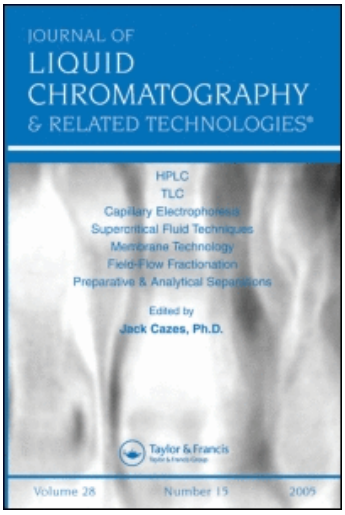

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SIMULTANEOUS DETERMINATION OF CREATININE AND URIC ACID IN HUMAN URINE BY CAPILLARY ZONE ELECTROPHORESIS

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ABSTRACT

A simple, fast and precise method for the simultaneous determination of creatinine and uric acid in human urine by capillary zone electrophoresis has been developed. The method only requires filtering urine sample and no other pretreatment. The effect of pH of the background buffer on the existent state of creatinine and uric acid was investigated. Photodiode array detection permitted the rapid identification of creatinine and uric acid in the sample analysed. The linear range, detection limit, precision, and recovery of the method was investigated.

INTRODUCTION

Creatinine and uric acid levels in human urine are important parameters of renal function. So, the determination of creatinine and uric acid is very important in clinical diagnosis. However, the routine methods for their measurements, such as photometric methods or enzymatic methods suffer from interference by various endogenous and exogenous compounds.

To avoid these interferences, high performance liquid chromatography (HPLC) methods were developed. Reversed-phase,[1] ion-exchange,[2] ion-pair,[3] column-switching,[4] and normal-phase[5] liquid chromatography were used for the simultaneous determination of creatinine and uric acid. HPLC method needs a lot of organic solvent and the chromatographic column is easily contaminated and hard to clean.

High performance capillary electrophoresis (HPCE) is a powerful separation and quantitation technique that often provides high resolving power, short analysis time, low operation cost, and very small sample volume. Guzman et al.[6] described a CZE method for determination of creatinine and urea. Jia et al. reported the determination of creatinine by CZE.[7] Masson et al.[8] analyzed uric acid in urine by CZE. Miyake et al.[9] separated creatinine and uric acid in urine within 18 min by micellar electrokinetic capillary chromatography(MEKC).

In this paper, we developed a simple, fast and precise CZE method for the simultaneous determination of creatinine and uric acid in urine. The method only requires filtering the urine sample prior to analysis. Compared with the MEKC method reported by Miyake et al.,[9] the analysis procedure reported by us is carried out in less than 10 min.

In this paper, the effect of pH of the background buffer on the existence state of creatinine and uric acid was studied. Photodiode array detection permitted the rapid identification of creatinine and uric acid in the sample analysed. The linear range, detection limit, precision, and recovery of the method was also investigated.

EXPERIMENTAL

Apparatus

An HP^{3D} CE system (Hewlett-Packard, Palo Alto, CA, USA), equipped with a diode array detector was used in all experiments. For data collection, data analysis, spectral identification, and system control, HP^{3D} CE software was used. Polyimide-coated fused-silica capillaries with 50 cm total length, 75 μm internal diameter were obtained from YongNian Photoconductive Fibre factory, Hebei, China.

The detection window was located 8.5 cm from the end of the capillary. Pressure injection (50 mbar, 5s) was used. The applied voltage was 15 KV. The capillaries were thermostated at 20°C. The absorbance values were recorded from 190 to 400 nm (using 234 nm as monitoring channel). The electroosmotic velocity was measured with dimethylformamide.

Chemicals and Solutions

Creatinine was purchased from Fluka. Dissolved 100 mg of creatinine in a little amount of distilled water, added 1.00 mL of concentrated hydrochloric acid, then diluted to 10 mL with distilled water. A 10 mg/mL of creatinine stock solution was prepared. The solution was stored at 4°C. Less concentrated standard solutions were prepared from the stock solution by dilution using distilled water.

Uric acid was obtained from Shanghai Chemical Reagent Company. Dissolved 100 mg of sodium carbonate in about 30 mL of distilled water, added 100 mg of uric acid, then diluted to 100 mL with distilled water. A 1 mg/mL of uric acid solution was prepared. The solution was stored at 4°C. Less concentrated standard solutions were prepared as needed from the stock solution by dilution using distilled water.

Sodium tetraborate, sodium phosphate, sodium monohydrogen phosphate, sodium dihydrogen phosphate and dimethylformamide were purchased in China.

The carrier electrolytes were prepared as to contain 25 mM of sodium tetraborate, whose pH was adjusted to 9.6 by addition of appropriate volume of concentrated sodium hydroxide solution. Unless otherwise specified, all chemicals were of analytical reagent grade. All solutions were prepared using filtered, degassed, and deionized distilled water.

Analytical Procedure

Urine samples were collected from healthy volunteers who were not taking any type of drug. These samples were passed through a 0.45 μm cellulose acetate syringe filter. The filtrates were then introduced directly into the CE system for the simultaneous determination of creatinine and uric acid. After each run, the capillaries were purged under vacuum for 3 min with 0.1 M NaOH, followed by electrophoresis buffer for 3 min. Because the urine samples were analysed directly without precleaning, it was necessary to use this rinsing procedure after each analysis to remove the impurities adsorbed on the CE column.

RESULTS AND DISCUSSION

Selection of Detection Wavelength

The UV spectrum of the standard creatinine was given in our previous work.[7] The maximum absorbance wavelength of creatinine was 234 nm. The

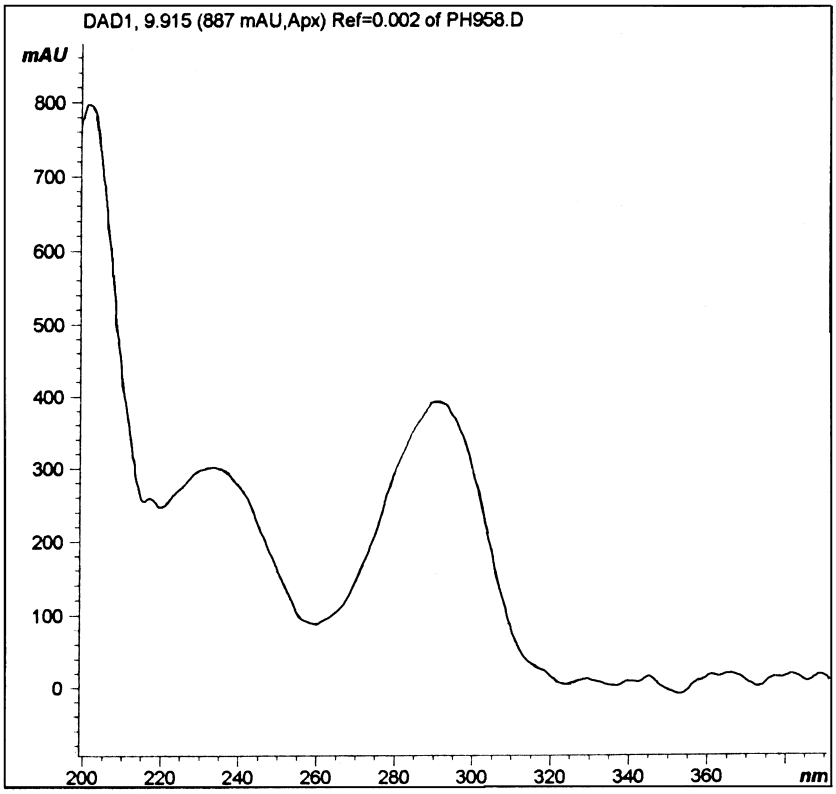

Figure 1. The UV spectrum of the standard uric acid at pH 9.6.

UV spectrum of the standard uric acid at pH 9.6, which was obtained by using the photodiode-array detector of HP^{3D} CE instrument, was shown in Figure 1. From Figure 1, we can see, uric acid has two maximum absorbance wavelength, which are respectively 234 nm and 290 nm. The absorbance at 290 nm is higher than that at 234 nm. In this paper, we use 234 nm as detection wavelength.

Effect of pH of the Background Buffer on the Existence States of Creatinine and Uric Acid

In our previous work,[7] the effect of pH of the background buffer on the existence state of creatinine was studied. When the pH of the background buffer is lower than 6.98, creatinine has a positive charge due to protonation. When the pH is higher than 6.98, creatinine is neutral.

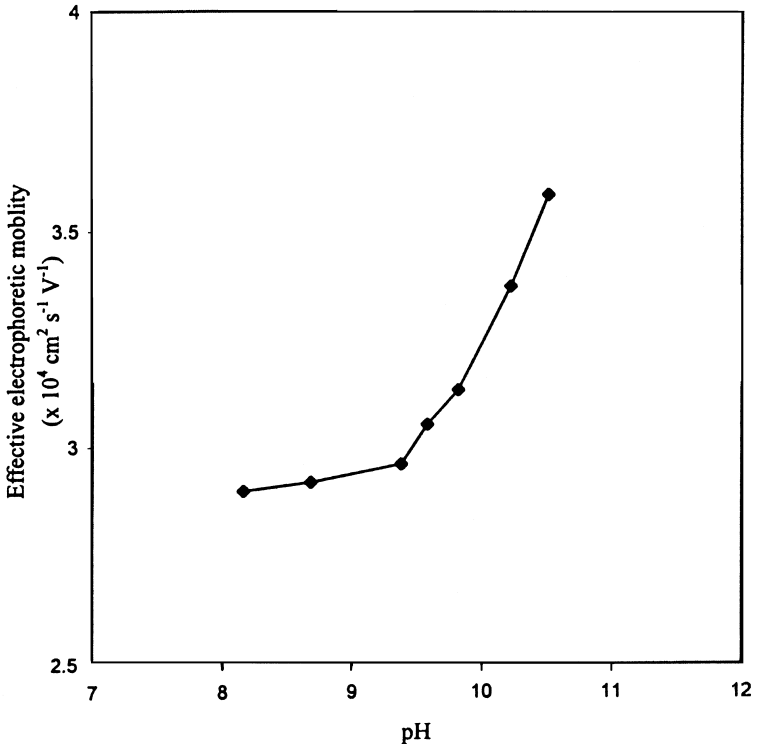

Figure 2. The relationship between pH of the background buffer and the effective electrophoretic mobility of uric acid.

In this paper, in order to study the effect of the background buffer on the existent state of uric acid, the solution containing 500 μg/mL of standard uric acid and 1% (v/v) of dimethylformamide as test solution, the pH of the background buffer containing 25 mM of sodium tetraborate was varied by addition of appropriate volume of 25 mM of phosphoric acid or concentrated sodium hydroxide solutions.

At first, we studied the effect of pH of the background buffer on the effective electrophoretic mobility of uric acid. Figure 2 showed the relationship between pH of the background buffer and the effective electrophoretic mobility of uric acid. In the basic buffer, uric acid eluted after neutral dimethylformamide. This illustrated that uric acid has a negative charge.

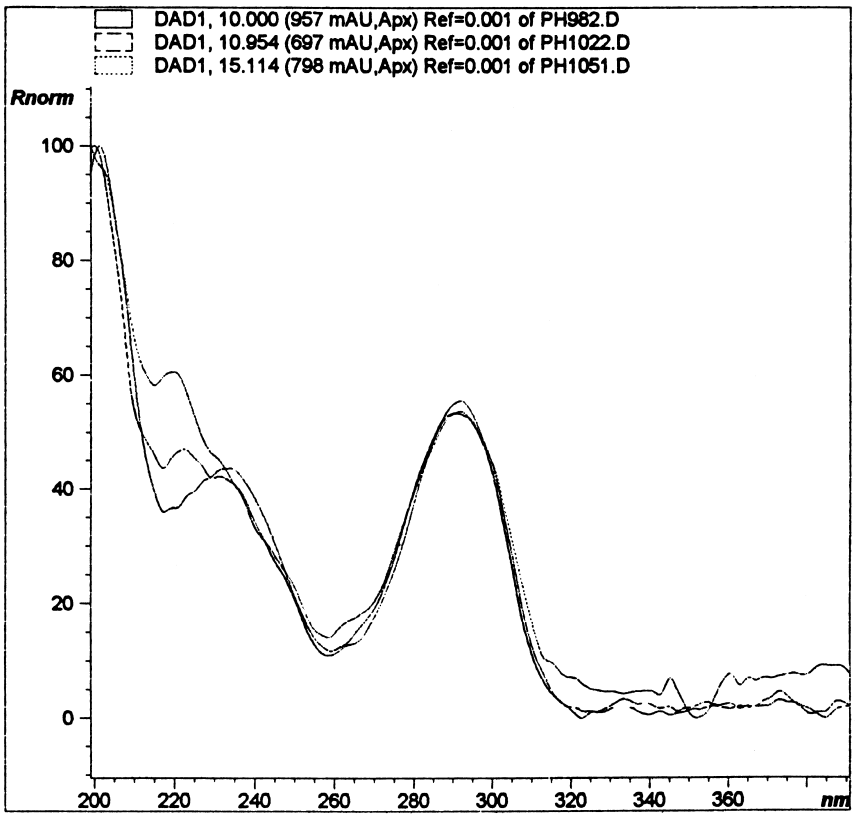

Figure 3. The effect of pH of the background buffer on the UV spectrum of uric acid.

From Figure 2, we can see that the effective electrophoretic mobility of uric acid increases with increasing pH of the background buffer. This is because uric acid has more negative charges when the pH of the background buffer increases.

Secondly, we studied the effect of pH of the background buffer on the UV spectrum of uric acid. Figure 3 gives the UV spectrum of uric acid at pH 9.8, 10.2, and 10.5. Our experimental results showed that the pH of the background buffer had no effect on the maximum wavelength at 290 nm. The maximum absorbance wavelength change at 234 nm took place when the pH of the background buffer increased.

When the pH of the buffer was lower than 9.8, the maximum absorbance wavelength at 234 nm had almost no change. When the pH of the buffer was higher than 10.2, the maximum absorbance wavelength at 234 nm moved to shortwave. When the pH of the buffer was 10.5, the maximum wavelength at 234 nm disappeared.

Effect of pH of the Background Buffer on the Separation of Creatinine and Uric Acid in Urine

At first, the pH of the background buffer solution was varied by mixing appropriate portions of 25 mM of sodium phosphate, 25 mM of sodium monohydrogen phosphate and 25 mM of sodium dihydrogen phosphate solutions. A urine sample was used as test solution.

The effect of pH of the background buffer on the separation of creatinine and uric acid in urine was investigated. When the pH was higher than 6.98, creatinine was separated from other substances in urine, but uric acid was not separated well. The peak shape of uric acid is fronting.

Next, in order to separate creatinine and uric acid in urine, a similar work was carried out using 25 mM of sodium tetraborate solution at different pH values as carrier electrolytes. When the pH of the background buffer containing 25 mM of sodium tetraborate was from 9.24 to 10.51, creatinine, uric acid, and other substances in urine were well separated, creatinine and uric acid were identified. The fronting of the peak of uric acid was minimized because uric acid and borate formed borate complex.

Figure 4 gives the representative electropherogram of urine at pH 9.24. In this paper, we used 25 mM of sodium tetraborate solution (pH 9.6) as a background buffer.

Identification of Creatinine and Uric Acid in Urine

Creatinine and uric acid in urine samples were identified by comparing their migration times with those of the standards. Pure standards of creatinine and uric acid were respectively added to the samples so that the peak areas of creatinine and uric acid were increased respectively. The UV spectrum of creatinine and uric acid in urine were taken and compared with those of their standards.

The peaks of creatinine and uric acid in the electropherogram of the urine sample have the same migration time as those of their standards. The UV spectra of the peaks of creatinine and uric acid in the urine samples obtained with the photodiode array detector were identical with those of their standards.

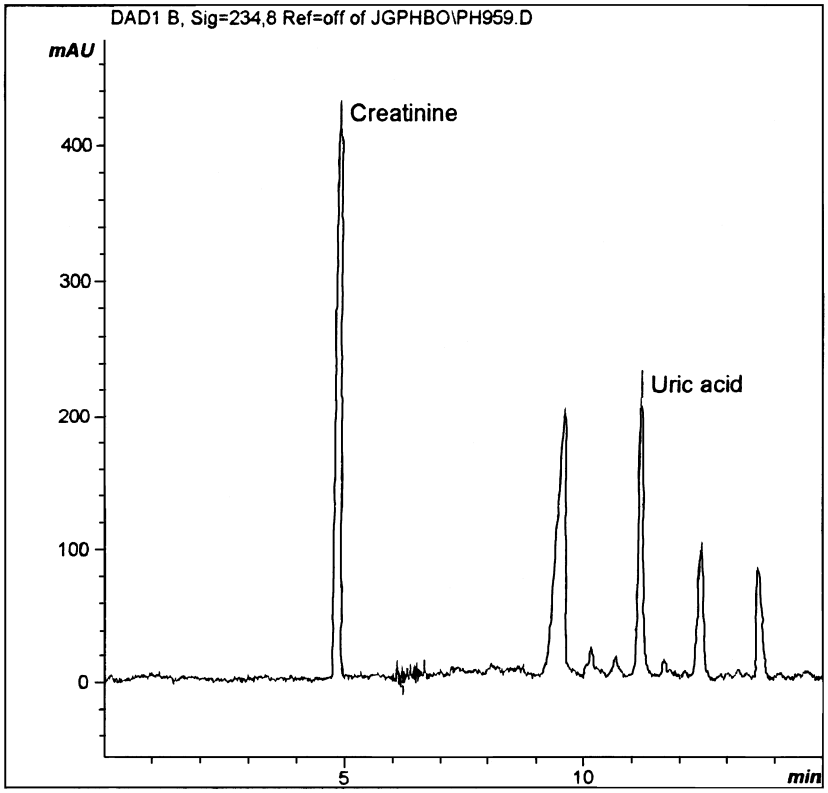

Figure 4. The typical electropherogram of urine at pH 9.6.

Quantitation

The linear calibration ranges for creatinine and uric acid are 0-2.0 mg/mL, 0-1.0 mg/mL respectively. The regression equations are as follows:

Creatinine, A=2.45C (r=0.9991);

Uric acid, A=3.94C (r=0.9997),

where A is the peak area in mAU*sec and C the concentration in μg/mL. The detection limits of creatinine and uric acid achieved at three times the signal-to-noise ratio are 10 μg/mL, 6 μg/mL, respectively.

Table 1

Analysis Results of Urine Samples

	Determined Content[a] (mg/mL)	RSD[b] (%)	Added (mg/mL)	Recovery[c] (%)
Creatinine	1.200	1.8	1.2	101 ± 2.5
	0.814	0.5	0.8	99 ± 1.0
	0.533	1.0	0.5	98 ± 1.6
	0.610	1.6	0.6	97 ± 0.6
	1.470	2.3	1.5	103 ± 1.2
Uric Acid	0.426	2.2	0.4	96 ± 2.1
	0.579	1.6	0.5	98 ± 1.0
	0.408	0.7	0.4	99 ± 1.4
	0.286	0.7	0.3	103 ± 1.2
	0.594	2.8	0.6	102 ± 2.4

[a] Average of three determinations. [b] Abbreviation of relative standard deviation. [c] Mean ± relative standard deviation (n=3).

Sample Analysis

The urine samples were analysed using the CZE method developed here. The analysis results are given in Table 1. For various amounts of creatinine and uric acid added to urine samples, the results of recovery studies are also listed in Table 1. These results indicate that this CZE method is suitable for the determination of creatinine and uric acid in urine.

CONCLUSIONS

The simultaneous determination of creatinine and uric acid by CZE was investigated. The method is simple, only requires filtering the sample solution, and takes only 10 min per sample. Photodiode-array detection permitted the rapid identification of creatinine and uric acid in the sample analysed.

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