Optimized Separation of Pharmacologically Active Xanthones from *Securidaca inappendiculata* by Capillary Electrophoresis

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Summary
A capillary electrophoresis (CE) method with diode-array detection (DAD) has been developed for the separation of the therapeutically important xanthones from *Securidaca inappendiculata* for the first time, based on the systematic optimization of such parameters as pH, concentration of running buffers, addition of sulfated β-CD, applied voltage and column temperature. Baseline separation was achieved for the nine xanthones in less than 15 min using a background electrolyte consisting of 200 mM borate (pH 9.5) and 10 mM sulfated β-CD. Apparent analyte - selector binding constants between sulfated β-CD and xanthones were calculated to elucidate the migration order and the separation mechanism.

Introduction
*Securidaca inappendiculata* Hassk. is a traditional Chinese herbal medicine, belonging to Polygalaceae family, mainly distributed in the south of China and the tropical regions of Asia. The roots and stems are used as anti-inflammatory, anti-bacterial and anti-rheumatism agents under the names “Chan yi teng” and “Wu wei teng” in the south of China [1]. Pharmacological investigations have shown that the xanthones, as main components accumulated in *S. inappendiculata* have many bioactivities, such as MAO inhibition, antitumor activity, cytotoxicity, antibacterial activity, antifungal activity, anti-inflammatory properties, antioxidant activity and tuberculoatatic activity [2]. Therefore, a simple and rapid method is highly desirable to quantitatively determine these bioactive components in herbal medicines.

Due to the diversity and similar structures of xanthones, they represent a rather difficult separation problem. Existing methods for the analysis of xanthones involved high performance liquid chromatography (HPLC) [3] and thin-layer chromatography (TLC) [4]. However, owing to the complicated mixture of components in Chinese herbal medicines, HPLC requires gradient elution that tends to be inconvenient, time consuming and often results in interference with unknown constituents. TLC is unlikely to give better resolution than HPLC and is not recommended for quantitative analysis. Recent improvements in capillary electrophoresis (CE) are attractive for the studies of natural products, because of high separation efficiency, short analysis time, less sample consumption, low cost, and ease of mode change-over and column regeneration. There has been a number of publications in the literature [5 - 7] in this respect, but the analysis of xanthones by CE has not been reported.

In this work, ten xanthones (see Figure 1 for their structures) were resolved for the first time by CE using a borate complexing running buffer, containing sulfated β-CD as an additive which enhances the resolution greatly. At the same time, apparent analyte - selector binding constants between sulfated β-CD and xanthones were calculated for elucidation of the separation mechanism.

Experimental
Apparatus and Conditions
All separations were performed on an Agilent 3D CE system with air-cooling and a diode array detector (Agilent Technologies, Palo Alto, CA, USA). A 58.5 cm × 50 μm I.D. fused silica capillary (Yongnian Optical Fiber Factory, Hebei, China) was utilized. with an effective length of 50 cm; the temperature was maintained at
Figure 1. The chemical structures of the ten xanthones.

Figure 2. Effect of pH on the separation of the xanthones. See Figure 1 for peak identification. Condition: buffer, 30 mM borate, (A) pH = 8 (B) pH = 8.5 (C) pH = 9 (D) pH = 9.5 (E) pH = 10 (F) pH = 10.5; applied voltage, 18 kV; temperature, 25 °C; detection, UV at 265 nm.

40 °C. The other conditions are as follows: applied voltage 30 kV, UV detection at 265 nm, samples were injected at 50 mbar for 10 second.

The capillary was conditioned daily by washing first with 0.5 M sodium hydroxide (10 min), then with water (10 min) and finally with the running buffer (15 min). Between consecutive analysis, the capillary was flushed with 0.5 M sodium hydroxide (1 min), then with water (2 min) and finally with the running buffer (3 min) in order to improve the migration time and peak-shape reproducibility.

Reagents and Materials

The xanthones were provided by Institute of Medicine Plant Development (Beijing, P.R. China). All chemicals were of analytical-reagent grade: boric acid, sodium hydroxide, methanol from Beijing Chemical Factory (Beijing, P.R. China); pure water prepared by Milli-Q system (Millipore, Bedford, MA, USA) was used for all buffer solutions. Sulfated β-CD was kindly presented by Bioanalytical System Inc. (West Lafayette, IN, USA).

Buffer and Standards

Solutions Preparation

Results and Discussion

Optimization of Electrolyte Solutions

Effect of pH

A series of borate buffers with the same borate concentration (30 mM) but at different pH values (8–10.5) were tested in initial CE experiments to resolve the xanthones. As shown in Figure 2, the migration times of all xanthones increased with the increase of pH from 9.5–10.5, due to greater ionization of the phenolic hydroxyl groups at higher pH resulting in greater mobilities in the opposite direction to the electro-osmotic flow (EOF). At pH 8–9, the peak shape and resolution was very poor, thus the pH 9.5 was selected for further optimization, at which the compounds 5–10 were separated with good resolution, but the compounds 1–4 were not separated.

Effect of Borate Concentration

The effect of borate concentration on the CE separation was studied from 30–200