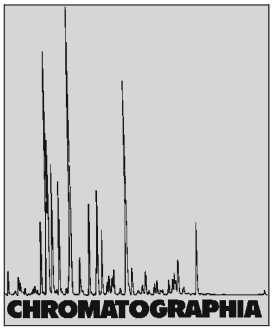
Differentiation of Resina Draconis from Sanguis Draconis by CE



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Abstract

A simple, accurate method based on capillary electrophoresis with electrochemical detection (CE–ED) has been developed to determine loureirin A, loureirin B and dracorhodin for differentiation of Resina Draconis from Sanguis Draconis. The effects of some important factors such as acidity and concentration of running buffer, separation voltage, injection time, and applied potential on the CE–ED working electrode were investigated. Under the optimum conditions, the three analytes could be well separated within 30 min in a 75 cm capillary at a separation voltage of 14 kV in a 80 mmol L^{-1} borate buffer (pH 9.24). The working electrode was a 300-μm-diameter carbon disc electrode positioned opposite the outlet of the capillary in a wall-jet configuration and was set at a potential of 0.90 V (vs. SCE). Excellent linearity was established over two orders of magnitude with detection limits (S/N = 3) ranging from 3×10^{-7} g mL^{-1} to 1×10^{-6} g mL^{-1} for all three analytes. The relative standard deviations of peak current and migration times of loureirin A, loureirin B and dracorhodin were 2.1, 1.7, 4.4 and 2.9, 2.8, 3.3% (n = 5), respectively. The recoveries of three constituents ranged from 98.8 to 101.8%. The methodology has been successfully applied to analyze and differentiate the actual samples with satisfactory assay results.

Keywords

Capillary electrophoresis
Electrochemical detection
Resina Draconis
Sanguis Draconis

Introduction

Sanguis Draconis is a resin exuded from the fruit of *Daemonorops draco* Bl., which belongs to the family of *Palmae*, genus of *Daemonorops*. Sanguis Draconis is a precious plant used in traditional chinese medicine (TCM) and is described in the Chinese Pharmacopoeia. It is beneficial for the treatment of blood circulation and blood stasis, saprophytic muscle tissue, inflammation and pain relief, wound healing and congestion [1]. Modern medical research indicates that Sanguis Draconis has antifungal immune stimulating properties [2]. Dracorhodin is the main constituent of Sanguis Draconis and is used as the marker for quality control [3].

Sanguis Draconis was mainly cultivated in Indonesia and Malaysia and had to be imported to China which resulted in high costs and limited availability of this medicinal plant. In 1972 Professor Cai and co-workers [4] reported that *Dracaena cochinchinensis* (Lour.) SC Chen, grown in the Yunnan and Guangxi province in China, could serve as a substitute for Sanguis Draconis. The crude drug was Resina Draconis, which belongs to the family of *Liliaceae*, genus of *Dracaena* which has been used as a Sanguis Draconis substitute for 20 years in China, and pharmacological, toxicological and clinical studies have shown similar effects [5]. By 1999 the national standard of Resina Draconis as the new TCM was authorized [6]. The main ingredients in Resina Draconis are loureirin A and B [3].

With pharmacological research improvement, Resina Draconis has been extensively exploited for clinical treatment. Correspondingly, more and more fake and defective goods appeared on the market. Although Resina Draconis resembles Sanguis Draconis in many aspects, one major differentiating feature is their financial value. It is therefore necessary to establish an accurate, reliable and convenient method for quality control in order to enable differentiation of both plant products.

Sanguis Draconis and Resina Draconis have a similar appearance, even approximate curative effects, but they belong to a

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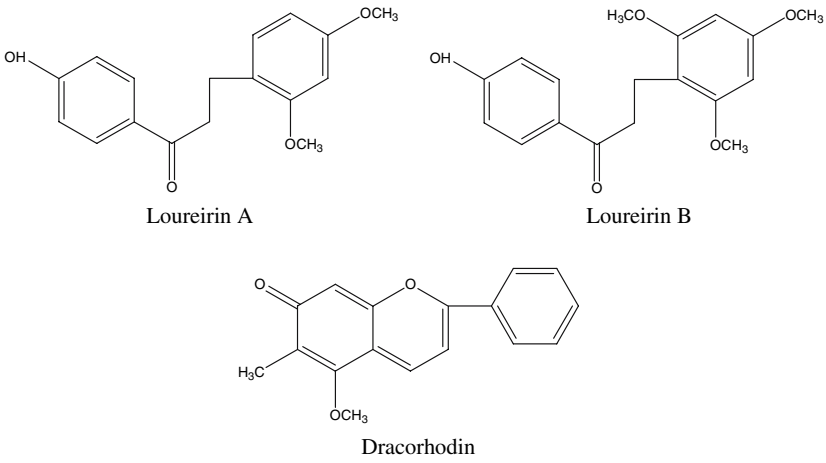

Fig. 1. Molecular structures of loureirin A, loureirin B and dracorhodin

different genus and family. Accordingly, their chemical constituents are definitely dissimilar. Dracorhodin is the quality marker of Sanguis Draconis which is not found in Resina Draconis. Furthermore, loureirin A and B are the most important ingredients of Resina Draconis which are absent in Sanguis Draconis. The determination of dracorhodin, loureirin A and loureirin B in both crude drugs should facilitate this differentiation.

Many methods including thin layer chromatography scanning (TLCS) [7, 8], thin layer chromatography (TLC) [9–12], ultraviolet–visible spectrophotometry (UV) [9–12], micro-observation [9], second derivative spectrophotometry [13], high performance liquid chromatography (HPLC) [14–22] have been applied to analyze loureirin A and B in Resina Draconis and dracorhodin in Sanguis Draconis. Among these methods, HPLC coupled with MS, UV, or photodiode array detection is still the most frequently analytical technique in the separation and analysis of traditional Chinese medicinal herbs. However, HPLC often has shortcomings, such as long analysis time, low resolution and short column lifetime, due to the introduction of contaminants. Capillary electrophoresis (CE) is becoming increasingly recognized as an important analytical separation technique. Compared with HPLC, CE provides high separation efficiency (theoretical plate numbers between 50,000 to 5,00,000), minimal sample quantity, short migration time and reproducibility. It also allows facile removal of contaminants. Electrochemical detection (ED) operated in the amperometric mode can be coupled with CE to provide high sensitivity and selectivity for electroactive analytes [23]. To date, however, CE–ED has not been applied to the analysis of Resina Draconis and Sanguis Draconis. In this work, we developed a sensitive and reliable method for the simultaneous determination of three active ingredients in these two TCM products by CE–ED after a relatively simple extraction procedure. Differentiation was based on the inspection of their electropherograms.

Experimental

Apparatus

In this work, a CE–ED system has been used similar to that described previously [23]. A + 30 kV high-voltage DC power supplied a separation voltage between the ends of the capillary (Shanghai Institute of Nuclear Research, China). The inlet of the capillary was held at a positive potential and the outlet was maintained at ground. The separation was carried out in a 75 cm fused silica capillary (25 μm ID and 360 μm OD) (Yongnian Co., Hebei, China). Samples were all injected electrokinetically, applying 14 kV for 8 s.

The design of CE–ED detector was based on the end-column approach in which the working electrode was simply placed at the outlet of the separation capillary. Detection was carried out in the same solution reservoir that contained the grounding electrode for the CE instrument. A three-electrode electrochemical cell consisting of a 300-μm-diameter carbon disk as the working electrode, a platinum auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode, was used in combination with a BASLC-3D amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA). Before use, the carbon disc electrode was successively polished with emery paper and sonicated in distilled water.

The data were analyzed by HW-2000 software (Qianpu software company, Shanghai, China).

Reagents and Solutions

Dracorhodin, loureirin A and B were all purchased from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and their molecular structures are shown in Fig. 1. All aqueous solutions were made up in double distilled water. Other chemicals were of analytical grade. Samples were obtained from different pharmaceutical factories in China. Shulong Resina Draconis crude drug was provided by MingSheng factory (YunNan, China); Huangguan Sanguis Draconis crude drug was purchased from ShanHe drugstore (WuXi, China); Sanjin longxuejie capsules, Yulin longxuejie capsules and Yunshan longxuejie capsules were provided by SanJin factory (NanNing, GuangXi); XiShuangBanNa factory (XiShuangBanNa, YunNan) and GeJiu factory (GeJiu, YunNan), respectively. Stock solutions of the three analytes (1×10^{-3} g mL^{-1}) were prepared in anhydrous ethanol and were diluted to the desired concentration with the running buffer (H_3BO_3–$Na_2B_4O_7$ buffer, pH from 8.0 to 9.24). Before use, all solutions were filtered through 0.25 μm nylon filters.

Sample Preparation

All samples were kept in a desiccator. About 1.0 g Resina Draconis and Sanguis Draconis was ground into powder in a mortar and accurately weighed. Each sample was extracted with 30 mL anhydrous ethanol in an airtight container for 20 min in an ultrasonic bath. After cooling, the sample solutions were filtered through a 0.25 μm nylon filter. A 0.1 mL volume of sample solution was diluted to 1 mL with running buffer, and then injected directly for analysis. Before use, all sample solutions were stored in the dark.

Results and Discussion

Optimum Conditions for the Determination of Loureirin A, Loureirin B and Dracorhodin

Loureirin A and B have similar molecular structures (Fig. 1) and since both of them are found in Resina Draconis a complete separation was carried out. Separation was achieved based on the difference in mass-to-charge ratio (q/m) between the analytes in CZE mode. The running buffer enabled ionization of both phenolic hydroxyl groups and resulted in almost equal negative charges. Both structures, however, differed by one additional methoxyl group (Fig. 1). The q/m values of the analytes are so close that it was a challenging task to obtain a good resolution between loureirin A and loureirin B.

Effects of the Running Buffer

The pH of the running buffer not only affected the electroosmotic flow but also the overall charge of the analytes, which had an impact on migration time and resolution. Borate buffer was employed as the running buffer in this work because borate can chelate the analytes to form more soluble complex anions. The pH dependence of the migration time was investigated in the pH range from 8.00 to 9.24. The migration time of all analytes increases with increasing pH value and separation of the analytes was achieved at pH 9.24. A pH above 9.24 led to oxidation of both and therefore pH 9.24 was selected as the optimum pH value in this work.

The concentration of the running buffer was another important factor. The effect of the running buffer concentration on resolution was studied, and the resolution achieved was optimal at a concentration of 80 mmol L^{-1}.

Effect of Separation Voltage and Injection Time

The influence of the separation voltage on the migration time of analyte was also studied in this work. Higher separation voltages gave shorter migration times for all analytes. However, when the separation voltage exceeded 14 kV, separation of loureirin A and loureirin B could not be achieved and baseline noise became larger. Therefore, the application of 14 kV resulted in good resolution within 30 min and was subsequently chosen as the separation voltage.

The injection time, determining the amount of sampling, affected both peak current and peak shape. The effect of injection time on peak current was studied by varying injection times from 6 to 10 s at 14 kV. When the injection time was longer than 8 s, peak current levels and shapes deteriorated. In this experiment, 8 s (14 kV) was selected as the optimum injection time.

Effect of the Potential Applied to the Working Electrode

The potential applied to the working electrode affected the sensitivity, detection limit and stability of this method. The effect of applied potential to the working electrode on peak current of the analytes was studied. When the potential was higher than 0.75 V, oxidation of the three analytes occurred. As the potential increased, peak heights of the three analytes increased stepwise between 0.75 and 0.85 V, but at 0.90 V and beyond, the increase slowed down and resulted in the formation of large background noise. Therefore, the applied potential to the working electrode was maintained at 0.90 V (vs. SCE) where the background current was not too high and the S/N ratio was highest. Moreover, the working electrode showed good stability and high reproducibility at this optimum potential.

Through the above experiments, the optimum conditions for this work have been identified. The applied potential to the working electrode was selected at 0.90 V (vs. SCE), injection time was 8 s (14 kV), and compounds could well be separated within 30 min in a 80 mmol L^{-1} borax running buffer (pH 9.24). A typical electropherogram for a standard solution of three analytes is shown in Fig. 2a. It can be seen that satisfactory separation can be achieved within 30 min.

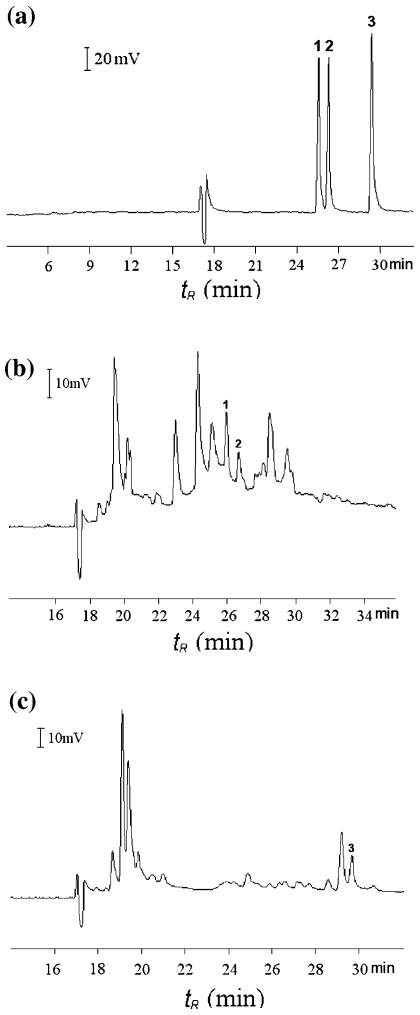

Fig. 2. Electropherograms of a standard solution (**a**) and actual samples: Resina Draconis (**b**) and Sanguis Draconis (**c**). Fused-silica capillary: 75 cm length, 25 μm ID; working electrode: 300-μm-diameter carbon disk electrode; concentration of three analytes: 5×10^{-5} g mL^{-1}; running buffer: sodium tetraborate (pH 9.24); concentration of running buffer: 80 mmol L^{-1}; injection time: 8 s (at 14 kV); working potential: 0.90 V (vs. SCE). Peak identification: **1** = loureirin B; **2** = loureirin A; **3** = dracorhodin

Regression Equations, Linear Ranges and Detection Limits of the Three Analytes

A series of the standard mixture solutions of loureirin A, loureirin B and dracorhodin with concentrations ranging from 1×10^{-7} to 2×10^{-4} g mL^{-1} were tested to determine the linearity for all analytes at the carbon disc electrode. The detection limits were evaluated on the basis of S/N = 3. The results of regression analysis on calibration curves, linear range and detection limits are summarized in Table 1.

System Suitability Test

The method was validated for reproducibility of migration time and peak current of the analytes. The reproducibility was estimated by making five replicate injections of a standard mixture solution (5×10^{-5} g mL^{-1} each) under the

Table 1. Calibration curves and detection limits

Compound	Linearity			Detection limit[b] (g mL^{-1})
	Regression equation[a]	Correlation coefficient	Linear range (g mL^{-1})	
Loureirin A	$Y = 6.07 \times 10^4 + 1.68 \times 10^7 x$	0.9994	5×10^{-6} to 1×10^{-4}	3×10^{-7}
Loureirin B	$Y = 2.76 \times 10^4 + 1.72 \times 10^7 x$	0.9996	5×10^{-6} to 1×10^{-4}	5×10^{-7}
Dracorhodin	$Y = 1.88 \times 10^4 + 2.60 \times 10^7 x$	0.9993	5×10^{-6} to 1×10^{-4}	1×10^{-6}

CE–ED conditions are the same as described in Fig. 2
[a] Y and X are the peak area (mV s) and concentration of the analytes (g mL^{-1}), respectively
[b] Detection limits based on signal-to-noise (S/N) ratio of 3

Table 2. Assay results for crude medicines and preparations ($n = 3$)

Sample brand	Ingredient	Found (mg g^{-1})	RSD (%)
Shulong	Loureirin A	3.8	3.3
	Loureirin B	9.6	3.3
Yunshan	Loureirin A	7.8	1.4
	Loureirin B	6.4	0.4
Yulin	Loureirin A	6.7	0.4
	Loureirin B	7.4	0.8
Sanjin	Loureirin A	12.2	1.2
	Loureirin B	6.8	2.3
Huangguan	Dracorhodin	4.4	3.9

CE–ED conditions are the same as in Fig. 2

selected optimum conditions. The RSDs of peak current were 2.1, 1.7, 4.4%, and RSDs of migration time were 2.9, 2.8, 3.3% for loureirin B, loureirin A and dracohodin, respectively. Recovery experiments under the chosen conditions were also conducted to evaluate the precision and accuracy of the method. Recovery was determined by the standard addition method. Accurate amounts (10 μL of stock solution) of loureirin A, loureirin B were added to a Shulong sample solution whereas dracorhodin was added to a Huangguan sample solution and results ranged from 98.8 to 101.8%, with RSDs below 3.8%.

Solution Stability

The stability of standard and sample solutions was determined by monitoring the peak currents of loureirin A, loureirin B and dracorhodin in sample solutions after 24 h. The results showed that migration time and peak current of each analyte was almost unchanged (RSDs < 5.0) indicating sample stability for at least 24 h.

Application

Under the optimum conditions, loureirin A, loureirin B in Resina Draconis and dracorhodin in Sanguis Draconis were determined by CE. The electropherogram of Resina Draconis is shown in Fig. 2b. Compounds could be identified in the electropherogram by the addition of standards. In case of Resina Draconis peaks 1 and 2 were determined as loureirin B and loureirin A, respectively which did not appear in Sanguis Draconis. The electropherogram of Sanguis Draconis is shown in Fig. 2c. Peak 3 did not emerge in Resina Draconis and was judged to be dracorhodin by comparison with added standard substance. Comparison of both electropherograms enabled facile differentiation between both medicines. Table 2 shows assay results determined for different sample brands.

Conclusions

Loureirin A, loureirin B and dracorhodin possess a phenolic hydroxyl group and all of them can be oxidized with the application of a moderate oxidization potential. The compounds are characteristic marker substances in Resina Draconis and Sanguis Draconis, respectively. The employed methodology enabled a qualitative and quantitative assay to be carried out for the determination of loureirin A, loureirin B and dracorhodin in TCM samples. It was also feasible to differentiate Sanguis Draconis from Resina Draconis for quality control purposes. The assay results indicated that this method was accurate, sensitive and reproducible, providing a useful quantitation and differentiation method for Sanguis Draconis and Resina Draconis without complicated prep-treatment.

Acknowledgements

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