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ORIGINAL ARTICLE

Analysis of arecoline in *Semen Arecae* decoction pieces by microchip capillary electrophoresis with contactless conductivity detection

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Abstract A new method for the determination of arecoline in *Semen Arecae* decoction pieces by microchip capillary electrophoresis with contactless conductivity detection (MCE-CCD) was proposed. The effects of various electrophoretic operating parameters on the analysis of arecoline were studied. Under the optimal conditions, arecoline was rapidly separated and detected in 1 min with good linearity over the concentration range of 20–1500 μM (r^2=0.9991) and the detection limit of 5 μM (S/N=3). The method was used for the analysis of arecoline satisfactorily with a recovery of 96.8–104%.

1. Introduction

Areca catechu L., belonging to the family Palmae (or Arecaceae), native to Malaysia, widely cultivated in Indonesia, Sri Lanka, Hainan province, Guangdong province, Yunnan province and other places in Southeast Asia, is one of the most widely used South-China medicine resources. Its dry and mature nut, namely *Semen Arecae* in traditional Chinese medicine (TCM) has antiparasitic, antifungal, antiviral effects, can dissolve food stagnation, move the Qi and promote urination. It has been used for tapeworm infestation, abdominal distension, diarrhea, edema with high medicinal values. The main ingredient known for *Semen Arecae* is arecoline, which is considered as the effective constituent. In the previous literature, many studies on the quality control of *Semen Arecae* focused on the extraction and isolation technology of arecoline and determination on its contents. The main extraction methods of arecoline in *Semen Arecae* were supercritical-CO_2 fluid extraction, ultrasonic extraction, and liquid–solid extraction and the determination of arecoline was performed by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection [1] or mass spectrometry (MS) detection [2] and capillary electrophoresis (CE) with ultraviolet (UV) detection [3]. There are no reports for

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the analysis of arecoline in *Semen Arecae* by microchip capillary electrophoresis (MCE).

MCE is a modern analytical technology rapidly developed in recent years. With the advantages of its high degree of integration, remarkable sensitivity, high resolution, rapid analysis, low reagent consumption and very cheap running, it has been applied in chemistry, biology, life sciences [4], pharmaceutical analysis [5], environmental analysis [6], clinical diagnosis [7], food security [8], and so forth.

In this study, a homemade microchip capillary electrophoresis system consisted of a high voltage supplier [9], a contactless conductivity detection (CCD) [10] and a thin microchip was developed. The system is successfully applied to detecting arecoline in *Semen Arecae* decoction pieces on the microchip.

Electrodes of the CCD did not contact with the solution inside the microchip capillary directly, so there was no special and troublesome interface required. It was only needed to push the microchip capillary through both the tubular electrodes, thus, electrode contamination could be avoided effectively, and its installation and manipulation were extremely easy and convenient. Now the CCD has been employed to the analysis of inorganic anions, cations and some organic compounds, especially amino acids [11], and alkaloids [12].

2. Materials and methods

2.1. Chemicals

Arecoline hydrobromide standard sample was obtained from National Institutes for Food and Drug Control (Beijing, China). 2-(N-morpholino) ethanesulfonic acid (MES) was purchased from AMRES Co., Hong Kong. 2-Amino-2-(hydroxymethyl)-1,3-propanediol (Tris) was from Sigma (St. Louis, MO). β-Cyclodextrin (β-CD) was purchased from Sinopharm Group Chemical Reagent Co. Ltd. (Shanghai, China). Sodium dodecyl sulfate (SDS) was obtained from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). *Semen Arecae* decoction pieces were obtained from Sri Lanka, Hainan province, and National South-China Medicine Plantation of Guangdong Food and Drug Vocational College (Guangzhou, China), and authenticated by Professor Yue-Wen Cai, Guangdong Traditional Chinese Medicine Institute (Guangzhou, China). All the other chemicals were from Guangzhou Chemical Reagent Co. (Guangzhou, China) and were all of analytical grade purity.

2.2. Preparation of the electrophoretic running buffers

All the binary acid–base buffers consisted of acid (A) and base (B). Stock solutions of A and B were prepared at the concentration of 0.1 M, and then appropriate quantities of A and B were diluted with an appropriate amount of redistilled water and mixed to obtain various electrophoretic running buffers according to its desired concentrations and ratios.

2.3. Preparation of standard solutions

A stock solution of arecoline hydrobromide standard sample was prepared at the concentration of 10.00 mM and appropriately diluted with an appropriate amount of the running buffer to obtain a series of working standard solutions for calibration curves.

2.4. Preparation of sample solutions

Semen Arecae decoction pieces were ground into powder at 200 mesh sieve before use. Accurately weighted 2 g powder was extracted with 30 mL redistilled water by continuously refluxing for 30 min, repeating twice. The extracted solution was combined, and then diluted with an appropriate amount of the running buffer to obtain a series of sample solutions.

All stock solutions and buffer solutions were prepared with redistilled water, and filtered through a 0.22 μm membrane filter before being loaded to the inlet and outlet reservoirs and all solutions were stored at 4 °C and left at room temperature before use.

2.5. Electrophoresis system and procedures

The homemade microchip capillary electrophoresis system consisted of a high voltage supplier, a contactless conductivity detector and a thin microchip. The high voltage supplier made of piezoelectric ceramics [13] was used to provide a separation voltage of 0–5 kV and an injection voltage of 0–500 V, consecutively. The contactless conductivity detector provided three waveforms (sine, square, and triangle) with oscillation frequency of 0–300 kHz and oscillation voltage of 0–300 V (in Vp–p). The detector was connected to a personal computer with an A/D converter (model PCL-711B, EVOC, Taiwan). The polymethylmethacrylate (PMMA) microchip consisted of double-T crossed channels and four reservoirs, including a four-way injection cross (connected to the four reservoirs). A schematic of the PMMA microfluidic devices is shown in Fig. 1.

The channels and four reservoirs of the new PMMA microchip were flushed with 30% ethanol, 1 M nitric acid, 0.1 M sodium hydroxide aqueous solution, and redistilled water for 10, 10, 30, and 30 min, respectively. Before use, the channels and four reservoirs were flushed again with 0.1 M sodium hydroxide aqueous solution, redistilled water, and the electrophoretic running buffer solution for 10, 5, and 20 min, respectively. Reservoirs including buffer reservoir (BR), waste reservoir (WR) and sample waste reservoir (SW) (Fig. 1) were filled with the electrophoretic running buffer solution, while

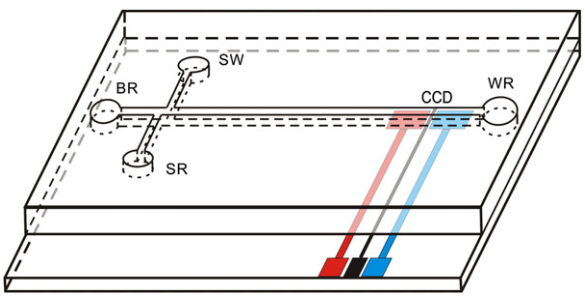

Figure 1 PMMA microfluidic-CCD schematic. BR: buffer reservoir; WR: waste reservoir; SR: sample reservoir; SW: sample waste; CCD: contactless conductivity detector. Separation channels dimension: 30 μm in top width, 100 μm in bottom width, 30 μm in depth, 44 mm in length and 43 mm in effective length. Electrodes dimension: 25 μm Pt wire working electrodes.