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## Note

# Determination of 2,8-dihydroxyadenine in urine by high-performance anion-exchange liquid chromatography

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A renal calculus of 2,8-dihydroxyadenine (DOA) is a rare disease and studies are limited [1, 2]. Various analytical techniques such as UV spectroscopy and fluorometry were used to characterize DOA and distinguish it from other purine metabolites [3, 4]. Recently, Ericson et al. [5] reported the measurement of renal excretion of adenine and DOA in plasma, after administration of adenine, using a cation-exchange column. To determine the DOA in urine, the photometric method, primary purification and concentration procedure are comparatively complicated, not so accurate and time-consuming. We have developed a simple and efficient method for measuring DOA in urine using high-performance anion-exchange liquid chromatography (HPLC). This method was applied in a case of DOA stones with a partial deficiency of adenine phosphoribosyltransferase (APRTase) after renal transplantation. The HPLC technique is herewith described and the results in a rare case of DOA stones are compared with those of some normal subjects.

## EXPERIMENTAL

### *Chemicals*

DOA and adenine were obtained from Sigma (St. Louis, MO, U.S.A.). Ammonium acetate and acetic acid were purchased from Wako Pure Chemicals (Osaka, Japan).

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*High-performance anion-exchange column chromatography*

A high-pressure piston pump (Mitsumi Scientific Industry Co., Tokyo, Japan) served to pump the column eluent through a six-port sample injection valve with a 100-$\mu$l sample loop. A jacketed, stainless-steel column, 25 × 0.46 cm I.D., was used, and prepacked with Diaion CDR-10 anion-exchange resin with a mean particle size of 7 $\mu$m (Mitsubishi Kasei Co., Tokyo, Japan) [6]. The column temperature was kept at 60°C with a Haake constant-temperature circulator (Haake Co., Berlin, F.R.G.). All buffer solutions were delivered by an automated buffer exchanger (Hijiri Seiko Co., Tokyo, Japan). A JASCO UVIDEC-100 UV spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan) was used at 305 nm. The UV signal was recorded using a JASCO RC-100 recorder (Japan Spectroscopic Co.) with a chart speed of 30 cm/h. Peak areas were determined with a digital integrator (Chromatopac C-E1B, Shimadzu Seisakusho Co., Kyoto, Japan).

*Eluent and reagent solutions*

All reagents were analytical grade, made up in double glass-distilled water. All aqueous reagents were filtered through a 0.45-$\mu$m microfilter (Fuji Photo Film Co., Tokyo, Japan), and degassed. Standard DOA solutions were made in 0.5 *M* hydrochloric acid at 5—20 $\mu$g/ml. A stepwise gradient for each 0.6 *M* rise in 6 *M* ammonium acetate buffer (pH 4.4) was used; the initial eluate was 0.006 *M* ammonium acetate buffer, and the final one was 6 *M* ammonium acetate buffer. Each buffer was pumped for 6 min through the column, which was operated at a flow-rate of 30 ml/h.

*Sample preparation*

Urine samples (24 h) from normal persons and patients were collected, acidified to pH 1—2 with hydrochloric acid and stored at —20°C until analysis.

## RESULTS AND DISCUSSION

DOA is an adenine metabolite. Normally adenine is converted into adenylic acid (AMP) by APRTase and can again be used in the nucleotide metabolic cycle [7]. In the case of low activity or deficiency of APRTase, adenine accumulates, and excess adenine is changed to DOA by xanthine oxidase and excreted into the urine. But because of its poor solubility [4] a high level of DOA in the urine is known to cause calculi.

In this study, high-performance anion-exchange liquid chromatography proved to be useful for the determination of DOA in urine. The macroreticular anion-exchange resin column was used for the separation of urinary components [6]. The UV absorption spectra of DOA and adenine showed maxima at 305 nm and 260 nm, respectively (Fig. 1). The wavelength selected for the detector was 305 nm, corresponding to maximum UV absorption for DOA. This condition indicated that there was no detectable amount of adenine.

A chromatogram of normal urine spiked with DOA at a concentration of 1.5 $\mu$g per 100 $\mu$l, is presented in Fig. 2. The retention time of DOA was 16.4 ± 0.3 min ($n$=10). The calibration curve was plotted in the concentration range 5—20 $\mu$g/ml and found to be linear with a correlation coefficient of

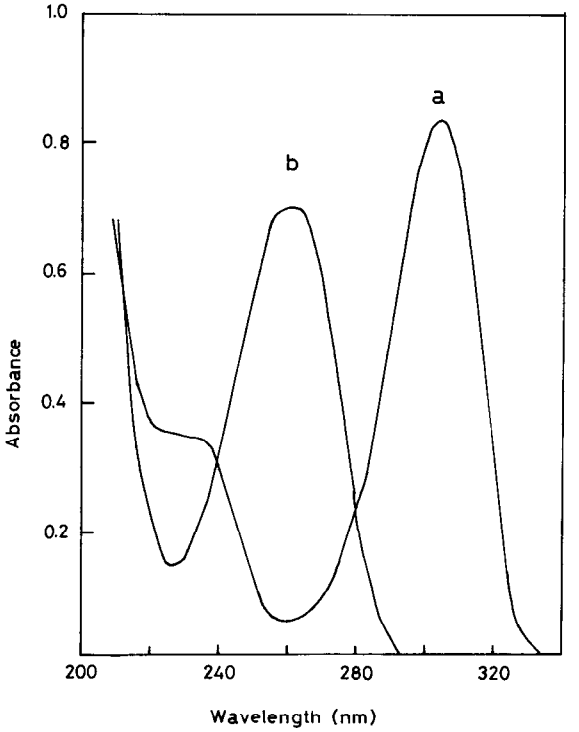


Fig. 1. The UV absorption spectra of DOA and adenine at a concentration of 10 $\mu$g/ml in 0.5 *M* HCl. Peaks: a = DOA, b = adenine.

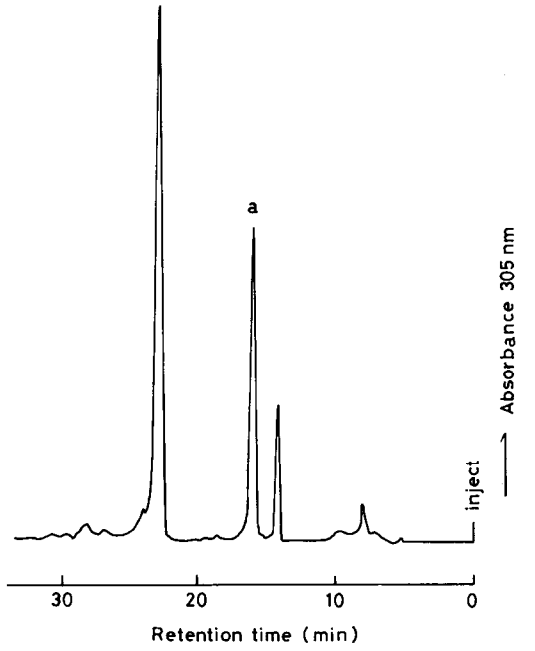


Fig. 2. Chromatogram of normal urine spiked with DOA, at a concentration of 1.5 $\mu$g per 100 $\mu$l. Chromatographic conditions: stationary phase, CDR-10 (average particle size, 7 $\mu$m), prepacked in a 25 × 0.46 cm I.D. stainless-steel column; mobile phase, 0.006—6 *M* ammonium acetate buffer (pH 4.4) with a stepwise gradient; flow-rate, 30 ml/h; detector, UV 305 nm; recorder chart speed, 12 cm/h. Peak: a = DOA.

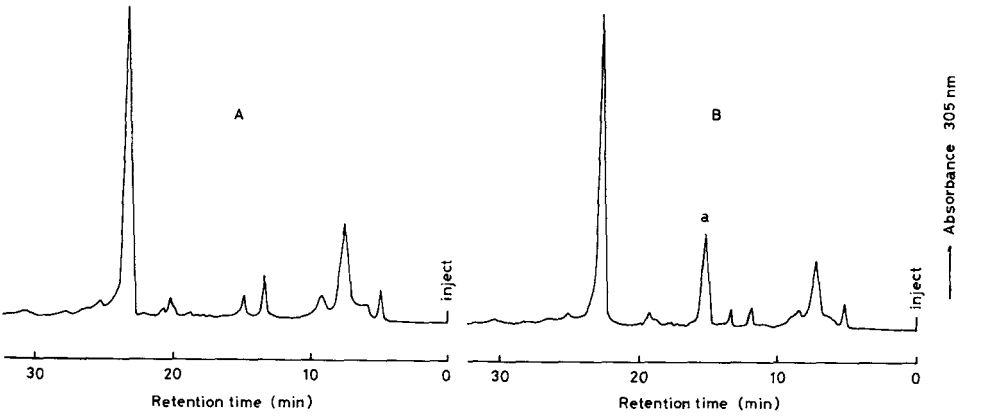


Fig. 3. Chromatograms of urine samples. The conditions are described under Experimental procedures. (A) A 100-$\mu$l aliquot of a normal urine was injected. (B) Urine of patient with a partial deficiency of APRTase after renal transplantation. Peak: a = DOA. The amount of DOA present = 14.3 $\mu$g/ml.

TABLE I

URINARY EXCRETION OF 2,8-DIHYDROXYADENINE

| Sample* | Urinary excretion (mg/day) |
|---|---|
| 1 | 40.6 ± 8.6** (mean ± S.D.) |
| 2 | N.D.*** |
| 3 | N.D. |

*1 = Patient with a partial deficiency of APRTase after renal transplantation; 2 = patient with normal APRTase after renal transplantation ($n$ = 3); 3 = normal persons ($n$ = 5).
**Urine of the same patient during 46 days ($n$=15).
***N.D. = not detected.

0.998. Chromatograms of urines of control and patient are shown in Fig. 3A and B, respectively.

Table I compares DOA levels in the urine of the patient with a partial deficiency of APRTase with that of a healthy person. DOA was not detected in the urine of either normal persons or patients with normal APRTase activity after renal transplantation. Excretion of DOA in the patient was observed to be 30—65 mg/day during 46 days immediately after renal transplantation. Since it was reported that the xanthine oxidase inhibitor allopurinol was effective for such therapeutic DOA [8], the present results suggest the possibility of recurrence of renal calculus and the necessity of prescribing xanthine oxidase inhibitor to prevent it.

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