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Separation and determination of the effective components in the alabastrum of *Edgeworthia chrysantha* Lindl. by micellar electrokinetic capillary chromatography

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Abstract

A rapid and efficient micellar electrokinetic capillary chromatography (MEKC) method was developed to analyze edgeworoside C (**1**), kaempferol-3-*O*-β-D-glucoside (**2**) and rutin (**3**) in the alabastrum of *Edgeworthia chrysantha* Lindl. for the first time. The factors that affect the separation were studied, such as the concentrations of the buffer, SDS, and organic modifier, the apparent pH, the applied voltage and temperature. The analytes were well separated within 15 min with an electrolyte containing 25 mM $Na_2B_4O_7$, 30 mM NaH_2PO_4, 60 mM SDS and 15% acetonitrile (pH* 9.1) at 25 kV and 15 °C. The correlation coefficients between the peak areas of analytes and the corresponding concentrations were 0.9976–0.9981 under the optimum conditions. The relative standard deviations (R.S.D.) of the migration time and peak area were in the range 0.6–1.7 and 1.9–5.3%, respectively. The contents of analytes in *E. chrysantha* Lindl. collected from the different places were successfully determined with the recoveries ranging from 95.9 to 104.3%. And, the results demonstrated that there was significant difference between the two real samples.

Keywords: *Edgeworthia chrysantha*; Edgeworoside C; Kaempferol-3-*O*-β-D-glucoside; Rutin; Micellar electrokinetic capillary chromatography

1. Introduction

Edgeworthia chrysantha Lindl. (Thymelaeaceae) is distributed in eastern Asia. While it is used to make paper in Korea and Japan, the alabastrum is often used as the succedaneum of traditional Chinese medicine "*Buddleja officinalis* Maxim (Chinese name: Mi Meng Hua)" in China for the treatment of swelling of eye, ophthalmalgia, delacrimation, nephelium of eye and nocturnal emission [1]. Investigation of chemical constituents showed that it contained edgeworoside C (**1**), kaempferol-3-*O*-β-D-glucoside (**2**), rutin (**3**) (their structures were shown in Fig. 1) and other compounds [2]. According to the literature, these constituents have the effect of anticoagulated blood, antimicrobial, anti-inflammation and antioxidant activity, and can be used to cure angiosclerosis, diabetes and amphiblestritis [3,4]. Up to now, there has been no report on the analysis of the effective components in *E. chrysantha* Lindl. Therefore, it is necessary to develop an efficient method for the separation and determination of these compounds in *E. chrysantha* Lindl.

So far, there has been no report on analysis of **1**. Some methods have been established to analysis of **2** or **3**, such as TLC [5], LC [6] and CE [6,7–12]. In addition, Gall et al. determined **2**, **3** and other compounds in tomato fruit by LC, but the analysis time was rather long (more than 30 min) [13]. Though Bjergegaard et al. separated **2**, **3** and other compounds by MEKC, they did not apply the method to the real samples [14].

CE has the advantages of excellent separation efficiency for the complex sample, rapid analysis and minimal use of the samples and solvents. Since it was first described by Jorgenson and Lukacs [15], its application to the separation and determination of various kinds of samples has been increasingly extensive. In this work, a simple, rapid, efficient and reproducible MEKC method was established for the separation and determination of **1**–**3** in the alabastrum of *E. chrysantha* Lindl.

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2. Experimental

2.1. Chemicals and materials

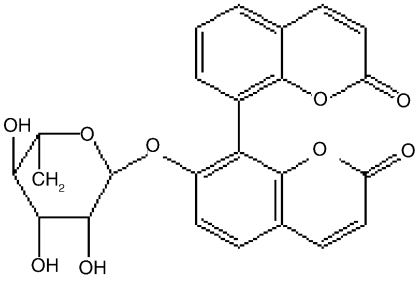
Edgeworoside C, kaempferol-3-*O*-β-D-glucoside and rutin isolated from *E. chrysantha* Lindl. were used as standards. The three compounds were identified by ^{1}H NMR, ^{13}C NMR, MS and comparing the data with the spectral information from the literature. Sodium borate ($Na_2B_4O_7{\cdot}10H_2O$), sodium dihydrogen phosphate ($NaH_2PO_4{\cdot}2H_2O$) and sodium dodecyl sulfate (SDS) were analytical grade. Methanol and acetonitrile was chromatographic pure (Merck, Germany). Purified water (Hangzhou Wahaha Group Co. Ltd., Zhejiang Province, China) was used in this study. The alabastrums of *E. chrysantha* Lindl. were collected from Suichang county (SA) and Hangzhou city (SB) of Zhejiang Province in China. The plants were identified by Lecturer Qing He (College of Pharmaceutical Science, Zhejiang University).

2.2. Apparatus

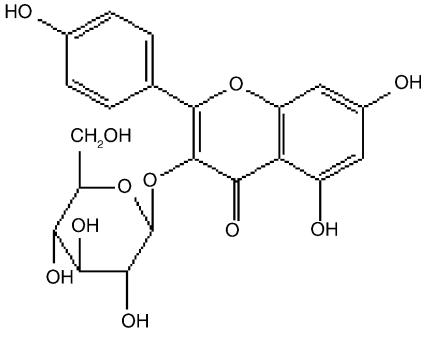
An HP^{3D} capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany) equipped with diode-array detector (190–600 nm) was used in this study. Data acquisition and analysis were carried out with ChemStation software (Agilent Technologies). Capillary electrophoresis was performed using a 60.0 cm (51.5 cm to the detector) × 50 μm i.d. fused silica capillary (Yongnian Photoconductive Fibre Factory, Hebei Province, China). Sample was injected by applying a pressure of 20 mbar for 5 s from the anodic end of capillary. The capillary was conditioned prior to its first use by consecutively flushing with acetonitrile for 10 min, 100 mM HCl for 10 min, H_2O for 5 min, 1000 mM NaOH for 10 min, H_2O for 5 min and the electrophoresis buffer for 15 min. After each run the capillary was rinsed with water for 1 min, followed by 100 mM NaOH for 2 min, water for 2 min and then the electrophoresis buffer for 3 min. The buffer was renewed after every three runs for good reproducibility. A PHS-3C acidity meter (Shanghai REX Instrument Factory, Shanghai, China) was used for the pH measurements. The pH system was calibrated with potassium dihydrogen phosphate–disodium hydrogen phosphate (pH 6.88) and sodium borate (pH 9.23) solutions. 100 mM NaOH and HCl were used to adjust the pH of buffer.

2.3. Solution preparation

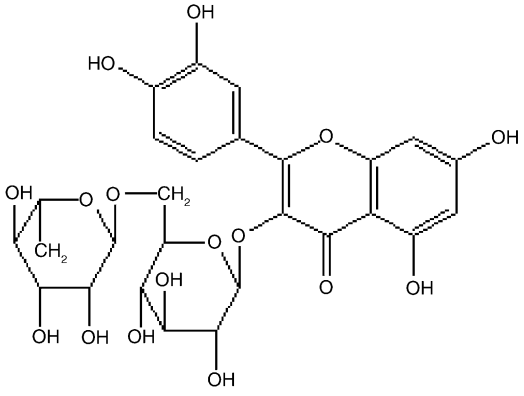
The stock solution containing 800 μg/mL of **1** and **3** and 400 μg/mL of **2** was prepared in methanol. Solutions of lower concentration were prepared by diluting the stock solutions with appropriate amounts of methanol. The running buffer solution was prepared by mixing 2.5 mL 100 mM $Na_2B_4O_7$, 3.0 mL 100 mM NaH_2PO_4, 1.5 mL 400 mM SDS and 1.5 mL



1 Edgeworoside C



2 Kaempferol-3-O-β-D-glucoside;



3 Rutin.

Fig. 1. The structures of the three analytes studied.

acetonitrile in a 10 mL flask and diluted to graduation line with water. All solutions for CE were filtered through a 0.45 μm microfilter (Shanghai Yadong Nulear Grade Resin Co. Ltd., Shanghai, China).

2.4. Sample preparation

The powdered alabastrum of *E. chrysantha* Lindl. (8.0 g) was extracted with 160 mL methanol for 30 min in an ultrasonic bath and then extracted repeatedly with 60 mL methanol for 30 min. The extracts were combined and then methanol was evaporated. The residue was redissolved with 5 mL methanol. The solution was passed through a 0.22 μm microfilter (Shanghai Yadong Nulear Grade Resin Co. Ltd., Shanghai, China) before being injected into the capillary electrophoresis system.

3. Results and discussion

3.1. Optimization of separation conditions

The structures of three analytes (Fig. 1) suggested that they could be determined either as neutral (**1**) or anions (**2** and **3**) compounds, so MEKC was selected as the separation mode. Originally, a buffer containing 10 mM $Na_2B_4O_7$ and 60 mM SDS was used as the electrolyte. But baseline separation of **2** and **3** could not be achieved with this buffer system even if organic modifier was added. Though **2** and **3** were well separated from each other by using a buffer of 10 mM $Na_2B_4O_7$–30 mM NaH_2PO_4–60 mM SDS, they were not completely separated from other components in the real samples. It is known that the addition of organic modifier can improve the separation efficiency. The migration behaviors of analytes were different when organic modifier was added in the buffer or not, which could influence the result of optimizing separation condition. So acetonitrile was added to the initial buffer, i.e. 10 mM $Na_2B_4O_7$–30 mM NaH_2PO_4 containing 60 mM SDS and 10% acetonitrile was selected as the initial buffer. The optimum separation was achieved by systematically optimizing the concentrations of the buffer, SDS, organic modifier, the apparent pH, the applied voltage and temperature.

3.1.1. Effect of buffer concentration

To investigate the $Na_2B_4O_7$ concentration on the separation, experiments were performed with the different $Na_2B_4O_7$ concentrations (5, 10, 15, 20, 25 and 30 mM). It was found that

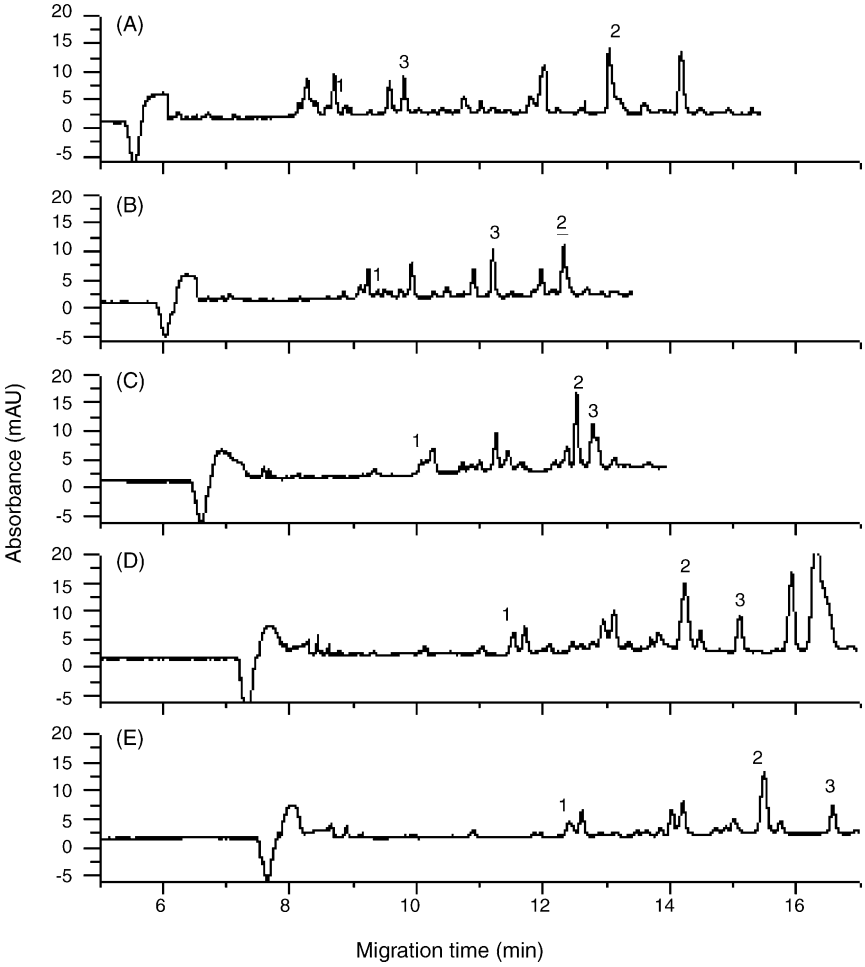


Fig. 2. Electropherograms of SA at different acetonitrile concentration. Buffer: 0–20% acetonitrile and 25 mM $Na_2B_4O_7$–30 mM NaH_2PO_4–60 mM SDS. (A) 0% acetonitrile; (B) 5% acetonitrile; (C) 10% acetonitrile; (D) 15% acetonitrile; (E) 20% acetonitrile. Capillary: 60.0 cm (51.5 cm to the detector) × 50 μm i.d. Applied voltage: 20 kV. Cartridge temperature: 25 °C. Detection wavelength: 272 nm. Symbols: 1, edgeworoside C; 2, kaempferol-3-*O*-β-D-glucoside; 3, rutin.

Table 1
The results of regression analysis on calibration curves

Analytes	Regression equation ($y = a + bx$)[a]	Correlation coefficient	Linear range (μg/mL)
1	$y = (-0.90 \pm 1.59) + (0.19 \pm 0.005)x$	0.9976	18.2–640
2	$y = (0.68 \pm 1.07) + (0.22 \pm 0.006)x$	0.9981	22.8–320
3	$y = (-0.11 \pm 1.62) + (0.18 \pm 0.006)x$	0.9979	22.8–456

[a] y and x stand for the peak area and the concentration (μg/mL) of the analytes, respectively.

the migration times of the analytes firstly decreased and then increased slightly with the increasing of $Na_2B_4O_7$ concentration. This was because the pH* of buffer would increase with the increasing of $Na_2B_4O_7$ concentration, which would increase the electroosmotic flow (EOF). But when the concentration was too high, the increase of ionic strength would decrease the EOF. It was found that the three analytes could be well separated from each other at all concentrations. But one or two of their peaks were overlapped with other components in the real samples. Finally, 25 mM $Na_2B_4O_7$ was selected in the following experiments for better separation.

Experiments with different NaH_2PO_4 concentrations (20, 25, 30 and 35 mM) in the electrolyte were carried out to investigate the NaH_2PO_4 concentration on the separation. It was found that at 20 and 35 mM NaH_2PO_4 the peaks of **2** and **3** was overlapped. They were both separated from each other at 25 and 30 mM NaH_2PO_4, but the resolution at 30 mM was greater. So 30 mM NaH_2PO_4 was selected.

3.1.2. Effect of SDS concentration

In this work, four electrolyte containing different SDS concentrations (40, 50, 60 and 70 mM) were used to study the effect of SDS concentration on the separation. It was found that the separations of **2** and **3** from other components in the real samples were improved with the increasing of SDS concentration. At 60 mM SDS, the separations of **2** and **3** in the real samples were better. Consequently, 60 mM SDS was chosen.

3.1.3. Effect of organic modifier

The addition of organic modifier to buffer can improve the separation efficiency by the modification of partition coefficient, the polarity of aqueous phase and EOF. In this work, methanol and acetonitrile were both attempted to use as the organic modifier, and narrower peak shape was obtained with the electrolyte containing acetonitrile. The electropherograms of the real sample at different acetonitrile concentrations were shown in Fig. 2. With the acetonitrile concentration increasing, the migration times of **1** and **3** increased, which was due to the decrease of the EOF. It was the same reason for the increase of migration time of **2** when the acetonitrile concentration increased from 5 to 20%. But, the migration time of **2** decreased when the acetonitrile concentration increased from 0 to 5%. There were two reasons for this phenomenon. One was that addition of acetonitrile would make for **2** partitioning from the micellar phase to the aqueous phase. The other was the addition of acetonitrile in buffer would restrain the formation of micelles, i.e. the addition of acetonitrile would increase the critical micelle concentration. As shown in Fig. 2, the separations of three compounds in the real sample were optimum when the acetonitrile concentration was 15%.

3.1.4. Effect of apparent pH

To investigate the effect of apparent pH (pH*) on the separation, experiments were performed with pH* ranging from 9.1 to 10.1. The result was shown in Fig. 3. pH* has dual effects on the migration times of analytes. On one hand, the rise of pH* increased the EOF, which would shorten the migration times of analytes. On the other hand, the rise of pH* increased the ionization degree of **2** and **3**, which would lengthen the migration time of **2** and **3**. Because the molecular weight of **2** was smaller, the latter effect on **2** was greater than that on **3**. So, the increase extent of the migration time of **2** was smaller than that of **3**. Therefore, the migration order of **2** and **3** was inversed at pH*

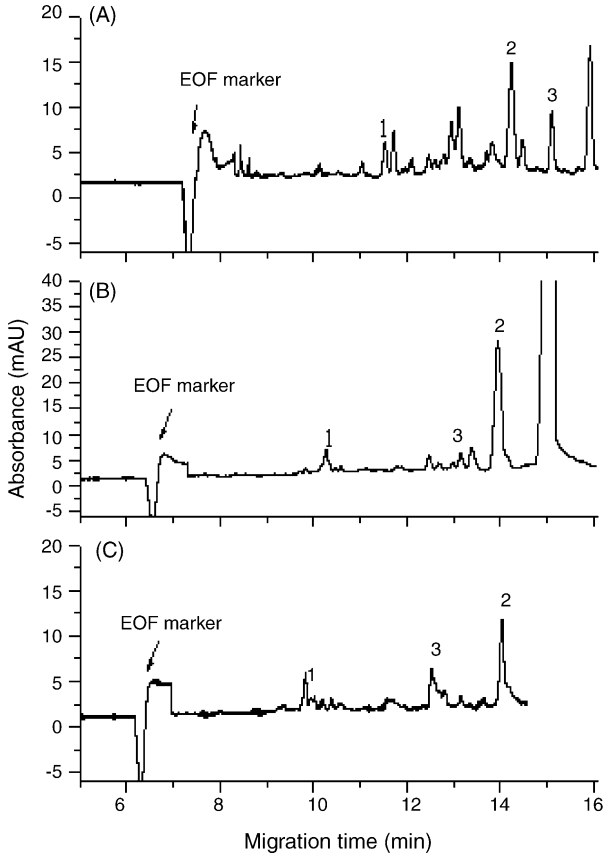


Fig. 3. Electropherograms of SA at different pH*. Buffer: 25 mM $Na_2B_4O_7$–30 mM NaH_2PO_4–60 mM SDS–15% acetonitrile with pH* ranging from 9.1 to 10.1. (A) 9.1; (B) 9.6; (C) 10.1. Other conditions and the symbols are the same as in Fig. 2.

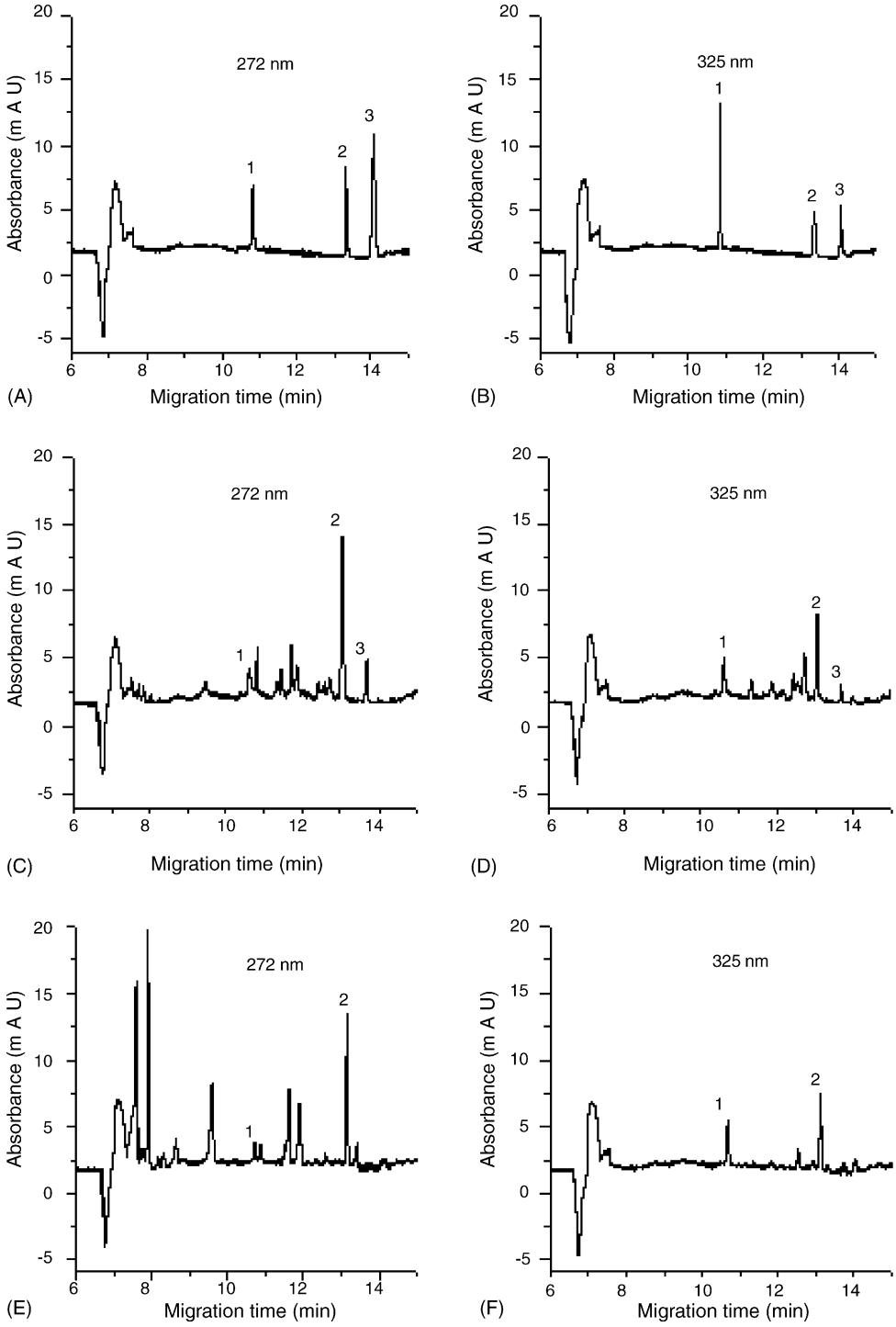


Fig. 4. Electropherograms of standards mixture (A and D), SA (B and E) and SB (C and F). Buffer: 25 mM $Na_2B_4O_7$–30 mM NaH_2PO_4–60 mM SDS–15% acetonitrile at pH* 9.1. Applied voltage: 25 kV. Cartridge temperature: 15 °C. Detection wavelength: 272 nm for (A), (C), (E) and 325 nm for (B), (D), (F). Other conditions and the symbols are the same as in Fig. 2.

9.6. As shown in Fig. 3, the separations of three analytes in the real sample were best at pH* 9.1.

3.1.5. Effect of applied voltage and temperature and choice for detection wavelength

Attempts were also made to optimize the separation conditions by using different applied voltage (17–25 kV) and temperature (10–25 °C). The separation voltage and temperature determine the migration time and influence the resolution. Based on experimental results, 25 kV and 15 °C were selected.

The maximum absorption peak of UV spectra of **2** and **3** was about 272 nm, so the determination of them was performed at 272 nm. While the detection wavelength for **1** was selected as

Table 2
Contents of the analytes in a *Edgeworthia chrysantha* Lindl. and the R.S.D. values calculated according to peak area ($n = 3$)

Sample	**1** (mg/g)	R.S.D. (%)	**2** (mg/g)	R.S.D. (%)	**3** (mg/g)	R.S.D. (%)
SA	0.0404 ± 0.0020	4.9	0.155 ± 0.0026	1.7	0.0481 ± 0.0017	3.5
SB	0.0398 ± 0.0017	4.3	0.153 ± 0.0054	3.5	Not found	–

325 nm, at which there is no interference and the UV absorbance was stronger than that at 272 nm.

According to the factors mentioned above, the optimum buffer was 25 mM $Na_2B_4O_7$–30 mM NaH_2PO_4 (pH 8.7) containing 60 mM SDS and 15% acetonitrile at pH* 9.1. The applied voltage and temperature was 25 kV and 15 °C, respectively. The detection wavelength was 325 nm for **1** and 272 nm for **2** and **3**. The typical electropherograms for a standard mixture under the optimum conditions were shown in Fig. 4A (the detection wavelength was 272 nm) and Fig. 4B (the detection wavelength was 325 nm). All the analytes were well separated within 15 min.

3.2. Method validation

The linear relationships between the peak areas of analytes and the corresponding concentrations were shown in Table 1. The calibration curves exhibited good linear behavior over the concentration ranges. The limits of detection (LODs) were calculated on the basis of a signal-to-noise (S/N) of **3**, and the results were 3.9 μg/mL for **1**, 4.1 μg/mL for **2** and 5.9 μg/mL for **3**, respectively. The limits of quantification (LOQ) calculated as 10 times the baseline noise level were 13.1 μg/mL for **1**, 13.8 μg/mL for **2** and 19.8 μg/mL for **3**, respectively. The repeatability of the proposed method was determined by repeated ($n = 5$) injection of a standard mixture solution containing 91.2 μg/mL of **1** and **3** and 45.6 μg/mL of **2** under the optimum conditions. The relative standard deviations (R.S.D.) of the migration time and peak area of each peak were 0.6–0.7 and 1.9–4.0% (for intra-day) and 1.1–1.7 and 3.5–5.3% (inter-day), respectively. The recovery experiments were performed by adding accurate amount of three analytes to the two real samples. The results were 95.9–103.4% for **1**, 101.9–104.3% for **2** and 97.5–102.7% for **3**, respectively.

3.3. Analysis of analytes in the alabastrum of E. chrysantha Lindl.

The typical electropherograms of two real samples with the detection wavelength of 272 and 325 nm were shown in Fig. 4C–F. Fig. 4C and E were used to determine the contents of **2** and **3** in the real samples SA and SB, while Fig. 4D and F were used to determine the content of **1**. The analytes in the real samples were identified by comparing the migration times and the UV spectra of the standards with that of the analytes in the real samples and spiking the standards to real sample solutions. Table 2 lists the contents of analytes in the real samples. The result showed that the contents of **1** and **2** in SA and SB were almost same, but **3** was not found in SB. As shown in Fig. 4C–F, there was a significant difference between the electropherograms of SA and SB, which included the peak numbers and peak height. There were more peaks in the electropherograms of SA and the peak heights for some peaks in the electropherograms of two real samples were also different.

4. Conclusion

The developed method is simple, rapid, efficient and reproducible. It has been successfully applied to analyze edgeworoside C, kaempferol-3-*O*-β-D-glucoside and rutin in the alabastrum of *E. chrysantha* Lindl. The analysis results demonstrated that there was significant difference between the medicinal plants from different places. In addition, the method can also be a promising alternative for the preliminary investigation of these compounds in *E. chrysantha* Lindl. from other places or in other *Edgeworthia* plants.

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