Asian Journal of Chemistry; Vol. 27, No. 2 (2015), 705-708

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.17792

Determination of Creatinine in Human and Rat Urine by Capillary Zone Electrophoresis

PINGPING ZHANG[1], XIAOLI WU[2], XIAOYU TIAN[2], MIN YAO[1], XIN LU[1], KAOQI LIAN[3,*] and LINGLING JIANG[1,*]

[1]Department of Biochemistry and Molecular Biology, Institute of Basic Medicine, Hebei Medical University, Shijiazhuang, P.R. China
[2]Department of Pediatrics, the Second Hospital of Hebei Medical University, Shijiazhuang, P.R. China
[3]Department of Sanitary Inspection, School of Public Health, Hebei Medical University, Shijiazhuang, P.R. China

*Corresponding authors: E-mail: guiyang1959@126.com; liankaoqi@126.com

Received: 10 April 2014; *Accepted*: 27 June 2014; *Published online*: 10 January 2015; AJC-16650

Creatinine is a very useful clinical marker of renal function and an internal standard to correct the excretion rate of other biological compounds in a urine sample. In this paper, a reliable, convenient and low-cost method for the determination of urinary creatinine by capillary zone electrophoresis was described. A typical calibration plot from 1 to 200 mg L^{-1} (R^2 = 0.9978) with a detection limit (LOD) of 0.15 mg L^{-1} (S/N = 3) is achieved. The relative standard deviation (RSD) was less than 4.2 % for peak area and 0.5 % for migration time, respectively. The proposed method was successfully applied to the determination of creatinine in both human urine and rat urine.

Keywords: Creatinine, Capillary zone electrophoresis, Human urine, Rat urine.

INTRODUCTION

Creatinine, with a chemical name 2-amino-1-methyl-1*H*-imidazol-4-ol, is a widely studied zwitterionic compound within the bioanalytical field[1]. It is the catabolic end product of creatine, being produced in muscle tissues and eliminated from body in urine[2]. Nowadays urinary creatinine has routinely been used as an indicator for evaluating the state of renal[3] and muscle function[4], and as an internal standard for normalizing the excretion rate of some biomolecules, such as desmosine (DES), isodesmosine (IDES)[5], glucose[6], albumin[7], and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in urine[8]. On this basis, urinary creatinine determination is very important not only in clinical evaluation but also in analytical research.

Since the first method for the determination of creatinine by spectrophotometry was introduced by Jaffe[9] in 1886, various measurement methods have been developed for creatinine determination including enzymatic method[10], high-performance liquid chromatography with low wavelength UV detection (HPLC-UV)[11], high-performance liquid chromatography-mass spectrometry (HPLC-MS)[12], gas chromatography-mass spectrometry (GC-MS)[13] and micellar electrokinetic chromatography (MEKC)[14]. These reports are collectively well suited for the assay samples and show good sensitivity for creatinine determination. However enzymatic method needs the enzyme with high purity, GC-MS often requires chemical derivatization, HPLC-MS costs much more. Although Jaffe techniques show more accurate at lower concentrations, a major limitation is that it is highly non-selective. Capillary electrophoresis (CE) is a powerful analytical technique with significant importance in drug discovery and life sciences because of its small sample size capability, unique separation mechanism, efficiency, speed, versatility and automation. Capillary electrophoresis has been used for separation and quantization of creatinine[14-17] in many reports. Among all kinds of capillary electrophoresis modes, capillary zone electrophoresis with UV detection (CZE-UV) is the most common, simple and popular mode. CZE-UV methods, without precolumn treatments, appear to be the simplest options for the analysis of creatinine compared with many other current analytical methods.

In capillary zone electrophoresis mode, the analyte molecules move freely through the capillary driven by both the electrophoretic mobility and the electroosmotic flow (EOF). Since creatinine has two pKa values[1] at 4.8 and at 9.2, and the isoelectric point (pI) of creatinine is at a pH value about 7, it can be separated from other urinary components using free-solution capillary zone electrophoresis at a pH below 7 (where it is cationic) or at pH above 7 (where it is anionic). But when the pH value of background electrolyte (BGE) is 7, creatinine is zwitterionic with zero net charge. It moves only with electroosmotic flow because the electrophoretic mobility is zero. Under such condition creatinine moves in the zone of electro neutral compounds together with other compounds from urine, accordingly it cannot be separated from other neutral compounds effectively. A number of the previous reports evaluated creatinine at a pH below 4[15-17], although the separation could

be better in the mentioned acidic background electrolyte, higher sensitivity and the maximum peak height were obtained at a pH value of 6 in present work.

A reliable, convenient and low-cost CZE-UV method for the determination of creatinine in human spot urine and 24 h rat urine was described in this paper. Method development efforts were focused on the optimization of a series of parameters affecting the capillary zone electrophoresis separation efficiency and sensitivity. Finally, the developed method has been validated for the analysis of the urinary creatinine in real samples with better results.

EXPERIMENTAL

Creatinine and dimethyl sulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, USA). Analytical reagent grade phosphoric acid (H_3PO_4), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), sodium phosphate monobasic dehydrate ($NaH_2PO_4 \cdot 2H_2O$) and sodium hydroxide were purchased from Tianjin General Chemical Reagent Factory (Tianjin, China). All solutions were prepared with ultra-pure water from a Milli-Q water system (Millipore Corp., Bedford, MA, USA) and were filtered through a 0.22 μm membrane before use. Stock solutions of creatinine (1 g L^{-1}) were stored at 4 °C until use. The pH measurements were performed with a PB-10 pH meter (Sartorius, Germany).

Capillary electrophoresis analysis: Capillary electrophoresis analysis was performed on a Beckman Coulter ProteomeLab PA 800 (Beckman Coulter, Inc., Fullerton, CA, USA) instrument, and operated in normal polarity mode. Data acquisition and instrument control were carried out using 32KaratTM software 8.0 (Beckman Coulter, Inc., Fullerton, CA, USA). An uncoated fused-silica capillary of 50 × 75 cm i.d., 40 cm from inlet to detector was used for the determinations. The new capillary column was flushed successively with methanol (5 min), 0.1 mol L^{-1} NaOH (20 min), ultrapure water (5 min) and then background electrolyte (5 min). The in-between runs rinsing cycles were carried out by pumping sequentially through the capillary: 0.1 mol L^{-1} NaOH (4 min), water (4 min), and background electrolyte (4 min). Separation was carried out by applying a voltage of 10 kV. The capillary cartridge temperature and sample storage temperature were maintained by a liquid cooling system at 20 and 15 °C, respectively. The injection was hydrodynamic at a pressure of 0.5 psi for 5 s. Detection was performed by an ultraviolet detector (UV) at 200 nm.

Urine sample collection and pretreatment: Eight female Sprague-Dawleys (SD) rats weighing 190-210 g were purchased from the Experimental Animal Center of the Hebei Medical University (Shijiazhuang, Hebei Province, China) and housed in a room with an ambient temperature of 20-23 °C, 12 h light and 12 h dark cycles, and relative humidity of 50 ± 10 %. The rats were given a normal standard chow diet and freely available tap water. Urine was collected individually at 9 AM after a period of 24 h using a stainless steel metabolic cage.

Human spot urine samples were obtained from seven healthy adult volunteers between the ages of 26 and 40 years (two men and five women).

All the urine samples were centrifuged within 2 h after collection at 4000 rpm for 5 min at 4 °C. Afterward, 100 μL of each urine supernatant was diluted to 1 mL with background electrolyte for capillary electrophoresis analysis directly.

RESULTS AND DISCUSSION

Effect of background electrolyte concentration: In capillary electrophoresis analysis, optimization of the background electrolyte composition is crucial for method development because the background electrolyte composition affects many important factors such as EOF, Joule heating and electrolyte viscosity. Different types and concentrations of background electrolyte, including citrate, acetate, borate, and sodium phosphate, were compared in terms of resolution, sensitivity and peak shapes. Citrate buffer and phosphate buffer provided better performance than the other electrolytes. Phosphate buffer is the most commonly used buffer in lab, with a wider pH buffer range compared with other electrolytes, consequently phosphate buffer was selected.

The effect of phosphate concentration in background electrolyte was studied within the range 10-60 mmol L^{-1}. The pH was adjusted to 6 and creatinine concentration was fixed at 50 mg L^{-1}. As the phosphate concentration increasing, the migration time was prolonged and the current value was increasing. In order to avoid high Joule heating caused by increased current value, the 50 mmol L^{-1} phosphate concentration was selected.

Effect of background electrolyte pH: The pH value of background electrolyte has been recognized as one of the most important parameters affecting capillary electrophoresis performance since it can influence mobility of analyte by adjusting EOF velocity and the ionic charge of analyte molecules. Accordingly, the influence of a running buffer with a pH ranging from 2.5 to 9 was studied (Fig. 1). Phosphoric acid (1 mol L^{-1}) was used as an acidifying agent and NaOH (0.1 mol L^{-1}) was used as an alkalizing agent respectively. The background electrolyte was 50 mmol L^{-1} phosphate buffer and creatinine concentration was fixed at 50 mg L^{-1}. The result demonstrated that the highest peak height and the biggest peak area were obtained at the pH value of 6. Interestingly, when the background electrolyte was at a pH value of 7, we got a flat-top shape peak, and a turning point of the peak area is emerged at pH 7 (Fig. 1). This due to the isoelectric point (pI) of creatinine is 7. When the pH value of background electrolyte is the same as its isoelectric point, creatinine molecule carries the same amounts of both positive charge and negative charge, and the net charge is zero. Under such condition, creatinine moves only with EOF because the electrophoretic mobility is zero. In addition, we carried out experiment to prove that the peaks of creatinine and EOF (detect by using DMSO) can be separated effectively (Fig. 2) at pH 6. Take all the factors into account; the optimum pH value of 6 was therefore applied to obtain better peak shapes and higher sensitivity.

Effect of detection wavelength: The PA 800 system includes 200, 214, 254, and 280 nm filters when installed. The effect of these wavelengthes on the detection sensitivity of creatinine was determined and finally 200 nm was selected as the most sensitive wavelength for the detection of creatinine in pH 6 phosphate buffer.

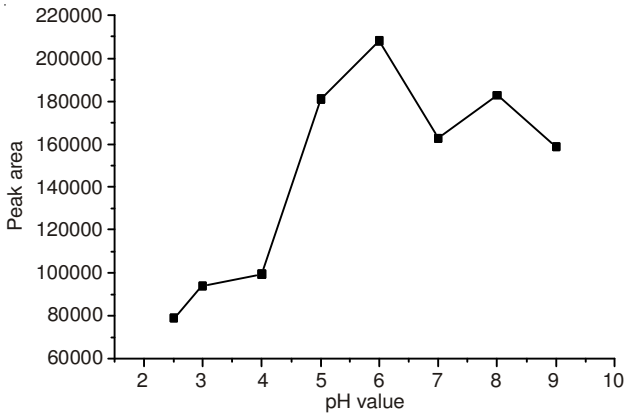

Fig. 1. Effects of pH values on capillary zone electrophoresis performance

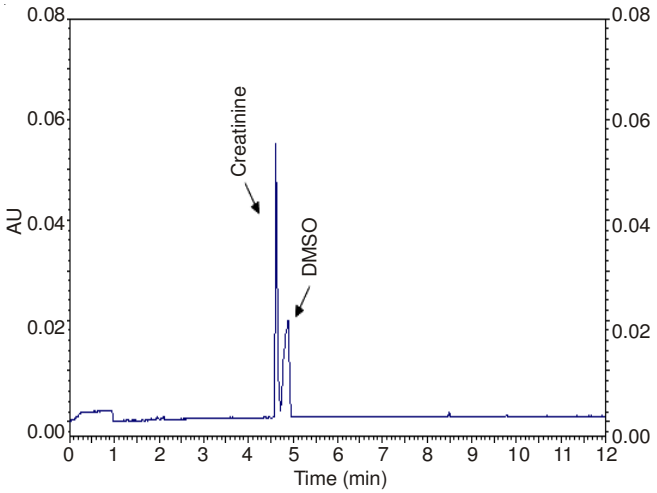

Fig. 2. Typical electropherograms of 50 mg L^{-1} creatinine and dimethyl sulfoxide (DMSO)

Effect of separation voltage: Effect of applied voltage was investigated over a range of 8-15 kV by three replicate injections at each voltage. As applied voltage increased, Joule heating, current value and peak area were all increased but the resolution efficiency was decreased. In account of satisfactory peak shapes, resolution values and migration times, 10 kV was considered to be the optimized applied voltage and selected for the experiment.

Method validation: Under the optimized capillary zone electrophoresis conditions (an ultraviolet detector at 200 nm, 10 kv separation voltage and pH 6, 50 mmol L^{-1} NaH_2PO_4-Na_2HPO_4 buffer), the calibration curve ($y = 0.4 \times 10^{-3} x + 2.8969$ mg L^{-1}) was linear within the range of 1 to 200 mg L^{-1} ($R^2 = 0.9978$). LOD (S/N = 3) was 0.15 mg L^{-1}.

As presented in Table-1, the intra-day and interday precision (%, RSD) of the measured concentration ranged from 2.6 to 3.1 % in rat urine and from 3.0 to 4.2 % in human urine, respectively. The RSD of migration time ranged from 0.4 to 0.5 % in both rat and human urine. Meanwhile, the per cent recovery of creatinine was determined by adding three creatinine concentrations (10, 50, and 100 mg L^{-1}) to a 1:10 dilute 24 h rat urine and a 1:10 dilute human spot urine. As shown in Table-2, the per cent recovery of creatinine ranged from 97.6 to 103.6 %. The results showed the method had good precision and accuracy.

Real sample analysis: The method was then applied to analyze the eight 24 h rat urine samples (the typical electropherogram, Fig. 3A) and seven human spot urine samples (the typical electropherogram, Fig. 3B). The result showed that the creatinine concentration of 24 h rat urine ranged from 402 to 1627 mg L^{-1} and the creatinine concentration of the human spot urine ranged from 458 to 769 mg L^{-1}, respectively.

Conclusion

This paper describes a methodology for the determination and quantification of creatinine by capillary zone electrophoresis in pH 6, 50 mmol L^{-1} NaH_2PO_4- Na_2HPO_4 buffer. The high separation efficiency and sensitivity of the capillary zone electrophoresis towards the creatinine enable the method to assay in real urine samples. The results obtained from the method have a good linearity, precision and accuracy. The developed capillary zone electrophoresis technique was also simple, low-cost, rapid and did not require complex sample-pretreatment.

TABLE-1
PRECISION OF CAPILLARY ZONE ELECTROPHORESIS FOR URINARY CREATININE ANALYSIS

	Samples	Measured concentration (μg mL^{-1})	RSD (%)	Migration time (min)	RSD (%)
Intra-day (n = 5)	[a]Urine 1	70.62 ± 1.84	2.6	4.65 ± 0.02	0.5
	[b]Urine 2	59.53 ± 1.85	3.1	4.71 ± 0.02	0.4
Inter-day (n = 5)	[a]Urine 1	71.12 ± 2.13	3.0	4.62 ± 0.02	0.5
	[b]Urine 2	60.23 ± 2.53	4.2	4.68 ± 0.02	0.4

[a]1:10 dilute 24 h rat urine; [b]1:10 dilute human spot urine

TABLE-2
RECOVERY OF CAPILLARY ZONE ELECTROPHORESIS FOR URINARY CREATININE ANALYSIS

Sample	Found (μg mL^{-1})	Added (μg mL^{-1})	Total found (μg mL^{-1})	Recovery (%)
[a]Urine 1	70.62	10.00	80.40	97.8
		50.00	121.47	101.7
		100.00	172.74	102.1
[b]Urine 2	59.53	10.00	69.29	97.6
		50.00	111.38	103.6
		100.00	158.41	98.9

[a,b]Same to Table-1

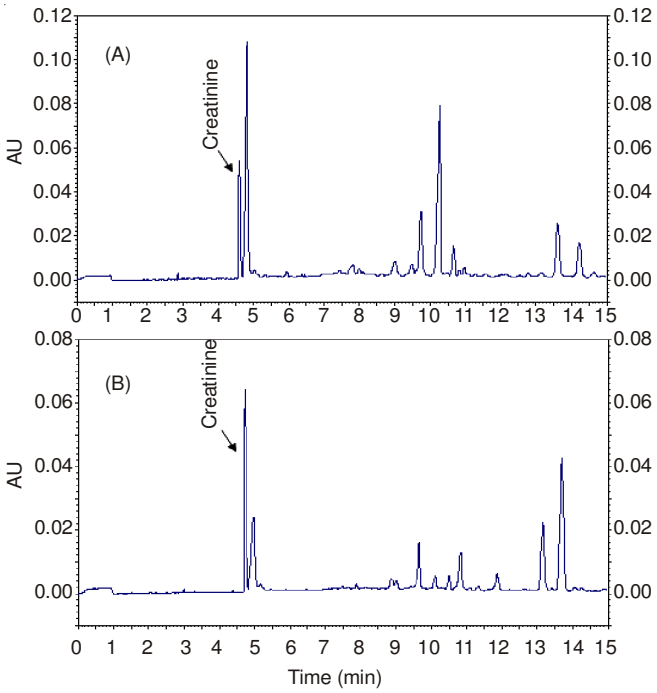

Fig. 3. Typical electropherograms of a 1:10 dilute 24 h rat urine sample (A) and a 1:10 dilute human spot urine sample (B)

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the Natural Science Foundation of Hebei Province (Nos. H2014206345) and Foundation of the Department of Health of Hebei Province (Nos. 20130454).

REFERENCES

1. T. Smith-Palmer, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **781**, 93 (2002).
2. L.A. Kaplan and A.J. Pesce, Clinical Chemistry: Theory, Analysis and Correlation, Mosby, New York, edn. 3 (1996).
3. H. Schiffl and S.M. Lang, *Mol. Diagn. Ther*., **16**, 199 (2012).
4. H. Borsook and J.W. Dubnoff, *J. Biol. Chem*., **168**, 493 (1974).
5. N.A. Devenport, J.C. Reynolds, V. Parkash, J. Cook, D.J. Weston and C.S. Creaser, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **879**, 3797 (2011).
6. M. del Carmen Hurtado-Sánchez, A. Espinosa-Mansilla, M.I. Rodríguez-Cáceres, E. Martín-Tornero and I. Durán-Merás, *J. Sep. Sci*., **35**, 2575 (2012).
7. S.S. Waikar, V.S. Sabbisetti and J.V. Bonventre, *Kidney Int*., **78**, 486 (2010).
8. C.W. Hu, C.J. Wang, L.W. Chang and M.R. Chao, *Clin. Chem*., **52**, 1381 (2006).
9. M.Z. Jaffe, *Physiol. Chem*., **10**, 39 (1886).
10. S. Mohr, A. Willing, E. Radtke, K. Wolf and H. Hartmann, *Kleintierpraxis*, **52**, 708 (2007).
11. V.F. Samanidou, A.S. Metaxa and I.N. Papadoyannis, *J. Liq. Chromatogr. Rel. Technol.*, **25**, 43 (2002).
12. Y. Jiang, X. Cheng, C. Wang and Y. Ma, *Anal. Chem*., **82**, 9022 (2010).
13. S.M. Pedersen, C. Nebel, N.C. Nielsen, H.J. Andersen, J. Olsson, M. Simrén, L. Öhman, U. Svensson, H.C. Bertram and A. Malmendal, *Eur. Food Res. Technol*., **233**, 1013 (2011).
14. E. Pobozy, A. Radomska, R. Koncki and S. Glab, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., **789**, 417 (2003).
15. A. Zinellu, C. Carru, M.F. Usai, S. Sotgia and L. Deiana, *Electrophoresis*, **25**, 1096 (2004).
16. A.C.O. Costa, J.L. da Costa, F.G. Tonin, M.F.M. Tavares and G.A. Micke, *J. Chromatogr. A*, **1171**, 140 (2007).
17. R. Paroni, I. Fermo, G. Cighetti, C.A. Ferrero, A. Carobene and F. Ceriotti, *Electrophoresis*, **25**, 463 (2004).