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Note

Rapid determination of urinary oxalate by high-performance liquid chromatography

A. MILLÁN

Department of Chemistry, Faculty of Sciences, Universidad de las Islas Baleares, 07071 Palma de Mallorca (Spain)

J.M. GRASES

Laboratoria Agrario, Generalitat de Catalunya (D A R.P.), Cabrils, Barcelona (Spain)

and

F. GRASES*

Department of Chemistry, Faculty of Sciences, Universidad de las Islas Baleares, 07071 Palma de Mallorca (Spain)

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Determination of oxalate in urine has aroused interest as a consequence of the important role of urinary oxalate in the genesis of calcium oxalate urolithiasis [1,2], as well as in other human deseases [3,4], and several procedures have been described and proposed [5–19]. The oxalate oxidase and oxalate decarboxylase methods have been the most widely used [5–9], but precipitation [10,11], immobilized enzyme [12,13], gas chromatographic (GC) [14,15] and high-performance liquid chromatography (HPLC) [16–18] techniques have also been widely applied. However, the precision and accuracy of the precipitation techniques are often poor, particularly at lower concentrations, and ascorbate interferes with the enzymic procedure. GC methods involve extraction and esterification or silylation, and direct HPLC methods are either too slow or present problems with respect to detection and sample preparation. In addition, gradient elution is required in most HPLC methods. A comparison of the urinary oxalate results determined by several laboratories indicated that only the use of the most sophisticated approaches (e.g. immobilized enzymes and HPLC) can be expected to reduce the unacceptable variation of results between laboratories [20].

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This paper describes a simple, rapid and accurate chromatographic method using an Aminex HPX 87H column, which has been widely employed for the separation of organic acids and post-column derivatization of oxalate based on its complexation with Fe^{III} ions and UV detection.

EXPERIMENTAL

Reagents

All the chemicals used were of anlytical-reagent grade. Distilled, deionized water was used in all the experiments. A 5% acetonitrile solution in 6 m*M* H_2SO_4 used as eluent was pressure-filtered through a 0.45-μm filter (Millipore, Molsheim, France) and degassed by sonication. The oxalate decarboxylase enzyme was obtained from a commercial kit purchased from Boehringer-Mannheim (Barcelona, Spain).

Apparatus

The liquid chromatograph consisted of a Waters 712 WISP injector (Milford, MA, U.S.A.), a Waters 510 pump, a Knauer 64 pump (Berlin, F.R.G.) for the derivatizing solvent, a Waters 490 multi-wavelength photometric detector and an IBM PC XT 286 loaded with a Waters Maxima 820 chromatography workstation program. The separation was carried out on an Aminex HPX 87H strong cation-exchange column (300 mm × 7.8 mm I.D., particle size 9 μm) (Bio-Rad Labs., Richmond, CA, U.S.A.) preceded by a Waters Resolve C_{18} precolumn. Out-of-use μBondapak (Waters) and Nucleosil 10 SA (Scharlau, Barcelona, Spain) columns connected in series were used to buffer the pulsation of the derivatizing pump. The eluents were mixed in a T-connection. A cation precolumn (Waters) was placed between the mixing-T and the detector.

Procedure

Urine samples were acidified with concentrated H_2SO_4 to a pH of 1.6, then filtered through a 0.45-μm filter. A 15-μl aliquot of the filtrate was injected. Constituents were developed by elution with the 5% acetonitrile solution in 6 m*M* sulphuric acid for 35 min at a flow-rate of 0.6 ml/min. A 0.44 m*M* Fe^{III} solution in 0.018 *M* sulphuric acid was used for derivatization at a flow-rate of 0.2 ml/min. Instructions contained in the commercial kit were followed for removing the oxalate from one urine sample.

RESULTS

A chromatogram of human urine detected at 210 nm without derivatization and chromatograms of the same sample detected at 210, 285 and 365 nm with derivatization are reproduced in Fig. 1. As can be observed, the resolution was

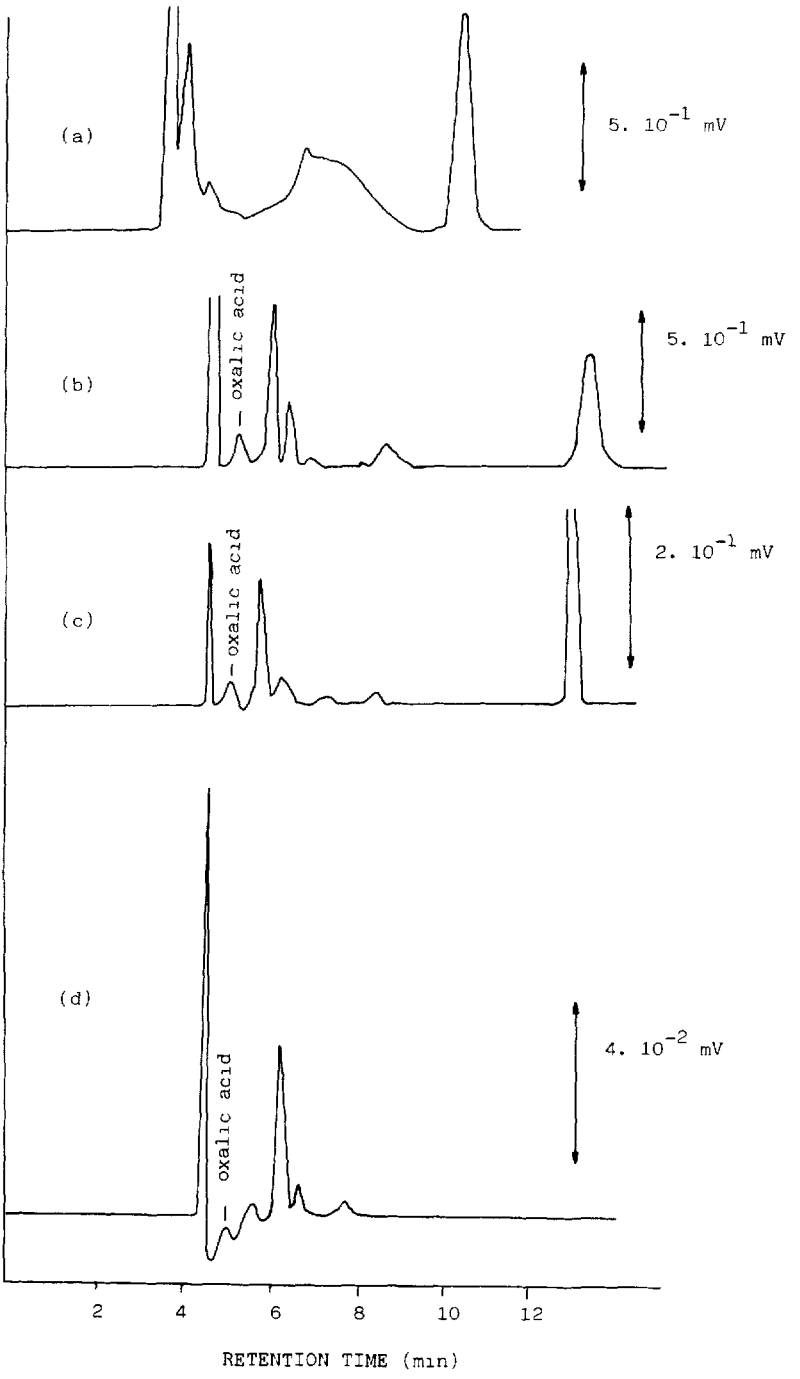

Fig. 1. Chromatograms of human urine: (a) at 210 nm without derivatization; (b) at 210 nm with derivatization; (c) at 285 nm with derivatization; (d) at 365 nm with derivatization. Eluent, 5 m*M* H_2SO_4; flow-rate, 0.8 ml/min; derivatization solvent, 1.8 m*M* Fe^{III} in 0.036 *M* H_2SO_4; flow-rate, 0.3 ml/min.

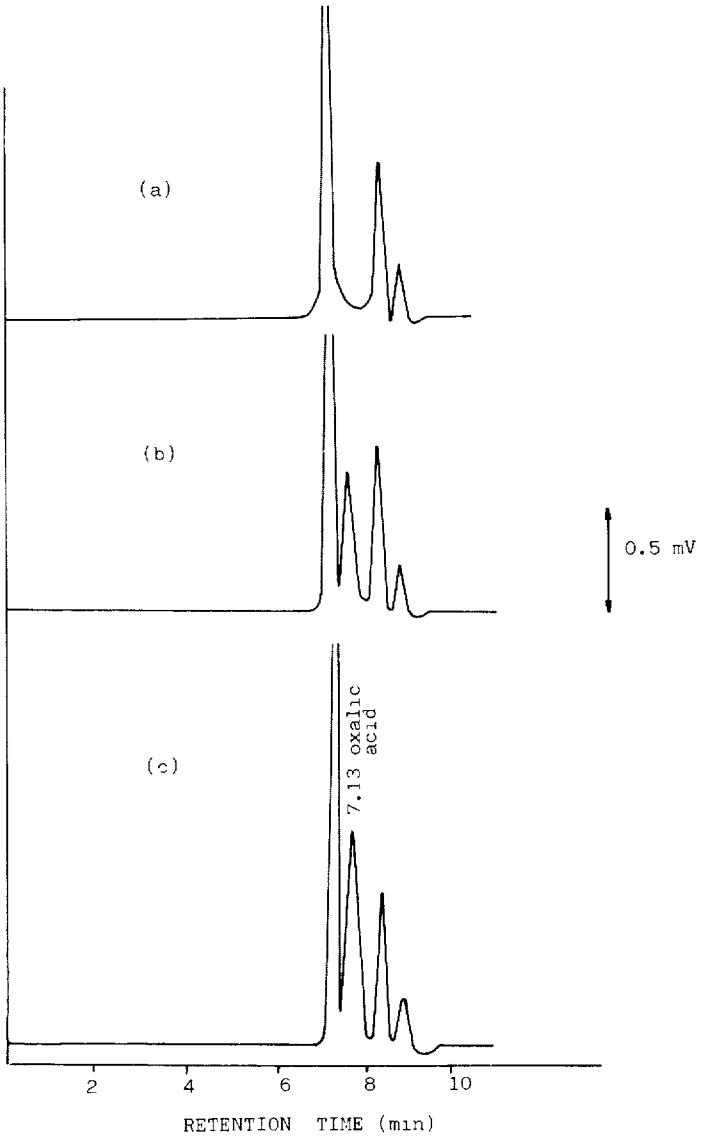

Fig. 2. Chromatograms of human urine (1:2 dilution) obtained according to the recommended procedure: (a) with oxalic acid removed; (b) standard urine; (c) urine with addition of 20 mg/l oxalic acid.

much improved by the derivatizing procedure; oxalate could not be detected without derivatization.

The oxalate peak was identified by spiking with a reference sample. In another experiment oxalic acid was totally removed from a human urine sample by means of the oxalate decarboxylase enzyme. The chromatograms obtained

after the enzymic reaction are shown in Fig. 2. It seems clear that no other substance is eluted at the same retention time as oxalic acid. The calibration curve was found to be linear over the range 5–100 mg/l. The recovery of six known amounts of oxalate ranging from 5 to 30 mg/l from urine samples was: 98.7 ± 6.074% (mean ± R.S.D.) at 210 nm and 99.5 ± 2.35% at 365 nm. Evaluation of the reproducibility gave an R.S.D. of 0.7% (36 mg/l) at 210 nm ($n=7$) and 1.9% (8 mg/l) at 365 nm ($n=7$). Over the observed concentration range the peak response was found to be proportional to the amount injected. The detection limit is 2 mg/l at a signal-to-noise ratio of 3. Finally, six samples of human urine and six of rat urine were analysed, yielding a mean (± S.D.) value of 16 ± 6.3 mg/l for human urine and 47 ± 9.7 mg/l for rat urine.

DISCUSSION

The formation of a cationic Fe^{III}–oxalate complex (absorbance maxima at 285 and 365 nm), stable in acidic media, has been used as a basis for a post-column derivatization procedure to determine oxalate in urine samples. The sensitivity of the method was higher at 210 nm than at 285 or 365 nm. Several concentrations of H_2SO_4 were assayed. An increase of the acid concentration of the eluent improves the resolution but widens the peak-width, making the quantification more difficult. Optimal results were obtained using 6 m*M* H_2SO_4. Addition of acetonitrile does not affect the resolution of the oxalic acid peak, but it helps the elution of the organic components and thus shortens the analysis time. Several sulphuric acid solutions of Fe^{III} were assayed to develop the derivatization process. Optimal results were obtained using a 0.44 m*M* Fe^{III} solution in 18 m*M* H_2SO_4. The flow-rate was also optimized.

The precision of the assay depends critically on the buffering of the pulsation of the pump delivering the derivatizing solvent. Previously the use of a cation-exchange column (Nucleosil 10 SA) and precolumn derivatization with Fe^{III} was studied. Although the resolution was very poor the cation-exchange column proved to be a good adsorbent for the formation of the Fe^{III}–oxalic acid complex. An oxalic acid solution containing an excess of iron was injected into the column, and 5 min after the elution of the corresponding peak a solution containing the same amount of oxalic acid, but without iron, was injected. The peaks of both samples detected at 365 nm matched each other. The excess of iron is thus retained by the column and it forms a complex with oxalic acid when it passes through the column. When a cationic column was placed after the mixing-T the resolution and precision of this method were greatly improved, but the pressure increased beyond the limit recommended for the Aminex column. However, the use of a cationic guard column gave satisfactory results without raising the pressure in the system. Without the cationic guard column, the baseline noise was four times greater than the signal from a 10 mg/l oxalate sample.

In summary, the proposed method can be used readily to monitor the concentration of oxalate in urine. It operates under isocratic conditions, no pretreatment of the sample is required, and it can be employed in clinical studies.

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REFERENCES

1 W.G. Robertson and M. Peacock, in H.J. Schneider (Editor), Urolithiasis: Ethiology, Diagnosis, Springer, Berlin, 1975, p. 185.
2 P.O. Schwille, M. Manoharan, G. Ruemenapf, G. Woelfel and H.J. Berens, Clin. Chem. Clin. Biochem., 27 (1989) 87.
3 H.E. Williams and L.J. Smith, Jr., in J.B. Stanbury et al. (Editors), Primary Hyperoxaluria in the Metabolic Basis of Inherited Disease, McGraw-Hill, New York, 1978, pp. 182–204.
4 T. Gerritsen, E. Kaveggia and H.A. Waisman, Pediatrics, 36 (1965) 882.
5 E. Straus, M. Meerking and J. Moshides, Clin. Chem., 33 (1987) 2301.
6 Q.E. Crider, Clin. Chem., 31 (1985) 1080.
7 J.D. Sallis, M.F. Lumley and J.E. Jordan, Biochem. Med., 18 (1977) 371.
8 J. Costello, M. Hatch and E. Bourke, J. Lab. Clin. Med., 87 (1976) 903.
9 J.H. Glick, Clin. Chem., 25 (1987) 419.
10 H.E. Archer, A.E. Dormer, E.F. Scowen and R.W.E. Watts, Clin. Sci., 16 (1957) 405.
11 G.H. Koch and F.M. Strong, Anal. Biochem., 27 (1969) 162.
12 S. Girotti, G. Carrea, A. Roda, R. Bocara, A.L. Piacentini, M.A. Angelotti, S. Ghini and S. Buscaroli, in J.W. Schoelmerich (Editor), Chemiluminescent Flow Assay of Hydrogen Peroxide and Urinary Oxalate with Immobilized Peroxidase and Oxalate Oxidase, Proceedings of the 4th International Symposium on Bioluminescence and Chemiluminescence, Chichester, 1986, p. 563.
13 M.A.S. Amini, J. Vallou and Bichou, Anal. Lett., 22 (1989) 43.
14 M. Yanagawa, H. Ohkawa and S. Tada, J. Urol., 129 (1983) 1163.
15 B.G. Wolthers and M. Hayer, Clin. Chim. Acta, 210 (1982) 87.
16 H. Hughes, L. Hagen and R.A.L. Sutton, Anal. Biochem., 119 (1982) 1.
17 W. Kooska, B.G. Wolthers, M. Hayer and H.M. Rutgers, Clin. Chim. Acta, 170 (1987) 237.
18 R.P. Singh and G.H. Nancollas, J. Chromatogr., 433 (1988) 373.
19 M.D. Luque de Castro, J. Pharm. Biomed. Anal., 6 (1988) 1.
20 C.T. Samuell, G.P. Kasidas and G.A. Rose, Urol. Res., 16 (1988) 1.