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Application of capillary zone electrophoresis in the separation and determination of the curcuminoids in urine

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Abstract

The major components of the plant *curcuma longa* are the curcuminoids that include curcumin, demethoxycurcumin and bisdemethoxycurcumin. It has been reported the curcuminoids have some important activities. A new CZE method with diode array detection has been developed for the separation and determination of the curcumin, demethoxycurcumin and bisdemethoxycurcumin. Three curcuminoids could be readily separated within 7 min with a 15 mM sodium tetraborate buffer containing 10% methanol (v/v) at pH 10.8, 25 kV and 30 °C. The method has been validated and shows good performance with respect to selectivity, reproducibility, linearity, limits of detection and recovery. The proposed method was successfully applied to determine the curcuminoids in urine.

Keywords: Curcuminoids; Capillary zone electrophoresis; Urine; *Curcuma longa*

1. Introduction

Turmeric is a widely cultivated tropical plant (Curcuma domestica) of Asia and Central America, having yellow flowers and an aromatic, somewhat fleshy rhizome. The powdered rhizome of this plant, used as a condiment and a yellow dye, has been also very popular in Asian medicine for the treatment of coryza, hepatic disorders and rheumatism [1]. It is valued for its yellow coloring components (curcuminoids) and the main coloring pigment is curcumin (CurI), accompanied by two related minor curcuminoids, demethoxycurcumin (CurII) and bisdemethoxycurcumin (CurIII) that are the major active constituents of turmeric (Fig. 1). Recently, curcuminoids have been reported to have very strong anti-inflammatory, anti-carcinogenic, anti-oxidant, antiallergic, anti-bacterial, and anti-tumor activities [1–4].

Although most of research regarded curcumin as the primary object, the activities of other two curcuminoids have been attracting scientists' interesting. Preclinical studies using cell cultures have shown curcumin has antiproliferative effects on human breast carcinoma cells and to induce apoptosis of the myelogenous leukemia HL-60 tumor cells [5,6].

Ruby compared mature curcuminoids for their cytotoxic, tumor reducing and antioxidant activities and found CurIII was more active than the other two as a cytotoxic agent and in the inhibition of Ehrhlich ascites tumor in mice [7]. The ability of these compounds to suppress the superoxide production by macrophage indicated that all the three curcuminoids inhibited superoxide production and CurIII produced the maximum effect.

The importance of monitoring relevant physiological and psychiatric parameters during all the period of treatment is obvious. Furthermore, it is necessary to check the amount of the drug or of the various drugs that the patients everyday have to ingest. Little is known about the absorption, distribution, and metabolism of curcuminoids in humans. The first step in in vivo physiological and pharmacokinetic studies is to develop a method to measure curcuminoids in urine.

Several methods have been developed to separate the curcuminoids: thin-layer chromatography (TLC) [8],

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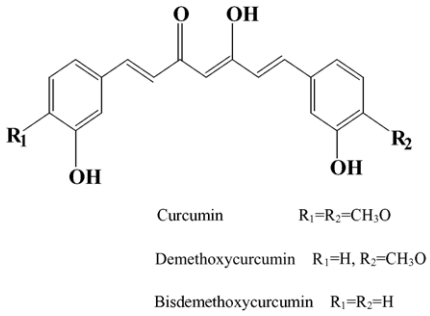

Fig. 1. Chemical structures of curcuminoids.

supercritical fluid chromatography (SFC) [9], gas chromatography (GC) [10], high performance liquid chromatography (HPLC) [11,12], and HPLC–mass spectrometry (LC–MS) [10,13]. Heath [14] developed a simple HPLC method for the analysis of curcumin in plasma and urine, only curcumin was determined and the other two active constituents were not mentioned.

Until now, there is no report on the separation and determination of the curcuminoids by capillary electrophoresis (CE). Nowadays, the application of CE for the separation of analytes in biological samples has become increasingly widespread because of its minimal sample volume requirement, short analysis time and high separation efficiency.

In this study, we have demonstrated for the first time that the curcuminoids from standards and urine samples can be simultaneously separated by CE with photodiode array detector (DAD). Hence, a method based on capillary zone electrophoresis (CZE) was envisaged for the separation and determination of three structurally comparable products, the influence of several parameters were investigated.

2. Experimental

2.1. Apparatus

All capillary electrophoresis experiments were carried out with a Beckman P/ACE system MDQ equipped with an on-column photodiode array detector (Beckman Coulter, Fullerton, CA, USA). Fused-silica capillaries (supplied by Hebei Yongnian optical fiber factory, China) of 50 cm (effective length 42 cm) × 50 μm i.d. × 375 μm o.d. were used. Spectroelectropherograms were registered in the range 200–800 nm. Date acquisition and processing were performed with Beckman P/ACE Station software (Version 1.5) running on a personal computer.

2.2. Chemicals and analytes

All other chemicals used for the buffer preparation, such as borate, NaOH, NaH_2PO_4 were of analytical grade. The reference standards of curcuminoids were purchased from Dalian Meiluo Pharmacy (curcumin) and Shichuan Tianyin Company (demethoxycurcumin and bisdemethoxycurcumin). Deionized water from a Milli-Q System (Millipore, Corp, Bedford, MA) had an electric resistance larger than 18 MΩ. HPLC-grade methanol was from Tedia (USA).

2.3. Preparation of standard solution and calibration curve

A curcumin stock solution was prepared by dissolving 10 mg curcumin in 10 ml methanol (other curcuminoids operated as the same). The standard solutions of various concentrations could be obtained by further dilution of the above prepared stock solutions. These solutions were used to obtain the calibration curve for quantitation. The autosampler temperature was maintained at 10 °C.

Appropriate dilutions (from 0.5 to 1000 μg/ml) for calibration curves were prepared. Since curcuminoids are slightly oxidized and photodecomposed, each solution was wrapped with black paper and stored in a freezer at −18 °C before use. The standard solutions are prepared everyday.

2.4. Preparation of the running buffer

Fifteen millimolar borate buffer, containing 10% methanol was prepared, and pH was adjusted to 10.8 by 1.0 M NaOH before dilution to the final volume. The buffer solutions were filtered through a 0.25 μm membrane filter before use.

2.5. Preparation of urine samples

Urine samples from fasted healthy individuals were collected into urine collection tube, centrifuged at 6000 rpm for 10 min and stored in −18 °C.

Two independent urine samples (1 ml) were prepared and one was spiked with varying amounts of curcuminoids from the previously prepared stock solutions. The tubes were mixed for 1 min at medium speed setting by vortex. Seven millilitres methylene chloride was added in each tube. The solutions were mixed by vortex for 2 min. Then tubes were centrifuged at 6000 rpm for 10 min. After centrifugation, the tubes were left in the dark for 20 min and the upper organic layer about 6 ml was carefully removed into a clean microcentrifuge tube. This organic layer was dried under a stream of nitrogen gas using low heat setting. Samples were dissolved in 100 μl of methanol for capillary electrophoresis analysis.

2.6. CE procedure

Each day, the capillary was conditioned by flushing with 0.1 M sodium hydroxide, deionized water and buffer for 10 min each and finally with the running buffer for 10 min each. Prior to each analysis, the capillary was rinsed with the buffer solution for 3 min. Samples were injected hydrodynamically at the anodic and in low pressure mode (0.5 psi) for 5 s. Electrophoresis was carried out at positive power supply