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Determination of flavonoids in *Houttuynia cordata* Thunb. and *Saururus chinensis* (Lour.) Bail. by capillary electrophoresis with electrochemical detection

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Abstract

Four flavonoids (rutin, hyperoside, quercitrin and quercetin) in *Houttuynia cordata* Thunb. and *Saururus chinensis* (Lour.) Bail. were determined by capillary electrophoresis with wall-jet amperometric detection. The working electrode was a 500 μm diameter carbon disc electrode and the detection potential was +0.95 V (versus Ag/AgCl). Effects of several important factors, such as the running buffer and its corresponding pH and concentration, separation voltage, injection time were investigated to acquire the optimum conditions for separation of these four flavonoids. Baseline separation for the four flavonoids was obtained within 21 min in a 60 cm length capillary at a separation voltage of 15 kV with a 60 mmoL/L $Na_2B_4O_7$–120 mmoL/L NaH_2PO_4 buffer (pH 8.8) as running buffer. The relationship between peak currents and analyte concentrations was linear over about two orders of magnitude with detection limits (defined as S/N = 3) ranging from 0.02 to 0.05 μg/mL for all analytes. This method was applied for the determination of the above four flavonoids in *H. cordata* Thunb. and *S. chinensis* (Lour.) Bail. with simple extraction procedures, and the assay results were satisfactory.

Keywords: Flavonoids; Capillary electrophoresis; Electrochemical detection; *Houttuynia cordata* Thunb.; *Saururus chinensis* (Lour.) Bail.

1. Introduction

Flavonoids, which exist abundantly in medicinal plants, exhibit a wide range of biological effects, such as anti-inflammatory, anti-tumor, anti-allergic, anti-virus, anti-bacteria and anti-oxidation [1], so flavonoids are main bioactive ingredients in medicinal plants and their analysis is of considerable interest. The main method used for the determination of flavonoids in medicinal plants is generally high-performance liquid chromatography (HPLC) [2–8], but HPLC has some shortcomings, such as relatively large consumption of materials, time-consuming sample pre-treatment, etc., which limit its application in this area. As a newly arisen separation method, capillary electrophoresis (CE) has been a useful tool for the analysis of bioactive constituents in medicinal plants for its high separation efficiency, minimal sample volume and rapid analysis. Until now, there are several reports on the determination of flavonoids in medicinal plants by CE with ultraviolet (UV) detection [9–14]. However, on-line sample pre-treatment is sometimes required to pre-concentrate and separate minor or trace flavonoids from complex plant matrix due to the poor sensitivity and selectivity of UV detector [9]. Compared to UV detection, electrochemical detection (ED) provides high sensitivity and good selectivity for the electroactive substances [15]. So CE–ED has been employed for the determination of flavonoids in some medicinal plants [16–20].

Houttuynia cordata Thunb. and *Saururus chinensis* (Lour.) Bail. are two medicinal plants of Saururaceae family. Saururaceae plants have been used to clear heat, counteract toxins, evacuate pus, relieve stranguria and alleviate edema

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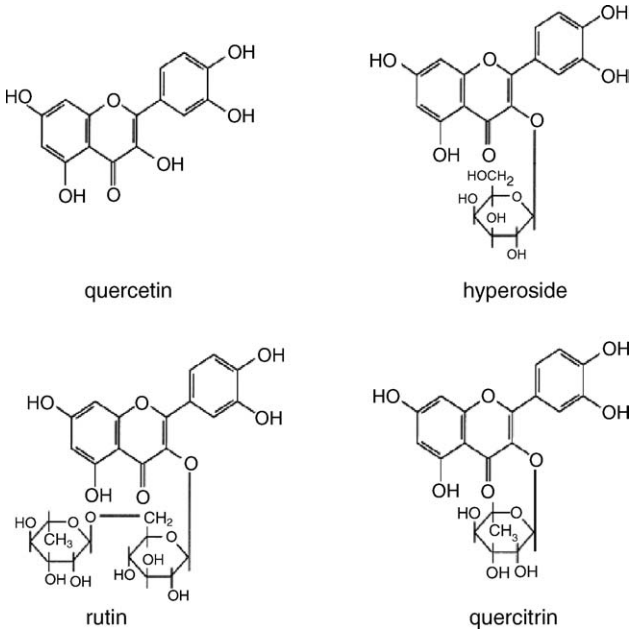

Fig. 1. The molecular structures of quercetin, hyperoside, rutin and quercitrin.

due to the presence of many bioactive ingredients, especially flavonoids (such as rutin, hyperoside, quercitrin and quercetin, and their molecular structures are shown in Fig. 1) [21], so it is necessary to develop a simple and reliable method to determine the above four flavonoids for estimating the quality of the two medicinal plants. Pulse polarography [22], coulomb titration [23] and spectrophotometry [24,25] have been used to determine the total amount of flavonoids in the above two medicinal plants. Quercetin and rutin in *S. chinensis* (Lour.) Bail. have also been determined by HPLC with UV detection [5]. In this paper, we developed a simple CE–ED system to determine the above four flavonoids in *H. cordata* Thunb. and *S. chinensis* (Lour.) Bail., and the assay results were satisfactory.

2. Experimental

2.1. Chemicals

Rutin, hyperoside, quercitrin and quercetin were obtained from Chinese Chemical and Biological Drugs Institute (Beijing, China), *H. cordata* Thunb. and *S. chinensis* (Lour.) Bail. were purchased from Laoyaogong Drugstore, Fuzhou, Fujian Province, China. All aqueous solutions were made up in doubly distilled water. Other chemicals were of analytical grade.

Standard stock solutions of the four flavonoids at concentration of 1.00×10^3 μg/mL were prepared in ethanol and diluted to the desired concentration with the running buffer just prior to use. All standard solutions were kept in a refrigerator and were stable for at least 2 months. The borate–phosphate running buffer was prepared by mixing $Na_2B_4O_7$ solution (concentrations ranging from 30 to 80 mmol/L) with NaH_2PO_4 solution (corresponding concentrations ranging from 60 to 160 mmol/L) and the pH value of the running buffer was measured by a pH meter. Before use, all solutions were filtered through a 0.22 μm polypropylene filter film.

2.2. Apparatus

A laboratory-built capillary electrophoresis system equipped with wall-jet amperometric detector was used in the experiment. The details of this system have been described in the previous works [26,27]. Separation capillary was an untreated fused silica capillary with 60 cm × 25 μm i.d. × 370 μm o.d. (Hebei Yongnian Optic Fiber Factory, China). The inlet end of the capillary was held at a positive potential and outlet end of capillary was kept at ground. A ±30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, China) provided a separation voltage between the ends of the capillary. A pre-aligned electrochemical cell, consisting of three-electrode (a 500 μm diameter carbon disc working electrode, a platinum auxiliary electrode and a Ag/AgCl reference electrode), was used in combination with a BAS LC-4C amperometric detector (Bioanalytical System, West Lafayette, IN, USA). The electropherograms were monitored using a Chromatogram workstation (Model HW-2000, Qianpu Software company, Shanghai, China). A PHS-3C meter (Shanghai Dapu Instrument Company, Shanghai, China) was used to measure the pH value of the running buffer. A CHI660 electrochemical system (CH instruments, Austin, TX, USA) was chosen to perform cyclic voltammograms.

2.3. Sample solution preparation

After being air-dried and crushed into powder, 0.5 g of the accurately weighed medicinal plant sample (stems or leaves of *S. chinensis* (Lour.) Bail. and *H. cordata* Thunb.) was extracted with 2 × 20 mL ethanol by sonication for 30 min. The extracts were combined and concentrated to about 3.5 mL at 50–60 °C, and then diluted to 4.0 mL with ethanol. The extract was six-fold diluted with a 60 mmol/L $Na_2B_4O_7$–120 mmol/L NaH_2PO_4 (pH 8.8) running buffer just prior to its analysis. Peak identification was performed by standard addition methods.

2.4. Procedures

The carbon disc working electrode was successively polished with sand emery paper and rinsed with doubly distilled water. CE was performed at the separation voltage of 15 kV using a 60 mmol/L $Na_2B_4O_7$–120 mmol/L NaH_2PO_4 (pH 8.8) running buffer. The potential applied to the working electrode was +0.95 V (versus Ag/AgCl). Sample was injected electrokinetically at 15 kV for 10 s.

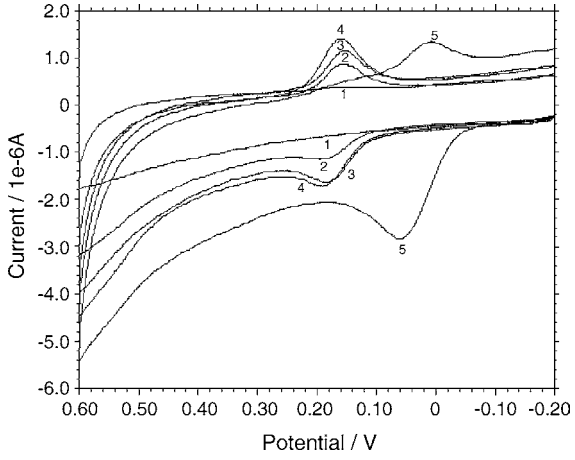

Fig. 2. Cyclic voltammograms of the four analytes in a 60 mmol/L $Na_2B_4O_7$–120 mmol/L NaH_2PO_4 solution (pH 8.8). Working electrode: glassy carbon electrode; auxiliary electrode: platinum wire; reference electrode: Ag/AgCl; scan rate: 100 mV/s. (1) Blank solution; (2) 9.9 μg/mL hyperoside; (3) 20.8 μg/mL rutin; (4) 14.8 μg/mL quercitrin; (5) 9.9 μg/mL quercetin.

3. Results and discussion

3.1. Electrochemistry

The electrochemical behavior of the four analytes at the glassy carbon electrode (GCE) in a 60 mmol/L $Na_2B_4O_7$–120 mmol/L NaH_2PO_4 (pH 8.8) running buffer was investigated by using cyclic voltammetry. As shown in Fig. 2, in the potential range of −200 to +600 mV, quercetin and its glucosides hyperoside, rutin and quercitrin produce an obvious anodic peak, respectively, due to the oxidation of the two catechol hydroxyl groups of analytes. It should be noted that quercetin exhibits a quasi-reversible electrode reaction whereas rutin, hyperoside and quercitrin show an almost reversible electrode process, respectively. This phenomenon maybe attributed to that the presence of the hydrophilic carbohydrate group in flavonoid glycoside would inhibit the adsorption of the oxidation products at the GCE. Thus, the four analytes are electroactive and can be determined by electrochemical detection.

3.2. Hydrodynamic voltammetry

In order to select an optimum detection potential, a hydrodynamic voltammetry experiment was performed and the results are illustrated in Fig. 3. The peak currents of all analytes increased with the detection potential, but when the applied potential was higher than +0.95 V (versus Ag/AgCl), both the background current and the baseline noise increased greatly, so +0.95 V (versus Ag/AgCl) was selected as detection potential in the subsequent experiments for a suitable compromise of high sensitivity and low background current.

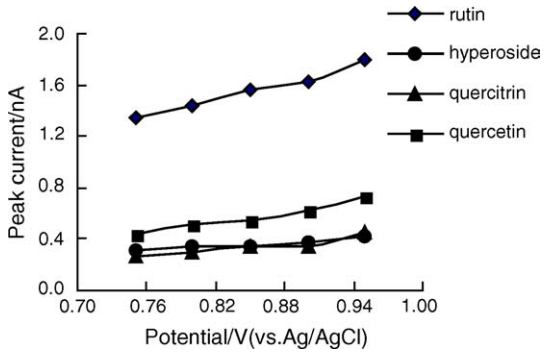

Fig. 3. Hydrodynamic voltammograms of the four analytes. Fused-silica capillary: 60 cm × 25 μm i.d. × 370 μm o.d.; working electrode: 500 μm diameter carbon disc electrode; running buffer: 60 mmol/L $Na_2B_4O_7$–120 mmol/L NaH_2PO_4 (pH 8.8); separation voltage: 15 kV; electrokinetic injection: 10 s (at 15 kV).

3.3. Selection of the running buffer

Since borate can chelate with flavonoids to form more soluble complex anions, borate buffer ($Na_2B_4O_7$–H_3BO_3) was first selected as running buffer to separate the four flavonoids. However, the baseline separation of the analytes was still difficult to achieve by adjusting the pH and concentration of the simple borate running buffer. In order to improve the resolution, another two buffer solutions, namely $Na_2B_4O_7$–NaH_2PO_4 and NaH_2PO_4–NaOH have been selected as the running buffer for testing their effects on the separation of analytes mentioned above. The experimental results showed that $Na_2B_4O_7$–NaH_2PO_4 buffer gave not only the best resolution but also the highest sensitivity under the same conditions. Therefore, $Na_2B_4O_7$–NaH_2PO_4 buffer was chosen as running buffer to separate the analytes.

3.4. Effects of the pH and concentration of the running buffer

The pH of the running buffer affects the electroosmotic flow as well as the overall charge of the analytes, which affects the migration time and the resolution of the analytes. Experiments were performed using a 60 mmol/L $Na_2B_4O_7$–120 mmol/L NaH_2PO_4 buffer with different pH. As shown in Fig. 4, the resolution of the four analytes was poor at pH 7.0, the migration time increased and the resolution was improved with increasing of pH value, but the peak currents decreased when pH was higher than 8.5. The four analytes could be well separated in the pH range of 8.0–9.0. In this paper, a 60 mmol/L $Na_2B_4O_7$–120 mmol/L NaH_2PO_4 buffer of pH 8.8 was chosen as the running buffer in considering the sensitivity, resolution (especially the resolution of the other coexist components in real samples) and analytical time.

Besides the pH value, the running buffer concentration is also an important parameter. The experimental results indicated that the migration time and the resolution increased,

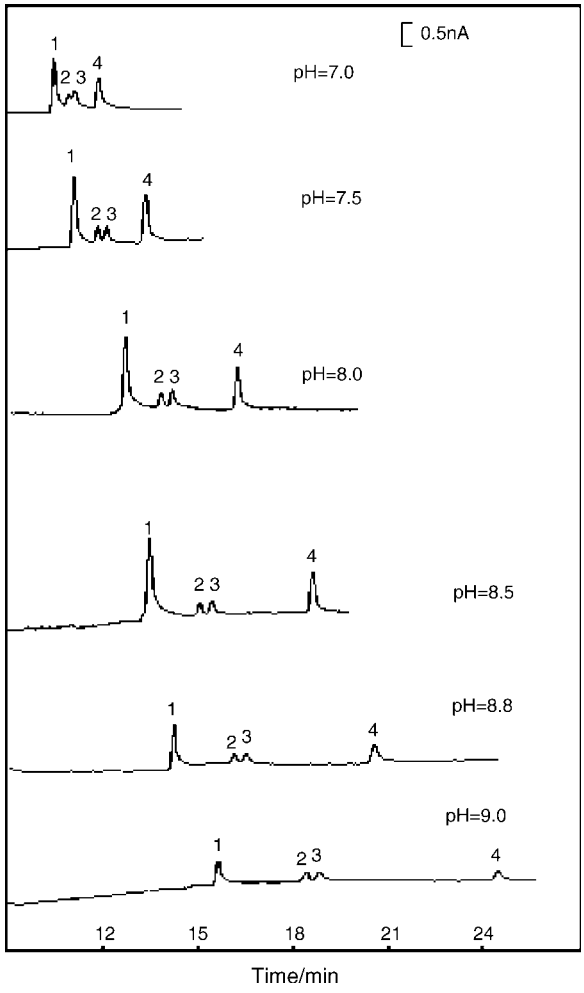

Fig. 4. Effect of pH on migration time. Working potential: +0.95 V (vs. Ag/AgCl); other conditions as in Fig. 3. (1) Rutin; (2) hyperoside; (3) quercitrin; (4) quercetin.

but the peak currents of all analytes decreased with increasing of buffer concentration, which was due to that the degree of chelation between flavonoids and borate increased with the increasing of buffer concentration. In addition, the high buffer concentration also produced more Joule heating, which resulted in poor sensitivity. So, a 60 mmol/L $Na_2B_4O_7$–120 mmol/L NaH_2PO_4 buffer of pH 8.8 was chosen as the running buffer in this work.

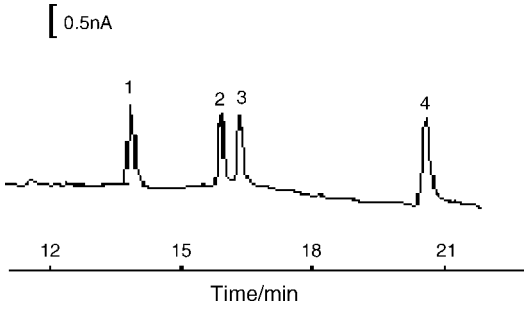

Fig. 5. Electropherogram of the standard mixture solution of flavonoids. Working potential: +0.95 V (vs. Ag/AgCl); other conditions as in Fig. 3. (1) Rutin (5.4 μg/mL); (2) hyperoside (5.4 μg/mL); (3) quercitrin (5.4 μg/mL); (4) quercetin (6.0 μg/mL).

3.5. Effect of separation voltage and injection time

The influence of separation voltage on the migration time was studied in this work. The results illustrated that the resolution of the four flavonoids was not improved with the separation voltage ranging from 12 to 21 kV. Although the high separation voltage gave shorter migration time for all analytes, it produced more baseline noise, which resulted in higher detection limits. Thus, the optimum separation voltage was 15 kV, at which good separation could be obtained for all analytes within 21 min.

The injection time affects both the peak current and peak shape. The effect of injection time on peak current was investigated by varying injection time from 5 to 15 s at 15 kV. The peak current increased with the injection time. However, when the injection time was longer than 10 s, the peak current increased slowly and the peak exhibited obvious broadening. Therefore, 10 s (at 15 kV) was chosen as the optimum injection time in this work.

Under the optimum conditions, the four flavonoids could be completely separated and detected within 21 min, and the typical electropherogram for a standard mixture solution is shown in Fig. 5.

3.6. Analytical performance

The repeatability of the peak current and the migration time for all analytes was estimated by making repetitive injections of a standard mixture solution under the optimum

Table 1
The results of regression analysis on calibration curves and detection limit[a]

Analyte	Regression equation, $Y = a + bX$[b]	Correlation coefficient	Linear range (μg/mL)	Detection limit[c] (μg/mL)
Rutin	$Y = 0.0292 + 0.2356X$	0.9976	0.20–16.0	0.05
Hyperoside	$Y = -0.0225 + 0.2615X$	0.9988	0.20–16.0	0.02
Quercitrin	$Y = -0.0518 + 0.2833X$	0.9958	0.20–16.0	0.03
Quercetin	$Y = -0.2157 + 0.2678X$	0.9981	0.43–35.0	0.02

[a] Working potential: +0.95 V (vs. Ag/AgCl); other conditions as in Fig. 3.
[b] Here, Y and X are the peak current (nA) and concentration of the analytes (μg/mL), respectively.
[c] The detection limits corresponding to concentrations giving signal-to-noise ratio of 3.

condition. The results show that the inter-day relative standard deviations (R.S.D.s) of peak current and migration time are 3.8 and 0.14% for rutin, 4.5 and 0.16% for hyperoside, 5.5 and 0.14% for quercitrin and 3.0 and 0.22% for quercetin, respectively.

A series of the standard mixture solutions were tested to determine the response linearity of the four analytes under the optimized condition. The detection limits are evaluated on the basis of signal-to-noise ratio of 3 and the results are presented in Table 1. The calibration curves exhibit excellent linearity over the concentration range of about two orders of magnitude with the detection limits ranged from 0.02 to 0.05 μg/mL for all analytes.

3.7. Sample analysis and recovery

Hyperoside, quercitrin, quercetin and rutin in the different parts of *H. cordata* Thunb. and *S. chinensis* (Lour.) Bail. were determined by CE–ED. The typical electropherograms of the samples are shown in Fig. 6. The comparisons of the above analytes in two different parts of the medicinal plant, respectively, leaves and stems are listed in Table 2. As shown

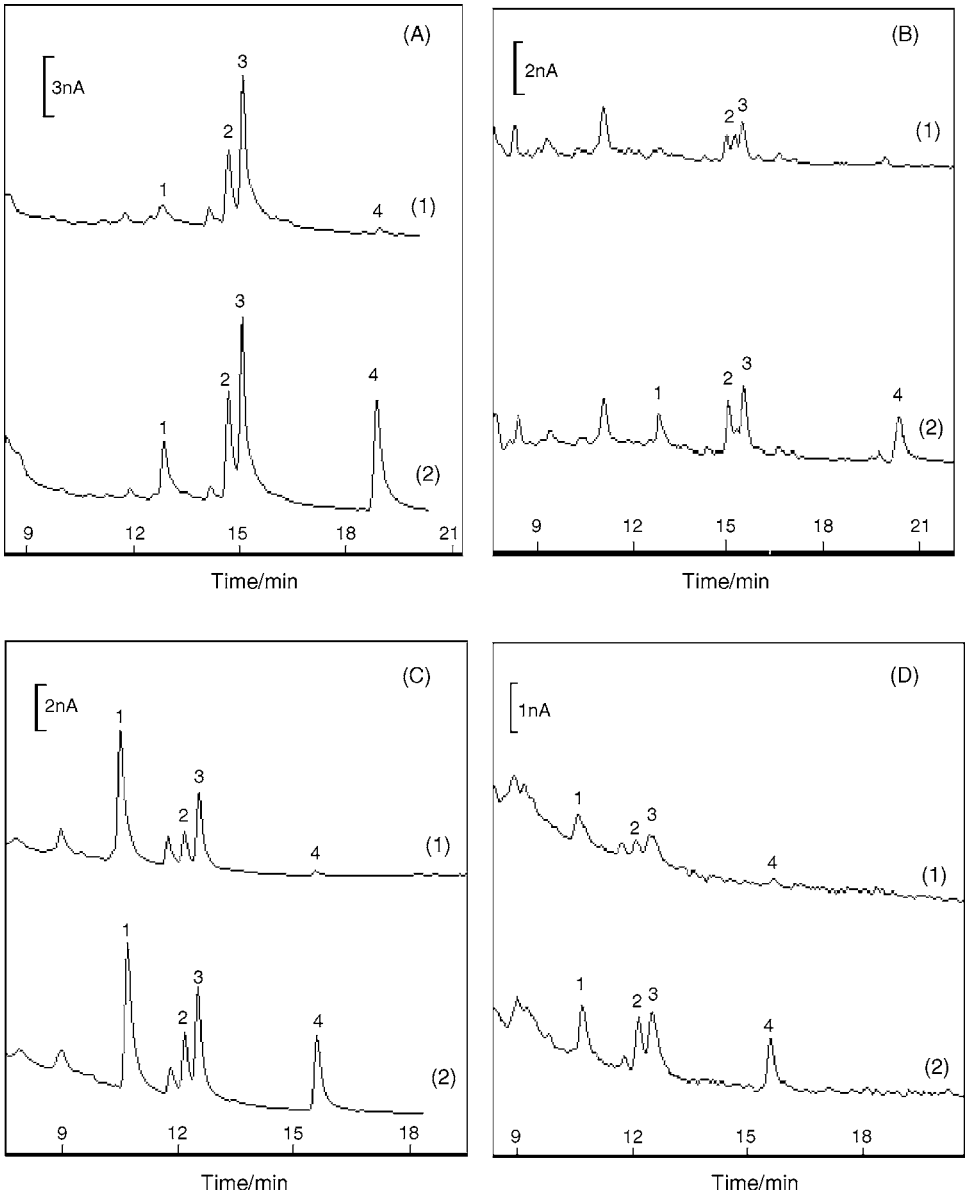

Fig. 6. Electropherogram of (A): (1) the extract of *Houttuynia cordata* Thunb. leaves and (2) the above extract + the accurately known amounts of the four flavonoids; (B): (1) the extract of *H. cordata* Thunb. stems and (2) the above extract + the accurately known amounts of the four flavonoids; (C): (1) the extract of *Saururus chinensis* (Lour.) Bail. leaves and (2) the above extract + the accurately known amounts of the four flavonoids; (D):(1) the extract of *S. chinensis* (Lour.) Bail. stems and (2) the above extract + the accurately known amounts of the four flavonoids. Fused-silica capillary: (A and B) 63 cm × 25 μm i.d. × 370 μm o.d.; (C and D) 60 cm × 25 μm i.d. × 370 μm o.d.; separation voltage:19.5 kV; working potential: +0.95 V (vs. Ag/AgCl); other conditions as in Fig. 3. (1) Rutin; (2) hyperoside; (3) quercitrin; (4) quercetin.

Table 2
Assay results of the analytes in plant samples ($n = 4$, μg/g)[a]

Sample	Rutin	Hyperoside	Quercitrin	Quercetin
Houttuynia cordata Thunb.				
Leaves	100.8	474.4	1054.0	112.4
Stems	N.F.[b]	26.4	53.3	N.F.
Saururus chinensis (Lour.) Bail.				
Leaves	N.S.[c]	276.0	761.5	69.4
Stems	56.1	16.1	28.4	10.0

[a] Working potential: +0.95 V (vs. Ag/AgCl); other conditions as in Fig. 3, R.S.D.s are from 1.2 to 7.7%.
[b] N.F. refers to not found.
[c] N.S. refers to not separated.

Table 3
Determination results of the recovery for this method ($n = 3$)

Samples	Compound	Added amount (μg/mL)	Determined amount (μg/mL)	Recovery (%)
Houttuynia cordata Thunb.				
Leaves	Rutin	7.69	7.60	98.8
	Hyperoside	7.69	7.65	99.5
	Quercitrin	7.69	8.10	105
	Quercetin	16.9	16.7	98.8
Stems	Rutin	4.76	4.38	92.0
	Hyperoside	4.76	4.47	93.9
	Quercitrin	4.76	4.85	102
	Quercetin	10.4	11.2	108
Saururus chinensis (Lour.) Bail.				
Leaves	Rutin	7.69	4.06	52.8
	Hyperoside	8.33	9.15	110
	Quercitrin	8.33	7.96	95.6
	Quercetin	18.3	17.5	95.6
Stems	Rutin	2.14	2.54	119
	Hyperoside	2.14	2.39	112
	Quercitrin	2.14	2.56	120
	Quercetin	4.71	4.30	91.3

Working potential: +0.95 V (vs. Ag/AgCl); other conditions as in Fig. 3.

in Table 2, the contents of the four analytes in these two different parts are quite different and leaves contain more flavonoids than stems.

Since there was no additional pretreatment process of the ethanolic extracts from the two plants prior to CE–ED analysis, accurately known amounts of the four analytes were directly added to the extracts of samples. The recovery values were obtained using their peak currents from the calibration curve under the same conditions. The average recoveries are listed in Table 3. The results indicated that this method is appropriate for the analysis.

4. Conclusions

The results demonstrated that the proposed CE–ED method was very suitable for the fast determination of flavonoids in the different parts of *H. cordata* Thunb. and *S. chinensis* (Lour.) Bail. with relatively simple extraction procedures. This work also showed that CE–ED was a powerful technique to determine flavonoids in the complex extracts of the medicinal plants.

Acknowledgements

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