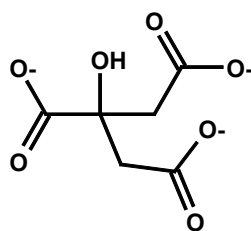
Experimental Sample Preparation

The samples were diluted 1:20 with distilled water and subsequently filtered through a 0.45 µm membrane. In addition, the sulfate can be precipitated out of solution during the sample preparation by the addition of BaCl₂. The insoluble BaSO₄ precipitate produced is easily removed by centrifugation. Since the stability of the sample solution is limited, the measurement should take place within 24 h of sample preparation. In order to prevent irreversible adsorption of the urine sample to the anion exchange column, the use of a precolumn is essential.

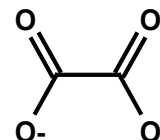
Experimental Preparation of Standard Solution

The calibration solutions for Citrate and Oxalate were prepared in a concentration range between 0.025 mmol/L and 2 mmol/L. The standard solutions for this calibration range must be prepared daily since the stability of the solutions is limited. The stock solutions are stable for several days and must be prepared fresh once a week.

Chemical Structures



Citrate



Oxalate

Method Parameters

Column	Anion exchange column A-2, 250 x 4 mm
Eluent	isocratic, 15 mmol Na ₂ CO ₃
Flow rate	1.0 ml/min
Injection volume	5 µl
Column temperature	column: 50 °C / detector cell: 45 °C
Detection	conductivity with suppression

Results

The analysis of Oxalate and Citrate can be carried out very accurately and reproducibly by means of ion chromatography. The retention of both acid anions can be significantly accelerated without overlapping the Oxalate peak with that of the ubiquitous sulfate peak in urine by adjusting the concentration of the buffer eluent (e.g. to 15 mmol Na₂CO₃). If the anorganic anions are also to be determined simultaneously, this is possible by lowering the buffer concentration. The retention times for both organic acid anions would be however thereby increased. The average concentration of Oxalate in Urine lies between 0.05 and 0.3 mmol/L. A higher concentration is an indicator for the potential formation of urinary stones. The maximum sulfate concentration which can be injected without problems is about 300 mg/L. Assuming an average sulfate concentration in Urine of 1700 to 2700 mg/L, a dilution in the range of 10 to 20 is suitable. In addition, the sulfate can be precipitated out of solution during the sample preparation by the addition of BaCl₂. The insoluble BaSO₄ precipitate produced is easily removed by centrifugation. The drawback is that chloride can no longer be determined once the sample has been so treated. In the case that a high sulfate concentration interferes with the Oxalate peaks, this sample preparation step presents itself as a practical alternative.

The detection limit for Oxalate under the given conditions is 0.006 mmol/L and 0.03 mmol/L for Citrate. Based on the 1:20 dilution, the detection limit in Urine for Oxalate is 0.125 mmol/L and 0.6 mmol/L for citrate. Since the injection volume was set at 5 µl, there is ample possibility for increasing the sensitivity if necessary. The donors who provided the urine samples were not known to have any disease, so an elevated Oxalate value was not expected from the Urine samples investigated. This was confirmed by the analyses carried out. The concentration range for Oxalate fluctuated between 0.1 mmol/L and 0.4 mmol/L. The Citrate content was markedly higher as expected. The values found were in the range of 0.9 mmol/L to 3.6 mmol/L. The reproducibility of this method was verified through 5 repetitive injections.

Fig. 1 (left)

Oxalate Calibration Curve

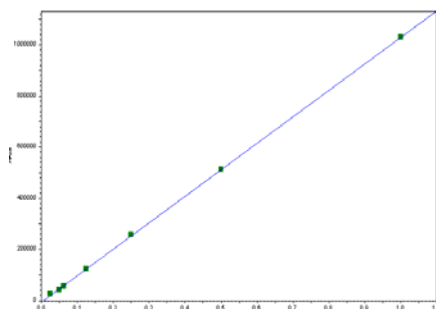
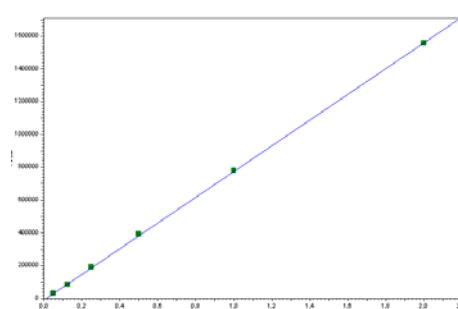


Fig. 2 (right)

Citrate Calibration Curve



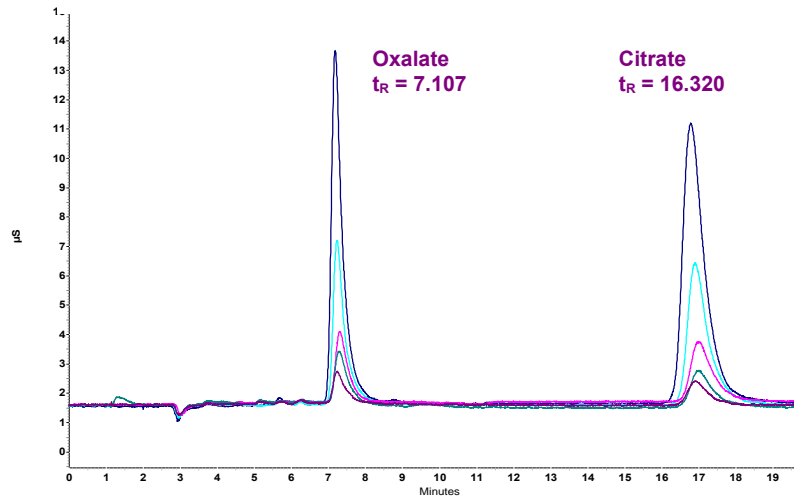


Fig. 3
Overlaid calibration standards (0.025 mmol/L – 2 mmol/L)

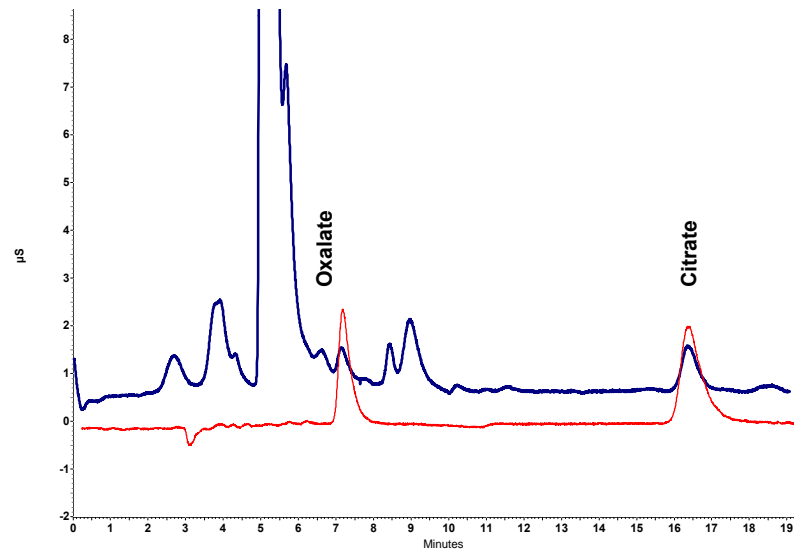


Fig. 4
Urine sample (blue) diluted 1:20, overlaid with standard

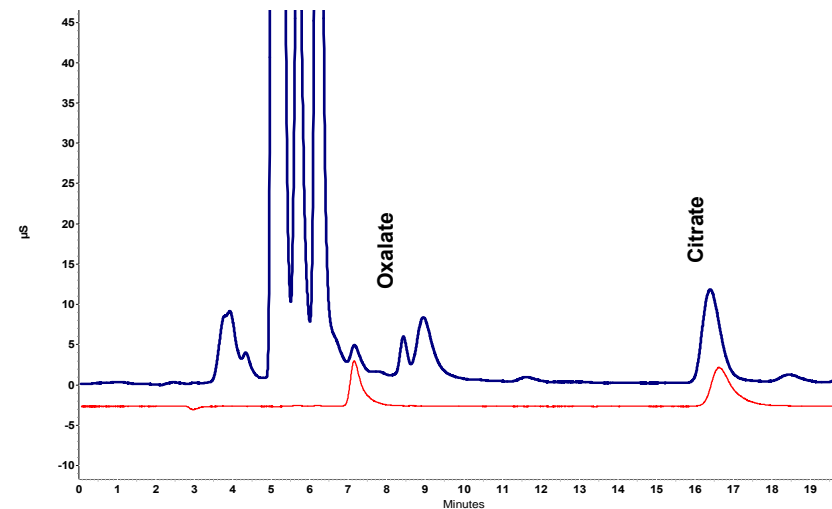


Fig. 5
Urine sample (blue) with sulfate precipitation

Method Performance

Limit of detection	ng range (S/N = 3)
Linearity (r²)	0.999923-0.999925
Linearity range	0.025 to 2 mmol/l
Retention time precision*	< 0.3 % RSD
Peak area precision*	< 2.8 % RSD

*repeatability calculated over 5 replicate runs

Conclusion

With a Smartline IC System from Knauer a simple and fast analysis of Oxalate and Citrate in physiological fluids in the presences of sulfate ions can be carried out. The sulfate precipitation can be helpful by analyzing undiluted urine samples.

References

- [1] N. Blau, A. Matasovic, A. Lukasiewicz-Wedlechowicz, C. W. Heizmann, and E. Leumann, *Clinical Chemistry* 44: 1554-1556, 1998
- [2] Bishop M, Freudinger H, Largiader U, Sallis JD, Felix R, Fleich H (1982) Conductivometric determination of urinary oxalate with oxalic decarboxylase. *Urol Res* 10:191

Physical Properties of recommended Column



The anion exchange column is based on polyvinyl alcohol with quarternary ammonium groups. The particle size is 5 µm. This column type is ideal for the separation of inorganic and organic anions.

Stationary phase	Novosep A-2 IC column
Particle size	5µm
Dimensions	250 x 4 mm
Order number	B92

Recommended Instrumentation



This application requires a isocratic HPLC system equipped with degasser, autosampler, column oven, and conductivity detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
Smartline Pump 1000, incl. 10 ml pump head	A50303
Smartline Manager 5000 with degasser and Interface	A5312
Autosampler 3900	A1508
Smartline Column Oven 4000	A5300
Conductivity Detector	A1252-1
Suppressor Kit for isocratic mode	A09412
ChromGate Software	A1493

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