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Journal of Chromatography B, 860 (2007) 127–133
JOURNAL OF CHROMATOGRAPHY B
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Microemulsion electrokinetic chromatography for the separation of arctiin and arctigenin in Fructus Arctii and its herbal preparations

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Received 13 April 2007; accepted 12 October 2007
Available online 22 October 2007

Abstract

A microemulsion electrokinetic chromatography method was used to separate arctiin and arctigenin in Fructus Arctii and its herbal preparations. The separation of arctiin and arctigenin was performed using a 1-butanol–SDS–ethyl acetate–water microemulsion in 10 mM sodium tetraborate buffer. The analytes were baseline-resolved within 4 min. In the concentration range 5–500 μg/mL, the calibration curves reveal linear relationships between the peak area for each analyte and its concentration (correlation coefficients: 0.9993 for arctiin and 0.9998 for arctigenin). The method was applied to the analysis of arctiin and arctigenin in herbal preparations, and the recoveries were 98.7–103.1% for arctiin and 97.6–103.2% for arctigenin, respectively.

Keywords: Microemulsion electrokinetic chromatography; Fructus Arctii; Arctiin; Arctigenin

1. Introduction

Fructus Arctii (Niubangzi in Chinese), the dried fruits of *Arctium lappa* L., is one of the most popular traditional Chinese medicines. It has been widely used for dispelling pathogenic wind-heat, promoting eruption, relieving sore throat, removing toxic substances and subduing swelling [1,2]. Arctiin and arctigenin (shown in Fig. 1) are major lignans in Fructus Arctii [3–11]. They possess many kinds of bioactivities [12] and a number of important pharmacological properties including demutagenic [13,14], cytotoxic, anti-proliferative [15,16], platelet activating factor (PAF) antagonist [17], calcium antagonist [6] and anti-carcinogenesis [18] activities. Furthermore, arctigenin inhibits HIV-1 in tissue culture systems and is also a potent inhibitor of DNA topoisomerase II and of HIV-1 integrase [9,10,19,20].

Most analytical methods for determining arctiin and arctigenin in Fructus Arctii were based on liquid chromatography [21–23]. However, HPLC methods present lower efficiency and have longer analysis time. Capillary electrophoresis has the advantage of high resolution and high speed of analysis and has been the subject of very active research in the area of separation science.

Microemulsions are nanometre-sized droplets of oil-in-water or water-in-oil, stabilized by a surfactant and often a short-chain alcohol as co-surfactant. The possibility of using microemulsions as separation media for capillary electrophoresis was demonstrated for the first time by Watarai [24], and the technique was termed microemulsion electrokinetic chromatography (MEEKC). MEEKC is a useful technique for the separation of both charged and neutral solutes, covering a wide range of solubility. Separation is based on both hydrophobic interactions and the electrophoretic mobility of solutes. Thus, substances that are not resolved by micellar electrokinetic chromatography (MEKC) can be separated using MEEKC [25]. Several authors have studied selectivity in MEEKC [26–28]. MEEKC has been used in the separation analgesics [29], neutral steroids [30], cardiac glycosides [31] and numerous drugs and pharmaceutical products [32–35].

In this work, a MEEKC method was first developed for separation and determination of arctiin and arctigenin in Fructus

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doi:10.1016/j.jchromb.2007.10.017

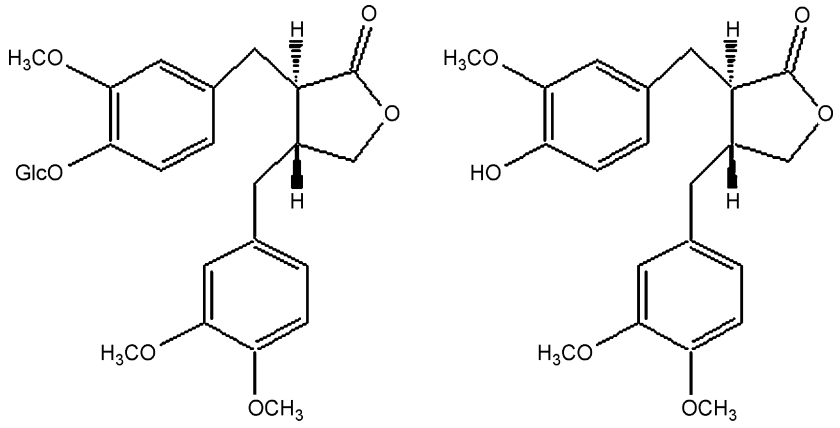

Fig. 1. Chemical structures of the analytes.

Arctii and its herbal preparation. The method was simple and rapid.

2. Experimental

2.1. Apparatus

A CL1030 Capillary system (Beijing Lucai Science Instrument Company, Beijing, China) was used. Uncoated silica separation capillaries of 53 cm (43.8 cm effective length) × 75 μm I.D. × 375 μm O.D. (Yongnian Optical Fiber Factory, Hebei, China) were used throughout the study. UV detection was carried out at 220 nm, the temperature was 18 ± 1 °C. The data acquisition was carried out with a HW-2000 Chromatography Workstation (Shanghai Qianpu Software Company, Shanghai, China). Samples were introduced into the capillary by hydrodynamic injection, where the sample vial was raised by 15.5 cm for 5 s. At the beginning of each working day, the capillary was flushed sequentially with 0.5 M NaOH for 10 min, distilled water for 10 min and the running electrolyte for 10 min. Moreover, the capillary was rinsed for 3 min with fresh buffer after each run, prior to the next injection. A PHS-10A acidity meter (Xiaoshan Science Instrumentation Factory, Zhejiang, China) was used for the pH measurement.

2.2. Chemicals and materials

Standards of arctiin and arctigenin (purity >99%) were obtained from Wuhu Delta Biotechnology Company, Anhui, China. Their structures were shown in Fig. 1. The crude drug of Fructus Arctii, Yinqiao jiedu tablet and Yinqiao shangfeng capsule were purchased from local drug store. All other chemicals were of analytical reagent grade. Ethyl acetate, SDS and 1-butanol were purchased from Tianjin Chemical Corporation (Tianjin, China), sodium tetraborate was purchased from Xi'an Chemical Corporation (Shanxi, China). All solutions and buffers were made in distilled water.

2.3. Solutions preparation

Stock standard solutions of arctiin and arctigenin (500 μg/mL) were prepared in methanol. Working standard solutions, ranging 5–500 μg/mL of arctiin and arctigenin, were prepared from various aliquots of stock standard solution, and diluted to 1 mL with methanol to give a final appropriate analyte concentration. A stock microemulsion solution containing 3.2% (v/v) ethyl acetate, 3.5% (m/v) SDS and 0.8% (v/v) 1-butanol was prepared [36]. The running buffer contains 10 mM sodium tetraborate and 10% microemulsion (pH 10.5). The running buffer was prepared daily from a stock solution of 0.1 M sodium tetraborate and microemulsion with distilled water. The running electrolyte was adjusted to the desired pH with 0.01 M NaOH.

2.4. Sample preparation

Five grams of Fructus Arctii were crushed to obtain a fine powder and mixed well; accurately weighed 0.5 g powder, dissolved with 25 mL methanol and ultrasonicated for 1 h, the filtrate solution was filtered through 0.45 μm syringe filters and diluted four-fold with methanol before injecting into the CE equipment. Twenty Yinqiao jiedu tablets were crushed to obtain a fine powder and mixed well; powder equivalent to the average weight of one tablet (0.5 g) was accurately weighed and dissolved in 10 mL methanol, and then ultrasonicated for 1 h. Powder of 20 Yinqiao shangfeng capsules were mixed well, weighed powder equivalent to the weight of two capsules (0.6 g), dissolved with 10 mL methanol and ultrasonicated for 1 h. The both filtrate solutions were filtered through 0.45 μm syringe filters and directly injected into the CE equipment without dilution.

3. Results and discussion

In this work, separation conditions of arctiin and arctigenin were optimized by studying the effects of pH, concentration of sodium tetraborate, microemulsion and applied voltage on the peak heights and resolution between adjacent peaks. The

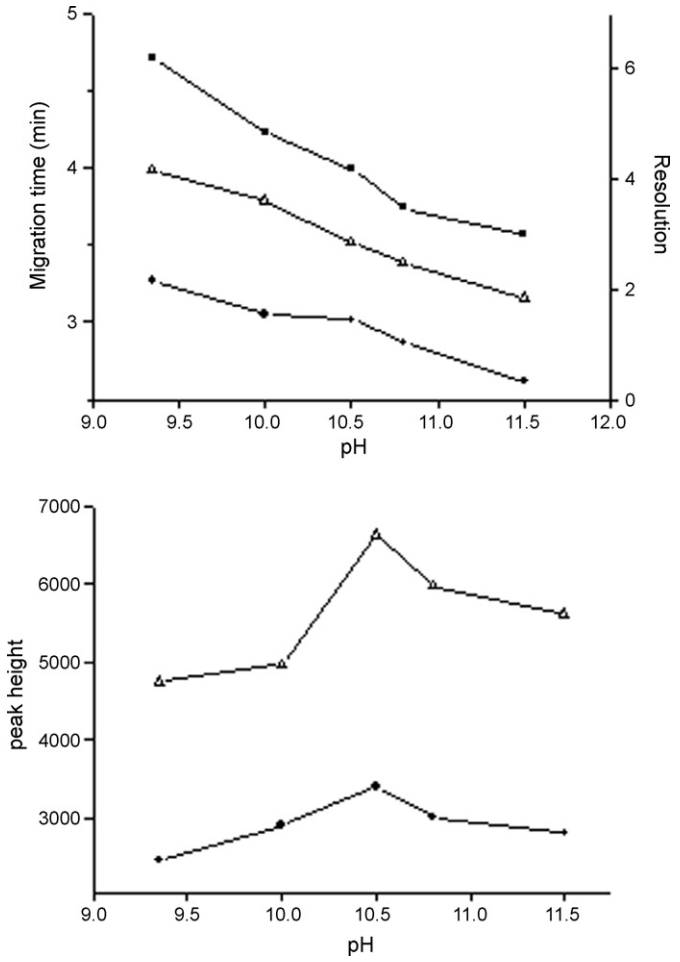

Fig. 2. Influence of pH on the migration time, resolution of the peaks and peak height (■, resolution of arctiin and arctigenin; ●, arctiin; Δ, arctigenin). Conditions: 75 μm I.D. × 375 μm O.D. × 53 cm length (43.8 cm effective length), uncoated; buffer: 10 mM sodium tetraborate and 10% microemulsion; voltage, 22.0 kV; detection wavelength, 220 nm; temperature, 18 ± 1 °C; sample: 80 μg/mL arctiin and arctigenin.

peak sequence of the compounds in MEEKC was arctiin and arctigenin. The identity of the recorded peaks was confirmed by comparing the migration time and adding the pure standard compounds.

3.1. Influence of the buffer pH on separation

The key factor of separation in capillary electrophoresis is based on the charge-to-mass ratio. The charge amount of particle varies with the pH of the running buffer. Therefore, the pH of the running buffer is an important factor in CE separation. To improve the resolution of arctiin and arctigenin, we investigated the effect of pH on the resolutions, migration time and peak height of analytes when 10 mM sodium tetraborate and 10% microemulsion was used as the running buffer in the pH range of 9.35–11.5. The plots of migration time, resolution and peak heights against the pH of the running electrolyte are shown in Fig. 2. As seen in Fig. 2, the results indicated that the resolution between arctiin and arctigenin decreased with the increasing of pH, the migration time of the two components also decreased dramatically. Although the analytes can be separated completely in the pH range of 9.35–11.5, the peak shape of the two analytes changed dramatically with the increasing of pH. Only at pH 10.5, the peak of the both analytes became spiculate and symmetrical. The reason may be that when pH < 10.5 arctiin molecules were not ionized completely and the charge of molecules were not the same, so the electrophoretic velocities of arctiin molecules were different, this led the peak of arctiin to spread forth. When pH 10.5, arctiin molecules were ionized completely and the electrophoretic velocities of arctiin molecules were the same, so then the peak of arctiin became spiculate and symmetrical, and the peak height was maximum. When pH > 10.5, arctiin as a phenolic glycoside compound may be hydrolyzed partly in the CE separation process and produce arctigenin, so the peak of arctigenin spread forth, this led the peak height and the symmetry of arctigenin to decrease. So at pH 10.5, the peak heights of both analytes were maximum. And the migration time was moderate at pH 10.5. Therefore, pH 10.5 was selected for the following experiments.

3.2. Influence of sodium tetraborate concentration on separation

The effect of sodium tetraborate concentration on the separation behavior of arctiin and arctigenin was investigated by changing the amount of sodium tetraborate from 5 to 25 mM using 10% microemulsion (pH 10.5). It was found in the experiments that there was a considerable influence of sodium tetraborate concentration on migration time of analytes and the resolution between arctiin and arctigenin. The migration time of arctiin and arctigenin increased when increasing the concentration of sodium tetraborate, and the resolution between arctiin and arctigenin increased when the concentration of sodium tetraborate reached 10 mM, but it began to decrease when concentration of sodium tetraborate was greater than 10 mM. Therefore, 10 mM sodium tetraborate was selected as the optimum concentration.

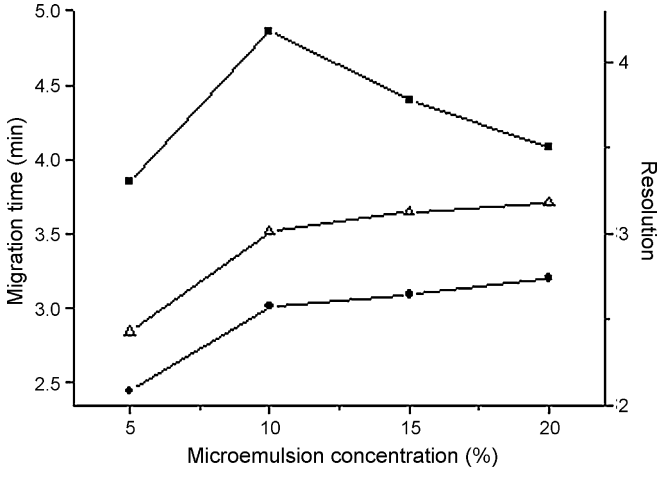

Fig. 3. Influence of microemulsion concentrations on the migration time and resolution of the peaks. Buffer: 10 mM sodium tetraborate (pH 10.5). Other conditions are the same as in Fig. 2.

3.3. Influence of microemulsion concentration on separation

The effect of microemulsion concentration on the separation behavior of arctiin and arctigenin was also investigated. Its concentration ranged from 5 to 20% using 10 mM sodium tetraborate (pH 10.5). The plot was shown in Fig. 3. It was found that there was a considerable influence of microemulsion concentration on migration time of analytes and the resolution between arctiin and arctigenin. The migration time of arctiin and arctigenin increased when increasing the concentration of microemulsion, but the resolution between arctiin and arctigenin began to decrease when concentration of microemulsion sodium tetraborate was greater than 10%. This can be explained in terms of the solubilization of these analytes by the microemulsion. Since lignans are highly hydrophobic, neutral species, the solubilization into the microemulsion will be probably increased with an increase of microemulsion concentration and this led to increasing partition of the analytes into the oil phase of microemulsion, resulting in degradation of the quality of the separation as reflected by poor resolution and longer migration times. Thus, 10% microemulsion was chosen considering the resolution and total analysis time.

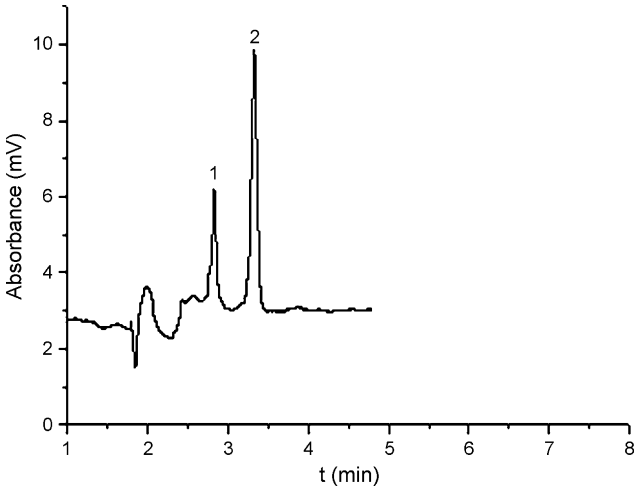

Fig. 4. Electrochromatogram of a standard mixture: 1, arctiin; 2, arctigenin. Buffer: 10 mM sodium tetraborate and 10% microemulsion (pH 10.5). Other conditions are the same as in Fig. 2.

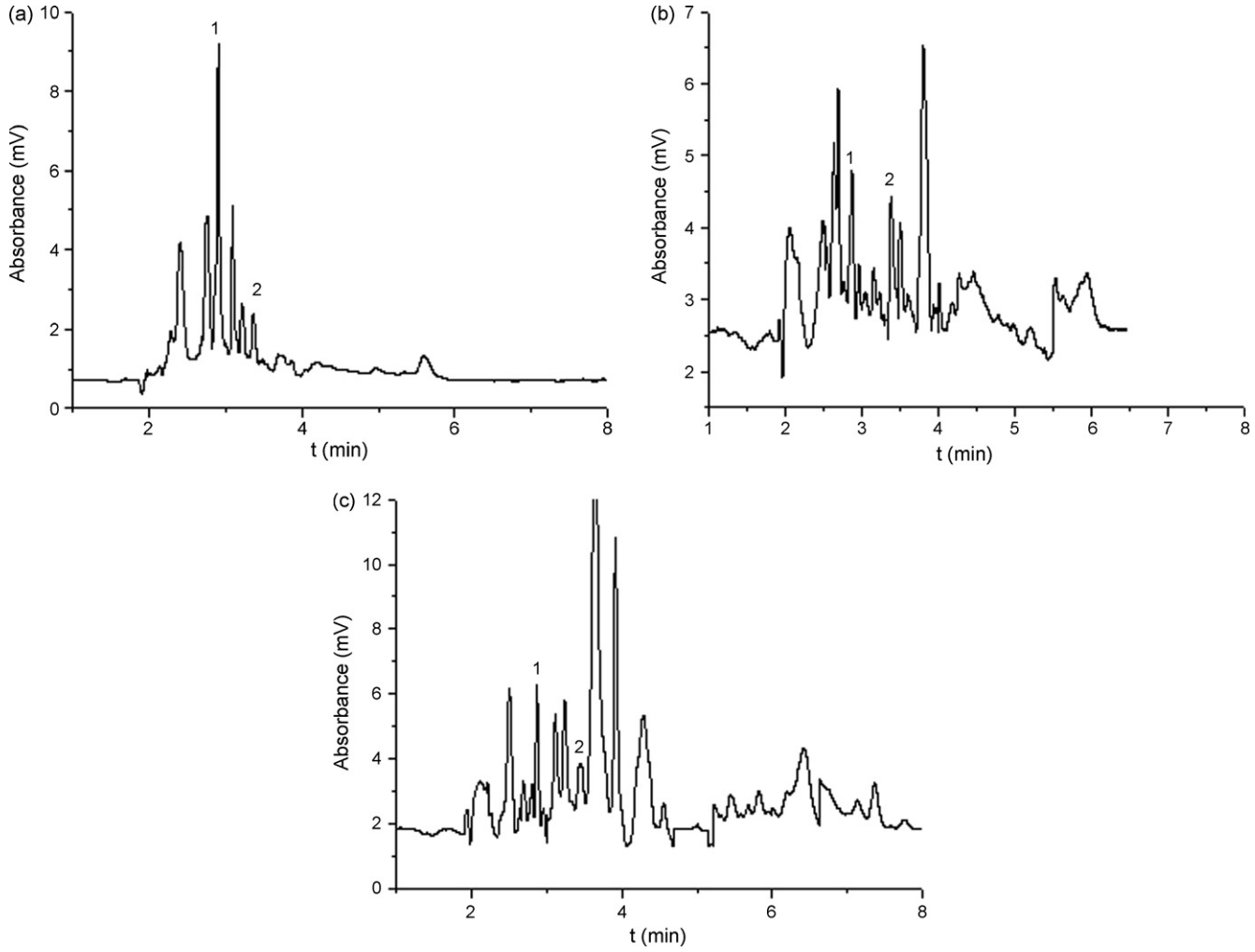

Fig. 5. Electrochromatogram of (a) *Arctium lappa* L., (b) Yinqiao jiedu tablet and (c) Yinqiao shangfeng capsule. Other conditions are the same as in Fig. 4.

Table 1
The results of regression analysis and the detection limits

Analytes	Regression equation, $y = a + bx$[a]	Correlation coefficient	Linear range (μg/mL)	Detection limit[b] (μg/mL)
Arctiin	$y = 825.85 + 72.19x$	0.9993	10–500	3.4
Arctigenin	$y = 725.34 + 231.87x$	0.9998	5–500	1.5

[a] y and x are the peak areas and the concentration of the analytes, respectively.
[b] The detection limit was defined as the concentration where the signal-to-noise ratio is 3.

3.4. Influence of separation voltage

Attempts were made to optimize the separation condition by using different applied voltages ranging from 15 to 25 kV. As may be expected, increasing the voltage gave shorter migration times, but this also caused the decrease of resolution. With consideration for the peak shape, migration time and resolution, the separation voltage finally chosen was 22 kV.

The final optimum separation condition was: 10 mM sodium tetraborate and 10% microemulsion (pH 10.5), 220 nm, UV detection, 22 kV applied voltage, temperature, 18 ± 1 °C.

3.5. Stability, linearity, limits and repeatability

The standard stock solutions of arctiin and arctigenin, which were protected from daylight, were stored under 4 °C for 6 weeks. During this period, the solutions were analyzed at 1, 5, 7, 14, 21, 42 days, and the peak areas were compared with the peak areas of daily prepared standard solution and no differences were obtained between the stored and freshly prepared samples, the percentage deviation from the nominal values were all below 10%. It was determined that arctiin and arctigenin were stable in the mentioned conditions for at least 6 weeks.

Under the optimum conditions, the linear relationships between the peak area of the analytes and the corresponding concentrations were established by running six standard solutions for the analytes were shown in Table 1.

The detection limit was estimated from the calibration curve of peak height *versus* standard concentration, and based on the concentration necessary to yield a net height equal to three times the SD of the baseline noise. The baseline noise was evaluated by recording the detector response every 5 s over a period of about 2 min. The detection limits were also given in Table 1.

The precision of the experiment were determined by repeated injection ($n = 6$) of a standard mixture solution containing 80 μg/mL arctiin and arctigenin under the optimum conditions, the relative standard deviation (RSD) values ($n = 6$) of the migration times and peak areas were 2.6 and 1.2% for arctiin, 2.1 and 2.0% for arctigenin, respectively. The electropherogram of a standard mixture was shown in Fig. 4. The repeatability of the experiment was determined by analyzing six independent series injection of Fructus Arctii extractions, the RSD values ($n = 6$) of the migration times and peak areas were 2.8 and 2.5% for arctiin, 3.1 and 2.8% for arctigenin, respectively.

During 24 h, the standard mixture solution was analyzed at 0, 2, 4, 8, 12 and 24 h, the RSD values ($n = 6$) of the migration times and peak areas were 3.6 and 3.2% for arctiin, 2.7 and 2.4% for arctigenin (intra-day), respectively. The inter-day precision was calculated during a 5-day period and the RSD values ($n = 6$) of the migration times and peak areas were 3.7 and 4.5% for arctiin, 3.3 and 3.6% for arctigenin, respectively.

Table 3
Recovery of the two analytes ($n = 3$)

	Fructus Arctii	Yinqiao jiedu tablet	Yinqiao shangfeng capsule
Arctiin			
Content (μg/mL)	110	72	120
Concentration spiked (μg/mL)	100	100	100
Concentration found (μg/mL)	212.6	170.7	123.1
Average recovery (%)	102.6	98.7	103.1
RSD (%)	3.8	4.3	4.9
Arctigenin			
Content (μg/mL)	21	19	24
Concentration spiked (μg/mL)	20	20	20
Concentration found (μg/mL)	41.6	39.4	43.5
Average recovery (%)	103.2	101.8	97.6
RSD (%)	1.9	2.7	3.4

3.6. Application and recovery

The optimum conditions were applied to the separation and determination of arctiin and arctigenin in the extract of Fructus Arctii and its herbal preparations. The electropherograms were shown in Fig. 5. The peaks were identified by comparing the migration time and adding the pure standard compounds to the real sample solution. In addition, the standard addition technique was applied to the same preparations which were analyzed by calibration curve of methods. There was no difference between slopes of the calibration curve and standard

Table 2
Contents of the two components in Fructus Arctii and its herbal preparation by calibration and standard addition methods ($n = 3$)

Samples	Arctiin				Arctigenin			
	Calibration	RSD (%)	Standard addition	RSD (%)	Calibration	RSD (%)	Standard addition	RSD (%)
Fructus Arctii	55.0 mg/g	1.7	56.4 mg/g	1.8	2.63 mg/g	1.9	2.71 mg/g	2.1
Yinqiao jiedu tablet	1.44 mg/g	2.5	1.50 mg/g	2.8	0.38 mg/g	2.8	0.40 mg/g	3.0
Yinqiao shangfeng capsule	0.60 mg/capsule	3.2	0.57 mg/capsule	3.2	0.12 mg/capsule	2.1	0.13 mg/capsule	4.1

Table 4
A comparison of deferent methods to determinate arctiin and arctigenin

	RP-HPLC	HPLC	HPLC	Presented method
Reference	[21]	[22]	[23]	–
Mobile phase/buffer condition	MeOH–H_2O (1:1)	Methanol–acetonitrile–water–tetrahydrofura (10:24:65:1 to 10:39:50:1)	33% acetonitrile	10 mM sodium tetraborate and 10% microemulsion (pH 10.5)
Analysis time (min)	16	15	16	4
λ (nm)	270	220	280	220
Precision (RSD, %)	–	0.8	1.01	1.2
Arctiin				
Arctigenin	–	1.0	0.89	2.0
Repeatability (RSD, %)	–	0.5	0.86	2.5
Arctiin				
Arctigenin	–	0.4	0.87	2.8
Content determined in Fructus Arctii (μg/mg)	43–119	26.4–80.2	53–78.5	55.0
Arctiin				
Arctigenin	1.6–5.4	1.3–24.4	1.1–15.1	2.63

addition techniques. These results showed that there was no interference from the matrix components. Therefore, it could be said that the method was highly selective. The contents of the analytes in real samples analyzed by calibration and standard addition methods together with their RSD ($n = 3$) are given in Table 2.

Recovery experiments were performed as follows: Accurately weighed powder of Fructus Arctii, Yinqiao jiedu tablets, Yinqiao shangfeng capsules (which contents of arctiin and arctigenin were known) 0.05, 0.5 and 0.6 g, accurately added 2.5, 1.0 and 1.0 mg standard of arctiin, respectively; accurately weighed powder of Fructus Arctii, Yinqiao jiedu tablets, Yinqiao shangfeng capsules 0.2, 0.5 and 0.6 g, accurately added 1, 0.4 and 0.4 ml stock standard solution of arctigenin (500 μg/mL), respectively. Then all the samples were extracted following the steps of Section 2.4. All the filtrates were directly injected into the CE equipment without dilution. The recovery of the method together with their RSD ($n = 3$) are given in Table 3 and the results were 98.7–103.1% for arctiin, 97.6–103.2% for arctigenin, respectively.

The comparison of the present method with others is shown in Table 4. It was concluded that the present method had high efficiency with very short analysis time and the quantitative results were consistent with references.

4. Conclusions

In this paper, the MEEKC method has been successfully used to analyze arctiin and arctigenin in Fructus Arctii and its herbal preparations for the first time. The analytes were completely separated within 4 min under the optimum conditions described above. The analysis time in the presented method was significantly shorter than HPLC methods. The resolution of the analytes and the recovery of the method were both satisfactory. The proposed MEEKC method could be a good alternative for simultaneous analysis of the two lignans in Fructus Arctii and its herbal preparations.

Acknowledgements

We kindly acknowledge the National Science Foundation of China (No. 20275014) for supporting this work. Also we would like to thank Allan Grey the English teacher for Ph.D. students at Lanzhou University for checking this manuscript.

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