Short Communication

Electrochemical Determination of Baicalein, Baicalin and Quercetin in *Scutellaria barbata*

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Scutellariae barbata, a Chinese traditional medicine plant, are composed of three important components (Baicalein, baicalin and quercetin). Nowadays, to develop a simple technique for determining the containing Baicalein, baicalin and quercetin accurately and economically is highly demanded. Herein, capillary electrophoresis (CE) was incorporated with electrochemical detection (ED) to provide more sensitive and selective determination method. Baicalein, baicalin and quercetin could be well separated within 12 min using the proposed CE-ED method with the optimum conditions. The detection limits (S/N=3) was in the concentration range of 0.214 to 0.495 μM for all three analysts. Owing to the high resolution, good reproducibility and excellent stability, the CE-ED method can be successfully employed for the determination of actual samples.

1. INTRODUCTION

Scutellariae barbata, a Chinese traditional medicine composed of active components baicalin and baicalein, has widely used as therapeutical medicine for heat-clearing, fire-purging, moistening, detoxifying and most importantly cancer-combating [1]. In China, the content of baicalin in *Scutellariae barbata* is required to be more than 8.0% by the Public Health Department, which was resulted from the easy hydrolysis of baicalin to baicalein and glucuronic acid by baicalinase. In addition, owing to the convenient usage, certain concentrated composite herbal that contain *Scutellariae barbata* are also widely used in oriental countries. Therefore, to develop a simple

technique for determining baicalin ((2*S*,3*S*,4*S*,5*R*,6*S*)-6-(5,6-dihydroxy-4-oxo-2-phenyl-chromen-7-yl)oxy-3,4,5-trihydroxy-tetrahydropyran-2-carboxylic acid), baicalein (5,6,7-Trihydroxy-2-phenyl-chromen-4-one) and quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one) in traditional Chinese medicines accurately and economically is highly demanded.

Several methods including pulse porlarography [2], ultraviolet (UV) spectrophotometry [3, 4], thin layer chromatography [5], electrochemistry [6-9] and liquid chromatography (LC) [10-14] have been proposed to determine baicalin, baicalein and quercetin in Scutellariae *barbata*. However, the detection limits of above-mentioned methods are unsatisfactory which cause the difficulty of determining baicalein, baicalin and quercetin at low concentrations. Capillary electrophoresis (CE), firstly presented by Joegenson and Luckas in 1981 [15, 16], has been widely employed in the separation of various samples owing to many attractive advantages such as fast analysis and excellent separation performance. Moreover, capillary electrophoresis (CE) can be incorporated with electrochemical detection (ED) to provide a new determination technique. And the proposed CE-ED method can determine the content of baicalin, baicalein and quercetin more sensitively and selectively [17].

In our work, the determination of baicalein, baicalin and quercetin (molecular structures in Fig. 1) in *Scutellariae barbata* was investigated by a developed CE-ED method. The CE-ED determination technique is simple, accurate, sensitive and effective, which makes it a reliable method for controlling the quality of pharmaceutical preparations that contain *Scutellariae barbata*, and the quality in medicinal manufacturers as well.

2. EXPERIMENTS

A CE-ED system that composed of capillary electrophoresis (CE) as separation system and electrochemical detection (ED) as detection system has been developped in the laboratory. A fused-silica capillary (75 cm long, 25 μm inside diameter and 360 μm outside diameter) which was purchased from Polymicro Technologies was used for separation. The separation voltage applied to the capillary (inlet end at positive and outlet end at ground) was supplied by a high-voltage power system (±30 kV) that was obtained from Shanghai Institute of Applied Physics. Samples were all injected by electrokinetic drive with 16 kV for 8 s. A three-electrode electrochemical cell was used with carbon-disc with diameter of 300 μm as working electrode, SCE as reference electrode and platinum as auxiliary electrode. The working electrode was laboratory made from graphite rod with diameter of 300 μm by polishing method. Before the usage of the carbon electrode, the surface was firstly polished using emery sandpaper, then washed in doubly deionised water under sonication. Finally, an Oriel micromanipulator (Stratford, CT, USA) was employed for installing the clean carbon electrode at the outlet of the capillary [18]. BAS LC-3D amperometric detector (Biochemical System, USA) and chart recorder (Shanghai Dahua Instrumental Factory, China) were also employed to achieve the electropherograms.

Baicalein, baicalin and quercetin were obtained from Sigma Aldrich and used as received. Milli-Q water was used in the entire experiments. Dry *Scutellariae barbata* was supplied by a Chinese

traditional drug store Tongren Tang. The solution of each analyst (baicalein, baicalin and quercetin) with concentration of 5.0 mM were prepared using the mixture of ethanol and 50 mM borate buffer (pH 8.0) with the volume ration of 1:1 as solvent. Running buffer was used to dilute the solution to desired concentration. Hydrodynamic voltammograms of each analyte were obtained separately by application of ten sequential potential pulses (from +0.1 to +1.1 V, 100 ms each) for triplicate injections of standard solutions through the system using the multiple-pulse amperometric technique. CV was performed in 0.1 M pH 7.0 PBS from 0 to 1.0 V at scan rate of 50 mV/s.

Purchased *Scutellariae barbata* was firstly dried in an oven at 60 °C for 4 h. Nearly 1 g of the powder (after pulverization) was refluxed in 70% ethanol solution (50 mL) at 80°C for 1 h. After cooling, the mixture was treated with filtration with a paper filter and the obtained solution was then treated under vacuum to make the volume to be 50 mL by evaporating amounts of solvent. Finally, 2.0 mL of above solution was taken out and then diluted to 50 mL with the running buffer.
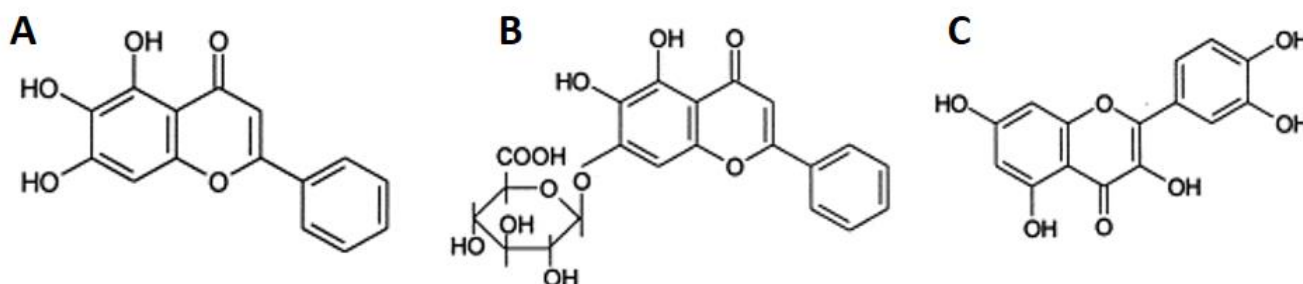

Figure 1. The molecular structures of (A) baicalein, (B) baicalin and (C) quercetin.

3. RESULTS AND DISCUSSION

The sensitivity of the proposed CE-ED technique for the determination of baicalein, baicalin and quercetin was greatly influenced by the applied potential for working electrode. As demonstrated in Fig. 2A, the peak current of each analyst increased rapidly with increasing potential when the applied potential was above +0.70 V. Nevertheless, the currents of the three analysts increased much slower as the potential was higher than +0.90 V. Despite the fact that larger peak currents achieved with potential larger than +0.90 V, the background current also increased strongly and the resultant unstable baseline hindered enormously the stable and effective determination. Therefore, the determined applied potential was +0.70 V with acceptable background current and highest *S/N* ratio.

Cyclic voltammograms (CVs) of all the three analysts (baicalein, baicalin and quercetin) were also investigated on carbon-disc electrode and the results were given in Fig. 2B-D. The oxidation peaks were observed for all mentioned analytes, probably ascribed to the oxidation of pyranoid rings and phenolic hydroxyl groups [13]. The oxidation was irreversible with the potential ranging from 0.70 to 0.90 V. In addition, more than two oxidation peaks were observed, indicating different electron transfer steps involved in the oxidation process. What's more, it was found that the oxidation of

solvent occurred as the potential above +0.90 V, further confirming the chosen potential of +0.70 V is highly suitable in CE experiments.
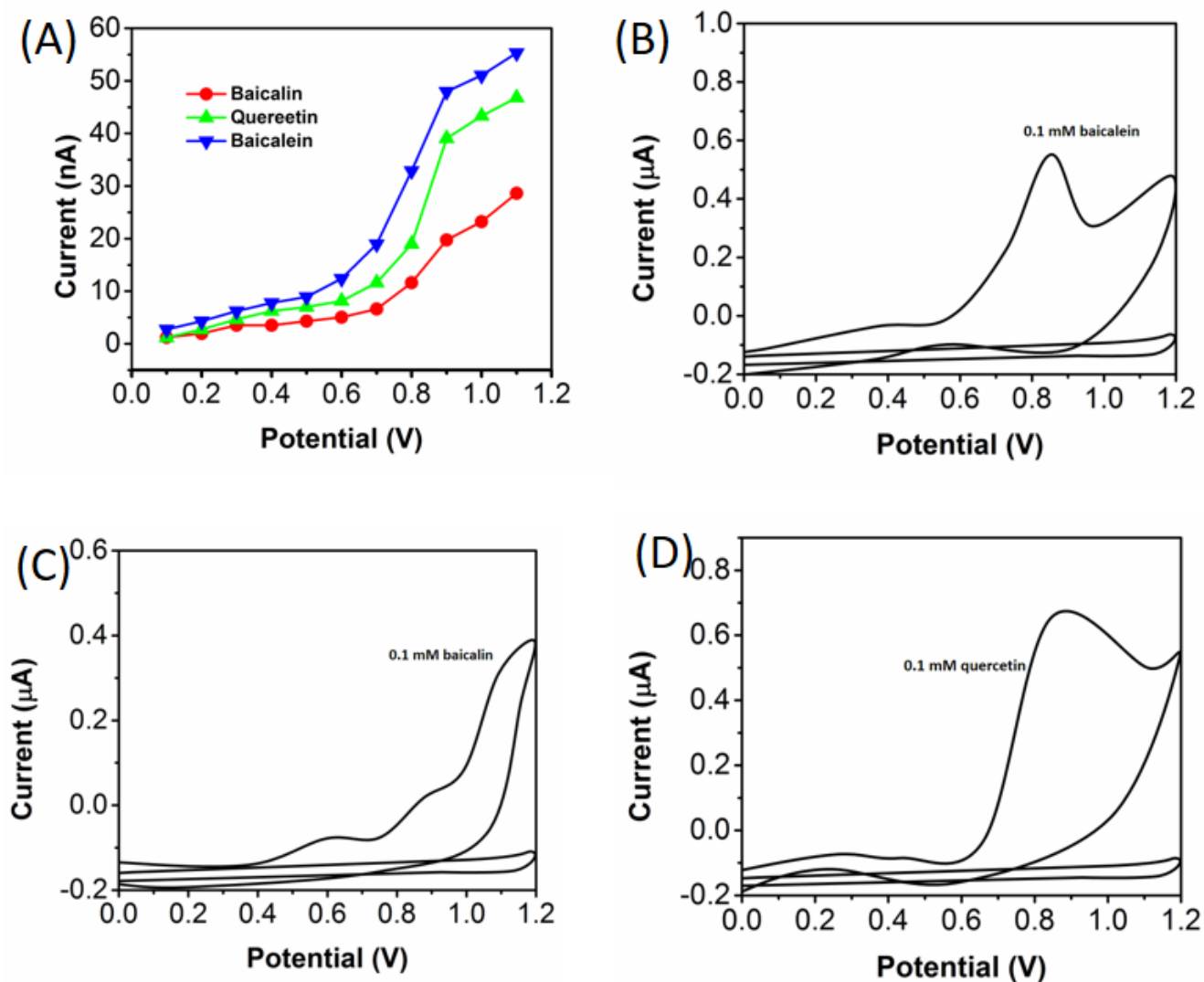

Figure 2. (A) Hydrodynamic voltammograms (HDVs) and (B-D) cyclic voltammograms (CVs) of all three analysts (baicalein, baicalin, quercetin) with concentration of 0.1 mM (Fused-silica capillary; electrokinetic injection at 12 kV for 6 s; carbon-disk working electrode; 0.1 M BB with pH of 9.0 as running buffer; 12 kV as voltage; 100 mV/s as scan rate).

The pH of the running buffer is of great importance to the performance of capillary electrophoresis (CE). Owing to the changeable zeta potential (ζ), overall charge and electroosmotic flow (EOF) of the running buffer with varying pH value, the migration time and the separation efficiency of the tested analyte will also undergo a revolution. The influence of pH value on the migration time of the tested analyte was investigated and the results were given in Fig. 3A. The tested seven different pH values were 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0, respectively. As can be observed clearly, the resolution for all analytes was poor at pH value of 8.0. As the pH increased, the migration time showed an increase accompanied with enhanced resolution, probably ascribed to the improvement

of dissociation process of the hydroxyl group. However, when the pH value was above 9.5, poor peak shape and low peak current were observed. Therefore, 9.0 was the most suitable pH value, at which condition the three analytes can be separated well in a short period of migration time. In general, the determined pH value of running buffer (100 mM BB) was 9.0 in consideration of migration time, peak current and resolution as well.

The concentration of the running buffer was also required to be considered carefully in order to achieve excellent performance (e.g., short migration time, high peak current and resolution) of capillary electrophoresis. This is mainly due to the change of the viscosity of the solution that greatly influenced the diffusion of analytes with different concentration of running buffer. Besides, the zeta potential (ζ) of the inner surface of capillary tube was also changed. As can be observed in Fig. 3B, the migration time showed an increase as the concentration of buffer increased Nevertheless, a negative effect was observed when the concentration of buffer was higher than 100 mM. The detection limits increased which was resulted from the decrease of the peak currents. In addition, the influence of Joule heat is more outstanding. Therefore, the determined concentration of running buffer was 100 mM in our study in consideration of migration time, resolution, peak current and buffer capacity as well.
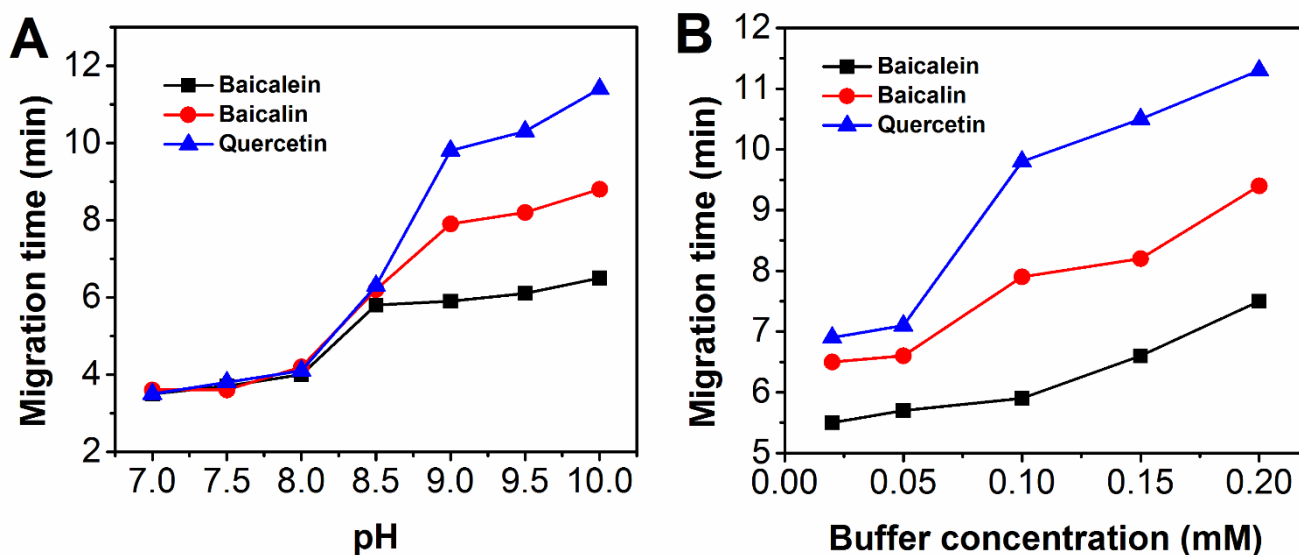

Figure 3. Effect of (A) acidity and (B) concentration of the running buffer on the peak current of the analytes.

Fig. 4A showed the influence of separation voltage on the performance of CE-ED technique. The migration time was shorter for all the three analytes with increasing separation voltage. However, poor detection limits was also achieved owing to the increased baseline noise with increasing separation voltage. In addition, the Joule heat was found to be more pronounced with higher separation voltage, which greatly reduced the resolution of all three analytes and further the separation efficiency of the proposed method. Nevertheless, the separation voltage could not be set too low due to the achievement of broad peak resulted from the increase of analysis time with lower separation voltage. The optimized separation voltage was 12 kV after comprehensive consideration of all factors.

The peak shape, current and resolution can be affected by the amount of samples which was changed by the injection time herein. Fig. 4B showed the effect of injection time (1, 3, 5, 7, 9, 11, 13 and 15 s at a voltage of 12 kV) on the separation performance of capillary electrophoresis. As the injection time increased, both the peak current and the width of the peak increased. The peak current increased and maintained at a constant value as the injection time was above 7 s. However, the broadening effect of the peak becomes more severe at this injection time. Therefore, the optimized sampling time was 7 s (at 12 kV) in our experiments.
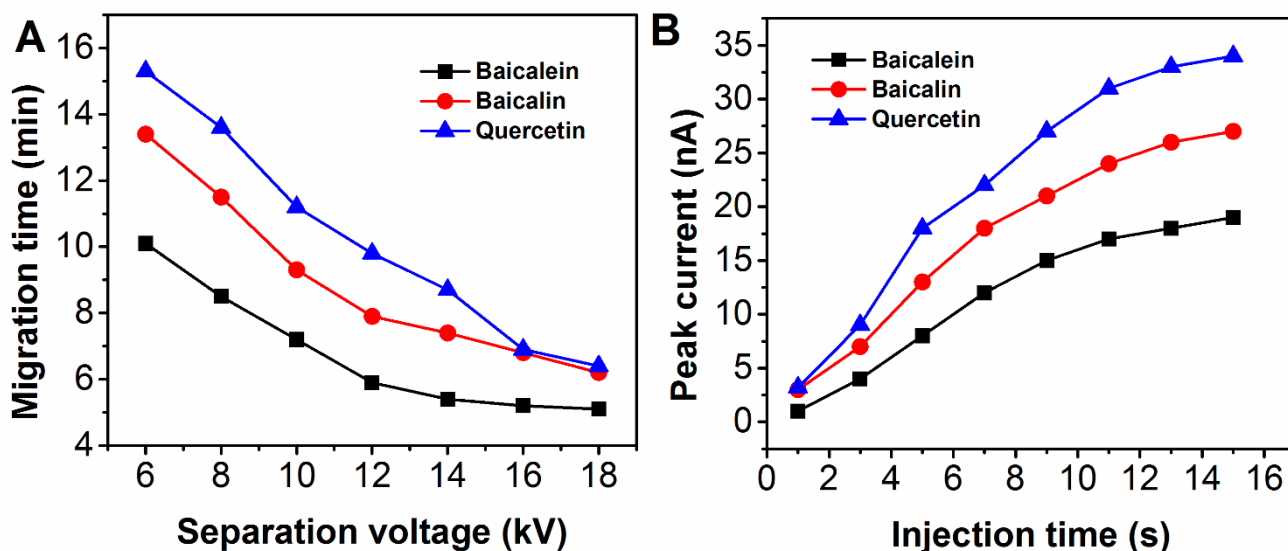

Figure 4. Effect of (A) separation voltage on the migration time and (B) effect of injection time on the peak current of the analytes.

Upon all performed experiments mentioned above, the optimizing conditions are as follows: the applied potential +0.70 V, the concentration of running buffer 100 mM, the separation voltage 12 kV and the injection time 7 s. Then typical electropherograms of the three analysts were obtained. Fig. 5A showed the typical electropherograms of standard mixture solutions of 0.05 mM of baicalein, baicalin and quercetin. Obviously, all the three analytes can be separated well within 12 min. Typical electropherograms of the diluted extracts from *Scutellariae barbata* using 70 % ethanol solution, 100 mM borate buffer and Milli-Q water were given in Fig. 5B, 5C and 5D, respectively. When 70 % ethanol solution was employed as the extraction solution, the mean contents of baicalein, baicalin and quercetin were 15.22, 87.17 and 2.421 mg/g, respectively. And the contents of baicalein and baicalin were very similar to previously reported. The corresponding R.S.D. were 1.77, 3.25 and 5.09%, respectively (*n*=5). When 100 mM borate buffer was employed as the extraction solution, the contents were 20.77, 51.23 and 1.879 mg/g with corresponding R.S.D. of 2.24, 2.07 and 3.52% (*n*=5) for baicalein, baicalin and quercetin separately. The content of baicalein was relatively high owing to the hydrolysis of baicalin in alkali borate buffer. As can be seen in Fig. 5D, when the Milli-Q water was employed as the extraction solution, the contents decreased to 10.68, 42.57 and 1.205 mg/g for baicalein, baicalin and quercetin separately corresponding R.S.D. of 3.57, 1.50 and 2.21%,

respectively. Obviously, as to the extraction efficiency of baicalin, 70% ethanol solution was the best solvent for extracting baicalin from Scutellariae Radix with the highest baicalin content. Table 1 illustrates the detail value collected using different extraction solvet.
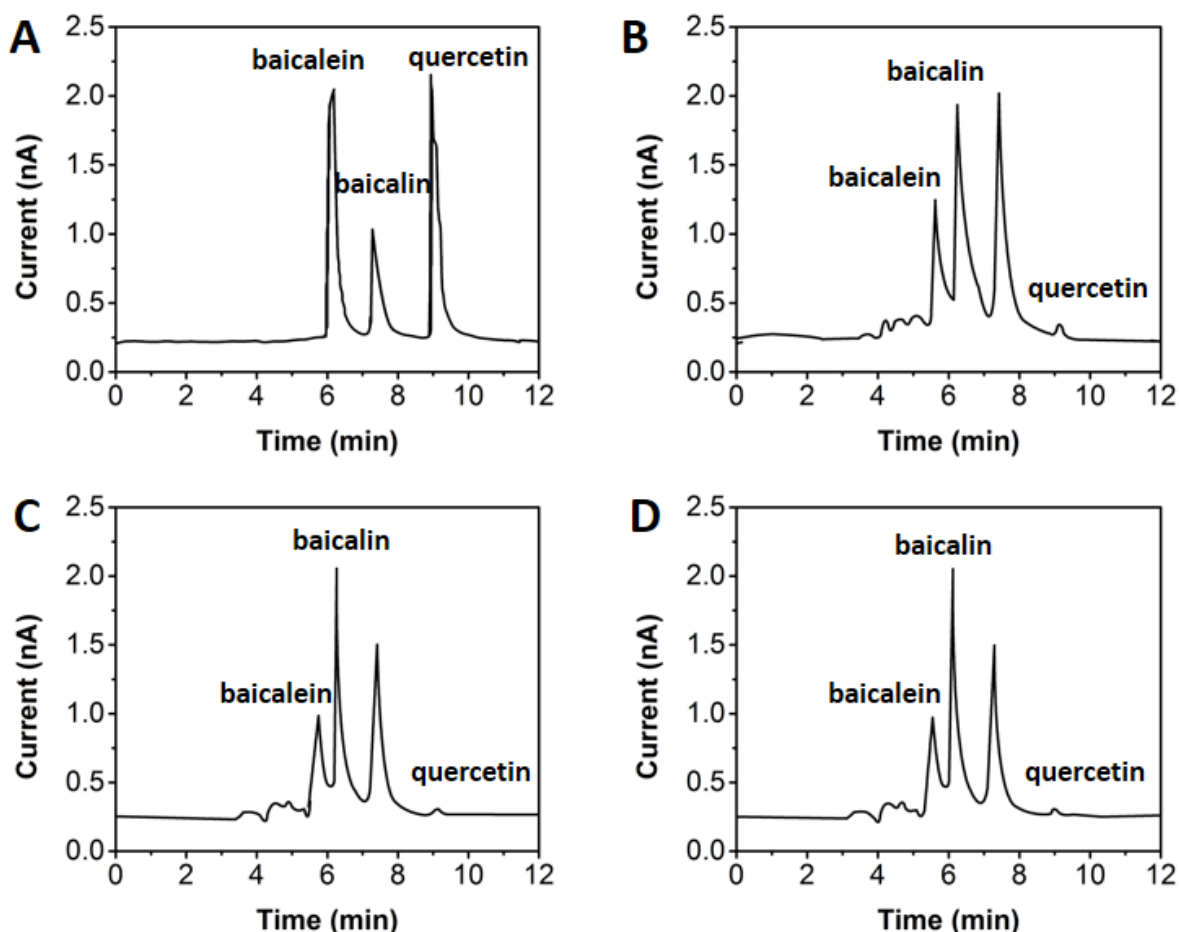

Figure 5. Electropherograms of (A) standard mixture solution and (B-D) the diluted extracts from *Scutellariae barbata* with different extraction solution (B) 70% aqueous ethanol, (C) 100 mM borate buffer and (D) doubly distilled water.

Table 1. Detection results of *Scutellariae barbata* extract using 70% aqueous ethanol, 100 mM borate buffer and doubly distilled water.

Solvent	Baicalein (mg/g)	RSD (%)	Baicalin (mg/g)	RSD (%)	Quercetin (mg/g)	RSD (%)
70% ethanol	15.22	1.77	87.17	3.25	2.421	5.09
100 mM borate buffer	20.77	2.24	51.23	2.07	1.879	3.52
Doubly distilled water	10.68	3.57	42.57	1.50	1.205	2.21

The reproducibility of the proposed method for the determination of the three analysts was investigated as to the migration time and peak current. Specific experimental steps were as follows: a standard mixture solution of baicalein, baicalin and quercetin with each concentration of 0.05 mM was

determined seven times. The R.S.D. of migration time and peak current were 1.51-1.93% and 2.23-4.05%, respectively.

The linearity was investigated using a series of the standard mixture solutions with the concentration varying from 0.1 μM to1 mM. The detection limits (S/N=3) was in the concentration range of 0.214 to 0.495 μM for all three analysts. Our proposed method is more sensitive than some specific target analyte sensor. According to the electrochemical sensor prepared by Gupta and co-workers [8], the NiO/CNTs nanocomposite modified ionic liquid carbon paste electrode could determine the quercetin as low as 0.3 μM. On the other hand, the performance of our method in baicalin determination is slightly lower than several reports [7, 19]. For example, Zhang and co-workers demonstrated an electrochemical baicaline sensor based on glassy carbon electrode modified with molybdenum disulfide nanosheets [7]. The detection limit of the fabricated sensor could reach to 0.05 μM.

The peak current was related linearly with the concentration for all the three analysts, and the correlation coefficients are all around 0.999.

4. CONCLUSIONS

In conclusion, a CE-ED technique has been proposed in this study for determination of baicalein, baicalin and quercetin in *Scutellariae barbata*. The most outstanding advantage of the analytical tool capillary electrophoresis (CE) is the very high reproducibility resulted from the much easier cleaning process of capillary. The optimum determination conditions are as follows: +0.70 V as the applied potential, 100 mM as the concentration of running buffer, 12 kV as the separation voltage, 7 s as the injection time and 70% ethanol solution as the extraction solvent. The peak current was related linearly with the concentration ranging from 0.1 μM to1 Mm for all three analysts. And the detection limits (S/N=3) was in the concentration range of 0.214 to 0.495 μM. The proposed CE-ED technique can be a competitive alternative for LC in the constituent study of natural plants owing to varieties of advantages such as high resolution, good reproducibility, low cost and excellent stability.

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