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Simultaneous determination of allantoin, choline and L-arginine in Rhizoma Dioscoreae by capillary electrophoresis

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Abstract

A rapid, easy and reproducible capillary electrophoresis (CE) method for the simultaneous determination of allantoin, choline and arginine in Rhizoma Dioscoreae was developed first time. Under the optimum condition, the three analytes could be well separated within 5 min in a 70 cm (60 cm effective length) × 75 μm i.d. capillary. The relative standard deviations for both migration time and peak height were less than 3.20%. The linear response range was 5.0–150, 0.9–100 and 1.0–200 μg/ml for arginine, choline and allantoin, respectively. The detection limit of three components was 2.0, 0.4 and 0.5 μg/ml for arginine, choline and allantoin, respectively. Contents of arginine, choline and allantoin in the crude drug of Rhizoma Dioscoreae could be easily determined by the proposed method with satisfactory results.

Keywords: Rhizoma Dioscoreae; Pharmaceutical analysis; Allantoin; Choline; Arginine

1. Introduction

Chinese traditional medicine, Rhizoma Dioscoreae, is the root of Dioscorea Opposita Thunb [1]. It is useful for treating the angiocardiopathy, controlling blood pressure and improving immunity ability and also can be used as an anti-decrepitude tonic [2–4]. Allantoin, choline and arginine (the structures of these three compounds are shown in Fig. 1) are the main bioactive compounds in Rhizoma Dioscoreae. Pharmacological test showed that allantoin was effective on treating cancer and ache. The choline can serve the regulatory function of peripheral and central nervous system. L-Arginine is useful for improving immunity ability and treating cancer. The bioactive constituents of Chinese traditional medicine are affected by many factors such as the growing area, the season of picking and the preparing method. In order to direct the growing of Rhizoma Dioscoreae and control its quality, a simple and rapid method for monitoring the contents of bioactive constituents of Rhizoma Dioscoreae is required.

So far, only a few literatures have reported the method for determining the components of Rhizomas Dioscoreae. Thin-layer chromatography (TLC) and HPLC have been used to determine allantoin individually [5–7]. Up to now, not many methods have been reported for the simultaneous separation and determination of allantoin, choline and arginine in Rhizoma Dioscoreae. Moreover, the application of TLC was limited by its complex preparation procedure, long determination time and poor resolution, and HPLC need consuming relatively large amounts of organic solvent, which is harmful to the environment.

Capillary electrophoresis (CE), as an important separation technique, offers the advantages of excellent separation efficiency, high resolution, and rapid analysis. In addition, the amount of sample, solvent consumption and hazardous waste solution produced are minimum for a CE method, therefore, CE would offer advantages over the TLC and HPLC method [8,9]. CE has been reported as a powerful tool for the drug analysis such as determination of the main component, estimation of the impurities, separation of the chiral etc. [10–13], and it has been also used for analysis of some Chinese herbal medicine [14–22].

A new CE method has been developed for the separation and determination of allantoin, choline and arginine in Rhizoma Dioscoreae in this paper. The proposed method is a

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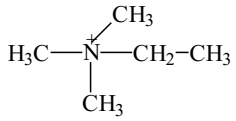
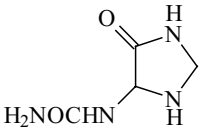
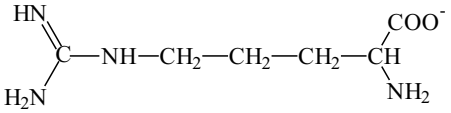


choline



allantoin



arginine

Fig. 1. The structures of arginine, choline and allantoin.

simple, rapid, reliable and efficient method, which has been successfully used for determination of allantoin, choline and arginine in some Chinese herbal medicine of Rhizoma Dioscoreae purchased from different area. Therefore, the developed method is not only a significant way for monitoring the quality of the medicine in the market from different area but also an excellent alternative method in quality control for the medicine manufacturers.

2. Experimental

2.1. Apparatus

All separations were carried out using a CAPEL 105 Capillary Electrophoresis System (Lumex, Russia) with a UV detector. Electrophoresis was performed in a 70 cm (60 cm effective length) $\times$75 μm i.d. bare fused-silica capillary (Yongnian Optical Fiber Factory, Yongnian, Heibei Province, China). A PHS-3c pH meter (Leizi Instrumentation Factory, Shanghai, China) was used for pH measurement of the electrolyte.

2.2. Chemicals and reference compounds

Choline was obtained from Sigma. Allantoin and arginine were obtained from Shanghai Chemical Reagent, China. Other chemicals were of analytical reagent grade. Two kinds of Rhizoma Dioscoreae were used in this experiment, one was obtained from a local Chinese traditional medicine store in Shandong, China, and another one was obtained from a pharmacy in Fuzhou. All water used was doubly distilled water.

All the stock solutions of allantoin, choline and arginine (1.0×10^{-3} mol/l) were prepared in 30 mmol/l phosphate buffer (pH 9.25) and diluted to the desired concentration. The stock solutions were kept at 4 °C, and diluted by running buffer solution for further application.

The sodium phosphate buffer solution (PBS) used throughout the experiment was prepared daily. The pH value of PBS was adjusted with diluted NaOH and/or HCl solutions. Each new capillary should be washed successively with 1 mol/l NaOH for 60 min, deionized water for 10 min and 0.1 mol/l NaOH for 30 min before using. Before the capillary electrophoresis system was used everyday, the capillary should be flushed sequentially with deionized water for 5 min, 0.1 mol/l NaOH for 10 min, and deionized water for 5 min and finally equibrated with background electrolyte solution (PBS) for 10 min.

2.3. Sample preparation

Rhizoma Dioscoreae samples were dried at 55 °C for 4 h in an oven and then gently pulverized. An accurate weight (about 5.0 g) of the sample powder was refluxed with 100 ml of 70% aqueous ethanol for 24 h. After cooling, the mixture was filtered and the residue was washed twice with 10 ml 70% aqueous ethanol. The washing solutions were mixed and concentrated to the final volume of about 80 ml under vacuum and then diluted to 250 ml with 30 mmol/l phosphate buffer (pH 9.25). The peak identification was performed by a standard additions method. Moreover, the sample solutions, standard solutions and running buffers all should be filtered through an injection cellulose acetate filter (0.22 μm) and ultrasonically degassed before using.

The sample was introduced by 30 mbar pressure for 10 s. The wavelength for UV detection was 200 nm. All experiments were performed at 25 °C.

3. Results and discussion

3.1. Optimization of separation conditions

The structure of these three analytes suggested that they could be determined as ions, so a capillary zone electrophoresis was selected as the separation mode. In this paper, five kinds of buffer solutions, i.e. citric buffer, boric acid buffer, phosphate buffer, Tris buffer and acetic acid buffer were used to examine the effect of buffer solution on separation efficiency. The experiment showed that the best sensitivity and resolution could be achieved when sodium phosphate was used as the running solution, because under this condition the Joule heat was lower and the shape of three peaks were better. Therefore, phosphate buffer was chosen as the background electrolytes in subsequent experiments.

3.2. Effect of electrolyte pH

It is considered that the pH of the buffer solution is the most important parameter for improving separation

efficiency. The dependence of the migration time of these three analytes on pH of phosphate buffer was investigated. The results demonstrated that the migration time of three compounds was increased with the pH in the range of 6.0–10.0, and arginine was easily separated from choline and allantoin in this pH range. In the pH range of 6.0–8.0, choline and allantoin could not be separated completely, but when pH was higer than 8.0, choline and allantoin could be separated completely. Therefore, in the range of pH 8.0–10.0, these three analysts could be separated completely, pH 9.25 was selected for subsequent experiments.

3.3. Effect of phosphate concentration

Because the concentration of buffer would influence the viscosity coefficient of the running solution, the diffusion coefficient of analytes and the ζ-potential of the inner surface of capillary tube, so it would further affect the resolution and migration time of the analytes [23]. The effect of phosphate buffer (pH 9.25) concentration on migration time and separation efficiency has been examined. The results demonstrated that when the concentration of phosphate was in the range of 20–40 mmol/l, the migration time of the three analytes was increased with the concentration of phosphate. However, when the concentration of phosphate was less than 25 mmol/l, the peak of allantoin and choline could not be separated completely. In order to obtain better resolution and save analytical time, 30 mmol/l phosphate solution at pH 9.25 was selected as the running buffer solution.

3.4. Effect of separation voltage and sampling

The effect of separation voltage on the migration time of the above mentioned analytes has been investigated, and the experiment demonstrated that the migration time was decreased with the increasing of voltage for all three compounds. So, lower voltage would result in longer analysis time and peaks broaden owing to the diffusion of the sample in the capillary. When separation voltage was 24 kV, both higher efficiency and better peak shape could be obtained.

We also examined the effects of sampling time in the range of 5–20 s at 30 mbar pressure to obtain the highest sensitivity and the best selectivity. The results indicated that peak height would be increased with the sampling time, however, peak broadening appeared obviously for injection time longer than 10 s. In this paper, 10 s was selected as the optimum injection time by considering the peak broadening and the sensitivity.

3.5. Choice of detection wavelength

Wavelength in the range of 190–350 nm has been examined for selection of the optimum UV detection wavelength for these three compounds, and the results showed that 200 nm was the most suitable wavelength for this method to achieve the optimum sensitivity.

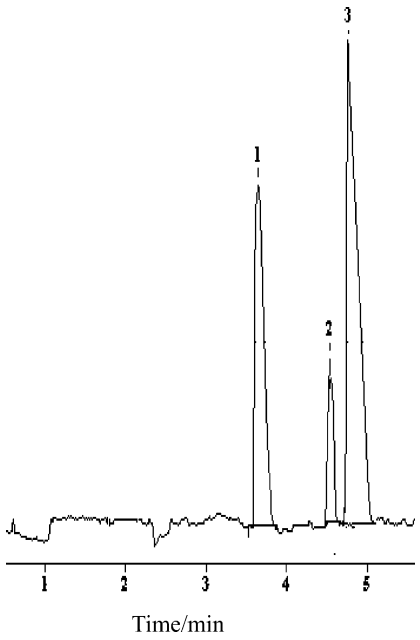


Fig. 2. Electropherogram of the standard mixtural solution. Analytical conditions: pH 9.25 phosphate (30 mmol/l); applied voltage, 24 kV; detection UV, 200 nm; 30 mbar pressure injection for 10 s: (1) arginine; (2) choline; (3) allantoin; the concentration of analytes is 5.0×10^{-4} mol/l.

To sum up, the optimum conditions for separation and detection of above mentioned compounds could be described as follows: 30 mmol/l phosphate (pH 9.25) as the running buffer solution, 24 kV as separation voltage, 10 s as injection time under pressure injection mode. Under the optimum conditions, a typical electropherogram for a standard mixture solution was shown in Fig. 2. The peak of number one, two and three was arginine, choline and allantion, reapectively, and the concentration of analytes were of 5.0×10^{-4} mol/l. It was clear that the three compounds could be completely separated within 5 min. The results showed the method was rapid and effective for separation allantoin, choline and arginine.

3.6. Reproducibility, linear response range and detection limit

A serious of the standard mixture solution of arginine, choline and allantoin were tested to determine the linearity for the analytes in this method. The linear relationships between the concentration of the three compounds and the corresponding peak height were obtained, and the regression equations and correlation coefficients are shown in Table 1. It can be known from Table 1 that the linear response ranges were 5.0–150, 0.9–100 and 1.0–200 μg/ml for arginine, choline and allantoin, respectively, and the detection limits were 2.0, 0.4 and 0.5 μg/ml for arginine, choline and allantoin, respectively. The detection limits are evaluated on the basis of a signal-to-noise ratio of 3.

A standard mixture solution of 5.0×10^{-4} mol/l of arginine, choline and allantoin were analyzed to determine the

Table 1
Linear response range of analytes[a]

Analyte	Regression equation, C (μg/ml)	Correlation coefficient (R^2)	Linear range (μg/ml)	Detection limit[b] (μg/ml)
Arginine	$A = 0.2981C + 1.1362$	0.9996	5.0–150	2.0
Choline	$A = 1.0678C + 0.3291$	0.9993	0.9–100	0.5
Allantoin	$A = 0.4610C - 1.2931$	0.9995	1.0–200	0.4

[a] CZE–UV conditions as in Fig. 2.
[b] The detection limits are estimated on the basis of a signal-to-noise ratio of 3.

reproducibility of the peak height and migration time under the opitimum conditions. The results showed that the R.S.D. ($n = 7$) of the migration times were 0.7, 2.60 and 2.30% for arginine, choline and allantoin, respectively, and the R.S.D. of peak heights for arginine, choline and allantoin were 1.63, 3.18 and 3.12%, respectively.

3.7. Applications to Chinese traditional medicine

The active components of the solutions of the Chinese traditional medicine Rhizoma Dioscoreae were analyzed according to the procedure described in experimental section. When the real samples were analyzed, some coexistent substances tended to affect on the inner surface of capillary, which would decrease the electroosomatic flow (EOF) and the peak height gradually. In order to improve the reproducibility of this method, when the capillary was used for analysis of real samples, it should be flushed sequentially with water for 0.5 min, 0.1 mol/l NaOH for 2 min, and water for 1 min and finally equilibrated with background electrolyte solution for 5 min before each injection.

Under the optimum conditions, the Chinese traditional medicine Rhizoma Dioscoreae was analyzed by capillary electrophoresis, and allantoin, choline and arginine were successfully separated. As shown in Fig. 3A and B, the content of Rhizoma Dioscoreae from two different producing areas is almost the same. Peaks were identified by addition of standard substance of allantoin, choline and arginine. The detection results for the real Rhizoma Dioscoreae samples are shown in Table 2. Table 2 indicated that the contents of the active components of Rhizoma Dioscoreae which obtained from the two different growing areas were almost the same, and the result of the determination of allantion is similar to that of the previous report [24].

Table 2
Content of analytes in Chinese medicinal preparation ($n = 5$)

Rhizoma Dioscoreae sample	Component	Determined (mg/g)	R.S.D. (%)
Shangdong	Arginine	0.30	2.6
	Choline	0.85	1.8
	Allantoin	0.91	2.2
Fuzhou	Arginine	0.29	2.4
	Choline	0.79	1.9
	Allatoin	0.92	1.6

CZE–UV conditions as in Fig. 2.

In order to examine the reliability of the method, the recoveries of arginine, choline and allantoin were investigated. The recoveries for the method were determined by addition of standard solution of allantoin, choline and arginine into the traditional herbal medicine of Rhizoma Dioscoreae samples under the same conditions stated above. The recoveries of these three compounds were found to be in the range of 96.8–104% (see Table 3).

The above results demonstrated that this method is a rapid, sensitive and reproducible method for determination of arginine, choline and allantoin in real Rhizoma Dioscoreae samples. This method promises to be applicable to quality analysis of arginine, choline and allantoin in Chinese pharmaceutical preparations of Rhizoma Dioscoreae.

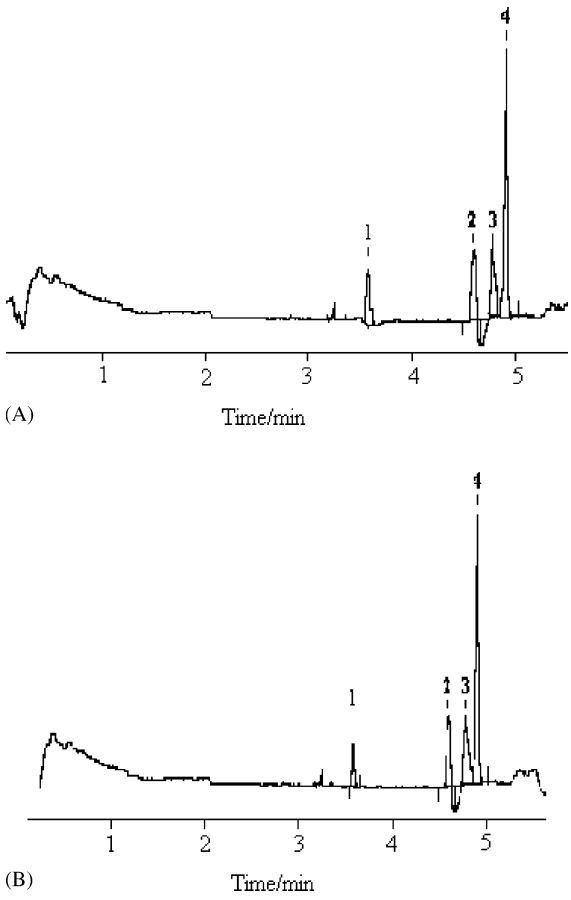


Fig. 3. Typical electropherograms of real samples of Rhizom Dioscoreae from (A) Shangdong and (B) Fuzhou. (1) Arginine; (2) unknown peak; (3) choline and (4) allantoin. Conditions as in Fig. 2.

Table 3
Determination of recovery for this method ($n = 5$)

Compound	Added amount (μg/mg)	Found amount (μg/mg)	Recovery (%)	R.S.D. (%)
Arginine	10	9.68	96.8	1.5
	20	19.4	97.0	1.9
Choline	10	9.82	98.2	2.3
	20	19.5	97.5	1.6
Allantoin	15	15.6	104	1.9
	30	30.8	103	1.6

CZE–UV conditions as in Fig. 2.

4. Conclusion

A capillary zone electrophoresis (CZE)–UV detection method has been developed for determination of arginine, choline and allantoin and successfully used for analysis of the real Rhizoma Dioscoreae samples. The results showed that this method is a rapid, simple and effective technique for identification, separated and determination of allantoin, choline and arginine in the Chinese traditional medicine Rhizoma Dioscoreae. In comparison to the chromatographic methods, the proposed method is a good alternative for simultaneous analysis of active components in Chinese traditional medicine. Moreover, the method is also possible to be applicable to the quality control and directing the producing of Chinese medicine.

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