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

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Abstract: A capillary electrophoresis (CE) method has been firstly used for the separation of the therapeutically important xanthones from *Securidaca inappendiculata.* The separation of the nine xanthones was systematically optimized with respect to 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 minutes using a background electrolyte consisting of 200 mmol/L borate (pH 9.5) and 10 mmol/L sulfated β-CD.

Keywords: Separation, xanthones, *Securidaca inappendiculata*, capillary electrophoresis, sulfated β-CD.

Introduction

Securidaca inappendiculata Hassk. is a traditional Chinese herbal medicine. Pharmacological investigations have shown that the xanthones, as main components accumulated in *S. inappendiculata* have many bioactivities, such as monoamine oxidase inhibition, antitumor activity, cytotoxicity, antibacterial activity, antifungal activity, anti-inflammatory properties, antioxidant activity and tuberculoatatic activity, *etc*[1]. The contents of xanthones are very important and key factors for quality control. Therefore, a simple and rapid method to quantitatively determine these bioactive components is highly desired.

Recent improvements in capillary electrophoresis (CE) are attractive for 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. The CE analysis of xanthones has not been reported. In this work, nine xanthones were firstly resolved by CE using a borate complexing running buffer, sulfated β-CD as an additive, which gave very satisfactory results.

Experimental

All separations were performed on an Agilent 3D CE system with air-cooling and a

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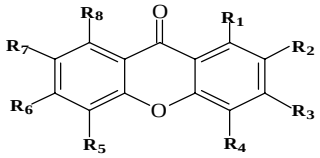
diode array detector (Agilent Technologies, Palo Alto, CA, USA). A 50 cm × 50 μm I.D. fused silica capillary was used. The UV detection wavelength was set at 265 nm. Samples were injected onto the capillary at 5000 Pa for 10 seconds.

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

The xanthones (for their structural formula, refer to **Table 1**) were provided by Institute of Medicine Plant Development (Beijing, China). Pure water prepared by Milli-Q system (Millipore, Bedford, MA, USA) was used for all buffer solutions. Sulfated β-CD was from Bioanalytical System Inc. All other chemicals were of analytical-reagent grade.

The buffers containing borate and sulfated β-CD were adjusted to the desired pH with 1 mol/L NaOH. All the buffers were filtered through a 0.45 μm membrane filter and degassed by ultrasonication for approximately 10 minutes before use. A standard solution of *ca.* 20 ppm of each xanthone was prepared in methanol, degassed in an ultrasonic bath and filtered through a 0.45 μm membrane filter.

Table 1 Structural formula of the xanthones



	Substituted groups							
Compd.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	HO	MeO	MeO	H	H	H	HO	H
2	MeO	H	H	MeO	H	H	HO	H
3	MeO	HO	H	H	H	H	MeO	H
4	MeO	HO	H	H	H	H	HO	H
5	HO	H	H	MeO	H	H	HO	H
6	HO	H	HO	MeO	H	H	HO	H
7	HO	MeO	HO	H	H	H	HO	H
8	HO	H	HO	H	H	H	HO	H
9	HO	MeO	MeO	H	H	HO	HO	H

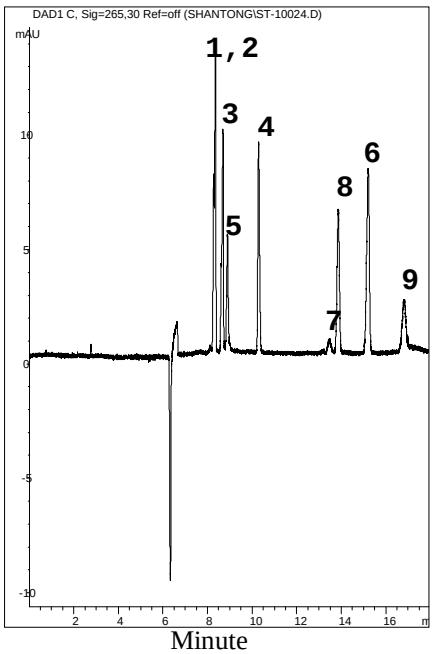
Results and Discussion

The pH and borate concentration in the CE analysis were optimized, and the optimum pH and borate concentration were 9.5 and 200 mmol/L under 18 kV applied voltage and 25ºC temperature, respectively, as indicated in **Figure 1**.

Sulfated β-CD was evaluated as an additive for the separation, the effect of which on CE selectivity was studied at pH 9.5 and 200 mmol/L borate concentration under 18 kV applied voltage and 25ºC temperature using several electrolyte systems of different

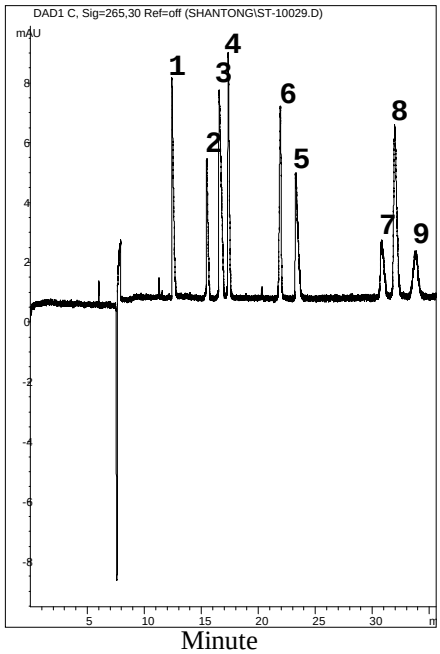
sulfated β-CD concentrations (6-14 mmol/L). Sulfated β-CD is a counter-migrating complexing agent and combines the properties of CD and a surfactant by providing a hydrophobic cavity and a negative charge. Analytes will form inclusion complexes with sulfated β-CD whose electrophoretic mobilities are in direction opposite to the electro-osmotic flow. What is more, surfactant can result in electric and hydrogen bond effects with the phenolic hydroxyl groups of the xanthones. The inclusive effects are different because of the different amount and position of the phenolic hydroxyl groups of the xanthones, which induces different electric and hydrogen bond effect with sulfated β-CD. So adding sulfated β-CD results in different mobilities of the xanthones. The optimum concentration of sulfated β-CD was 10 mmol/L, according to the results shown in **Figure 2** and **Figure 3**.

Figure 1 Electropherogram of nine xanthones



Conditions see Experimental

Figure 2 Electropherogram of nine xanthones



Conditions see Experimental

The effects of applied voltage (15 - 30 kV) and temperature (20 - 40ºC) on the separation were also studied in the buffer at pH 9.5, which contained 200 mmol/L borate and 10 mmol/L sulfated β-CD. The optimum voltage and temperature were found to be 30 kV and 40ºC, respectively, which combined sufficient resolution with a moderate analysis time.

Therefore, the 200 mmol/L borate buffer containing 10 mmol/L sulfated β-CD (pH 9.5) under 30 kV applied voltage, 40ºC column temperature was proved to be the optimized condition for the separation and the final result is illustrated in **Figure 4**. The repeatability of the migration time and the peak area of each xanthone was very good

with relatively standard deviation of less than 5%, and the theoretical plate number is in the range of 132989-397038 m^{-1}.

Figure 3 Migration time *vs* sulfated β-CD concentration

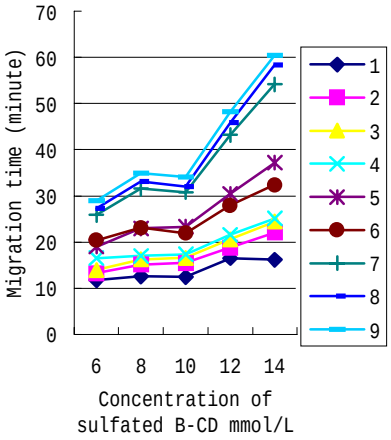
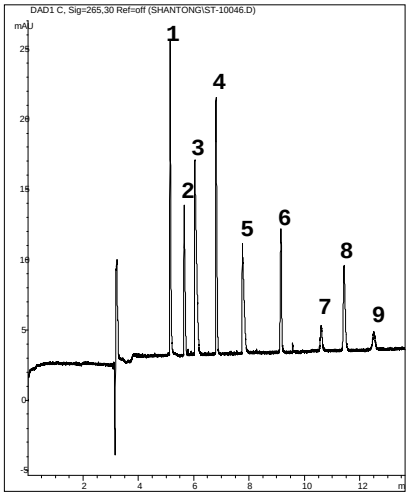


Figure 4 Electropherogram of nine xanthones



Conditions see Experimental.

Conclusion

This work demonstrates that the nine xanthones can be baseline separated in a relatively short time (< 15minutes) by CE with satisfactory migration time and peak area repeatability. The method can be used to determine the xanthones in the study of the Chinese traditional medicine of *Securidaca inappendiculata*, which is in progress.

Acknowledgments

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Reference

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