Journal of Chromatography, 386 (1987) 11–17
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 950

MULTI-COMPONENT ANALYSIS OF URINE OF DOGS WITH LIVER DISEASES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

YOSHIKO ABE, ERIKO KOBAYASHI, NORIKO SUZUKI, AKIKO KANEKO, TADAO FUJIE, YOSHIKO NAGATA and YOSHIKAZU MATSUSHIMA*
Kyoritsu College of Pharmacy, Shibakoen 1-5-30, Minato-ku, Tokyo 105 (Japan)
NOBUHARU TAKAI
Institute of Industrial Science, University of Tokyo, Roppongi 7-22-1, Minato-ku, Tokyo 106 (Japan)
and
TOSHIFUMI WATANABE and OSAMU OTSUBO
Institute of Medical Science, University of Tokyo, Shirokanedai 4-6-1, Minato-ku, Tokyo 108 (Japan)

SUMMARY

Components of the urine of normal dogs and dogs with liver diseases were analysed by high-performance liquid chromatography (HPLC) using a macroreticular anion-exchange resin with UV detection. The dogs with liver diseases were experimentally prepared by either Eck fistula by portal–caval anastomosis, dimethylnitrosamine-induced liver failure or total hepatectomy by operation. Eighteen chromatographic peaks were numbered and the cross-correlation coefficient of the intensities of every pair of peaks was computed. There were high correlations between several pairs of peaks according to the types and degrees of the hepatic failures, whereas no significant correlation was observed for the peak pairs of normal urine samples. The results showed that HPLC analysis of urine is useful in the diagnosis of the physiological state in liver dysfunction.

INTRODUCTION

High-performance liquid chromatography (HPLC) on macroreticular anion-exchange resins has allowed the simultaneous and rapid analysis of multi-component materials, such as urine[1–3]. For the purpose of diagnosing various diseases, cross-correlation analysis by microcomputer has been applied to the chromatograms obtained by this technique. Its theory, principle and applications have been described[4–7].

We have analysed urine samples of dogs with depatic failure in order to obtain information on the correlation between the components of urine and hepatic disorder and to establish a routine analytical method for urine applicable to the diagnosis of hepatic diseases.

0021-9673/87/$03.50

EXPERIMENTAL

Urine samples

Urine samples were obtained from 27 adult mongrel dogs (weight 10–15 kg; male, 15; female, 12) considered to be normal. Of these 17 dogs were employed for preparing three models with an experimental hepatic failure through one of the following treatments: (1) Eck fistula by portal–caval anastomosis and the ligation and severance of the proper hepatic artery and the common bile duct; (2) drug-induced liver failure by intravenous injection of 30 mg/kg of dimethylnitrosamine (DMNA); and (3) total hepatectomy after portal–caval anastomosis and the ligation and severance of the proper hepatic artery and the common bile duct.

Urine was sampled from each of these dogs through a catheter. Samples from the ten untreated dogs and from the model dogs before treatment were taken as control samples. After the treatment, samples were collected from the model dogs every hour. Immediately after the sampling, the urine was centrifuged at 800 *g* for 15 min, filtered through a 0.45-μm Millipore (Bedford, MA, U.S.A.) filter, frozen and stored at -20°C. Immediately before analysis, it was thawed in a water-bath and then filtered through the Millipore filter. A 25-μl volume of the sample was injected into the chromatograph.

Analytical procedure

A Shimadzu (Kyoto, Japan) Model LC-3A HPLC system equipped with a gradient device was used. The analytical column (250 mm × 4 mm I.D.) was packed with anion-exchange resin of a macroreticular type, Hitachi (Tokyo, Japan) Gel 3013-N. The column was connected to a thermostat, maintaining the column temperature at 60°C.

The sample was eluted with a gradient of water to 0.25 *M* ammonium perchlorate–acetonitrile (85:15) at a rate of 2%/min and then with 0.25 *M* ammonium perchlorate–acetonitrile (85:15) for 20 min. The 0.25 *M* aqueous ammonium perchlorate solution had been adjusted to pH 4.90. The flow-rate was 0.8 ml/min. The eluent was monitored with a Shimadzu Model SPD-2A UV detector, operated at 254 nm. Reproducible chromatograms were always obtained in this procedure.

The cross-correlations and principal-component matrix of the intensities of the main chromatographic peaks were computed with an NEC Model 9801E personal computer (Nippon Denki, Tokyo, Japan).

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram of a control urine sample. The separability of the peaks was inferior to those reported for human urine[1–7], which may reflect the difference between human and dog samples, as well resolved chromatograms were always obtained for human urine under the present conditions. Most of the peaks in Fig. 1 were common to the control urine samples, collected from 27 dogs, but their relative intensities varied widely from sample to sample. Among them, 18 main peaks were numbered. The unequivocal numbering of the peaks can be made according to the procedure outlined in ref. 5. The assignment of some of the peaks was carried out by comparison of retention times and UV spectra with those of standard com-

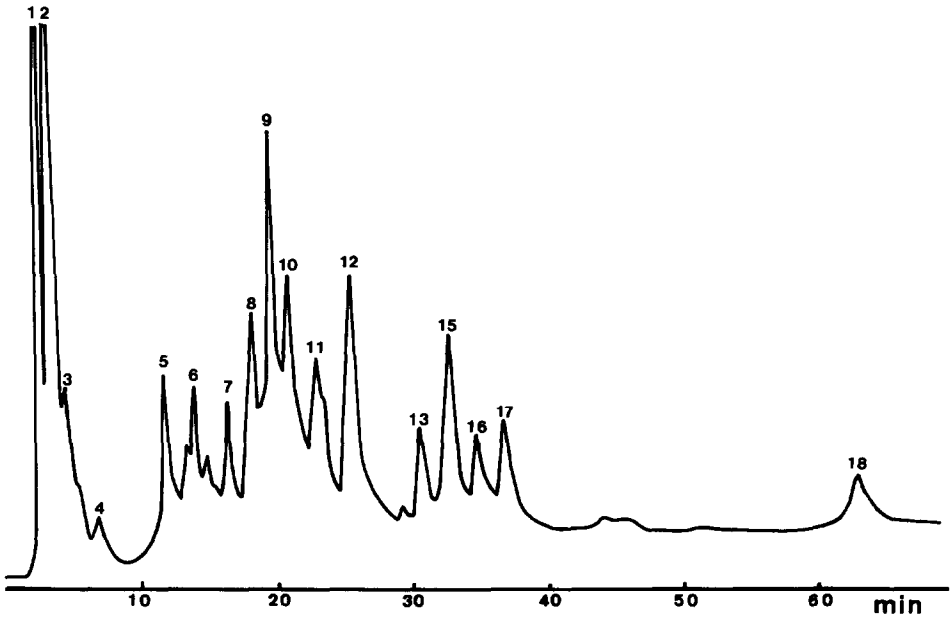

Fig. 1. Chromatogram of a control urine sample. Assignment of peaks: 1, creatinine; 4, L-tryptophan; 5, uric acid; 10, hippuric acid; 11, bilirubin; 12, urea; 13, 3-indoleacetic acid.

pounds. The cross-correlations between the intensities of the main peaks were calculated. No pairs of peaks gave a correaltion coefficient above ± 0.9.

The chromatograms of samples from the Eck fistula group, collected 3–10 h after the treatment, were similar to each other but slightly different from those col-

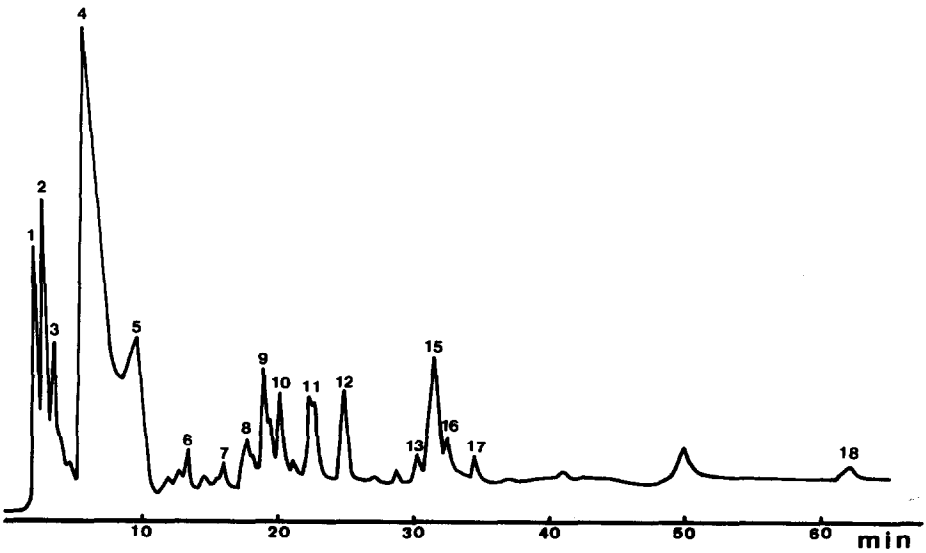

Fig. 2. Chromatogram of a urine sample from the Eck fistula group (7 h after the treatment).

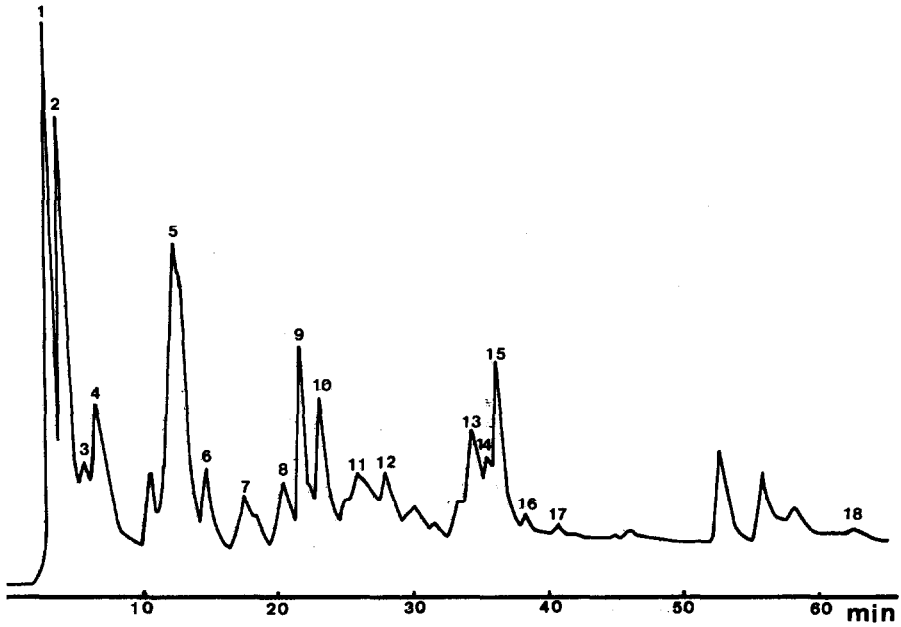

Fig. 3. Chromatogram of a urine sample from the DMNA-induced liver failure (10 h after the treatment).

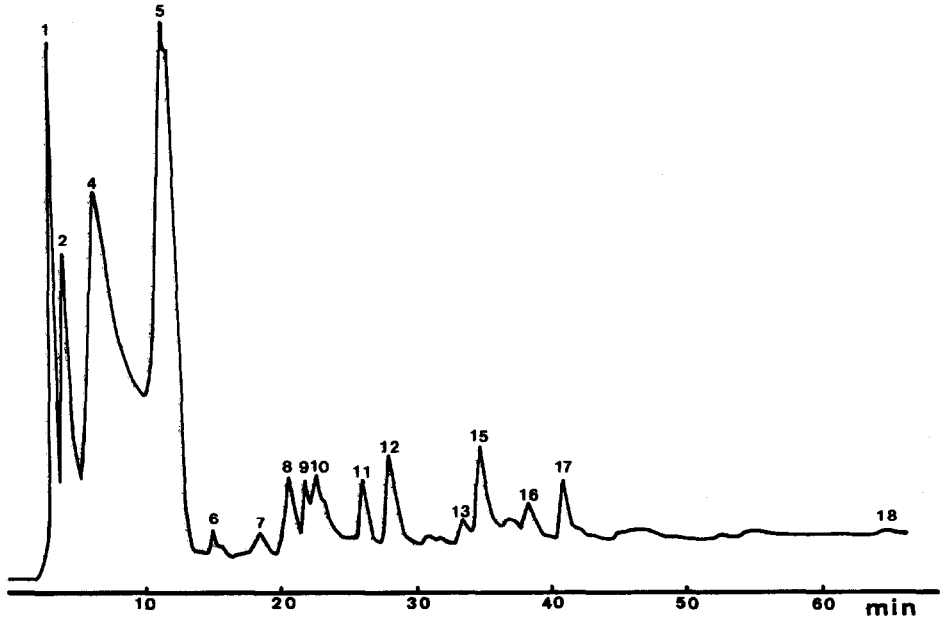

Fig. 4. Chromatogram of a urine sample from the total hepatectomy (3 h after the treatment).

lected later. The same was true for the other two groups. Slight changes in the chromatograms occurred 10 h and 7 h after the treatments in the DMNA and hepatectomy groups, respectively. Chromatograms of urine samples in the experimental hepatic failure group are shown in Figs. 2–4. They may be regarded as representative of each of the hepatic failures.

The dogs were anaesthesized with thiopental sodium before the operation. We confirmed that no significant effect was observed in the chromatograms of dog urine collected more than 1 h after administration of the agent. As the retention time of DMNA was 9 min under the chromatographic conditions used, the peak between peaks 4 and 5 in Fig. 3 can be assigned to the administered drug.

High correlations of the intensities were observed for several pairs of the main peaks in the samples of hepatic failures, whereas no significant correlation was observed in the control urine. Fig. 5 shows the pairs of peaks having correlation coefficients above ±0.9 within each type of sample.

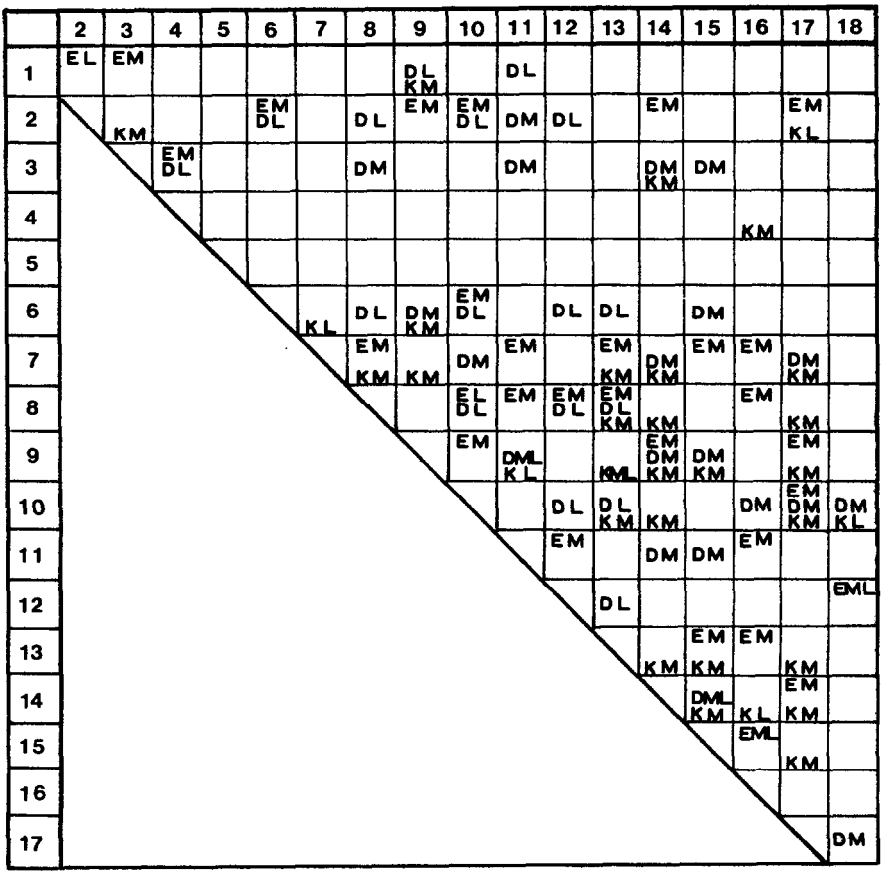

Fig. 5. Correlation coefficient matrix, showing the values above ±0.9 in experimental hepatic failure. EM, Eck fistula group (3–10 h after the treatment); EL, Eck fistula group (10–20 h after the treatment); DM, DMNA-induced liver failure group (3–10 h after the treatment); DL, DMNA-induced liver failure group (10–26 h after the treatment); KM, total hepatectomy group (3–7 h after the treatment); KL, total hepatectomy group (7–14 h after the treatment).

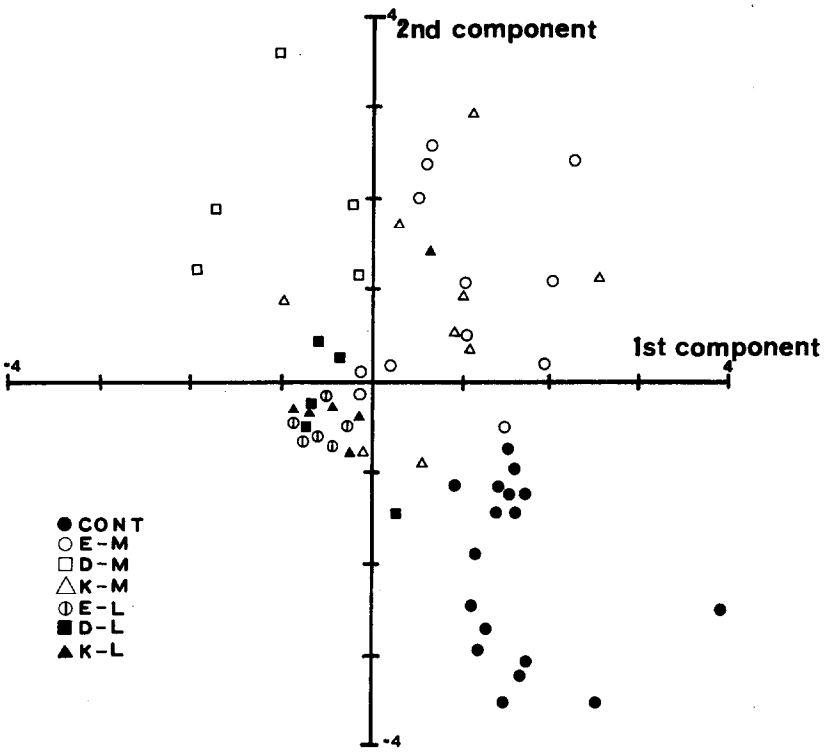

Fig. 6. Principal-component analysis. Groups as in Fig. 5.

The results may be interpreted as follows: components of control urine were affected by individual and external conditions, such as species, age, breeding and moving, while the hepatic damage might induce some unform metabolic changes, giving rise to similar chromatograms.

Fig. 6 shows the distribution of the scores of the first and second components, obtained by the principal-component analysis employing five peaks (Nos. 7, 8, 13, 14 and 15). The heights of these peaks were highly correlated in the hepatic failure samples. The first and second principal components were gathered in the fourth quadrant for control urine, in the first and second quadrants for advanced hepatic failure and in the third quadrant for further advanced stages. The changes in the scores of the first and second principal components may reflect the degree of hepatic damage.

CONCLUSION

The results indicate that the cross-correlation coefficients of HPLC peaks for samples of experimental hepatic failures are different to those of the control group. Principal-component analysis may provide information on the degree of liver disease. It can be concluded that HPLC analysis of urine is useful in the diagnosis of the physiological state in liver dysfunction. However, more work will have to be undertaken on the method to establish its position among routine diagnostic procedures.

REFERENCES

1 H. Miyagi, J. Miura, Y. Takata and S. Ganno, *Clin. Chem.*, 25 (1979) 1617.
2 K. Seta, M. Washitake, T. Anmo, N. Takai and T. Okuyama, *J. Chromatogr.*, 181 (1980) 311.
3 K. Seta, M. Washitake, I. Tanaka, N. Takai and T. Okuyama, *J. Chromatogr.*, 221 (1980) 215.
4 H. Miyagi, J. Miura, Y. Takata, S. Kamitake, S. Ganno and Y. Yamagata, *J. Chromatogr.*, 239 (1982) 733.
5 J. Miura, H. Miyagi, Y. Takata and Y. Yamagata, *Jpn. J. Clin. Chem.*, 12 (1983) 54.
6 H. Sakurai, M. Okana and N. Takai, *J. Med. Technol.*, 26 (1982) 995.
7 Y. Nagata, Y. Yokoyama, S. Nagura, Y. Matsushima and N. Takai, *J. Liq. Chromatogr.*, submitted for publication.