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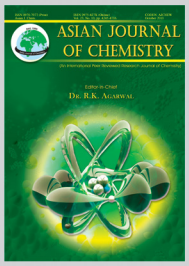

Determination of Glutamine and Arginine in Human Serum by Capillary Electrophoresis with UV Detection

JIA SHANSHAN[1,*], LUO BIN[1,*], ZHAO YING[2], LI ZENGNING[1,*] and KANG WEIJUN[2]

[1]Department of Nutrition, First Hospital of Hebei Medical University, Hebei, P.R. China
[2]The Public Health College of Hebei Medical University, Hebei, P.R. China

*Corresponding authors: Tel: +86 311 85917009; E-mail: zengningli@hebmu.edu.cn

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A rapid method for the determination of glutamine and arginine in serum is presented. Derivatization of glutamine and arginine using phenyl isothiocyanate were separated and detected in 8 min by high performance capillary electrophoresis with UV detection on 254 nm. The separation and determination condition were as follows: gravitation sampling, the time is 15 s, the height is 25 cm; running voltage 15 kv; bouncy silica capillary: 75 μm i.d., total length 70 cm, 58 cm from the inlet to UV detection; electrolyte, 20 mmol/L sodium tetraborate, 166 mmol/L lauryl sodium sulfate and acetonitrile, pH 9.33. The linear ranges of glutamine is 0.03-1.90 mmol/L and arginine is 0.03-1.50 mmol/L, r is 0.996-0.998. This new method could be used to determine the glutamine and arginine in human serum. The result was proved to be satisfactory.

Key Words: High performance capillary electrophoresis, UV detection, Serum, Glutamine, Arginine.

INTRODUCTION

The most free amino acid is glutamine in human body. The determination of glutamine is of great value not only to clinical diagnosis and treatment of metabolic disease, nephropathy and hepatopathy[1-3], but also to the evaluation of nutritive status of patient in recent years. It is reported that glutamine supplementation on immune response in anorectic patients had received the good effect[4]. Moreover, infectious complication could be reduced and shortening hospital stay would be achieved by glutamine supplementation[5]. Arginine is one of the indispensable amino acids to body in stress state. Functionally, it affected protein metabolism and was involved in protein synthesis after stress. Arginine has attracted great interest for its numerous important role. In recent years, many researchers devoted themselves to the study of enteral nutrition supplement with arginine[6-11]. A result of study on mouse suggested that arginine supplement had changed the cytokine concentrations and changed lymphocytes in severely burned mice[6] and the studies also conferred that the enteral diet supplemented with arginine might suppress excessive inflammatory responses in critically ill patients[9]. It is indicated that considering supplementation of this nutrient in clinical trials in burn patients was very important[9]. Therefore to establish a rapid and accurate determination of amino acids is increasingly important.

Common methods for the separation of physiological amino acids were ion exchange chromatography[12], high-performance liquid chromatography[13,14]. Even though these methods could identify or detect the amino acids, they often require complex procedures, high cost or long cycle. Therefore, developing a simple, economical, rapid and accurate method for the simultaneous determination of arginine and glutamine is highly desirable.

Capillary electrophoresis is a new analytical separation method after RP-HPLC. It is sensitive, economical and rapid. It has become a rapidly expanding area of analytical chemistry. Recently, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) have become a popular and useful tools for the separation and determination of amino acids due to their efficiency and separation of analytes from various interfering matrices. Komarova *et al.*[15] optimized capillary zone electrophoresis for separation of phenylthiohydantoin amino acids. Zhou and Lunte[16] explored a direct determination of amino acids by capillary electrophoresis. However, only a few capillary electrophoresis protocols have been established for biological amino acid analysis to date. Because the concentrations of these analytes especially in body fluids are very low, the highly sensitive detection methods are essential in order to accomplish a precise and accurate determination.

The aim of the present paper is to establish a rapid method for determination of glutamine and arginine in serum, which can be used for determining amino acids in clinical practice and scientific research. Arginine can be detected by UV determination, but low sensitivity. Glutamine can not be detected by UV determination. It was reported that it can be detected by laser-induced fluorescence detector (LIF)[17]. It has high sensitivity, but high cost. Most often, derivatives of amino acids are exposed to separation and detection[18], widespread reagents being: *o*-phthaldialdehyde (OPA), phenylisothiocyanate (PITC), dinitrobenzene (DNP) *etc.* Derivatization of glutamine and arginine acids using phenylisothiocyanate were detected by UV determination. It has high sensitivity and low cost. The result of determination in human serum was proved to be satisfactory.

EXPERIMENTAL

Experiments were performed with a CL1020 capillary electrophoresis system equipped with a UV detection system and a high-voltage power system, operated under chromatography date handling system for control, data acquisition and analysis, from Beijing Cailu Instrument Company (Beijing, China). The column used for the determination of glutamine and arginine was a bouncy silica capillary, from Yongnian Optical Conductive Fiber Plant (Yongnian, China).

All chemicals were of analytical reagent grade. Ultra-high purity water was used throughout. Standard of glutamine was purchased from Beijing Baierdi Biotechnology Company (Beijing, China). Standards of arginine was from Tianjin Guangfu Fine Chemicals Institute (Tianjin, China). Phenyl isothiocyanate for derivatization was from Sigma (St. Louis, MO, USA). Triethylamine was from Beijing Chemical Reagent Company (Beijing, China). Acetonitrile was from Honeywell International Inc. (USA). Buffer components [sodium tetraborate (borax), sodium lauryl sulfate] were from Tianjin Jinke Fine Chemicals Institute (Tianjin, China) and Tianjin Baishi Chemical Company (Tianjin, China).

Preparing stock solutions of amino acids: Standard solution of the pure drug was prepared by dissolving accurately each 0.0182 and 0.0219 g of glutamine and arginine, respectively in a 25 mL volumetric flask using ultra-high purity water, obtains the concentration of 5 mmol/L. Appropriate volume from this stock solution was further diluted to get different concentration levels according to the requirement.

Preparing samples of serum: Human serum was collected at the First Hospital of Hebei Medical University. 2 mL of serum sample was treated with heparin. After centrifugation (4 ºC 3000r/minX20min), supernatant 0.5 mL was transferred to a 1.5 mL centrifuge tube with 0.5 mL acetonitrile. After centrifugation (4 ºC 3000r/minX10min), supernatant was transferred to centrifuge tube. Then it was derived using phenylisothiocyanate or kept at -20 ºC.

General procedure: 100 µL treated serum or standard solution of glutamine or arginine, 150 µL triethylamine (3.5 mL triethylamine was settled to the volume of 25 mL using acetonitrile, 1 mol/L), 100 µL phenylisothiocyanate (0.3 mL phenylisothiocyanate was settled to the volume of 25 mL using acetonitrile, 0.1 mol/L) were added together and left for 1 h, then were weathered by nitrogen, settled to the volume of 1 mL using ultra-high purity water.

A new capillary was activated by washing with 1 mol/L and then 0.1 mol/L for 0.5 h each, with distilled water for 0.5 h and then rinsing for 0.5 h with running buffer. The capillary was prewashed with 1.0 mol/L NaOH for 10 min and running buffer for 20 min an the beginning of each working day and with running buffer for 2 min prior to each analysis. Gravitation sampling, the time is 15 s, the height is 25 cm; running voltage 15 kv; bouncy silica capillary: 75 µm i.d., total length 70, 58 cm from the inlet to UV detection;electrolyte, 20 mmol/L sodium tetraborate,166 mmol/L lauryl sodium sulfate and acetonitrile (10:10:3,v/v/v), pH 9.33.

Linearity: The calibration curve of glutamine and arginine were constructed using 5 series of standard copper solutions (0.03, 0.50, 1.00, 1.50, 1.90 mmol/L and 0.03, 0.30, 0.70, 1.00, 1.50 mmol/L) with UV-Visible spectrophotometer at 254 nm. The regression equation and the detection limit were determined.

RESULTS AND DISCUSSION

The retention time for glutamine and arginine were found to be 5.19 and 4.05 min, respectively. Previous studies[19,20] showed that the migration time of glutamine and arginine were 7-10 min. The results show that the capillary electrophoresis method was rapid. The method has been successfully applied to test glutamine and arginine in human serum. The retention time for glutamine and arginine in serum is coordinated with standard solution of glutamine and arginine. When standard solution of glutamine and arginine were added to serum, the peak areas were higher. Typical chromatogram of glutamine and arginine is shown in Fig. 1.

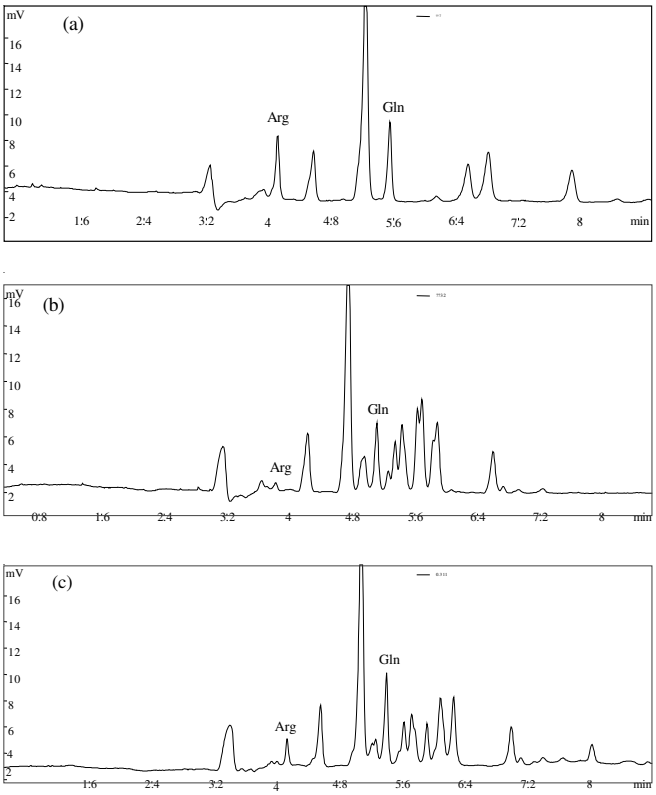

Fig. 1. Typical chromatogram of Gln and Arg (a)standard solution of Gln and Arg(b) Gln and Arg are in human serum(c)serum with standard solution of Gln and Arg

Calibration curve and detection limit: The calibration curves were constructed according to calibration curve procedure. The correlation coefficient (R^2) was 0.996-0.998 showing good linearity of calibration curve. The limit of detection (LOD) was based on the blank average signal plus two times the standard deviation of blanks. The results are summarized in Table-1.

TABLE-1
DATA OF CALIBRATION CURVES OF GLUTAMINE AND ARGININE

Parameters	Glutamine	Arginine
Linearity (mmol/L)	0.03-1.90	0.03-1.50
Regression equation	Y = 19873X + 37.82	Y = 13642X + 349.9
Correlation coefficient	0.996	0.998
LOD (mmol/L)	0.01	0.01
LOQ (mmol/L)	0.03	0.03

Accuracy and Reproducibility: Accuracy and reproducibility were determined by analyzing on the same day and on three different days over one week. The CV of accuracy was 2.37-4.04 %. The CV of reproducibility was 2.26-2.85 %. These data indicated that the capillary electrophoresis method for determination of glutamine and arginine had a good precision and accuracy.

Recovery studies: The extraction recovery of glutamine and arginine in serum were determined at three different concentrations (0.30,0.70,1.00 mmol/L) of glutamine and arginine. Recovery data from the study is reported in Table-2.The high percentages of recovery of the amino acids indicate that the method is highly accurate.

TABLE-2
RECOVERY STUDIES

Amino acids	Lable claim (mmol/L)	Amount added (mmol/L)	Amount recovered (mmol/L)	Recovery (%)
Gln	0.79	0.30	1.07	92.29
Gln	0.79	0.70	1.56	110.28
Gln	0.79	1.00	1.72	92.77
Arg	0.08	0.30	0.36	91.33
Arg	0.08	0.70	0.74	94.20
Arg	0.08	1.00	1.23	114.40

Determination of glutamine and arginine in human serum: One human serum sample was tested for glutamine and arginine level. Glutamine was 0.79 mmol/L. The result is coordinated with work of Alexander[21]. Arginine was 0.08 mmo/L. Shen and Wu[22] reported that arginine level was 0.11 ± 0.03 mmol/L in normal human serum. The result showed that good agreement was obtained between the estimated content by the capillary electrophoresis method and certified value.

Conclusion

The proposed capillary electrophoresis with UV detection method was found to be simple, rapid, low cost, precise and accurate for the estimation of glutamine and arginine in human serum. Hence, it can be easily, conveniently and economic adopted for routine quality control analysis.

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