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Analytical Isotachophoresis: A Rapid and Sensitive Method for Determination of Urinary Oxalate

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Summary. A variety of procedures have been described in the literature for quantitative determination of urinary oxalate concentrations. However, a rapid and sufficiently simple technique for routine determinations is lacking. This study presents an isotachophoretic technique for measurement of urinary oxalate. Complete separation of the oxalate anion from accompanying ions is achieved in a PTFE-capillary between two electrodes according to differences in the net mobility. Short analysis time, little pretreatment of the urine samples, and extremely high resolving power and accuracy are the significant advantages of this newly developed method. This new technique was shown to be of significant value in studying the role of oxalate in urinary stone disease.

Key words. Urinary oxalate - Oxalate analysis - Stone disease - Isotachophoresis.

A large number of different methods have been described for the analysis of oxalate. Volumetric procedures such as permanganate titration (10, 11) or cerate oxidimetry (4, 13) were found to be nonspecific since the oxidant reacts with many reducing substances. A variety of colorimetric methods have been used (6) for the analysis of oxalic acid in biological materials but most are of limited specificity due to the fairly critical quantitative reduction of oxalic acid to glyoxylic acid. High sensitivity can be achieved by formation of fluorescent complexes (5, 21). Methods involving enzyme reactions use oxalate oxidase (EC 1.2.3.4) or oxalate decarboxylase (EC 4.1.1.2) (9, 14). The reactions are highly specific but have the disadvantage that the enzymes are difficult to isolate. In addition catalytic activity is inhibited by various urinary constituents such as phosphate or sulphate (12). Gas-liquid chromatographic methods (7, 15, 19) are sensitive but oxalic acid must be transformed into a sufficiently volatile derivative. Calcium (1), lead (16), or europium salts (7) have been employed in the preliminary separation from interfering urinary constituents. Analytical isotachophoresis recently attracted attention as a simple, rapid, and sensitive method. It has been successfully applied to organic acids extracted from human urine. This paper demonstrates the separation and quantitation of oxalate in urine by means of analytical isotachophoresis.

MATERIAL AND METHODS

The isotachophoretic runs were made in a Tachophor 2127 (LKB-Produkter, Bromma, Sweden) equiped with an 800 mm PTFE capillary of 0.5 mm internal diameter. The separated compounds were detected by thermosignal and UV-absorbance at 254 nm. Current at detection was 75 μA. The design and function of the instrument have been described by Arlinger (2) and Everaerts (8). All chemicals used were suprapure or of analytical grade (E. Merck, Darmstadt, Germany; Fluka AG, Buchs, Switzerland and Sigma Chemicals, St. Louis, USA). Water was twice distilled in an all quartz apparatus.

The leading electrolyte was 0.01 M hydrochloric acid titrated with 0.01 M beta-alanine to pH 3.68. 0.3% methylcellulose (methocell MC 4000 cP) was added in order to reduce electroendosmosis.

The terminating electrolyte was 0.01 M n-hexanoic acid.

Urine samples were obtained from healthy male and female volunteers and kept frozen (-30°C) until analysis. Freezing had no significant effect on the results obtained.

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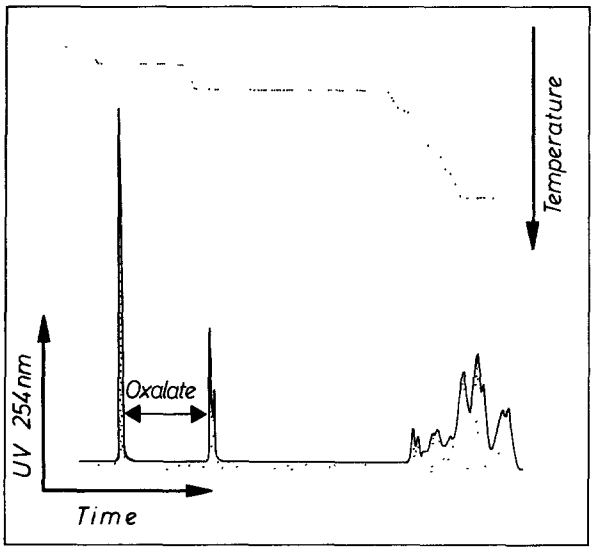

Fig. 1. Isotachophoretic separation of the urinary Ca^{++} precipitate dissolved in hydrochloric acid

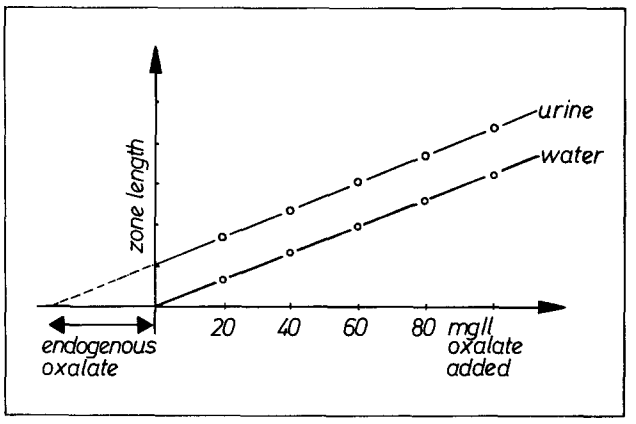

Fig. 2. Standard calibration curves of oxalate dissolved in water or urine

In order to lower the ionic strength of the urine samples and thus to reduce analysis time oxalate was precipitated by addition of calcium chloride. After centrifugation (3000 x g, 15 min) the precipitate was dissolved in 0.2 N hydrochloric acid and 10 µl were injected.

The width of the UV peaks corresponding to the zone length in the capillary was used for quantification of the separated ions. Chart speed was 10 cm/min and time required for a single determination was 40 min.

Identification and calibration of the oxalate was made with oxalate and ^{14}C-oxalate as internal standards. An internal oxalate standard was added to all runs to avoid errors caused by varying recovery rates at the precipitation step.

RESULTS

A typical result of an isotachophoretic separation of dissolved precipitate is given in Figure 1. The thermal step height representing the temperature of the corresponding zone produced by Joule's heat in the capillary was used for identification. The temperature is related to the electrical field strength in each zone and is therefore a measure of the mobility of the ions in the zone. In our electrolyte system the step height of oxalate amounts to 8% of the total thermosignal obtained with chloride-caproate. The UV recordings exhibit the characteristic absorbance of the oxalate anion. Various internal standards were added to the urine samples for calibration purposes. A standard calibration curve is drawn showing linearity in the range from 20 mg/l to 100 mg/l of oxalate added to the urine (Fig. 2). This curve parallels the one obtained with pure oxalate when dissolved in water. The concentration of the intrinsic urinary oxalate is measured on the negative abscissa. From the measurements a diurnal urinary oxalate excretion of 29 ±12 mg can be derived (n=30) which is in the range of the enzymatic methods. With an injection volume of 10 µl minimum measurable oxalate concentration was approximately 20 nmol/ml. Up to an oxalate concentration of 150 mg/l equilibrium can be obtained with the 800 mm capillary. In the case of a very high urinary oxalate concentration injection volume can be reduced. The recovery rates after precipitation of the oxalate with calcium ions are comparable to those reported by Baadenhuijsen (3). Our recovery studies confirm the hyperbolic precipitation curve found by this author.

Isotachophoretic separations of some urine samples showed a component with a very small difference in ionic mobility from oxalic acid. Although a number of substances chemically or metabolically related to oxalic acid were run, identification of this component has not been achieved so far. Isolation of the component in a micro fraction collector (Tachofrac, LKB, Sweden) and mass spectrometric identification is currently in progress. It is interesting that heating of the urine causes decomposition of the cyclic parabanic acid to oxaluric acid which yields oxalic acid and urea. The decomposition reaction can easily be followed by isotachophoresis (Fig. 3). All related compounds or metabolites tested, such as oxaluric acid, glycolic acid, glyoxylic acid, ascorbic acid (Fig. 4) were well separated from oxalic acid.

DISCUSSION

In isotachophoresis the sample ions are separated according to their net mobility (net mobility = ionic mobility x degree of dissociation). The technique described here for determination of urinary oxalate provides several advantages over other

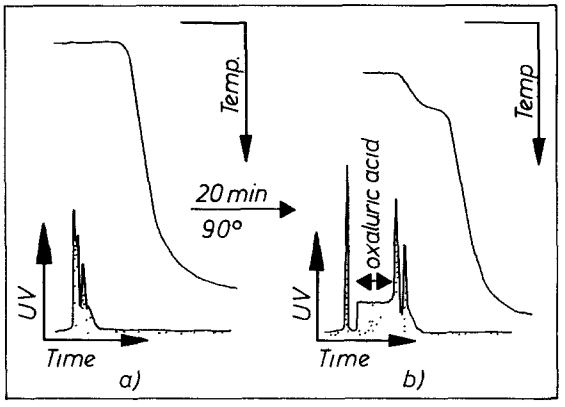

Fig. 3. Thermal decomposition of parabanic acid followed by isotachophoresis

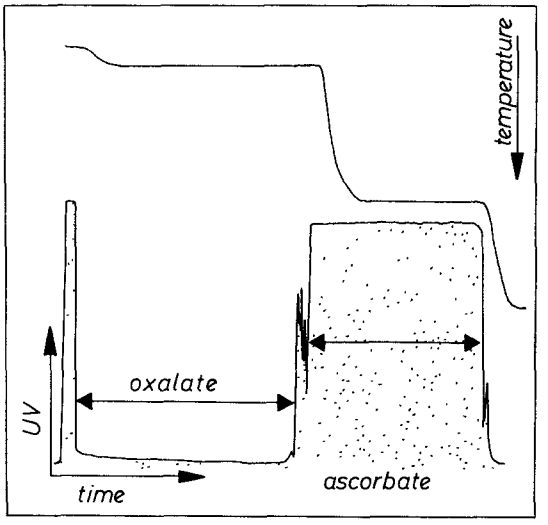

Fig. 4. Isotachophoretic separation of oxalate and ascorbate

methods. The reproducibility of the isotachophoretic analysis is less than 3%. The simple pretreatment of the urine samples allows the rapid handling of a large number of samples routinely. The use of internal standards avoids errors due to varying recovery rates. The method is sufficiently sensitive since the detection limit (20 nmol/ml) is far below the normal urinary concentration of oxalate. A principle problem of quantitative evaluation after isotachophoretic separation is that the pattern of preceding ion-zones can influence the zone length of ions with lower mobility. For oxalate, however, this problem is of minor importance due to the extremely high mobility of this ion at the pH chosen for separation.

REFERENCES

1. Archer, H. E., Dormer, A. E., Scowen, E. F., Watts, R. W. E.: Studies on the urinary excretion of oxalate by normal subjects. Clinical Science 16, 405 (1957)
2. Arlinger, L.: Analytical Isotachophoresis. Principle of Separation and Detection, Proteides of Biological Fluids, Vol. 22, Ed. H. Peeters: Pergamon Press 1975
3. Baadenhuijsen, H., Jansen, A. P.: Colorimetric determination of urinary oxalate recovered as calcium oxalate. Application of a simple correction factor for incomplete precipitation. Clinica Chimica Acta 62, 315 (1975)
4. Blanka, B.: Determination of oxalic acid in urine. Vnitrni Lekarstvi 6, 1301 (1960)
5. Britton, D. A., Guyon, J. C.: Fluorimetric determination of oxalate ion. Analytica Chimica Acta 44, 397 (1969)
6. Burrows, S.: A colorimetric method for the determination of oxalate. Analyst 75, 80 (1950)
7. Chalmers, R. A., Watts, R. W. E.: The quantitative extraction and gas-liquid chromatographic determination of organic acids in urine. Analyst 97, 958 (1972)
8. Everaerts, F. M., Beckers, J. L., Verheggen, T. P.: Isotachophoresis. Theory, Instrumentation, and Applications. J. Chromatogr. Library Vol. 6, Amsterdam: Elsevier 1976
9. Hallson, P. C., Rose, G. A.: A simplified and rapid enzymatic method for determination of urinary oxalate. Clinica Chimica Acta 55, 29 (1974)
10. Harcourt, A. V.: On the observation of the course of chemical change. Journal of the Chemical Society 20, 460 (1867)
11. Harcourt, A. V., Esson, W.: On the Laws of Connexion between the Conditions of a Chemical Change and its Amount. Philosophical Transactions of the Royal Society of London 156, 193 (1866)
12. Knowles, C. F., Hodgkinson, A.: Automated enzymic determination of oxalic acid in human serum. Analyst 97, 474 (1972)
13. Koch, G. H., Strong, F. M.: Determination of oxalate in urine. Analytical Biochemistry 27, 162 (1969)
14. Mayer, G. G., Markow, D., Karp, F.: Enzymatic oxalate determination in urine. Clinical Chemistry 9, 334 (1963)
15. Mee, J. M. L., Stanley, R. W.: A rapid gas-liquid chromatographic method for determining oxalic acid in biological materials. Journal of Chromatography 76, 242 (1973)
16. Pernet, J. L., Pernet, A.: Dosage colorimétrique de l'acide oxalique dans les milieux biologiques. Annales de Biologie Clinique 23, 1189 (1965)
17. Powers, H. H., Levatin, P.: A method for the determination of oxalic acid in urine. Journal of Biological Chemistry 154, 207 (1944)

18. Vittu, Ch., Lemahieu, J.C.: Determination de l'oxalurie par methode polarographique. Annales de Biologie Clinque 23, 913 (1965)
19. Von Nicolai, H., Zilliken, F.: Gaschromatographische Bestimmung von Oxalsäure, Malonsäure und Bernsteinsäure aus biologischem Material. Journal of Chromatography 92, 431 (1974)
20. Zarembski, P.M., Hodgkinson, A.: The fluorimetric microdetermination of glyoxylic acid in blood, urine and bacterial extracts. Biochemical Journal 96, 218 (1965a)
21. Zarembski, P.M., Hodgkinson, A.: The fluorimetric determination of oxalic acid in blood and other biological materials. Biochemical Journal 96, 717 (1965b)

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