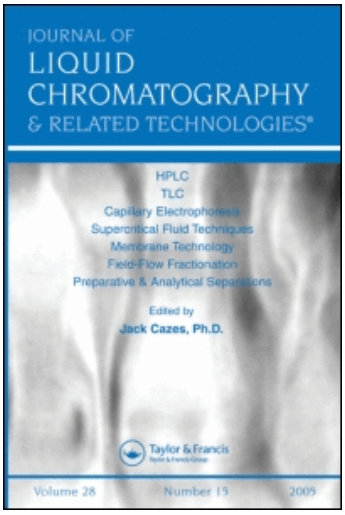


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# ANALYSIS OF ULTRAVIOLET-ABSORBING COMPOUNDS IN HUMAN URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Ultraviolet(UV)-absorbing compounds in human urine were analyzed by means of high-performance liquid chromatography using the column of macroreticular anion exchange resin maintained at 60°C. The sample was eluted with a linear gradient of water to 0.25 M ammonium perchlorate-acetonitrile (85:15) at 0-50 min and with 0.25 M ammonium perchlorate-acetonitrile (85:15) at 50-70 min and monitored with absorption at 254 nm. Reproducible chromatograms were obtained and 68 well resoluted peaks were numbered. The storage of a urine sample at -20°C for 7 weeks did not significantly affect the chromatogram. The correlation coefficients of every pair of the numbered peaks were calculated to examine the daily variations and the individual difference of the UV-absorbing urine components.

## INTRODUCTION

High-performance liquid chromatography (HPLC) using macroreticular anion exchange resin offers much savings in time and tedium in analysis of body fluid over the conventional analytical methods. The technique has become practical in clinical laboratories. The separation of ultraviolet(UV)-absorbing compounds in human and animal urine with this technique has been reported by a number of workers (1-7) and several elution systems were proposed.

We examined these systems with a view to obtaining a routine analytical method of human urine applicable to diagnostic purposes. A method based on gradient elution with water to 0.25 M ammonium perchlorate-acetonitrile (85:15) as the mobile phase (1) was found satisfactory. Using a modified elution method, we analyzed urine samples obtained from healthy females. The present paper describes the results of the studies which are necessary preliminaries to clinical applications.



## MATERIALS

Chemicals and solvents used in the study were of reagent grade and obtained from commercial sources. Water used as solvent was freshly distilled deionized water.

The strongly basic anion exchange resin of a macroreticular type, Diaion CDR-10, was obtained from Mitsubishi Chemical Industries, Tokyo, Japan. Its particle size distribution was 5-7 μm. The resin was packed into a 250 x 4 mm I.D. stainless-steel column using a high pressure-slurry technique.

A Hitachi Model 655 high-performance liquid chromatograph equipped with a Hitachi solvent programming system (a Model 655 proportioning valve and a Model 655-66 controller) and a Rheodyne Model 7125 injector was used. The chromatograph was coupled to a

Hitachi Model 150-20 double beam spectrophotometer with a flow cell and a Hitachi Model 150-20 data processor. The column was connected to a Shimadzu Model CTO-2A thermostat maintaining the column temperature within $\pm 0.2^{\circ}C$ of the selected setting.

## METHODS

Urine samples were obtained from healthy females of 22 and 23-years old. The urine sample was filtered through a 0.45-μm Milipore filter immediately after collection. Unless otherwise noted, the sample was refrigerated, completely frozen and stored at $-20^{\circ}C$. It was thawed immediately before analysis and a 100-μl volume of the sample was injected into the chromatograph.

The sample was eluted with a linear gradient of water to 0.25 M ammonium perchlorate-acetonitrile (85:15) at 0-50 min and with 0.25 M ammonium perchlorate-acetonitrile (85:15) at 50-70 min. 0.25 M Ammonium perchlorate-acetonitrile (85:15) was prepared by mixing 0.25 M ammonium perchlorate solution adjusted at pH 4.90 and acetonitrile in a volume ratio of 85:15.

The spectrophotometric detection was operated at 254 nm. The flow-rate was 0.8 ml/min and the temperature of the column was maintained at $60^{\circ}C$.

Correlations between intensities of peaks were computed with an NEC Model 9801E personal computer.

## RESULTS AND DISCUSSION

Under the chromatographic conditions described above, reproducible chromatograms of a urine sample were obtained. Sixty eight well resoluted peaks within 60 min were numbered among all peaks of the chromatograms. Figure 1 shows a typical chromatogram and the peak numbers. Assignment of the peaks are

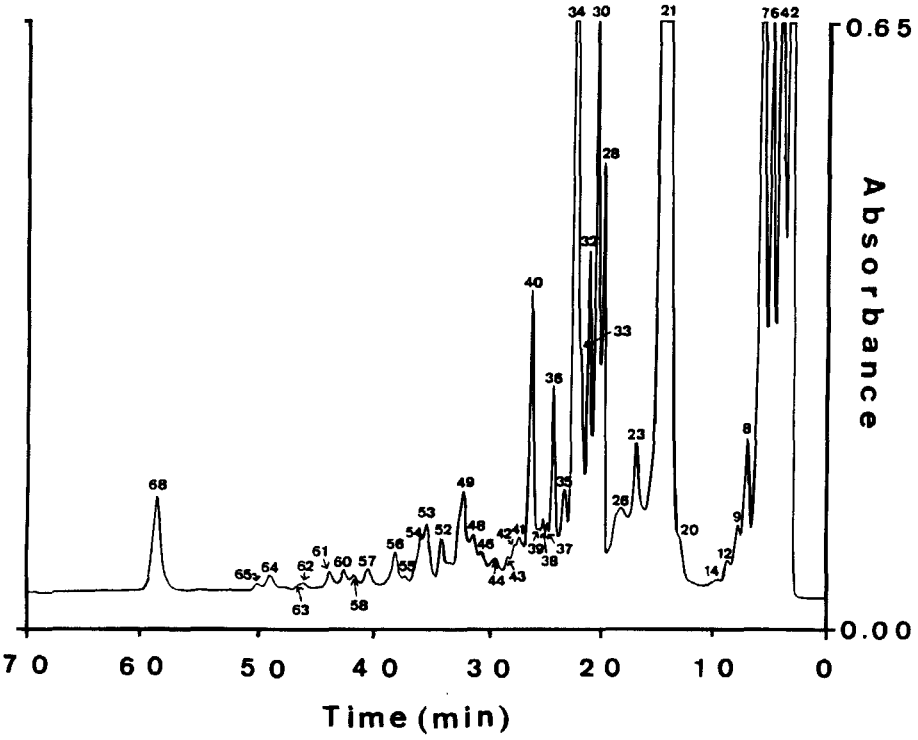


FIGURE 1. A chromatogram of human urine eluted with a linear gradient of water to 0.25 M ammonium perchlorate-acetonitrile (85:15) at 0-50 min and with 0.25 M ammonium perchlorate-acetonitrile (85:15) at 50-70 min and monitored at 254 nm.

in progress by separation of each compound. Detail will be reported separately.

Effect of storage conditions of a urine sample on the chromatogram was examined. Keeping the sample at 20°C for 8 days brought considerable changes in the chromatogram. The faster-eluting peaks underwent a decrease of the peak height. Considerable decrease in the height was also noted on the peaks Nos. 21 and 34, which are assigned to uric acid and hippuric acid, respectively, and on the peaks in their vicinity. Storage at 5°C

brought similar but less pronounced changes. No such change in the chromatogram was observed in 7 weeks under the conditions described in the section of method.

The urine samples fluctuate widely in composition. Though the numbered peaks were observed in every chromatogram, there was a large difference in their intensities between individuals. The chromatograms of urine samples of an individual were influenced by external conditions such as diet, sleeping and moving. With respect to intensity of a single peak, it is hard to differentiate clearly between effects of external conditions or between groups of individuals. Then correlations between intensities of two peaks among the numbered peaks were analyzed to recognize the chromatographic patterns.



Daily variations were studied with samples obtained from ten peoples. Seven samples in a day were collected before lunch, after lunch, before supper, after supper, before sleep, before breakfast and after breakfast. With the 70 chromatograms, the absolute values of the correlation coefficients of intensities of every pair of peaks among the numbered peaks were calculated. The values above 0.7 were observed for two pair of peaks. Chromatograms of the samples collected after meals were greatly different from those of other samples, indicating the effects of diets. With the 40 samples collected before meals and before sleep from the ten peoples, the values above 0.8 were observed for 2 pair of peaks, and those between 0.8-0.7 for 4 pairs of peaks.

Samples collected before supper from 30 people were analyzed similarly. The values above 0.8 for 2 pairs and those between 0.8-0.7 for 5 pairs were observed. The correlation of these pairs were random in the daily variation study.

We are now collecting basic data to clarify the difference between peoples of two different clinical states and the effects of medication by correlation analysis of the chromatographic peaks.

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