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A Simple Ion – Chromotographic Method for Determination of Urinary Oxalate

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Summary. A simple and rapid technique for the determination of oxalate in urine by ion chromatography has been described. Although there is difficulty in separating the oxalate peak from the sulfate peak on the conductivity chromatogram of unprocessed urine, it is possible to eliminate the sulfate peak by the addition of barium chloride to the urine. Using this technique, the author has estimated the urinary oxalate in 33 urolithiasis patients and in 40 non-urolithiasis patients. The means of 50 urinary oxalate determinations in 33 urolithiasis patients and of 42 urinary oxalate determinations in 40 non-urolithiasis patients were 21.5 ± 11.4 and 19.5 ± 13.0 mg/gCr, respectively. Of the 33 urolithiasis patients, 17 were calcium stone formers and 6 were non-calcium stone formers whose stones had been analyzed by infrared spectrometry, and the mean urinary oxalate values were 19.4 ± 6.9 and 21.3 ± 8.2 mg/gCr, respectively. The urinary oxalate was significantly higher in children under the age of 10 years.

Key words: Urinary oxalate, Ion chromatography, Urolithiasis.

Introduction

Many factors related to the occurrence of urolithiasis have been considered. Since oxalate is an important constituent in urolithiasis, various procedures for the determination of urinary oxalate have been developed. They include a chemical method [7], an enzymatic method [3], isotope dilution [4], atomic absorption spectrophotometry [9], a radioenzymatic procedure [1], an isotachophoretic technique [14], gas chromatography [5] and liquid chromatography [11].

Ion chromatography, which was described in 1975 [15], was first used for the determination of urinary oxalate in 1982 [10]. Although this technique was simple and accurate and required no preliminary separation or complicated treatment, the proximity of the oxalate peak to the sulfate peak on the conductivity chromatogram,was problematic.

Therefore, the author tried to decrease the sulfate peak by adding barium chloride to urine samples. Since the sulfate in the urine was taken off in sediment as barium sulfate, the oxalate peak appeared more clearly. This technique and its clinical application are reported in this work.

Materials and Methods

An ion-chromatographic analyzer Model IC100 (Yokogawa-Hokushin Electric Works) was used for measuring the oxalate. 0.004 M Na_2CO_3 and 0.004 M $NaHCO_3$ were used as eluents, and the flow rate of eluents was 2 ml/min. The eluents were made by dissolving 0.848 g of sodium carbonate and 0.672 g of sodium bicarbonate in 2,000 ml of deionized water. 0.05 M dodecylbenzenesulfonic acid was used as the scavenger at the same flow rate. The scavenger was made by dissolving 32.65 g of dodecylbenzenesulfonic acid in 2,000 ml of deionized water. The main analytical column was filled with low-capacity anion-exchange resin made of styrendivinyl benzene beads coated with anion latex particles. The suppressor was made of a catio-exchange membrane tube. The flow diagram of the ion-chromatographic analyzer is illustrated in Fig. 1. Barium chloride granules and 0.1 N standard oxalate solutions were obtained from Wako Pure Chemicals, Ltd.

The urinary oxalate of thirty-three urolithiasis patiens aged 20 to 71 years (22 males and 11 females) and forty non-urolithiasis patients aged 2 to 77 years (31 males and 9 females) was determined by the ion-chromatographic method. Morning urine specimens or 24-h urine specimens from these patients were collected in plastic bottles without any preliminary treatment, and were kept at 4 °C until the measurement was done. About 0.2 g of barium chloride granules were added to 8 ml aliquots of these urine samples. Then the urine samples were mixed and centrifuged at 4,000 RPM for 5 min. 0.5 ml of the supernatant were transferred to a scaled test tube and diluted 20fold with deionized water. This sample was injected into a loop injector of the ion-chromatographic analyzer, and the oxalate peak on the conductivity chromatogram appeared from 8 to 9 min after the injection.

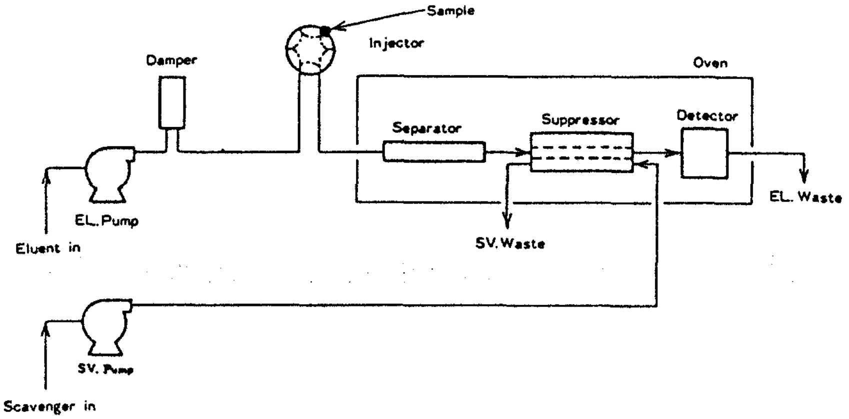


Fig. 1. Flow diagram of ion-chromatographic analyzer

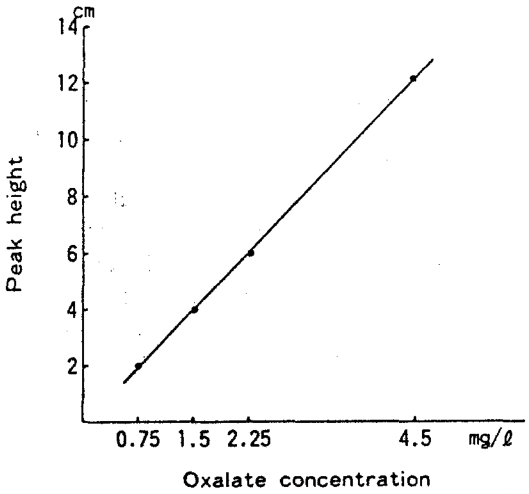


Fig. 2. Standard curve for oxalate

Results

Figure 3 shows the ion chromatogram of a 20fold dilution of urine (3 μs/cm) and Fig. 4 shows the ion chromatogram of a 20fold dilution of the same urine after the addition of barium chloride. Note the decrease in the sulfate peak.

When the oxalate solution was added to urine, the height of the supposed oxalate peak on the conductivity chromatogram was increased. When calcium chloride was added to urine, the height of the supposed oxalate peak was decreased. This confirmed that the peak represented oxalic acid.

The standard curve for oxalate was obtained by injecting standard oxalate solutions ranging from 0.75 to 4.5 mg/l into the ion-chromatographic analyzer and measuring the peak height on the conductivity chromatogram with a sensitivity range of 3 μs/cm (Fig. 2).

For the determination of the recovery rate of oxalic acid added to the urine, 1 ml of 3 different concentrations of oxalic acid solution (90 to 450 mg/l) was added to 9 ml of each of 3 different urine samples. The average recovery rate of the added oxalic acid was 84.0 ± 14.5% (mean ± S.D.) (Table 1).

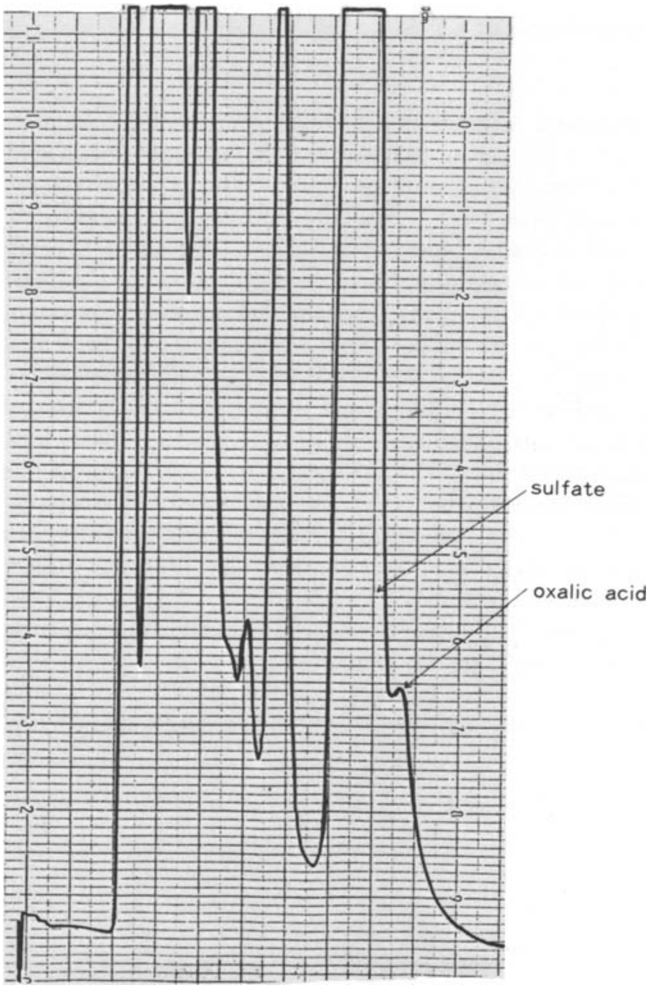


Fig. 3. Ion chromatogram of 20fold dilution of urine (3 μs/cm)

The mean urinary oxalate values in 50 analyses in the 33 urolithiasis patients and in 42 analyses in the 40 non-urolithiasis patients were 21.5 ± 11.4 and 19.5 ± 13.0 mg/gCr (mean ± S.D.) (Table 2).

Of these 33 urolithiasis patients, 23 patients underwent the stone analyses by infrared spectrometry. The mean urinary oxalate values in 24 analyses in 17 calcium stone formers

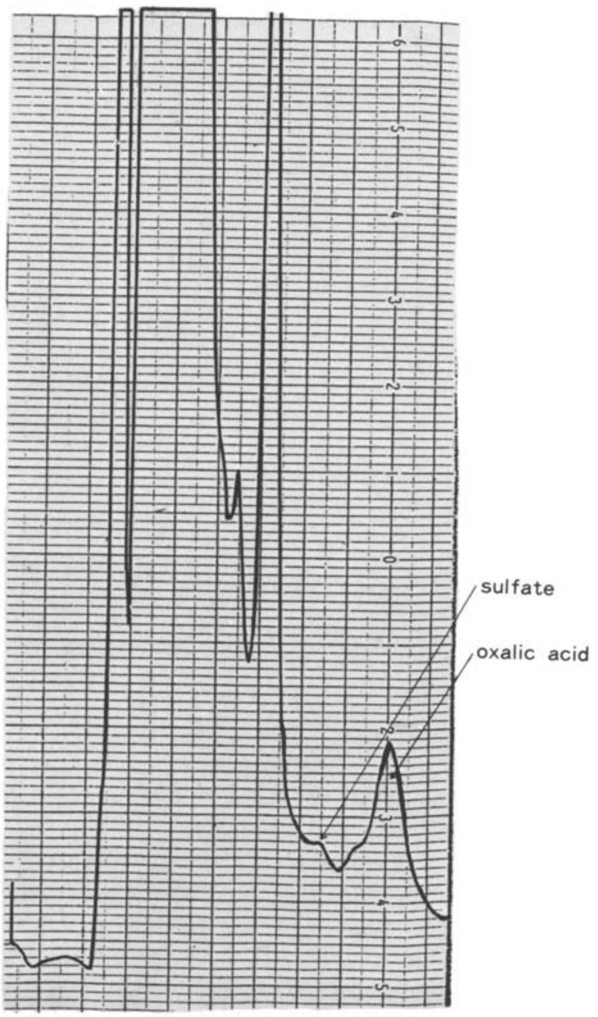
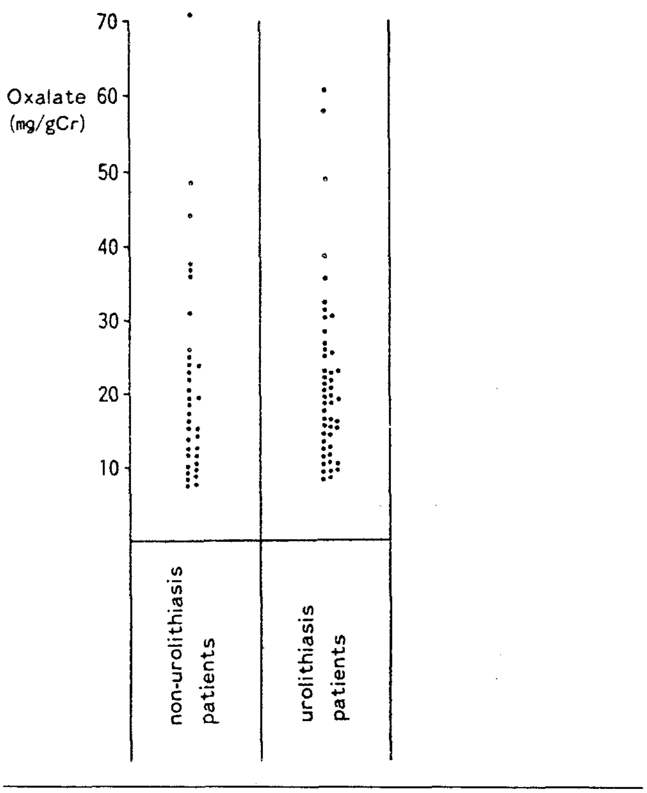


Fig. 4. Ion chromatogram of 20fold dilution of urine after adding barium chloride (3 μs/cm)

Table 1. Recovery rate of oxalate added to urine

Pt. No.	Content (mg/l)	Added (mg/l)	Measured (mg/l)	Recovery (%)
1	11.3	9.8	21.0	107.8
		18.8	24.8	71.8
		45.0	43.5	71.6
2	3.8	9.0	11.3	83.3
		18.8	16.5	67.6
		45.0	33.8	66.7
3	6.8	9.0	15.0	100
		18.8	23.3	92.0
		45.0	48.8	95.1

Table 2. Urinary oxalate in urolithiasis patients and non-urolithiasis patients



aged 20 to 71 years (13 male and 4 females) and in 9 analyses in 6 non-calcium stone formers aged 34 to 56 years (4 males and 2 females) were 19.4 ± 6.9 and 21.3 ± 8.3 mg/gCr (mean ± S.D.), respectively (Table 3). There were no statistically significant differences.

The variation in urinary oxalate with age in non-urolithiasis patients is shown in Table 4. The mean value under the age of 10 years was 39.4 ± 7.0 mg/gCr (mean ± S.D.) and was significantly higher than at other ages.

24-h urinary oxalate excretion was determined in 5 urolithiasis patients and in 5 non-urolithiasis patiens, and these mean values were 38.3 ± 32.4 (ranging from 6 to 92.9) and 37.9 ± 34.3 (mean ± S.D.) (ranging from 10.1 to 91.6) mg per day. There was no statistically significant difference between these values.

The urinary oxalate concentration in 20 urinary samples was measured by both ion chromatography and by gas chromatography [12]. There was fairly good correlation ($r = +0.746$, $P < 0.001$) between the two values (Fig. 5). These mean values were 17.2 ± 8.1 and 28.8 ± 13.4 mg/l (mean ± S.D.), respectively.

Discussion

Since more than 70 per cent of human urinary calculi are composed of calcium oxalate [18], it is very important to estimate urinary oxalate for the investigation of urolithiasis. The recovery of oxalate in urine with the ion-chromato-

Table 3. Urinary oxalate in calcium stone formers and non-calcium stone formers

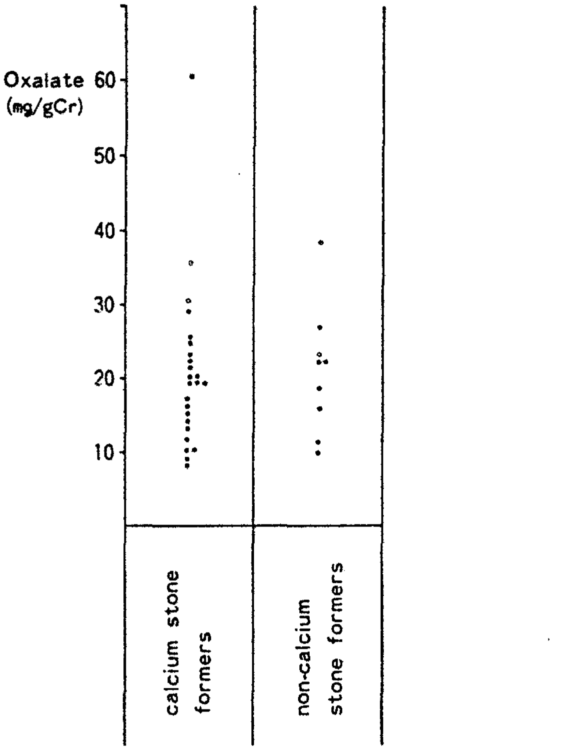


Table 4. Urinary oxalate in non-urolithiasis patients with age

Age	Oxalate concentration (Mean ± S.D. mg/gCr)		
0 ~ under 10 yr.	39.4 ± 7.0	($n = 3$)	(1)
10 ~ under 20 yr.	16.2 ± 6.2	($n = 3$)	(2)
20 ~ under 30 yr.	17.7 ± 10.9	($n = 4$)	(3)
30 ~ under 40 yr.	11.5 ± 2.2	($n = 4$)	(4)
40 ~ under 50 yr.	13.3 ± 4.9	($n = 4$)	(5)
50 ~ under 60 yr.	15.0 ± 6.3	($n = 7$)	(6)
60 ~	22.1 ± 15.6	($n = 17$)	(7)

(1)>(2) $P<0.02$
(1)>(3) $P<0.05$
(1)>(4),(6) $P<0.001$
(1)>(5) $P<0.01$
(1)>(7) $P<0.1$

graphic method by the author's technique was slightly lower than with the other methods previously reported [1, 7, 12, 13, 21]. This could be due to the formation of barium oxalate after the addition of barium chloride to the urine samples, but the infrared spectrograms of the sediment revealed no oxalate. Another possibility is that the oxalate in the urine samples is not completely ionized. Ogawa and Kitagawa [13] have stated that calcium oxalate crystals formed in an alkaline eluent in oversaturated samples will eventually pass through the column, because the conductivity of oxalic acid in the urine is higher in the 100fold diluted samples than in the 20fold diluted samples. Further investigations are required on this problem.

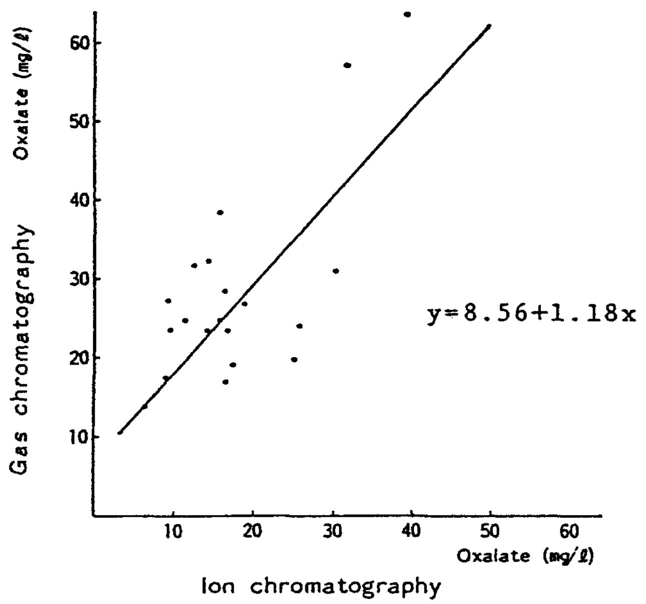


Fig. 5. Comparison of urinary oxalate concentration with gas-chromatographic analysis

In this study there was no significant difference in urinary oxalate excretion in urolithiasis patiens and non-urolithiasis patients. This result agrees with the previous report of Tiselius [19], although in other articles [8, 20, 22] the urinary oxalate excretion in calcium oxalate stone formers was higher than in normal subjects, especially in multiple and recurrent stone formers [16, 17]. The mean normal urinary oxalate excretion in this study was 37.9 mg per day, and was slightly higher than previously reported normal ranges [1, 2, 7, 9, 19, 21].

As for the change in urinary oxalate with age, Gibbs and Watts [6] have stated that the value is highest in early childhood and decreased with increasing age, and the results in the present study are almost in accord with their description. Ito and his associates [8] have stated that the urinary oxalate excretion per square meter of body surface area does not differ with age.

Since the technique described in this paper does not require preliminary separation, complicated treatment, or a long time for the determination, the author believes that this technique will be useful for routine clinical examinations.

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