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# Simultaneous determination of urinary creatinine, calcium and other inorganic cations by capillary zone electrophoresis with indirect ultraviolet detection

X. Xu, W.Th. Kok, J.C. Kraak, H. Poppe*

*Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, Netherlands*

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**Abstract**

A method for the rapid analysis of calcium and creatinine in urine has been developed using capillary zone electrophoresis without sample pretreatment other than dilution. Cations of $NH_3$, K, Na, Mg, Ca, Li, Ba and creatinine in human urine samples could be separated within 7 min. Background electrolyte (BGE) was composed of 5 mmol/l pyridine (chromophore), 3.6 mmol/l tartaric acid and 2 mmol/l 18-crown-6, having a pH of 4.05. An indirect UV detection mode was employed at 255 nm. Efforts were made to eliminate the interaction between cations and proteins and to minimize the electromigrative dispersion (EMD) for the analytes of interest by selecting a suitable BGE and sample dilution solution. Quantitative analyses were performed for calcium and creatinine. The calibration plots showed good linearity over the concentration range of interest to clinical analysis. Data on recoveries and reproducibilities are also reported. Results for urine samples (previously collected and frozen) from a variety of healthy and pathological individuals were in good agreement with those obtained by the Technicon SMA II calcium and creatinine methods.

## 1. Introduction

Calcium and creatinine occurring in urine have long been recognized as important metabolic products of diagnostic significance. Urinary calcium levels may be altered under several conditions [1] such as bone diseases, hyperparathyroidism, dietary calcium intake and vitamin D supplement. In some cases the variances in urinary calcium excretion are closely related to changes in bone status [2]. Urinary creatinine is not only used as an index of renal function [3] but also as a reference compound to compare excretion rates of other metabolites [1]. Usually the urinary calcium excretion is expressed as the calcium/creatinine ratio which is used to replace 24-h urine collection [2].

Urinary calcium and creatinine levels are measured routinely in many clinical laboratories. The most commonly used techniques today are the calcium method and the creatinine method performed on the Technicon SMA II computer-controlled, multichannel biochemical analyzer [4,5].

In the Technicon SMA II calcium method, calcium is separated from some interfering substances by dialysis under acidic conditions and determined colorimetrically using a metal com-

* Corresponding author.

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plexing dye. Although this method has wide clinical acceptance, disposal of the calcium waste (130 ml/h) containing potassium cyanide, which is used to eliminate the interference of heavy metals, presents a problem. The Technicon SMA II creatinine method is based on the Jaffe reaction, which produces a red color when picrate in an alkaline solution is added to creatinine. In this method, dialysis is also used to separate creatinine from proteins. However, there are a number of metabolites that may interfere with the red-color reaction.

Ion chromatography (IC) and high-performance liquid chromatography (HPLC) have been extensively used for analysis of creatinine in blood, serum, and urine [6,7] but less for calcium [8,9], probably due to the low sensitivity of the method, the complicated composition of the biological fluids and the strong interaction between calcium and proteins. In particular, the simultaneous separation of both monovalent and multivalent ions by those methods is possible only with specially designed columns [10,11].

There are also some other methods available to analyze both total metabolites in urine, including atomic absorption (AA) [12] and a turbidity test [13] for calcium, and an enzymatic technique [14] and micellar electrokinetic chromatography [15] for creatinine. However, in principle it is not possible to analyze both metabolites simultaneously with one of these methods.

Capillary zone electrophoresis (CZE) was recently introduced as an alternative for the simultaneous separation of ionic solutes (from amines to inorganic cations) in a simple and rapid way with high efficiency [16–18]. Sensitive detection of alkali and alkaline, metal ions, aliphatic amines and amino alcohols is based predominantly on indirect UV [18,19] or fluorometric modes [20] since those simple ions lack a specific chromophore. In one published method 8-hydroxyquinoline-5-sulfonic acid was used as a complexing agent for Ca and Mg and detection of the formed fluorescent complexes was accomplished [21]. Creatinine is usually electrophoresed at such a pH that it occurs as a monovalent cation. It can be detected directly because it shows strong UV absorbance in the range 200–300 nm [22,23].

Separations in CZE are based on differences in the electrophoretic mobilities of the analytes. Because some cations have very similar ionic mobilities, it is sometimes required to manipulate the mobilities by complexation to improve separation. Several papers have described the use of different complexing agents, e.g. $\alpha$-hydroxyisobutyrate (HIBA) [16] and tartrate [24], to increase the difference in electrophoretic mobility of the metals. By adding 18-crown-6, the resolution of ammonia and potassium has been improved [25]. It has been also found that optimal resolution and sensitivity could be obtained by matching the mobility of the analyte to that of the co-ion in the BGE [26–29].

Although in some papers the separation of calcium [8] and creatinine in biological fluids has been reported [21], very little attention has been given to the quantitative aspects of the analysis.

This paper describes our attempt to develop a practical CZE method for the simultaneous analysis of calcium and creatinine, as well as other cations, in urine collected from normal or pathological individuals.

## 2. Experimental

### 2.1. Apparatus

An ABI Model 270A-HT capillary electrophoresis system (Applied Biosystems, Foster City, CA, USA) was used throughout this study. The buffer volumes of the vials at the injection end and at the detector end were 4 and 10 ml, respectively. Plastic sample vials of 500 $\mu$l were used in all experiment. The polyimide-coated fused-silica capillary of I.D. 100 $\mu$m and O.D. 350 $\mu$m was obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was 81 cm and the effective length was 60 cm. The window for the on-column detector cell was created by burning off a small section (ca. 0.4 cm) of the polyimide-coating, and the excess residue was then wiped off with methanol. Indirect UV detection was performed at 255 nm. The electropherogram was recorded and evaluated on a Hewlett-Packard 3394 integrator.

### 2.2. Reagent and samples

High-purity grade creatinine, calcium chloride, 18-crown-6, and L-tartaric acid were purchased from Aldrich-Europe (Beerse, Belgium) and pyridine from Janssen (Beerse, Belgium). All other chemicals used were of analytical-reagent grade. Deionized and triply distilled water was used for the preparation of all solutions.

Background electrolytes (BGE) or solution were used as follows: BGE I: 5 mmol/l pyridine, 3.6 mmol/l L-tartaric acid and 2 mmol/l 18-crown-6 at pH 4.05, used for cation separations of urine; BGE II: 5 mmol/l pyridine, formic acid, varying concentrations of 18-crown-6 at pH 3.75, used for the evaluation of the influence of 18-crown-6 on the mobilities of the cations; BGE III: 5 mmol/l formic acid and sodium hydroxide at pH 4, used for the measurement of the effective mobility of pyridine; solution TC: 2.5 mmol/l L-tartaric acid, 2 mmol/l 18-crown-6, used for diluting urine samples. The BGEs were degassed under vacuum before use.

Urine specimens used for the determination of cations were collected from pathological individuals in the Academic Medical Center (Amsterdam, Netherlands) and stored at −20°C until analysis. The urines for the dilution factor tests were collected from a normal person and stored at 2°C. Standard solutions of a cation mixture were prepared daily from individual standard stock solutions of 100 mmol/l, which were stored at 2°C.

### 2.3. Procedures

*Urine sample dilution*

The frozen urine specimen was first thawed at room temperature. (The urine usually contains precipitates. However, the experiments were performed without removal of the precipitates.) The thawed urine specimen was shaken vigorously. Then immediately a fraction of the urine specimen (including the precipitates) was put into a sample vial, diluted with TC solution, and mixed well on a vibrator. The dilution factor [urine/(urine + TC)] usually was 1:50; this can be varied depending on the concentration of the analyte of interest in the sample. The fresh urine was diluted following a procedure similar to that described above.

*Electrophoresis*

A new capillary was flushed first with potassium hydroxide solution (pH 10) for 20 min, with water for 10 min and then with BGE I for 10 min. Each sample was introduced into the capillary by vacuum for 1 s at 12.7 cmHg. The components in the urine sample were separated at +25 kV (11 μA) for 9 min, maintaining the capillary at constant temperature (30°C). Separated components were monitored indirectly at 255 nm. As the used integrator can not deal with negative peaks, it is necessary that the peaks were recorded as positive by changing the polarity of the input signal. To ensure an optimal performance the capillary was flushed with BGE for 1 min at 50.8 cmHg after each electrophoretic run. The BGE was renewed after every 20 sample injections and the capillary was again treated with the potassium hydroxide–water–BGE cycle. Blank analyses were carried out with pure TC solution.

*Quantification and identification*

Calibration curves for both calcium and creatinine standards were performed under conditions identical to those used for the urine samples. The analytes of interest in urine were identified by comparing their migration times with those of the standards, or by spiking the urine sample with known standards and comparing their migration times. Quantitation was carried out by comparing the peak area of the analyte of interest in the urine with that of a standard solution. The recovery study was performed by measuring the difference in the concentration of the analyte in the urine and in the same urine spiked with known standard and comparing this with the added known concentration.

## 3. Results and discussion

The best resolution, separation efficiency and sensitivity can be achieved by choosing an appropriate detection mode and BGE composition,

through which the mobilities of the analytes can be manipulated. The selection of detection mode and BGE are based on the properties of the analytes and the sample matrix, which are summarized in Table 1.

### 3.1. Selection of detection mode

In the present CZE method, creatinine can be directly detected by UV detection at 200 or 234 nm. For calcium, however, due to the absence of a chromophore, indirect UV detection has to be used. In principle, the indirect UV detection method also allows the simultaneously detection of other inorganic cations in urine. Initially, indirect and direct UV detection at 205 nm were combined to measure calcium and creatinine in one run using 4 mmol/l copper(II) acetate and 8.5 mmol/l chloroacetic acid (pH 3.5) as BGE. In this system, calcium appeared as a negative peak and creatinine as a positive peak. However, data handling of peaks with different signs appears to be inconvenient, especially with the integrator presently used which can only integrate positive peaks. For this reason we decided to use indirect UV detection for both calcium and creatinine by selecting a wavelength where creatinine does not show UV absorption.

The principle of indirect detection is based on the displacement of UV-absorbing ions present in the BGE by UV non-absorbing sample ions [30]. This displacement causes a decrease of the background signal in the sample zone resulting in a negative peak. A higher sensitivity of the indirect UV detection can be obtained by a chromophore with a stronger UV absorption and a higher displacement ratio. Generally, the lower the charge of the background chromophore, the higher the displacement ratio. Pyridine was selected as the background chromophore in this experiment not only because it exhibits a sufficiently high UV absorption ($\epsilon = 3000$) at 255 nm where creatinine is UV transparent, but also because it appears as a mono-charged ion in the pH range used.

Table 1
Urine and BGE data

| No. | Substances | Typical concentration in urine [1] (mmol/l) | Effective mobility ($cm^2/V/s \cdot 10^{-5}$) | | $pK_a$ |
|---|---|---|---|---|---|
| | | | BGE II[a] | BGE I[a] | |
| 1 | Ammonia | | 78.66 | 78.23 | |
| 2 | Potassium | 70 | 78.50 | 71.83 | |
| 3 | Sodium | 200 | 54.49 | 54.45 | |
| 4 | Magnesium | 3 | 53.92 | 48.18 | |
| 5 | Calcium | 3 | 60.96 | 43.84 | |
| 6 | Lithium | | 42.52 | 42.68 | |
| 7 | Barium | | 65.33 | 40.95 | |
| 8 | Creatinine | 10 | 35.36 | 33.22 | 4.7 [33] |
| 9 | Pyridine | | 48.21[b] | 48.21[b] | 5.7 [33] |
| 10 | L-Tartaric acid | | | | 3.2<br>4.3 [34] |
| 11 | System peak | | 30.71 | 23.43 | |
| 12 | Electroosmotic flow | | 13.56 | 12.70 | |

[a] Composition is described in Experimental.
[b] Measured by BGE III.

### 3.2. BGE selection

*Concentration overloading*

In electrophoresis one is often confronted with the problem of concentration overloading, which can result in broad triangular shaped peaks and hence impairs the resolution and the sensitivity. Overloading, also termed electromigration dispersion (EMD), results from the dependence of the migration velocity of a sample constituent on its concentration in the sample zone [25]. The effect of EMD becomes more pronounced when the effective difference in mobility between the analyte ions and the co-ion of the BGE is large or when the ratio of the sample concentration to the BGE concentration is large. Hence EMD can be minimized either by keeping a sufficiently low concentration ratio of the analyte to the co-ion of the BGE (typically less than 1:100) in the analyte zone, or by matching the effective mobility of the analyte of interest to that of the co-ion of the BGE. However, increase of the concentration of the BGE will lead to an insufficient heat dissipation in the capillary and a smaller sensitivity of the indirect detection [31]. In Table 1, cations possibly occurring in urine are listed. As can be seen, the mobilities of these cations differ significantly. It is not possible to use only one BGE chromophore to match the mobilities of all analytes. Although more symmetrical peaks can be realized by using a BGE containing multichromophore co-ions with different mobilities [32], the disturbances of the system peak which occurs somewhere between every two symmetric peaks, as has been predicted by the computer simulation program for electrophoresis [28], are undesirable. Therefore EMD is inevitable and a compromise has to be found.

The mobilities obtained with BGE II are comparable to the mobilities of the free ions [20]. This indicates that the counter-ion of the BGE, formic acid, shows very little interaction with all cations. The use of BGE II presents two problems. First the differences in the mobilities of ammonia and potassium are too small to separate these analytes and secondly the large difference in mobility between calcium and creatinine made it very difficult to select a background co-ion to match them. As mentioned before, pyridine is a suitable ion for monitoring in the indirect detection method. It has a mobility close to that of sodium and not too different from that of creatinine. This implies little peak deformation for sodium and an acceptable peak shape for creatinine. This is particularly advantageous since sodium is usually present in large quantities in urine and its overloading will seriously impair the resolution of peaks close to it, e.g calcium.

*Necessity of complexing agent*

Complexing agents are added to BGE I in order to (1) manipulate the mobilities so as to improve the separation and minimize overloading, (2) release calcium from the calcium–protein complexes.

Adding 18-crown-6 [25] to the BGE resulted in the separation of potassium from ammonia and in a change of the migration order for barium (Fig. 1a). By using tartaric acid instead of formic acid in BGE I, the migration order of calcium, magnesium and sodium was altered while the order of barium and lithium was reversed as well (Fig. 1b). The optimal concentration of tartaric acid for the separation of the eight ions listed in Table 1 was 3.6 mmol/l. Fig. 2 shows the separation of the cations in standard solution (Fig. 2a) and in urine specimens (Fig 2b–d) with BGE I. The fact that magnesium shows the sharpest peak indicates that it has the smallest difference in mobility with pyridine, which also can be seen in Table 1. Due to complex formation between calcium and tartrate, the calcium peak has moved from the left to the right of the sodium peak. This leads to a narrower peak for calcium since its effective mobility is closer to that of pyridine than in the case where BGE II was employed. Moreover, overlap by the potassium or sodium peaks is avoided when these analytes are present in a considerable quantity and hence are overloaded in the sample concentration range where calcium can be detected (Fig. 2d).

Cations in urine mainly exist in two forms, i.e. free cation and protein-bound cation [8,9]. In a primary experiment, the earlier mentioned BGE I, composed of copper(II) acetate–chloroacetic

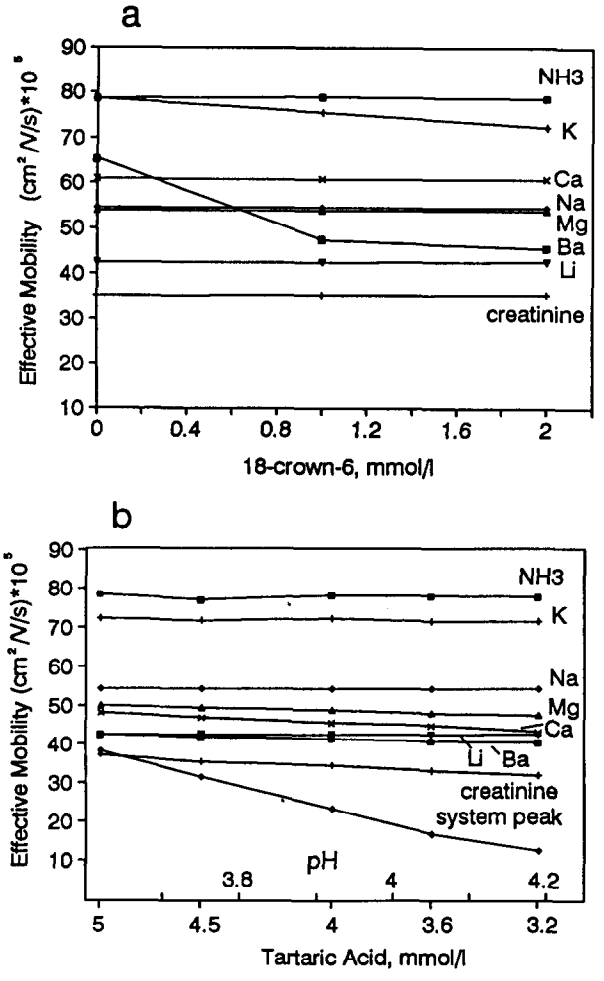


Fig. 1. The influence of 18-crown-6 (a) and tartaric acid (b) on mobilities of cations. BGEs are BGE II (a) and BGE I (b) but with varying amounts of 18-crown-6 and tartaric acid, respectively. All other details are as described in Table 1.

acid, and BGE II were used for urine samples. For fresh urine, the area of the calcium peak linearly increased only with an increase of the dilution factor up to 1:100 [urine/(BGE + urine)]. For precipitate-containing aged urine, only a little calcium peak appeared or it did not show at all. These phenomena remained when different solutions with different pHs were used to dilute the urine. Since the Ca–protein binding in the presence of copper(II) is small, the effects observed with BGE I and BGE II might be attributed to strong absorption of calcium on the precipitate or even inclusion. This assumption is supported by the fact that with BGE I, containing tartaric acid which readily forms complexes with Ca, these above mentioned effects did not occur and a linear relationship was found between the peak area of calcium and creatinine and the dilution factor (Fig. 3).

*Other considerations*

The concentration of pyridine is another important parameter of the BGE since indirect detection is used. A lower concentration of the BGE constituents will improve the detection sensitivity but may lead to overloading and less stable conditions, which e.g. could lead to changes in the electroosmotic flow (EO). A higher concentration decreases the signal-to-noise ratio and increases the heat effect, especially in this experiment where a 100-$\mu$m I.D. capillary is used to increase the detection sensitivity in order to meet the clinical analysis range required for the urine samples. The optimum pyridine concentration in BGE I was found to be 5 mmol/l.

The pH not only has an effect on the mobility of the creatinine and the system peaks but also on the baseline noise level. With decreasing pH, the effective mobility of creatinine increased and peak shape was improved. However, as shown in Fig. 1b, the system peak moved closer to the creatinine peak (pH 3.75) or even merged with this peak (pH 3.62) while at the same time the low-frequency noise level increased for unknown reasons. At pH 4.05, a good compromise between creatinine peak shape and noise level was obtained.

### 3.3. Urine sample preparation

The freshly collected clear urine samples become turbid after standing for a few minutes to a few days or soon after they have been put into the refrigerator. Usually urine specimens are kept at low temperature or frozen to prevent urine deterioration or modification [1] when they are not analyzed immediately after collection. The turbidity is most probably caused by the precipitation of proteins. Removal of the precipitate leads to a loss of calcium [1,8,9]. This was

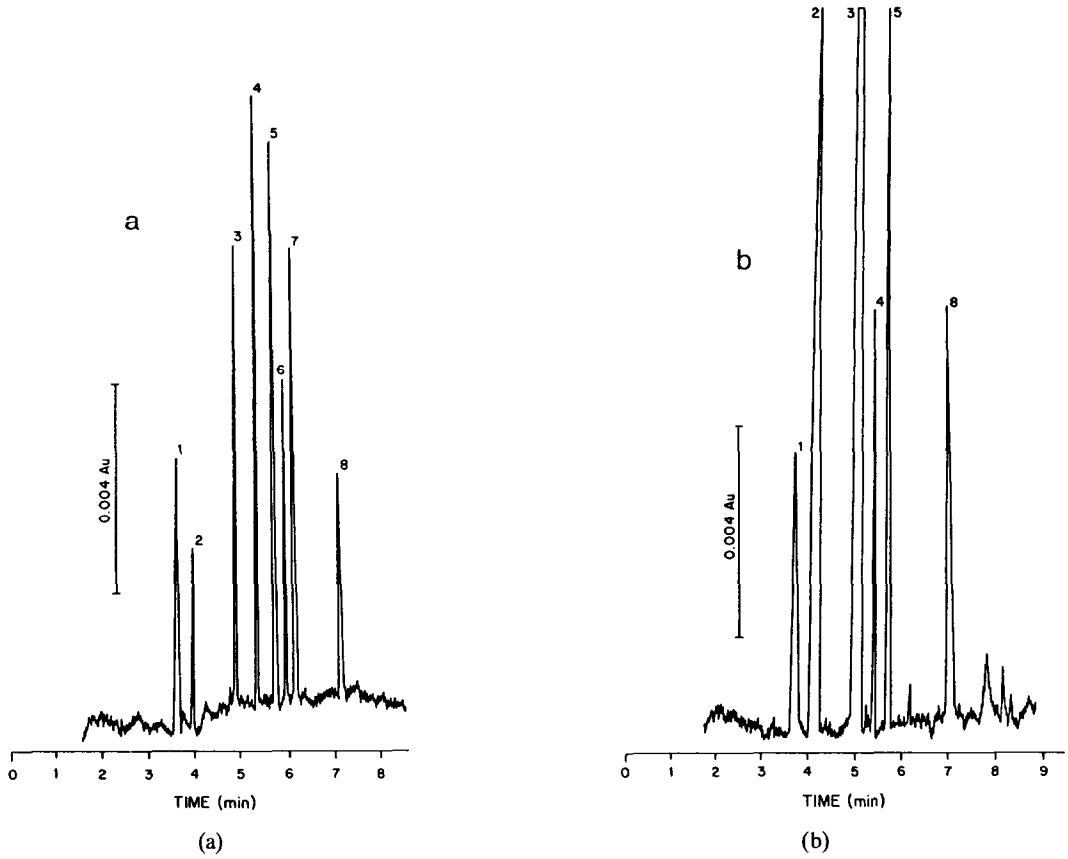


Fig. 2. Electropherograms of standard solution (a), urine samples from a normal person (b), a patient with complicated urinary composition (c), and a patient with extremely low urinary calcium level (d). Electrophoresis was performed with BGE I. The concentration of each cation in the standard solution is 0.1 mmol/l. Dilution factors and urinary levels (mmol/l) of calcium and creatinine for each urine specimen are 1:50, 4.94, 6.06 (b); 1:50, 3.03, 6.56 (c); 1:25, 0.29, 17.85 (d). The numbers of the peaks are according to Table 1. Conditions are as described in Experimental.

evidenced by a much low recovery of calcium when a known quantity of calcium was added to urine and only the clarified liquid was taken for analysis.

In the CZE method used in this paper, an aliquot of a urine specimen with precipitate was diluted with TC solution and directly injected onto the capillary. The use of TC solution, consisting of 2.5 mmol/l tartaric acid and 2 mmol/l 18-crown-6 (pH 2.9), has several advantages. Firstly, the TC solution has a much lower conductivity than the BGE. By stacking, the analytes are first concentrated in the injection zone and then electrophoresis is started; thus a narrower and higher peak is achieved. Secondly, the acidification of the urine samples (with a pH in the range 4.5–8.2) and the complexing effect of tartrate, decrease the cation–protein interaction. Concentrations of tartaric acid higher than 2.5 mmol/l have to be avoided, since these were found to lead to double peaks for ammonia and potassium. It should be noted that without 18-crown-6 broader calcium peaks are observed. The reason is not clear.

### 3.4. Reproducibility

Table 2 shows the reproducibility of the peak areas and the migration times of calcium and creatinine for two different injection times.

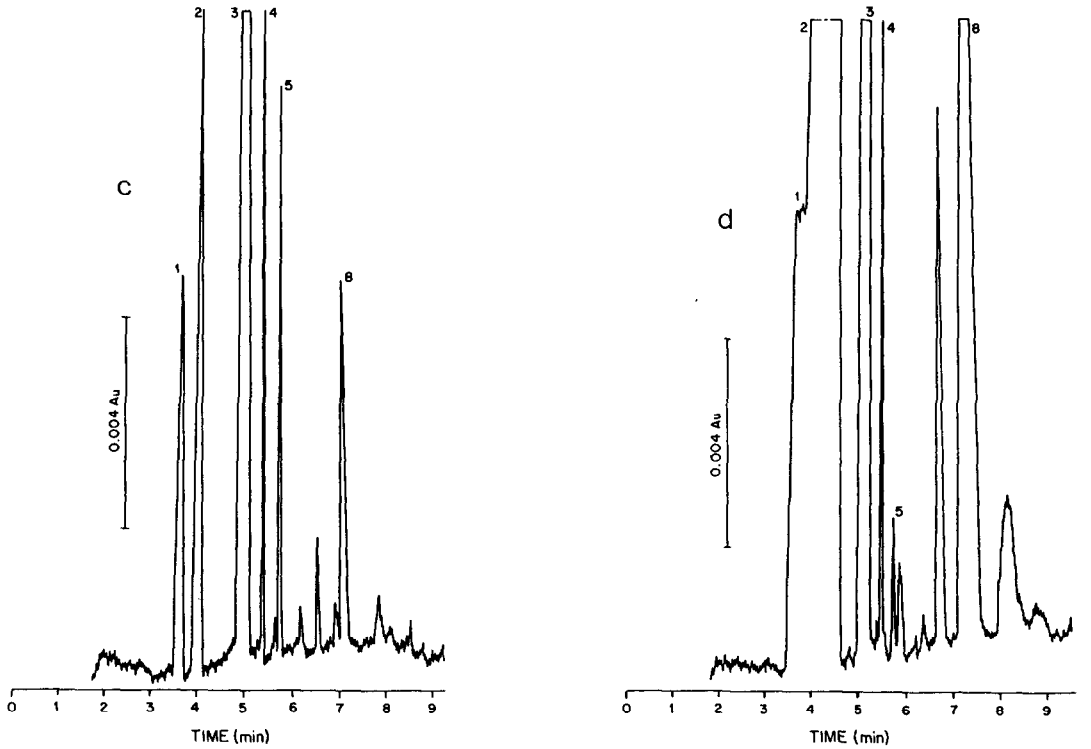


Fig. 2. (Cont).

The differences in migration time in all likelihood are caused by changes in the electroosmotic flow. Normally a very stable EO (S.D. of the migration time $< 1\%$) can be maintained for 20 injections just by flushing the capillary with BGE after each run. In one experiment a significant change in the EO was observed when a dilution factor of 1:10 was used. This probably resulted from contamination of the capillary wall by absorbing proteins. However, the EO can be easily restored by the potassium hydroxide–water–BGE flushing cycle. It was found necessary to check whether extra $NH_4^+$, $K^+$, $Na^+$, or $Ca^{2+}$ ions have been introduced after the cleaning cycle. This can be done by injecting the TC solution and measuring the peak heights of these cations. Under optimal conditions the peak heights of these cations (blank values) should not exceed 0.001–0.002 AUFS. The EO is also dependent on the pH. The pH of the BGE slowly but continuously decreases during electrophoresis and so the EO decreases. To keep a

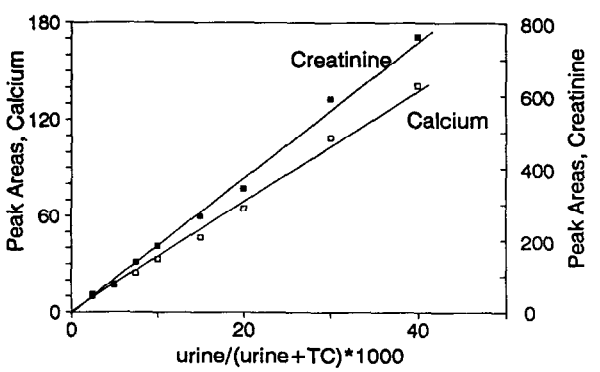


Fig. 3. Peak area as function of dilution factor for urinary calcium and creatinine. Other conditions as in Fig. 2.

Table 2.
Comparison of the reproducibility of migration times and peak areas for calcium and creatinine for different injection times

| Injection time | C (mmol/l) | Calcium ($n = 12$) | | | Creatinine ($n = 12$) | | |
|---|---|---|---|---|---|---|---|
| | | Plate number | Migration time[a] (min) | Peak area[a] | Plate number | Migration time[a] (min) | Peak area[a] |
| 0.4 s | 0.04 | 214 000 | 5.73 ± 0.035 (0.6%) | 98.1 ± 9.5 (9.7%) | 122 000 | 7.19 ± 0.064 (0.9%) | 69.6 ± 7.0 (10.0%) |
| | 0.10 | 107 000 | 5.69 ± 0.035 (0.6%) | 226 ± 19.2 (8.5%) | 74 000 | 7.10 ± 0.089 (1.3%) | 171 ± 14.8 (8.6%) |
| 1.0 s | 0.04 | 140 000 | 5.74 ± 0.010 (0.17%) | 201 ± 6.6 (3.3%) | 73 600 | 7.13 ± 0.014 (0.19%) | 144 ± 9.05 (6.2%) |
| | 0.10 | 71 500 | 5.70 ± 0.006 (0.11%) | 499 ± 16 (3.2%) | 34 800 | 7.08 ± 0.012 (0.17%) | 387.6 ± 20.3 (5.2%) |

[a] Migration time and peak area are given as mean ± S.D. Values in brackets represent relative standard deviation.

constant migration time of the analytes fresh BGE should be used after approximately every 20 injections.

In order to eliminate the influence of the sample matrix, injection of the samples was performed by introducing the sample solution into the capillary under vacuum to ensure introduction of a certain amount of analytes, although higher sensitivity could be obtained by electrokinetic injection [17]. Injection with 0.4 s (ca. 0.29 mm in injection length) led to a higher plate number but poorer area reproducibility and less sensitivity. Injection with 1.0 s (ca. 0.73 mm in injection length) resulted in both acceptable separation efficiency and reproducibility.

### 3.5. Calibration and detection limit

The calibration range for calcium and creatinine was found to be linear up to 0.6 mmol/l. The regression line parameters were $y = -7.04 + 4601x$ ($r^2 = 0.9993$) for calcium and $y = -16.12 + 4028x$ ($r^2 = 0.9971$) for creatinine ($n = 5$ for both cases). In order to minimize

Table 3.
Recovery of calcium and creatinine from spiked urine samples

| Sample No. | Present in urine (mmol/l) | Added (mmol/l) | Found (mean ± S.D.) ($n = 3$) (mmol/l) | Recovery (%) |
|---|---|---|---|---|
| *Calcium* | | | | |
| 1 | 4.00 ± 0.03 | 4 | 8.07 ± 0.19 | 101.8 ± 0.05 |
| 2 | 0.62 ± 0.02 | 1 | 1.60 ± 0.01 | 103.0 ± 0.02 |
| 3 | 1.54 ± 0.03 | 2 | 3.43 ± 0.03 | 94.5 ± 0.02 |
| 4 | 4.08 ± 0.05 | 5 | 9.33 ± 0.14 | 105.0 ± 0.03 |
| *Creatinine* | | | | |
| 1 | 5.88 ± 0.12 | 4 | 9.84 ± 0.26 | 99.0 ± 0.07 |
| 2 | 6.53 ± 0.08 | 4 | 10.30 ± 0.07 | 94.3 ± 0.03 |
| 3 | 2.34 ± 0.06 | 2 | 4.25 ± 0.06 | 95.5 ± 0.04 |
| 4 | 14.71 ± 0.60 | 10 | 24.86 ± 0.22 | 101.5 ± 0.06 |

concentration overloading and to obtain high resolution for complicated urine samples, it is essential to keep the concentration of calcium and creatinine in the injection liquid below 0.2 mmol/l (preferably around 0.1 mmol/l).

Using a signal-to-noise ratio of 3, the dynamic ranges for the detection of calcium and creatinine by the CZE method for the standard sample are $1.04 \cdot 10^{-3}$–0.5 mmol/l and $2.3 \cdot 10^{-3}$–0.5 mmol/l, respectively and for the urine sample at dilution factor 1:50 the ranges are 0.052–25 mmol/l and 0.12–25 mmol/l, respectively. Compared to the clinical ranges of calcium (0.1–20 mmol/l) and creatinine (2–25 mmol/l), most urine samples can be analyzed at a fixed dilution factor 1:50 except when concentrations are too low. This is of a great advantage when a large number of urine samples have to be analyzed in an automated manner.

### 3.6. Quantitation and interference

Four urine specimens collected from pathological individuals with different urinary calcium and creatinine levels have been used for the recovery study. The results listed in Table 3 indicate that recoveries of calcium and creatinine from urine samples ranged, respectively, from 94.5 to 105% and from 94.3 to 101.5%.

The method described in this work has been compared with Technicon SMA II calcium and creatinine methods. In Fig. 4, the concentrations in urine samples collected from 17 pathological individuals as determined with the alternative assays were compared with the CZE results and a reasonable agreement was obtained. Correlation coefficients of 0.996 and 0.967 were found for calcium and creatinine, respectively. An exception was observed for sample no. 9, in which a dark yellow precipitate developed other than the usually white one. The urinary calcium level measured by the Technicon SMA II method for this sample was 20.64 mmol/l, where the result from the CZE method was 8.09 mmol/l. These data were not included in the comparison. It should be noted that the assays with the Technicon SMA II methods were performed soon after the urine specimens had been collected, while the analysis by the present CZE method was carried out two months later. Between the two analytical operations, the urine specimens were frozen and kept at −20°C.

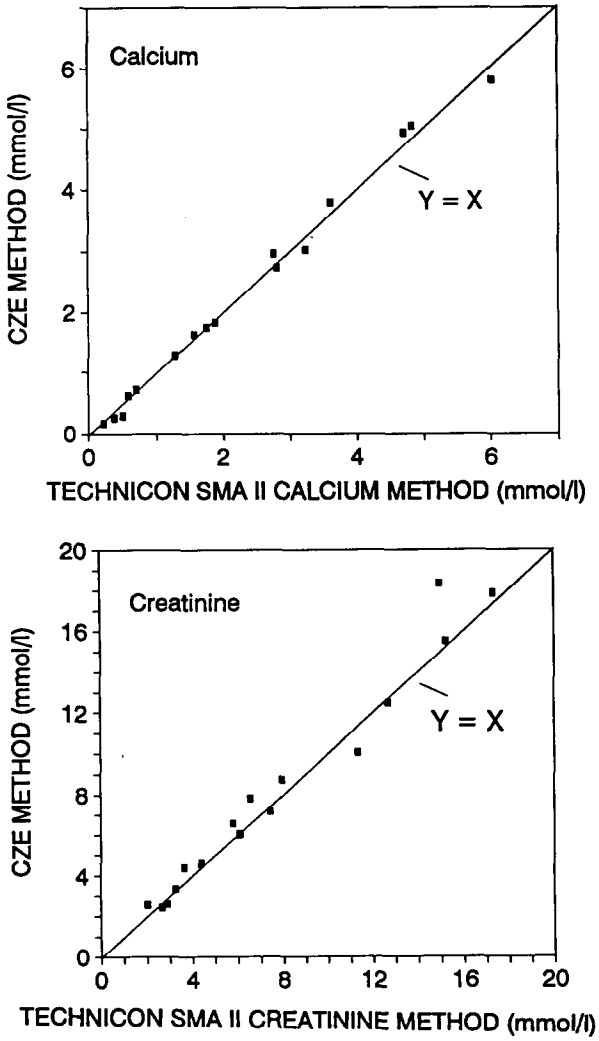


Fig. 4. Correlations between CZE method and Technicon SMA II methods. The regression line parameters obtained from this study are (calcium) $y = -0.03 + 1.013x$, $r^2 = 0.9960$, $n = 16$; (creatinine) $y = -0.03 + 1.052x$, $r^2 = 0.9671$, $n = 17$.

Calcium and creatinine, as well as ammonia, potassium, sodium and magnesium, were well separated from other peaks in all 17 urine samples, except for the case shown in Fig. 2d. In this single case a dilution factor of 1:25 had to be used to obtain a large enough signal. As a result

the ammonia and potassium peaks overlapped; this is caused by overloading. The sample shown in Fig. 2c is the most complicated one among the group of 17 urine samples. There are more small peaks appearing after the sodium peak. However usually those components are present in urine only in a small amount. Their interferences will be negligible in most cases.

## 4. Conclusions

Using the CZE method developed in this work, calcium, creatinine, and some other cations in urine can be determined simultaneously in a simple and rapid manner. Urine samples are prepared only by dilution. The quantitative study for calcium and creatinine indicates that this method is well suited to the clinical range. The good reproducibility and a wide dynamic range of detection allow the analysis of urine samples to be performed automatically on a large scale.

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