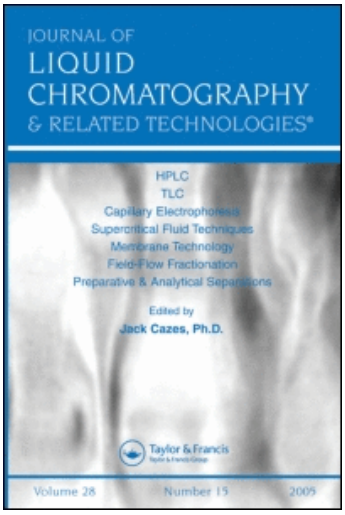

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Capillary Electrophoretic Analysis of Pharmacologically Active Xanthone Compounds from *Swertia przewalskii pissjauk* Extract

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ABSTRACT

Pharmacologically active xanthone compounds isolated from *Swertia przewalskii pissjauk* were well separated by capillary electrophoresis (CE) within 5 min, using a running buffer of 25 mM disodium tetraborate at pH 9.0. Quantitative determination was shown to be possible because regression equations revealed a linear relationship between the peak area of each constituent and its concentration, with correlation coefficients of 0.9972–0.9994. The relative standard deviations were between 0.44%–0.73% for migration times and 2.52%–4.28% for peak areas.

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The dissociation constant of 1,7-O-*β*-*D*-glucopyranosyl-8-hydroxy-3,7-dimethoxyxanthone, 1,8-dihydroxy-3, 7-dimethoxy-xanthone and 1,7-dihydroxy-3,8-dimethoxyxanthone were also measured by the CE method, giving a value of 9.04, 8.94, and 8.59, respectively.

Key Words: Capillary electrophoresis; Xanthones; *Swertia przewalskii*.

INTRODUCTION

Swertia przewalskii pissjauk, belonging to the family of *Swertia*, is a peculiar herbal medicine growing on Qinghai-Tibet Plateau. There are about 170 species of this genus recorded in the world and about 79 species are found in China. *Swertia* has been proven effective in the treatment of heptitis, cholecystitis, and gastroenteritis.[1,2] The major and pharmaceutically active constituents in *S. przewalskii pissjauk* include gentiopicroside, xanthones, xanthone-glycosides, and triterpenic acid.[3] Pharmacological studies indicate that xanthones have various biological effects such as anti-inflammatory, anti-virus and hepatoprotective activity, and exciting the central nervous system.[4] They are also inhibitory to the activities of hypertension and xanthine oxidase.

Separation of two xanthone-glycosides by high-performance liquid chromatography (HPLC) has been performed.[5] High performance liquid chromatography is an effective method, but it consumes a large amount of samples and organic solvent. Moreover, the chromatographic column can easily be contaminated by the unknown ingredients from natural products and can be hard to clean up. Alternatively, capillary electrophoresis (CE), with its high resolving power, is well suited for separating the complex mixture of molecules found in a natural product or extracts, without the column problem.[6–13] Xanthones from *securidaca* were separated by CE using backgrounds of 200 mM borate and 10 mM sulfated *β*-CD.[14,15] The separation and determination of xanthones, and xanthone-glycoside in *S. przewalskii pissjauk* by CE has not yet been reported. We have, hence, tried and as expected, a simple and fast method was established.

EXPERIMENTAL

Reagents and Solutions

Standards of 1,7-O-*β*-*D*-glucopyranosyl-8-hydroxy-3,7-dimethoxyxanthone (**1**), 1,8-dihydroxy-3,7-dimethoxyxanthone (**2**) and 1,7-dihydroxy-3,8-dimethoxyxanthone (**3**) were isolated from *S. przewalskii pissjauk*.

Their structures were confirmed by comparing their melting points, ^{1}H-NMR, IR, UV, and MS data with those given in the literature.[3] Disodium tetraborate (borax), boric acid, and sodium hydroxide were analytical reagent grade from Beijing Chemicals and Reagents Plant (Beijing, China).

Stock solution of analytes (1.0 mg/mL for **1**, 0.75 mg/mL for **2**, and 0.372 mg/mL for **3**) was prepared in methanol. Working standards were prepared by dilution of stock solutions at suitable concentration. The separation buffer was composed of 25 mM borax, adjusted to pH 9.0 by 1.0 M H_3BO_3.

Sample Preparation

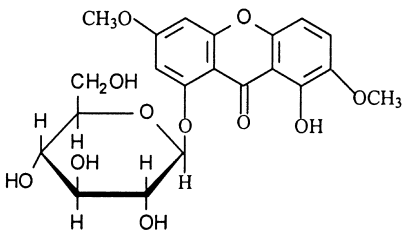
Swertia przewalskii pissjauk was collected from Qinghai-Tibet Plateau. It was ground into powder and then extracted with methanol under refluxing for 5 h. The extraction was repeated twice, combined, and condensed in vacuo. Part of the condensed product (83.76 mg) was dissolved in 10 mL methanol. After centrifugation for 10 min, the supernatant was collected and used for electrophoretic analysis.

Capillary Electrophoresis Separation

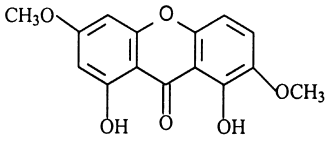
All separations were performed on Beckman P/ACE 2050 systems (Beckman Instrument, Fullerton, CA). Control of the instrumentation, data acquisition, and analysis were performed with the software of a P/ACE station. A 47 cm × 50 μm (40 cm to the detector) fused-silica capillary tube (Yongnian Optical Fiber Factory, China) was used. Prior to each injection, the capillary was rinsed under pressure with 0.1 mol/L NaOH, distilled water, and running buffer for 2 min each. The peak height is higher using electrokinetic injection than hydrodynamic injection in our experiment. Samples were injected electrokinetically at 10 kV for 2 s and temperature of the capillary tube during electrophoresis was maintained at 25°C. The applied voltage of the electrophoresis separation was 12 kV and the detection was performed with UV absorption at 254 nm. A diode array detector was used for on-line measuring of UV spectra between 190 nm–600 nm.

RESULTS AND DISCUSSION

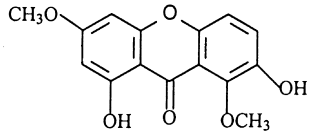
Figure 1 illustrates the molecular structure of analytes. The xanthone compounds are a kind of weak acid due to the presence of phenolic hydroxyl groups and migrate as anions in basic solution. Since the analytes probably



1-O- β-D-glucopyranosyl-8-hydroxy-3,7-dimethoxyxanthone (**1**)



1,8-dihydroxy-3,7-dimethoxyxanthone (**2**)



1,7-dihydroxy-3,8-dimethoxyxanthone (**3**)

Figure 1. Molecular structure of analytes.

form negative complexes with borax,[12,14] alkaline borax buffer system is chosen in order to obtain better resolution.

Borax Buffer pH

The effect of buffer pH on migration behavior was studied with 25 mM borax (Fig. 2). As expected, the migration times of all the xanthones increased with pH. This is clearly due to the dissociation of the hydroxy groups on xanthone compounds. Among the three compounds, xanthone-glycoside has the least negative charge-to-mass ratio, and it migrates in front of the other two xanthones.

According to Fig. 2, good separation can be achieved at pH between 8.5 and 10.5. For more even distribution of the peaks, the pH should be selected between 9 and 10. In this paper, pH 9.0 was adopted and baseline resolution of standards was reproducibly obtained.

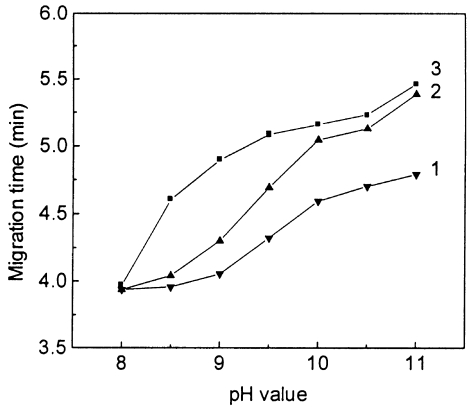


Figure 2. pH-Dependent elution behavior of 1,7-O-*β*-*D*-glucopyranosyl-8-hydroxy-3,7-dimethoxyxanthone (**1**), 1,8-dihydroxy-3,7-dimethoxyxanthone (**2**) and 1,7-dihydroxy-3,8-dimethoxyxanthone (**3**). Capillary: 47 cm (40 cm to detector) × 50 μm I.D.; applied voltage: 12 kV; detection wavelength: 254 nm; running buffer: 25 mM borax; injection: 10 kV for 2 s.

Running Buffer Concentration

Borax concentration was investigated in the range of 5–40 mM at pH 9.0. Figure 3 shows that the resolution, and elution time as well, increases with borax concentration. Analytes **2** and **3** comigrated at 5 mM tetraborax and can

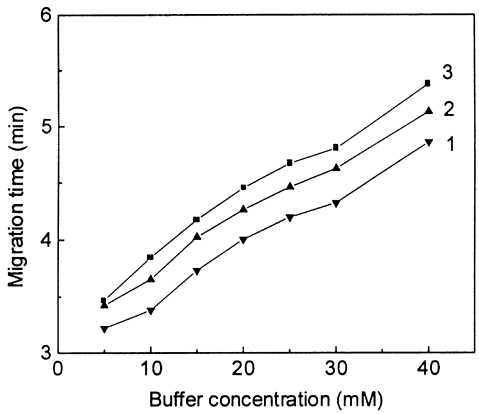


Figure 3. Influence of buffer concentration on the elution of analytes. Running buffer: borax at pH 9.0. Other conditions and peak identity as in Fig. 2.

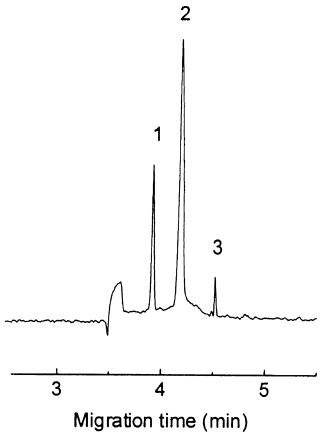


Figure 4. Electropherogram of standard solution. Running buffer: 25 mM borax, pH 9.0. Other conditions and peak identity as in Fig. 2.

be separated from each other at the borax concentration higher than 10 mM. For stable resolution, the borax concentration was selected at 25 mM. Higher concentration is also acceptable but at the cost of prolonging the running time. Figure 4 depicts the electropherogram of the analytes under the selected conditions. The three bioactive components were well separated within 5 min, with roughly the same distance from each other.

Linearity and Reproducibility

Repeatability was determined by carrying out six successive injections of standard solution within one day. The relative standard deviations (RSD) of migration times and peak areas are listed in Table 1. The linear relationships between the concentration of analytes and the corresponding peak area are

Table 1. Relative standard deviations (RSD) of migration time and peak area.

Analytes	RSD (migration time)	RSD (peak area)
1	0.65%	2.52%
2	0.44%	3.14%
3	0.73%	4.28%

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Table 2. Quantitative equations and their linear range.

Analytes	Linear regression	Linear range (μg/mL)	Regression coefficients
1	$y = -2737.3 + 211.4x$	20–500	0.9985
2	$y = 4306.9 + 379.7x$	5–500	0.9994
3	$y = 528.3 + 5.32x$	50–372	0.9972

listed in Table 2. The data show that the explored approach is suitable for quantification.

Separation of Extracted Sample

Methanol extracted solutions of *S. przewalskii pissjauk* were injected directly into the capillary. Baseline separation of the three bioactive components from each other and the unknown chemicals, was achieved within 8 min (Fig. 5). The peaks were identified by spiking standards. The on-line UV spectra of xanthones in *S. przewalskii pissjauk* (lower) well matched the standard xanthones (upper) (Fig. 6). The contents of **1**, **2**, and **3** in condensation methanol extraction of *S. przewalskii pissjauk* were 0.36%, 0.49%, and 2.01% (mg/mg), respectively. The recovery was determined by addition of a known amount of standards into the methanol extracts. The results were 97.6%, 103.4%, and 96.8% for **1**, **2**, and **3**, respectively.

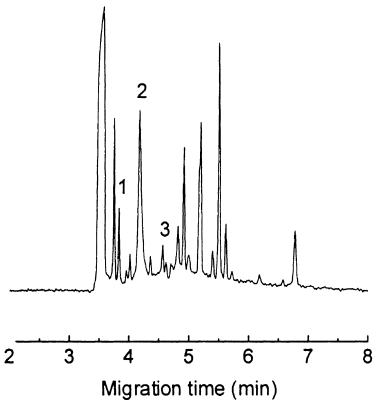

Figure 5. Electropherogram of methanol extract of *S. przewalskii pissjauk*. Conditions and peak identity as in Fig. 4.

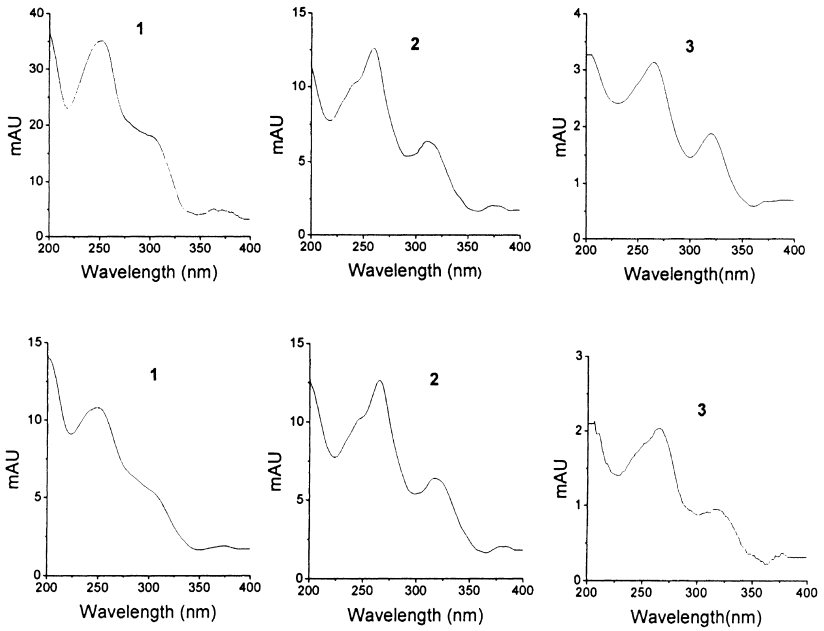

Figure 6. The on-line UV spectra of standard xanthones (upper) and xanthones in *S. przewalskii pissjauk* (lower). Diode array detector; injection time: 5 s. Other conditions and peak identity as in Fig. 2.

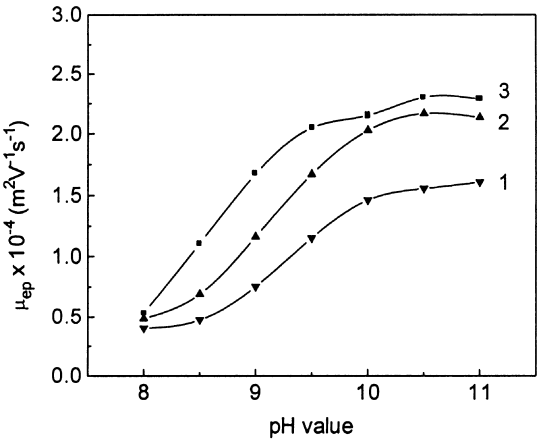

Figure 7. Plots of the electrophoretic mobilities of xanthones and xanthone-O-glycoside. Running buffer: 10 mM borax. Other conditions and peak identity as in Fig. 2.

Table 3. Measured pKa and μ_A^- for xanthones and xanthone-O-glycoside.

Analytes	pKa	$\mu_A^- \times 10^{-4}$ ($cm^2 V^{-1} s^{-1}$)
1	9.04	1.608
2	8.94	2.227
3	8.59	2.350

Determination of pKa Values

The aqueous ionization (pKa) is a very important physicochemical property in the pharmaceutical industry[16,17] and phytochemistry.[18] Recently, CE has been shown to be a convenient method for precise aqueous pKa determination.[19–21] Because the pKa values of the active molecules are not available at this moment, they were, thus, measured using the method from Ref.[22] Figure 7 depicts the influence of the pH of the running buffer on the effective mobilities of xanthones. The resulted pKa values of xanthones were calculated and listed in Table 3. The magnitude of pKa values of analytes are **1** > **2** > **3**, while the orders of migration times of analytes are **1** < **2** < **3**.

CONCLUSION

Capillary zone electrophoresis was explored for the measurement of xanthone compounds extracted from *S. przewalskii pissjauk*. The result revealed that this was a simple approach for the analysis of such a complicated mixture. It can be used as an assay for the mentioned compounds and for the determination of their pKa values.

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