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Determination of urinary oxalate by high-performance liquid chromatography monitoring with an ultraviolet detector

K. Kataoka, M. Takada, Y. Kato, M. Iguchi, K. Kohri, and T. Kurita

Kinki University School of Medicine, Osaka, Japan

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Summary. High-performance liquid chromatography (HPLC) monitoring with an ultraviolet detector was carried out to measure urinary oxalate levels in urolithiasis. Interfering substances in urine were removed by anion exchange prior to chromatography. This procedure was found excellent with respect to sensitivity, reproducibility, and analytical recovery. The findings were in agreement with colorimetric date. The mean oxalate level in 24-hour urine was 30.5 ± 15.1 mg in patients with a single episode and 36.3 ± 9.8 mg in recurrent stone formers. The latter values was significantly higher than the normal control level (27.4 ± 3.8 mg).

Key words: Urinary oxalate – Anion exchange resin – High-performance liquid chromatography – Ultraviolet detector

Calcium oxalate is the most common component of urinary stones and measurement of oxalate in urine is considered important in the investigation of stone formation. Various methods for determining urinary oxalate levels, including direct precipitation [1, 2], solvent extraction [3], gas chromatography [4, 5], isotope dilution [6], and enzymatic method [7], have been described hitherto. These conventional methods, however, are time-consuming, complicated and/or inaccurate, and are unappropriate for routine clinical use.

Recently, some investigators attempted urinary oxalate measurement by high-performance liquid chromatography (HPLC), but the urine contained a variety of organic acids, whose peaks appeared in close vicinity to the oxalate peak on the chromatogram. Consequently, oxalate could not be clearly separated from the interfering substances. In this study we used strongly basic anion exchange resin, before the oxalate was eluted with sulfuric acid ion. The interfering substances were eliminated by this pretreatment and the oxalate peak was identified satisfactorily.

Materials and method

Reagents and apparatus

The analytical grade chemicals required for HPLC were obtained from Wako Pure Chemicals, Ltd. Muromatic 1×8 (200–400 mesh), which is prepared by purifying Dowex 1×8 (strongly basic anion exchange resin, 200–400 mesh), and a disposable polypropylene minicolumn (7×50 mm) were purchased from Muromachi Chemical Industries, Ltd. The chromatoanalyzers used were a 206 Compact Model (Waters, Ltd) and a 710 Autoanalyzer (Waters, Ltd.) equipped with Unicorder U-228 (Nippon Denko Kagaku Co., Ltd). Chromatographic separation was carried out a Sephadex KC118 column (Showa Denko Co., Ltd.).

Analytical procedure

After addition of 0.1 ml of 4 M HCL, 1 ml of a urine sample was allowed to stand for 1 hour. The mixture was diluted to a volume of 10 ml by adding 8.8 ml of deionized water and 0.1 ml of 4 M NaOH. A 5 ml aliquot of the dilution was filtered through a minicolumn packed with Muromac 1×8 up to the height of 1.5 cm, then the resin was washed with 2 ml of 150 mM NaCl. After that, oxalate adsorbed onto the resin was extracted by passing 3 ml of 0.6 M Na_2SO_4/0.5 H_3PO_4. A 25 µl aliquot of the extract was offered for HPLC. Elution was performed with 0.5% H_3PO_4 at a rate of 0.7 ml/min at 60°C. The ultraviolet (UV) detector was operated at 214 nm. The oxalate peak appeared 8 min after injection of the solvent.

Results

Our procedure is characterized by the pretreatment of urine samples with anion exchange resin for the removal of interfering substances. Figure 1 shows chromatograms of two urine samples: one was untreated (Fig. 1a) and the other was pretreated (Fig. 1b). Whereas the oxalate peak overlapped extensively with one of the other peaks in the upper chromatogram, it was discrete in the lower picture. When urine was treated oxalate decarboxylase, no peak was formed at the correspondent site. Thus, identity of the oxalate peak was established.

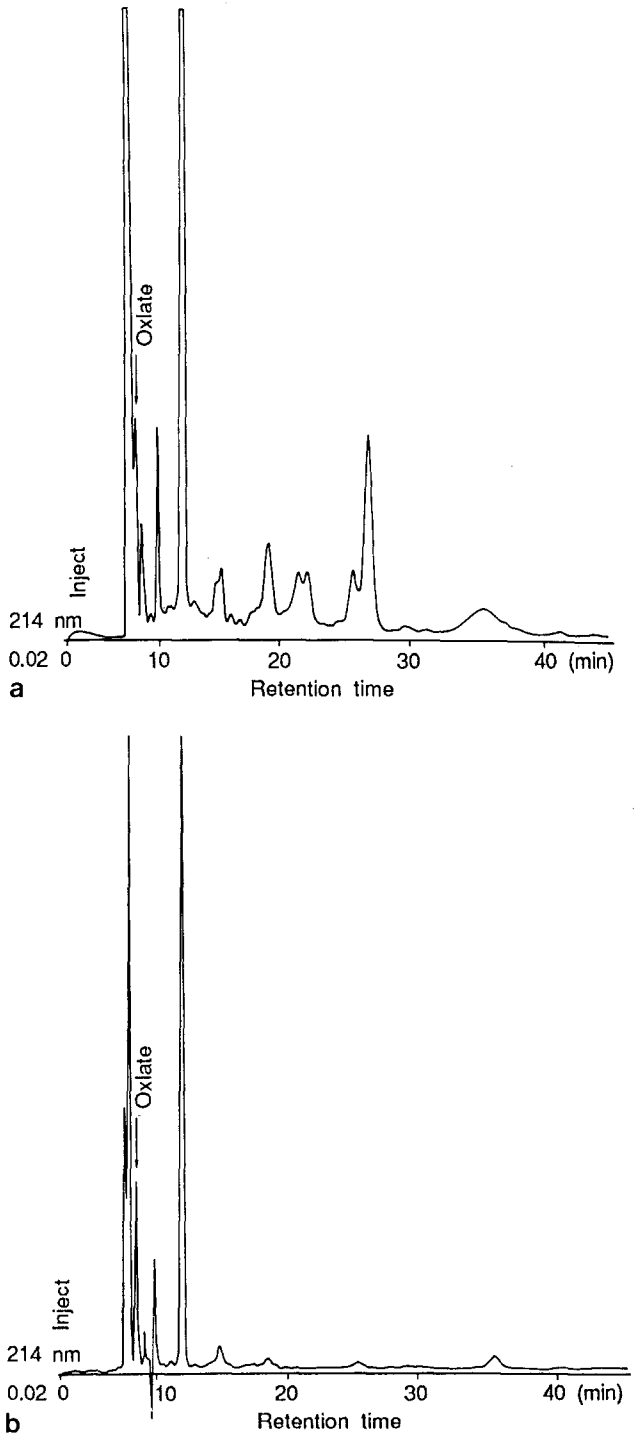

Fig. 1 a and b. Chromatogram of urine sample. **a** Untreated, **b** pretreated

The standard curve for oxalate was drawn by plotting peak height 10 to 200 μg/ml. As seen from this curve, there was linear relationship between the two parameters (Fig. 2).

The recovery of oxalic acid added to urine samples (1 ml, $N=5$) was studied with the results summarized in Table 1. The mean recovery rate $100.2 \pm 10.4\%$ (mean $\pm$ S.D.) after addition of 10 μg and $86.7 \pm 6.4\%$ after addition of 40 μg.

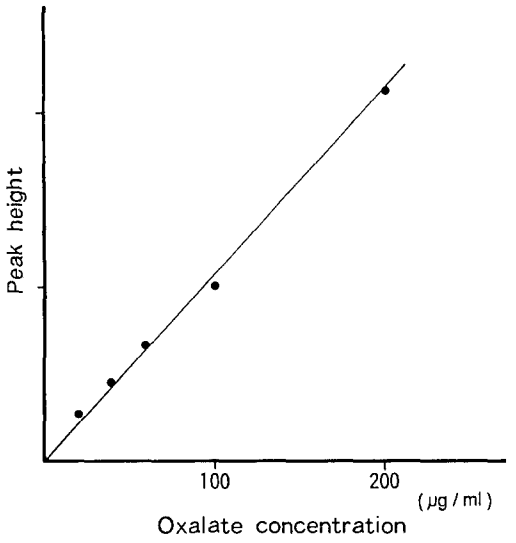

Fig. 2. Standard curve for oxalate

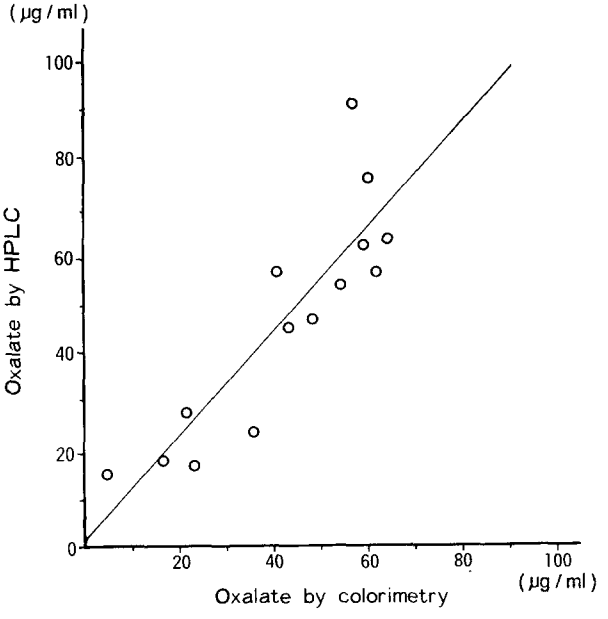

Fig. 3. Comparison of urinary oxalate concentration with colorimetric method. $r=0.870$, $P<0.01$, $N=14$

The reproducibility of determinations was excellent with a variation coefficient of $1.7 \pm 0.7\%$ (mean $\pm$ S.D.) (Table 2). Fourteen urine samples, whose oxalate concentrations had been determined by the colorimetric method of Yachiku [8], were examined for oxalate concentration by our procedure. As a result, a close correlation ($r=0.870$) was recognized between the two modalities (Fig. 3).

The amount of oxalate excreted into 24-hour urine was 27.4 ± 3.8 mg (mean $\pm$ S.D.) in healthy volunteers (normal control group: 10 males and 4 females, age 51.2 ± 9.7 years). The mean urinary oxalate excretion was 30.5 ± 15.1 mg/day in urolithiasis patients with a single episode of stone formation (single-episode group: 14 males and 10 females, age 45.6 ± 14.8 years) and

Table 1. Recovery rate of oxalate added in urine

	Sample No.	Oxalate concentration (μg/ml)			Recovery rate (%)
		Original urine	Added urine	Added-Original	
10 μg added	1	17.2	26.0	8.8	88
	2	57.5	68.5	11.0	110
	3	18.3	29.1	10.8	108
	4	16.1	25.1	9.0	90
	5	42.9	53.4	10.5	105
				Mean ± S.D.	100.2 ± 10.4
40 μg added	1	17.2	54.8	37.6	94.0
	2	57.5	88.9	31.5	78.8
	3	18.3	54.4	36.1	90.3
	4	16.1	52.4	36.3	90.8
	5	42.9	77.5	34.6	94.5
				Mean ± S.D.	86.7 ± 6.4

Table 2. Reproducibility of the determination of urinary oxalate

Sample No	Oxalate concentration (μg/ml)			Mean ± S.D.	C.V. (%)
	1	2	3		
1	32.4	32.4	32.0	32.3 ± 0.2	0.6
2	31.8	32.4	31.3	31.8 ± 0.45	1.4
3	54.2	53.8	56.9	55.0 ± 1.38	2.5
4	19.1	20.0	19.1	19.4 ± 0.42	2.2
5	27.3	26.2	26.4	26.6 ± 0.48	1.8
				Mean ± S.D.	1.7 ± 0.7

36.3 ± 9.8 mg/day in those with multiple episode (recurrent group: 18 males and 3 females, age 43.6 ± 9.9 years). Although there was no significant difference in urinary oxalate excretion between the single-episode group and normal control group, the urinary oxalate excretion was significantly greater in the recurrent group than in the control group.

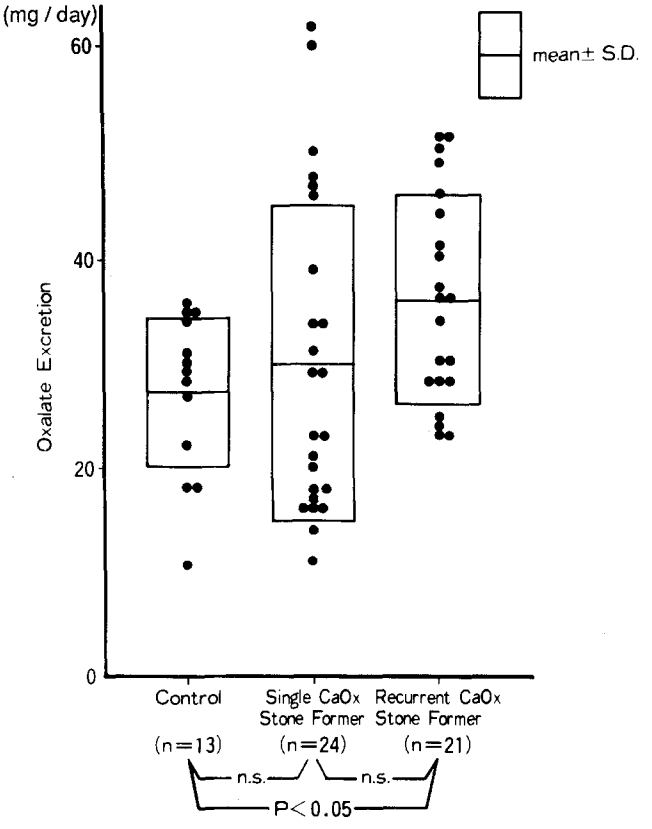

Fig. 4. Urinary oxalate in normal controls and calcium stone formers. CaOx = Calcium oxalate, n.s. = not significant

Discussion

HPLC is an efficient technique for the separation and quantitation of compounds in many different samples. Recently, it has also been applied in the assay of urinary oxalate, but the initial results were disappointing. Urine contains organic acids which are close to oxalate in terms of chromatographic retention time and they interfere with assay. Therefore, the necessity arises of binding oxalate to a UV-absorbing [9] or fluorescent compound [10]. Some outhors introduced a procedure, which purported to measure urinary oxalate directly without converting it to any derivative [11]. No solution to the removal of interfering substances prior to chromatography has been proposed. HPLC that does not require such pretreatment has also been described [12], but this procedure required several chromatographic columns and the entire system becomes unrealistically complicated.

In this work we performed pretreatment using Muromac 1 × 8. This anion exchange resin completely adsorbed urinary oxalate, when its concentration was not higher than 300 μg, and fully eluted sulfuric acid ions. Interfering substances were almost completely removed, so that a clear-cut peak of oxalate was formed. The reproducibility

of data and the recovery of added oxalate were both favorable.

The urinary excretion of oxalate in the normal controls was 27.4 ± 3.8 mg/day. This ratio was much the same as those reported elsewhere. There was no significant difference between the single-episode group and the control group with respect to the urinary excretion of oxalate, but it was significantly greater in the recurrent group than in the control group. These findings were compatible with the observations of Takasaki and coworkers [13].

A conductimetric detector is often used in HPLC. In this study, however urinary oxalate was monitored with a UV detector which is more easily available. HPLC combined with this detector requires only one column and can be performed routinely without special equipment in most laboratories.

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Kiyonori Kataoka, MD
Kinki University School of Medicine
377-2 Ohnohigashi
Osakasayama City
Osaka
589 Japan