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Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 105–116
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Separation and quantitative analysis of coumarin compounds from *Angelica dahurica* (*Fisch. ex Hoffm*) *Benth. et Hook. f* by pressurized capillary electrochromatography

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Received 26 July 2005; received in revised form 21 October 2005; accepted 22 October 2005
Available online 28 November 2005

Abstract

A pressurized capillary electrochromatography (pCEC) method with post-column detection cell has been developed for the therapeutically important coumarins from *Angelica dahurica* extract. The separation of five major coumarins (xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate, byakangelicin) was optimized with respect to composition of the mobile phase, ionic strength of buffers, pH, and applied voltage. Baseline separation was achieved for the five coumarins in less than 25 min using a mobile phase of methanol–acetonitrile–phosphate buffer (pH 4.8; 15 mM) (22.5:15:62.5, v/v/v). The method showed satisfactory retention time and peak area repeatability with the first use of post-column detection cell in the pCEC instrument. Comparing to capillary high performance liquid chromatography (capillary HPLC) and conventional high performance liquid chromatography (HPLC), higher column efficiency, and shorter analysis time were achieved in pCEC. The five coumarins in the extract samples representing different stages of traditional extraction of *A. dahurica* were also quantitatively analyzed by pCEC. The calibration curves were linear in the range 37–129, 36–126, 12–41, 88–306, 20–69 μg/ml of the standard solutions containing the five coumarins with correlation coefficients between 0.9976 and 0.9994.

Keywords: Pressurized capillary electrochromatography; Post-column detection cell; Coumarin; *Angelica dahurica*; Chinese herb medicine

1. Introduction

The alcohol extract of *Angelicae Dahuricae Radix*, the dried roots of *Angelica dahurica Benth. et Hook*. (*Umbelliferae*), have been used as a natural remedy since ancient times in Chinese herbal medicine. *A. dahurica* has been reported as having the protective activity against dexamethasone-induced disorders, liver protective activity, antimicrobial activity, anti-inflammatory activity and anti-mutagenic activity [1]. Main active components of *A. dahurica*, xanthotoxol (Fig. 1A), osthenol (Fig. 1B), imperatorin (Fig. 1C), oxypeucedanin hydrate (Fig. 1D), byakangelicin (Fig. 1E) are coumarins, which have been reported to have pharmacological effects such as activation of ACTH-induced lipolysis [2], inhibition of insulin-induced lipogenesis, inhibition of compound 48/80-induced histamine release [3] and inhibitory effect on cytochrome P-450 activity [4].

Several methods have been developed for the determination of coumarins in *A. dahurica*. The majority of these have been performed by using thin-layer chromatography (TLC) [5,6], UV spectrophotometry [7], reversed-phase high performance liquid chromatography (RP-HPLC) [6–11], bioassay-linked high performance liquid chromatography–mass spectrometry (HPLC–MS) [12], gas chromatography–mass spectrometry (GC–MS) [13], and high-speed counter-current chromatography [14].

Capillary electrochromatography (CEC) is a relatively new microcolumn separation technique, which combines the advan-

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doi:10.1016/j.jpba.2005.10.033

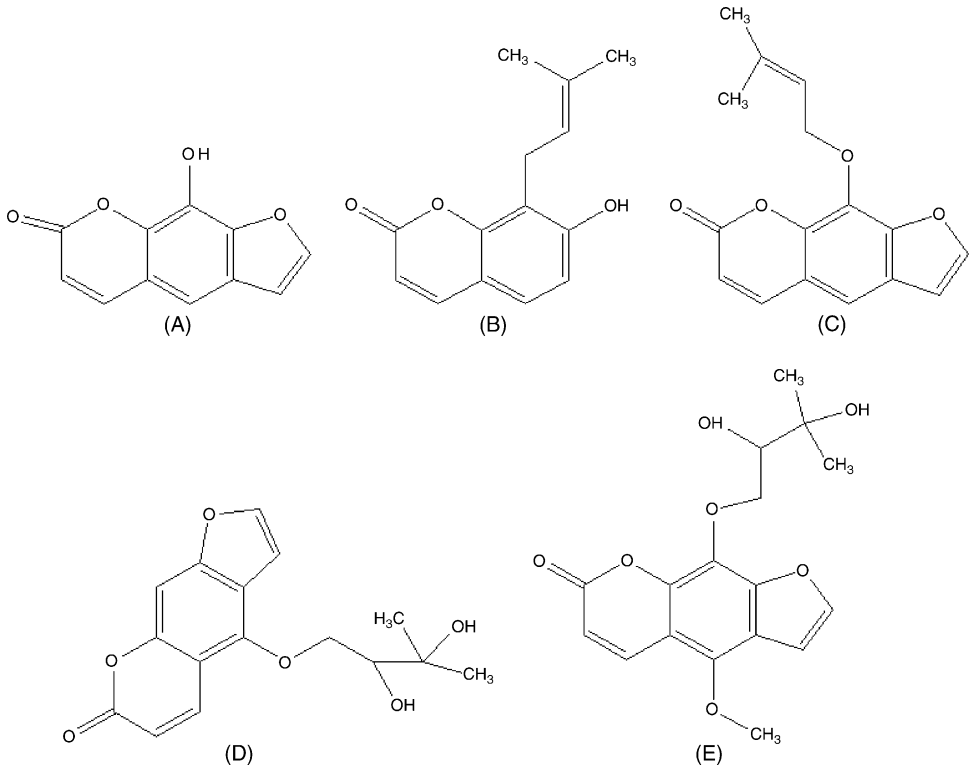

Fig. 1. Molecular structures of the five coumarins: (A) xanthotoxol, (B) osthenol, (C) imperatorin, (D) oxypeucedanin hydrate, and (E) byakangelicin.

tages of high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) [15–17]. It has the potential to become a powerful separation tool for complex mixtures. However, in practice, when CEC was used without pressure, often on a commercial CE instrument, there were problems and difficulties associated with bubble formation and column dry-out. These problems can be solved by a pressurized capillary electrochromatography (pCEC) system, in which a mobile phase is driven by both a pressurized flow and an electroosmotic flow (EOF). In such a system, pressure is applied to the capillary column to suppress bubble formation. Quantitative sample introduction in pCEC can be achieved through a rotary-type injector. The EOF

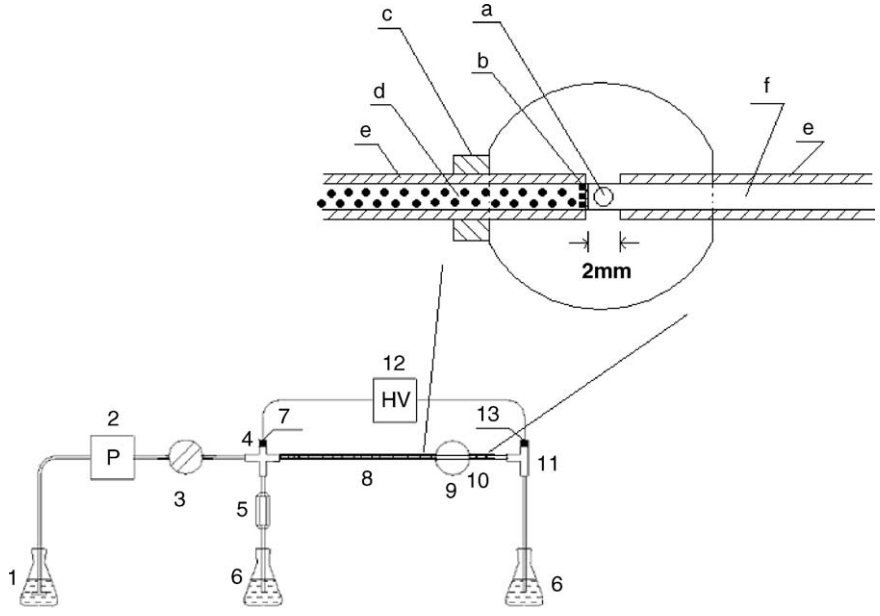

Fig. 2. Schematic diagram of experimental apparatus used for separation by pCEC: (1) mobile phase vial, (2) pump, (3) rotary-type injector, (4) splitting cross, (5) backpressure regulator, (6) waste reservoir, (7) ground, (8) capillary column (with peek tubing sleeve), (9) UV–vis detector, (10) uncoated fused-silica capillary, (11) tee, (12) high-voltage power supply, and (13) electrode. (a) UV–vis detector, (b) interface between two capillaries, (c) peek screw, (d) packed capillary column, (e) peek tubing sleeve, and (f) uncoated fused-silica capillary.

can either be in the same direction as, or against the pressurized flow. Therefore, the sample elution order may be manipulated. Most importantly, it is amenable for a solvent gradient mode, similar to that in HPLC, by programming the composition of eluents [18,19].

With pCEC [19–24], the promises of CEC can be fully exploited. A pCEC instrument is comprised of a solvent delivery system, a capillary column, a high-voltage power supply, a UV–vis detector and a data acquisition system. CEC or pCEC have the potential of applying to the separation of active con-

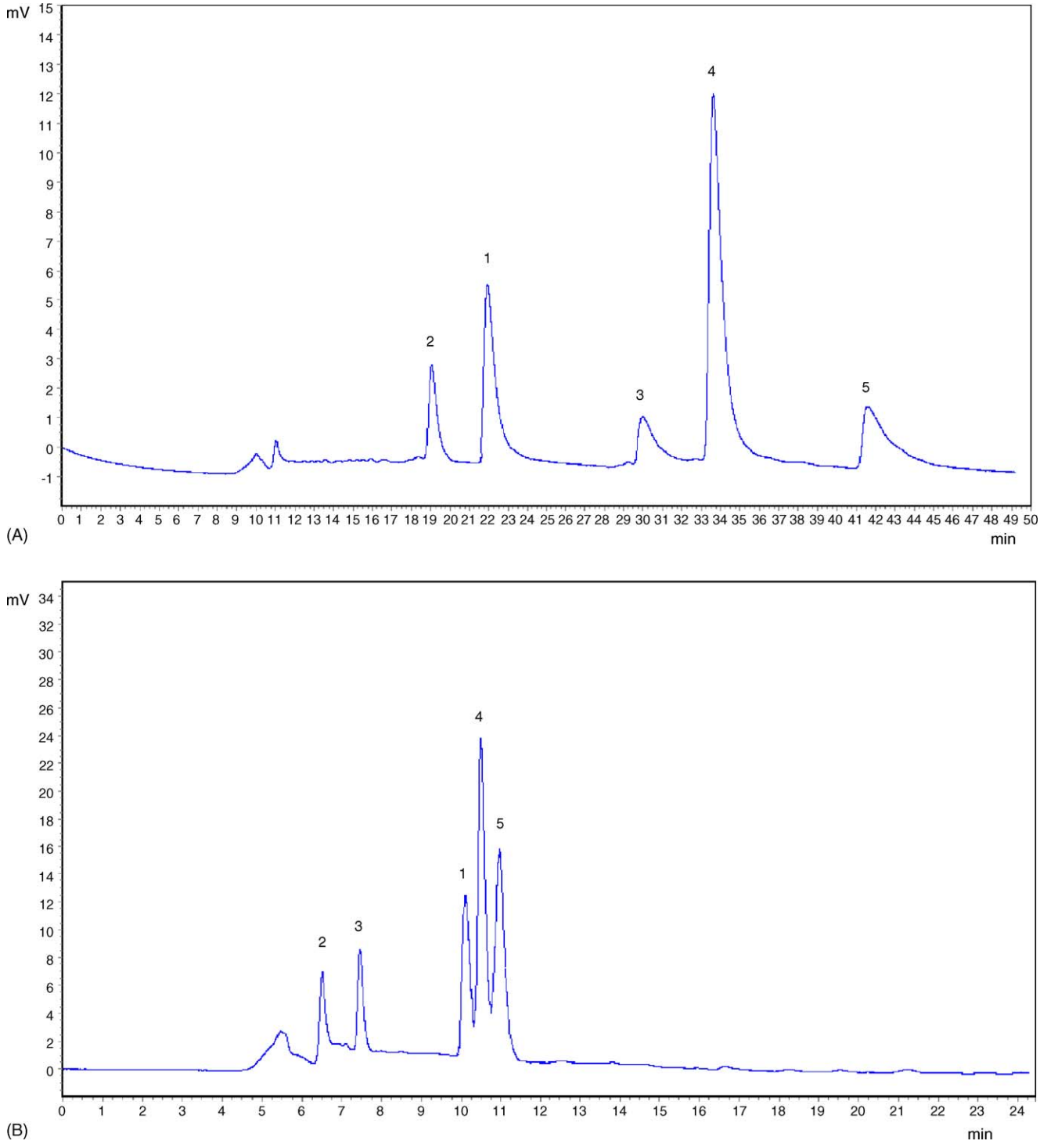

Fig. 3. Effect of mobile phase on separation. (A) Methanol–phosphate buffer (pH 4.8; 10 mM) (45:55, v/v), (B) acetonitrile–phosphate buffer (pH 4.8; 10 mM) (30:70, v/v), (C) methanol–acetonitrile–phosphate buffer (pH 4.8; 10 mM) (30:10:60, v/v/v), (D) methanol–acetonitrile–phosphate buffer (pH 4.8; 10 mM) (15:20:65, v/v/v), and (E) methanol–acetonitrile–phosphate buffer (pH 4.8; 10 mM) (22.5:15:62.5, v/v/v). Flow rate: 50 μl/min, voltage: 0 kV, backpressure: 13.8 MPa, injection: 4 nl, detection: 216 nm, temperature: 20 °C, and sample: standard coumarins solution. Peaks: (1) xanthotoxol, (2) osthenol, (3) imperatorin, (4) oxypeucedanin hydrate, and (5) byakangelicin.

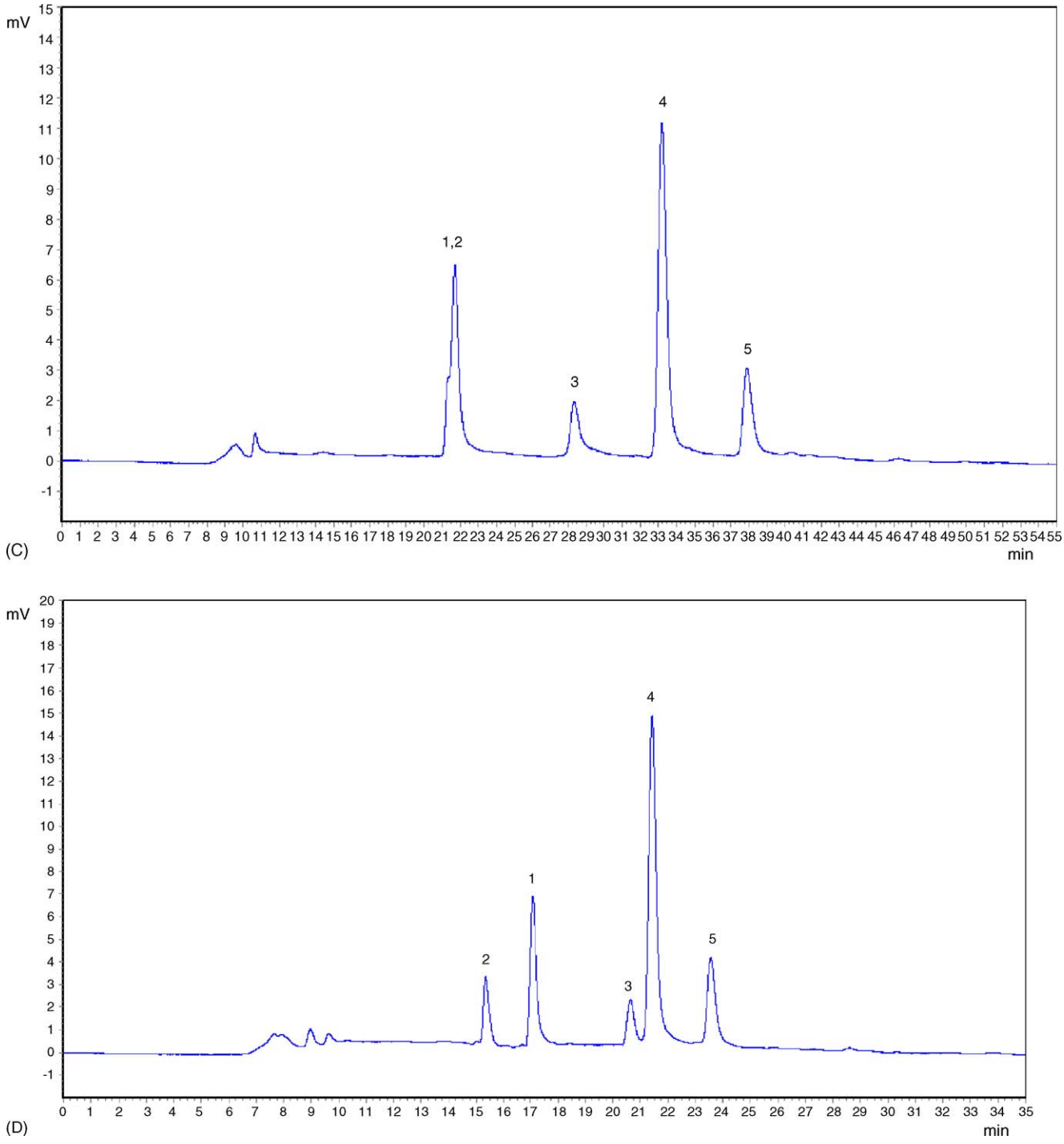

Fig. 3. (*Continued*)

stituents from Chinese herbal medicines [25–36]. However, the packed capillary column applied in pCEC contains an on-column detection window about 2 mm, which always leads to problems such as fracture of capillary and limited optical path of UV–vis detection. In this study, a CE system was transformed to pCEC and a post-column detection cell was added. Since CE and other microcolumn separation methods have not yet been successfully applied for the simultaneous analysis of xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate, and byakangelicin in *A. dahurica* due to the similarity in structure and charge-to-mass ratios of these compounds, the aim of the current study was to develop a pCEC method to separate and simultaneously quantify the five main coumarins in the extract samples.

2. Experimental

2.1. Materials and reagents

Polyamide-clad fused-silica capillary tubing with 100 μm inner diameter (i.d.) and 375 μm outer diameter (o.d.) was pur-

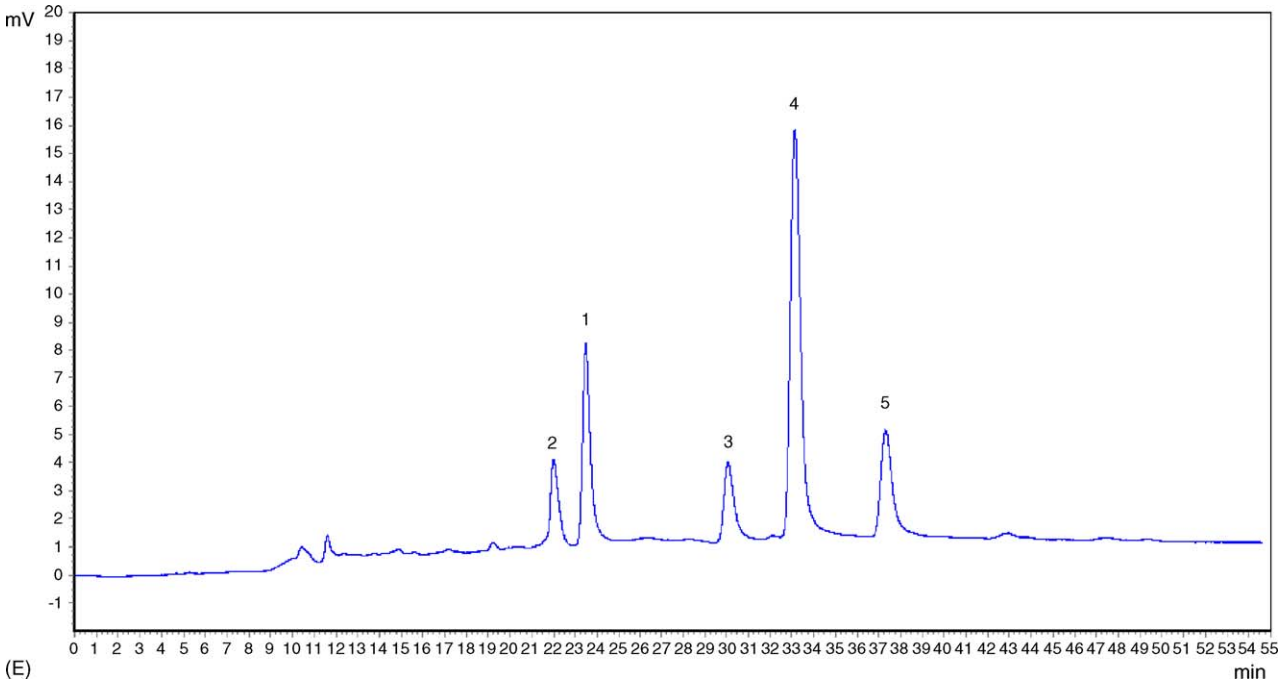

Fig. 3. (*Continued*).

chased from Hebei Yongnian Optical Filter Factory (Hebei, PR China). The 3 μm spherical octadecylsilica (ODS) particles were purchased from Synchrom (Lafayette, IN, USA). The 1 μm bare silica particles were purchased from Phase Separation (Norwalk, NJ, USA). The 5 μm silica particles used for frit fabrication were a gift from Waters (Milford, MA, USA). *A. dahurica* root (collected from Hangzhou, PR China) was obtained from Shanghai Lei Yun Shang Pharmaceutical Co. Ltd. Xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate, byakangelicin were extracted and obtained from *A. dahurica* root by preparative HPLC, and identified with NMR, IR, UV, and MS comparing with literature data [37–39]. The purities were 98.8 % for xanthotoxol, 98.8% for osthenol, 98.2% for imperatorin, 99.3% for oxypeucedanin hydrate, 99.1% for byakangelicin by RP-HPLC determination. 1300 macroporous resin was from Shanghai Institute of Pharmaceutical Industry. 95% ethanol of chemical grade; sodium dihydrogen phosphate, phosphoric acid, and sodium hydroxide of analytical grade were from China Medicine (Group) Shanghai Chemical Reagent Corporation (Shanghai, PR China). Acetonitrile and methanol were of chromatographic grade (Merck, Darmstadt, Germany). Pure water prepared by Milli-Q System (Millipore, Bedford, MA, USA) was used for all buffer solutions and mobile phases.

2.2. Instrumentation

pCEC was performed on reconstructed pCEC system which comprised a pump (Waters, USA), CE system (Bio-Rad, USA, containing a high-voltage power supply and a variable wavelength UV–vis detector), a microfluid manipulation module with a 2 μl injector (Unimicro Technologies, Inc.) and a data acquisition module. A packed capillary column was sleeved with a peek tubing with the inlet connected with a four-way valve and the outlet connected with the detector which contained an uncoated fused-silica capillary to be a detection flow-cell. The schematic diagram of the pCEC system is shown in Fig. 2.

The HPLC system consisted of LC-10AT pumps (Shimadzu, Japan), a SPD-10A UV–vis detector (Shimadzu, Japan), and a 20 μl sample loop. An Ultrasphere ODS C-18 (4.6 mm × 15 cm, 5 μm) (Beckman Coulter, USA) column was used with a precolumn filled with the same stationary phase. The temperature of the column was kept constant at 20 °C by using a CTO-10ASVP column oven (Shimadzu, Japan). The mobile phase (methanol–acetonitrile–phosphate buffer (pH 4.8; 15 mM), 22.5:15:62.5, v/v/v) was delivered to the column at a flow rate of 0.3 ml/min and the eluate was monitored at 216 nm. Twenty microliters of sample solution were injected into the HPLC system. Chromatograms were processed by using a model N2000 workstation (Zhejiang University, Hangzhou, China).

2.3. Preparation of packed capillary column

The capillary column employed in this study had an i.d. of 100 μm and an o.d. of 375 μm, which was packed for about 25 cm length using the electrokinetic packing method described previously [40]. First, an inlet frit was made at one end of a capillary by sintering 5 μm silica particles. Second, a suspension of 3 μm ODS (90%, w/w) and 1 μm bare silica particles (10%, w/w) in methanol containing 4 mM phosphate (pH 6.5) was sonicated for approximately 10 min, then electrokinetically packed into the column for approximately 1 h. (The role of the bare silica particles was to increase the EOF.) Third, after packing, a second frit (the outlet frit) was made in the column by sintering the packing material. The column was pressurized at 20.7 MPa during the fabrication of this second frit to minimize disturbance of the adjacent ODS particles. The column was then inspected

carefully under a microscope (at magnifications of 10×–40×) to verify the packing density and the structural appearance of the frits.

2.4. Sample preparation and pCEC separations

Pulverized dried root of *Angelicae Dahuricae* was extracted by reflux with tenfold amount of 70% alcohol twice (2 h each time). After filtration, the crude extract was combined and evaporated to dryness by rotary vaporization at 60 °C under reduced pressure and redissolved in water. The solution was then chromatographed on 1300 macroporous resin by eluting stepwise with water and 10, 30, 50, 70% ethanol. Water was first used to remove some un-target chemicals, which have no or little retention on 1300 macroporous resin, 30–70% ethanol was then used to yield target samples, and 95% ethanol was finally used to activate the resin for another use. Every fraction was evaporated to dryness by rotary vaporization at 60 °C under reduced pressure and redissolved in methanol. After centrifuged at 12,000 rpm for 10 min, each fraction was filtered through a 0.45 μm membrane filter, and diluted to suitable concentration with the mobile phase. Five standard coumarins, xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate, byakangelicin were weighed, dissolved in methanol, degassed in an ultrasonic bath, and filtered through a 0.45 μm membrane filter. Then they were diluted with methanol in a volumetric flask to obtain standard solutions for the calibration curves. All the solutions were stored at 4 °C. The ranges of calibration curves were 37–129 μg/ml for xanthotoxol, 36–126 μg/ml for osthenol, 12–41 μg/ml for imperatorin, 88–306 μg/ml for oxypeucedanin hydrate, 20–69 μg/ml for byakangelicin, respectively. The contents of the five coumarins in the extract samples were calculated using the respective calibration curves.

The buffers containing NaH_2PO_4 were adjusted to the desired pH with either 10% H_3PO_4 or 0.1 M NaOH. After the pH was adjusted, the buffer and methanol or/and acetonitrile were mixed. All buffer solutions were filtered through 0.45 μm membrane filters and degassed by ultrasonication for approximately 5 min before used as mobile phase and transferred to the outlet vial. A negative voltage was added on the column outlet and the column inlet was grounded. Pressure was applied to the column inlet during the separation. Flow rate of the pump was set at 50 μl/min. The wavelength of the UV–vis detector was set at 216 and 254 nm. The backpressure regulator was set at 13.8 MPa. Both the pump flow and the sample in the injection loop were split, and therefore, the actual flow rate in the capillary column was estimated to be 100 nl/min and the actual injection volume of sample was about 4 nl at the split ratio 1:500.

3. Results and discussion

3.1. Choice of mobile phase

The mobile phase of methanol buffer, acetonitrile buffer, and methanol–acetonitrile buffer with 0.01 M phosphate were used to optimize separation of standard solution containing the five coumarins. Several reports indicate that methanol–water [6,7], acetonitrile–water [8–10], methanol–phosphate buffers (pH 5.4; 0.01 M) (45:55, v/v) [11] were often used as mobile phase in HPLC with ODS C18 packed column. To increase the resolution and reduce the analysis time, the pCEC method was optimized with the composition of the mobile phase with different contents of methanol, acetonitrile, and buffer. As shown in Figs. 3 and 4, methanol–acetonitrile–phosphate buffer (pH 4.8; 10 mM) (22.5:15:62.5, v/v/v) was selected as the optimum mobile phase. Higher concentration of acetonitrile shortened the analysis time of the coumarins and sharpened their peaks, while higher concentration of methanol among the tri-phase system (methanol–acetonitrile–phosphate buffer) enhanced the resolution. Since ε/η ratio of acetonitrile is higher than that of methanol, an increase of the EOF velocity was observed, which owed to the higher ε/η ratios exhibited by the higher content acetonitrile-containing mobile phases. This ratio influences the EOF in the electrodriven system according to Eq. (1):

$$V_{EOF} = \frac{\varepsilon \xi E}{\eta} \quad (1)$$

where ε is the permittivity, ξ the zeta potential, E the electric field strength, η the solvent viscosity, and V_{EOF} is the EOF velocity. It is obvious that separation of the coumarins is faster in a mobile phase containing a higher content of acetonitrile. From Figs. 3 and 4, it can be seen that the resolution was decreased with increasing the acetonitrile content in the mobile phase, which could be explained as the change of retention factors.

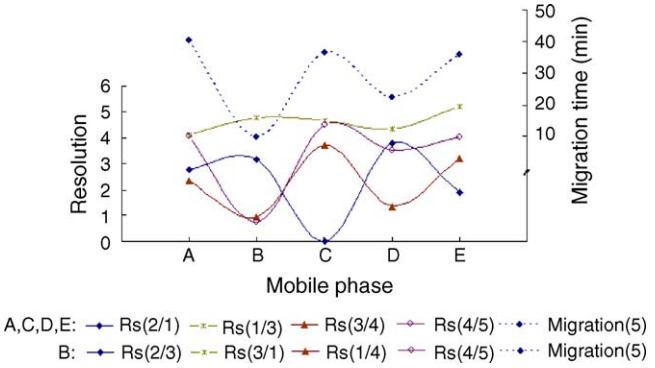

Fig. 4. Effect on migration time and resolution of various composition of mobile phase. A, B, C, D, and E represent the different composition of mobile phase, as in Fig. 3. Peaks as in Fig. 3. Rs(2/1) means resolution between peak of osthenol and xanthotoxol.

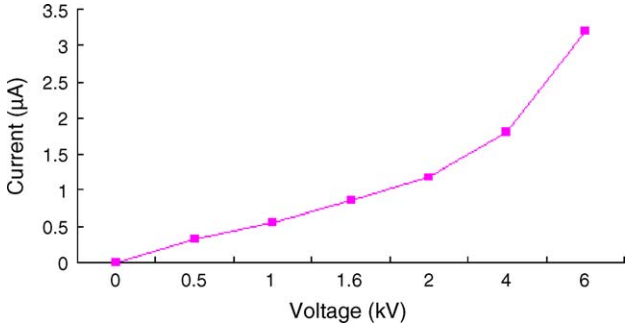

Fig. 5. Ohm's plot.

Table 1
Retention time (min) of coumarins at various applied voltages

Coumarins	Voltage (kV)						
	0	0.5	1	1.6	2	4	6
Xanthotoxol	16.63	14.39	14.14	14.05	14.79	13.62	13.20
Osthenol	15.60	16.06	16.16	16.87	18.50	20.32	23.10
Imperatorin	19.77	18.36	17.92	17.83	18.22	17.22	16.63
Oxypeucedanin hydrate	22.07	20.56	20.02	19.97	20.57	19.26	18.50
Byakangelicin	25.00	23.22	22.69	22.60	23.06	21.76	20.87

Table 2
Repeatability of retention time and peak area of coumarins in *Angelica dahurica*

Coumarins	t_R (min)[a]	RSD1 (%)[b]	RSD2 (%)[c]
Xanthotoxol	14.81	1.88	3.83
Osthenol	17.08	1.75	3.91
Imperatorin	18.78	1.42	3.78
Oxypeucedanin hydrate	20.75	1.99	3.60
Byakangelicin	23.38	2.01	4.96
Coumarins	**A[d]**	**RSD1 (%)[e]**	**RSD2 (%)[f]**
Xanthotoxol	243493	2.75	3.45
Osthenol	46765	2.85	3.67
Imperatorin	89474	3.01	3.64
Oxypeucedanin hydrate	588800	2.79	3.18
Byakangelicin	183242	2.94	3.20

Conditions: Mobile phase: methanol–acetonitrile–phosphate buffer (pH 4.8; 15 mM) (22.5:15:62.5, v/v/v), flow rate: 50 μl/min, voltage: 1 kV, backpressure: 13.8 MPa, injection: 4 nl, detection: 216 nm, temperature: 20 °C, and sample: standard solution.
[a] Retention time.
[b] Run to run relative standard deviation of retention time.
[c] Day to day relative standard deviation of retention time.
[d] Peak area.
[e] Run to run relative standard deviation of peak area.
[f] Day to day relative standard deviation of peak area.

In RP-CEC, the logarithmic retention factor, k, is correlated by a linear relationship involving the vol% (φ) of organic modifier in accordance with Eq. (2):

$$\ln k = \ln k_0 + S\varphi \quad (2)$$

where k is the retention factor, k_0 the retention factor extrapolated to 100% aqueous mobile phase, S the slope of the dependence function, and φ is the volume fraction of organic modifier in the mobile phase. So the resolution is decreased with increasing the acetonitrile content in the mobile phase. The lower acetonitrile content would benefit the separation, but longer analysis time was needed [41].

When the applied voltage was set at 0 kV, the chromatograms of coumarins were simply the behavior of capillary HPLC (CLC). We also applied 3 kV voltage to the separation, the analysis time was shortened when acetonitrile in the mobile phase increased compared with the mobile phase only containing methanol and buffer according to Eq. (1). The same as the previous experiment [42], increasing the acetonitrile content in the mobile phase increases both the theoretical plates and the speed of the EOF. These results show the importance of the organic modifier for the zeta potential and dielectric constant, which affect the EOF and the ionic migration through the column.

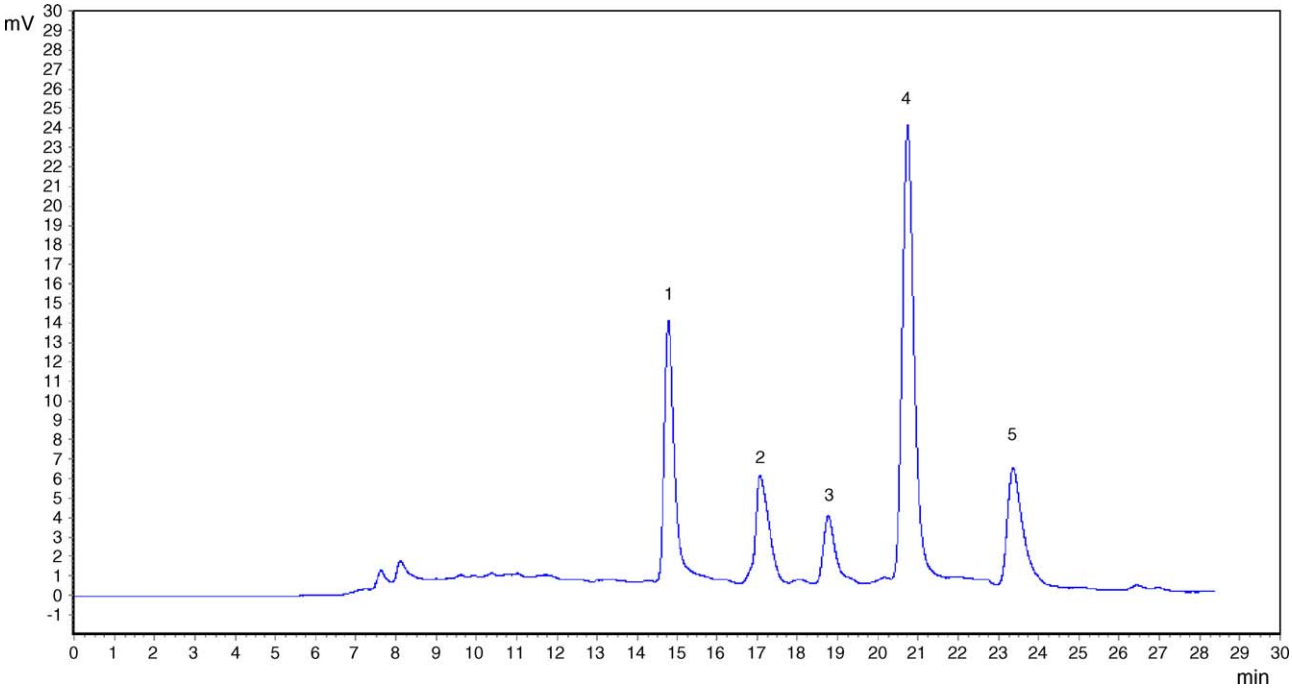

Fig. 6. Representative electrochromatograms of coumarins with optimized conditions. Mobile phase: methanol–acetonitrile–phosphate buffer (pH 4.8; 15 mM) (22.5:15:62.5, v/v/v), flow rate: 50 μl/min, voltage: 1 kV, backpressure: 13.8 MPa, injection: 4 nl, detection: 216 nm, temperature: 20 °C, and sample: standard coumarins solution; peaks as in Fig. 3.

3.2. Choice of electrolyte solutions

3.2.1. Effect of buffer ionic strength

To optimize the method, the concentration of phosphate was changed from 5 to 20 mM. Higher concentration led to higher ionic strength and thereby higher current, which increased from 0.18 μA (5 mM phosphate) to 0.72 μA (20 mM phosphate). Retention time of the coumarins was reduced with the increasing current while resolution declined to some extent. Combining the effect of EOF, ionic strength and chromatographic behavior, a reasonable good separation of the coumarins was obtained when using 15 mM phosphate for shorter run time and well-distributed peaks.

3.2.2. Effect of pH

The chemical structures of the coumarins indicate that they are hardly charged in conditions of acidity or alkalinity. Therefore, the effect of pH is almost solely based on changing the speed of EOF. The retention times of the five coumarins were approximately on the same level when changing pH from 3.45 to 6.45 (3.45, 4.8, 5.55, 6.45). In this range, the resolution was better at pH 4.8 and 5.55.

3.3. Effect of applied voltage

The influence of applied voltage on the resolution, selectivity and column efficiency was studied. In Fig. 5, the current is depicted as a function of the applied voltage. Increase of the applied voltage can increase the EOF, and consequently decrease the retention time of the neutral compounds. The chemical structures of the five coumarins (Fig. 1) show osthenol to be weak acid due to the presence of phenolic hydroxyl group. The deprotonation of the phenolic hydroxyl group, increase the absolute value of the electrophoretic mobility of osthenol. Since the direction of the electrophoretic mobility of the negatively charged osthenol is opposite to the EOF, the overall linear velocity of this compound in the column was still decreased. As can be seen in Table 1, an increase in the voltage resulted in reductions in analysis time of the coumarins except osthenol, while the resolution of them remained almost unaffected.

3.4. Final optimization

Detection was initially performed at two wavelengths, 216 and 254 nm, which were the wavelengths of maximum UV absorption of the standards. Two hundred and sixteen nanometer was selected for the detection because, under this condition, good signal-to-noise ratio and larger peak areas were obtained. Finally, a mobile phase containing methanol–acetonitrile–phosphate buffer (pH 4.8; 15 mM) (22.5:15:62.5, v/v/v) under conditions of 1 kV applied voltage, 20 °C column temperature, 216 nm UV detection, 50 μl/min flow rate, 13.8 MPa backpressure, and 4 nl injection was found to produce the best resolution (Fig. 6).

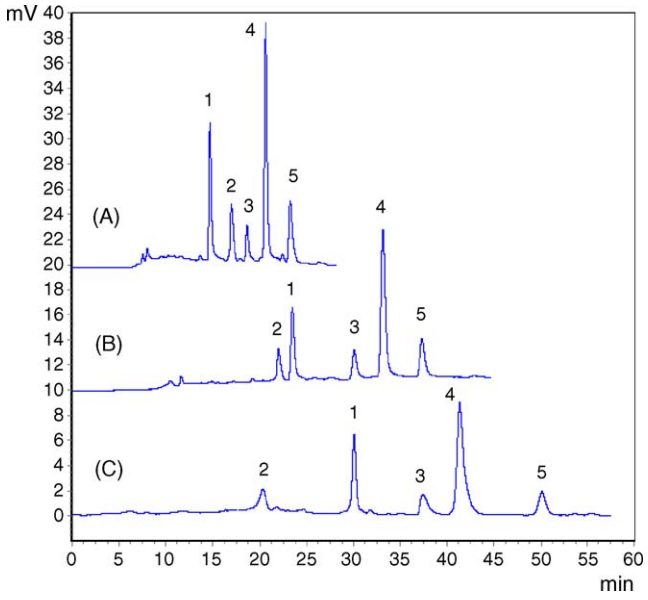

Fig. 7. Separation of coumarins with (A) pCEC, (B) capillary HPLC, and (C) HPLC. Conditions pCEC: as in Fig. 6. Conditions capillary HPLC: voltage, 0 kV; other conditions as in pCEC. Conditions HPLC: column: Ultrasphere ODS C18 (4.6 mm × 15 cm, 5 μm) (Beckman Coulter, USA), mobile phase: methanol–acetonitrile–phosphate buffer (pH 4.8; 15 mM) (22.5:15:62.5, v/v/v), flow rate: 0.3 ml/min, injection: 20 μl, detection: 216 nm, temperature: 20 °C, and sample: standard coumarins solution; peaks as in Fig. 3.

3.5. Method evaluation

The repeatability in these experiments was tested for consecutive analysis. The within-day repeatability as well as the between-day repeatability of the retention time and peak area were good (Table 2). For further quantitative determination of coumarins in Chinese herbal medicines, the external standard method could be used.

Table 3
Comparison of column efficiency and retention time among pCEC, capillary HPLC, and HPLC

Coumarins	Number of theoretical plates (*N*)/m			Retention time (min)		
	pCEC	Capillary HPLC	HPLC	pCEC	Capillary HPLC	HPLC
Xanthotoxol	108928	74800	21416	14.81	23.48	30.08
Osthenol	101800	68288	22500	17.08	21.10	20.52
Imperatorin	103000	82092	10444	18.78	30.10	37.55
Oxypeucedanin hydrate	105096	79472	23608	20.75	33.17	41.31
Byakangelicin	113440	81524	30896	23.38	37.33	52.88

Table 4
Results of regression analysis on calibration curves and detection limits

Analyte	Regression equation $y = a + bx$[a]	Correlation coefficient	Linear range (μg/ml)	Detection limit (μg/ml)[b]
Xanthotoxol	$y = -3110.2 + 329.6x$	0.9981	37–129	0.69
Osthenol	$y = -3443.6 + 332.6x$	0.9976	36–126	0.77
Imperatorin	$y = -1979.7 + 350.3x$	0.9982	12–41	0.86
Oxypeucedanin hydrate	$y = -11995.0 + 341.1x$	0.9994	88–306	0.96
Byakangelicin	$y = -683.8 + 414.1x$	0.9979	20–69	0.63

[a] y and x stand for the peak area and the concentration (μg/ml) of the analytes, respectively.
[b] The detection limit was defined as the concentration at the signal-to-noise of 3.

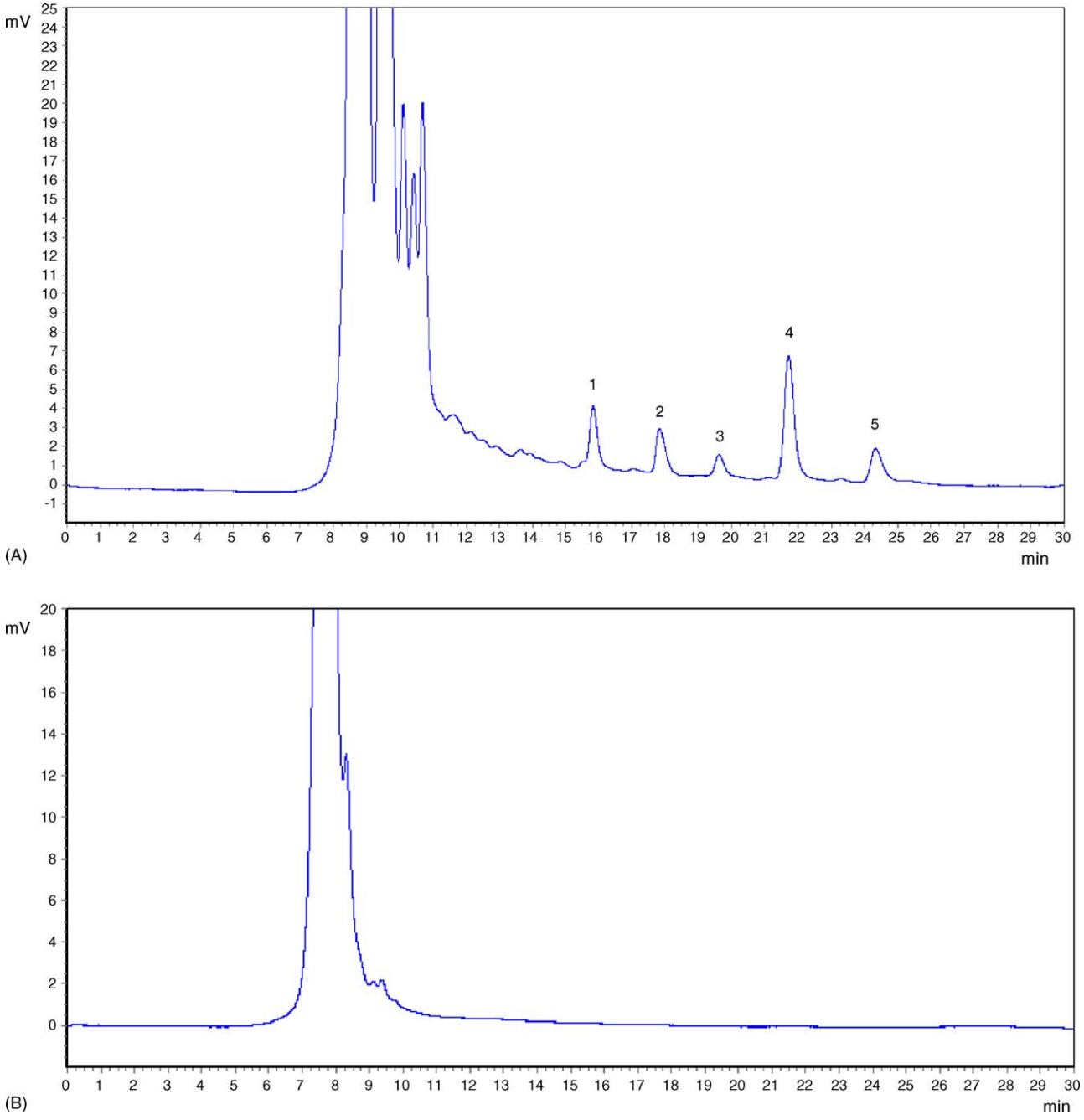

Fig. 8. Electrochromatograms of coumarins in *A. dahurica*. Mobile phase: methanol–acetonitrile–phosphate buffer (pH 4.8; 15 mM) (22.5:15:62.5, v/v/v), flow rate: 50 μl/min, voltage: 1 kV, backpressure: 13.8 MPa, injection: 4 nl, detection: 216 nm, and temperature: 20 °C. Sample: (A) crude extract, (B) fraction 1 of eluting stepwise from 1300 macroporous resin, (C) fraction 2, (D) fraction 3, (E) fraction 4, and (F) fraction 5; peaks as in Fig. 3.

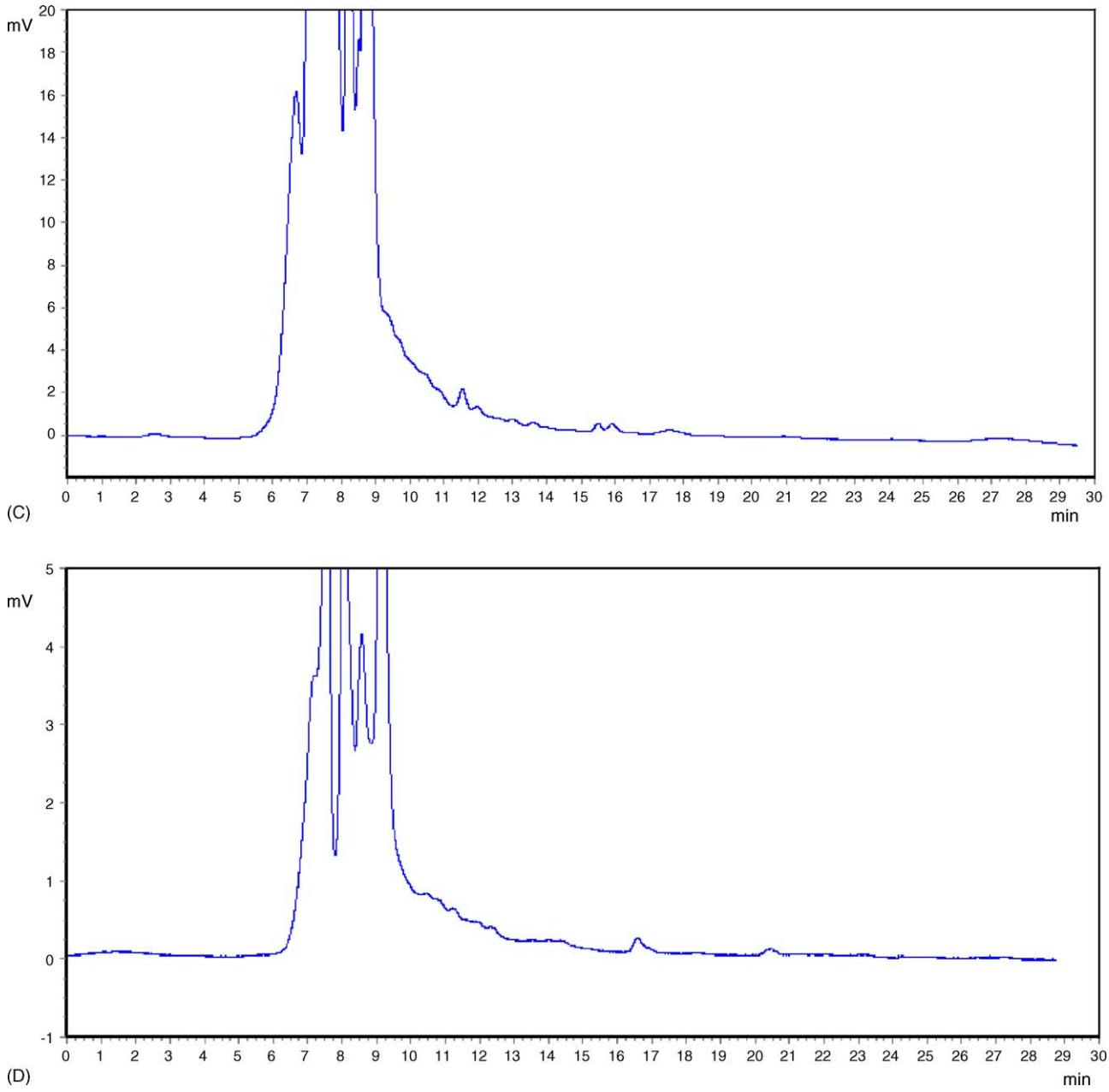

Fig. 8. (*Continued*)

3.6. pCEC, CLC, and HPLC separation

Separation of the standard coumarins solution performed with pCEC, CLC, and HPLC was also compared. The peak efficiency and retention time are described in Table 3 and Fig. 7. Higher column efficiency and shorter analysis time were obtained by pCEC.

3.7. pCEC for extract samples and quantitative analysis

Coumarin analysis by pCEC was conducted using a Chinese species of *A. dahurica* as a sample. The results indicate that the optimized pCEC conditions described above are applicable to extract samples of *A. dahurica* (Table 4; Fig. 8). From the crude extract, the contents of xanthotoxol, osthenol, imperatorin, oxypeucedanin hydrate, byakangelicin were 0.495, 0.340, 0.201, 1.230, 0.225 (mg/g dried medicinal herb), respectively. The recovery of the method was determined with the standard addition method for the five coumarins in the three sample solutions, respectively, with results of 96.3–103.6% for xanthotoxol, 95.4–104.3% for osthenol, 94.8–103.8% for imperatorin, 96.5–99.6% for oxypeucedanin hydrate, and 96.5–104.7% for byakangelicin, respectively. From the chromatograms of fractions 1–5, it can be concluded that the five coumarins were mainly contained in the eluates of 50% ethanol, partially in 70% ethanol and hardly in the eluates of water, 10 and 30% ethanol. Therefore, the pCEC method can be used for quality control of intermediate extracts and active fraction

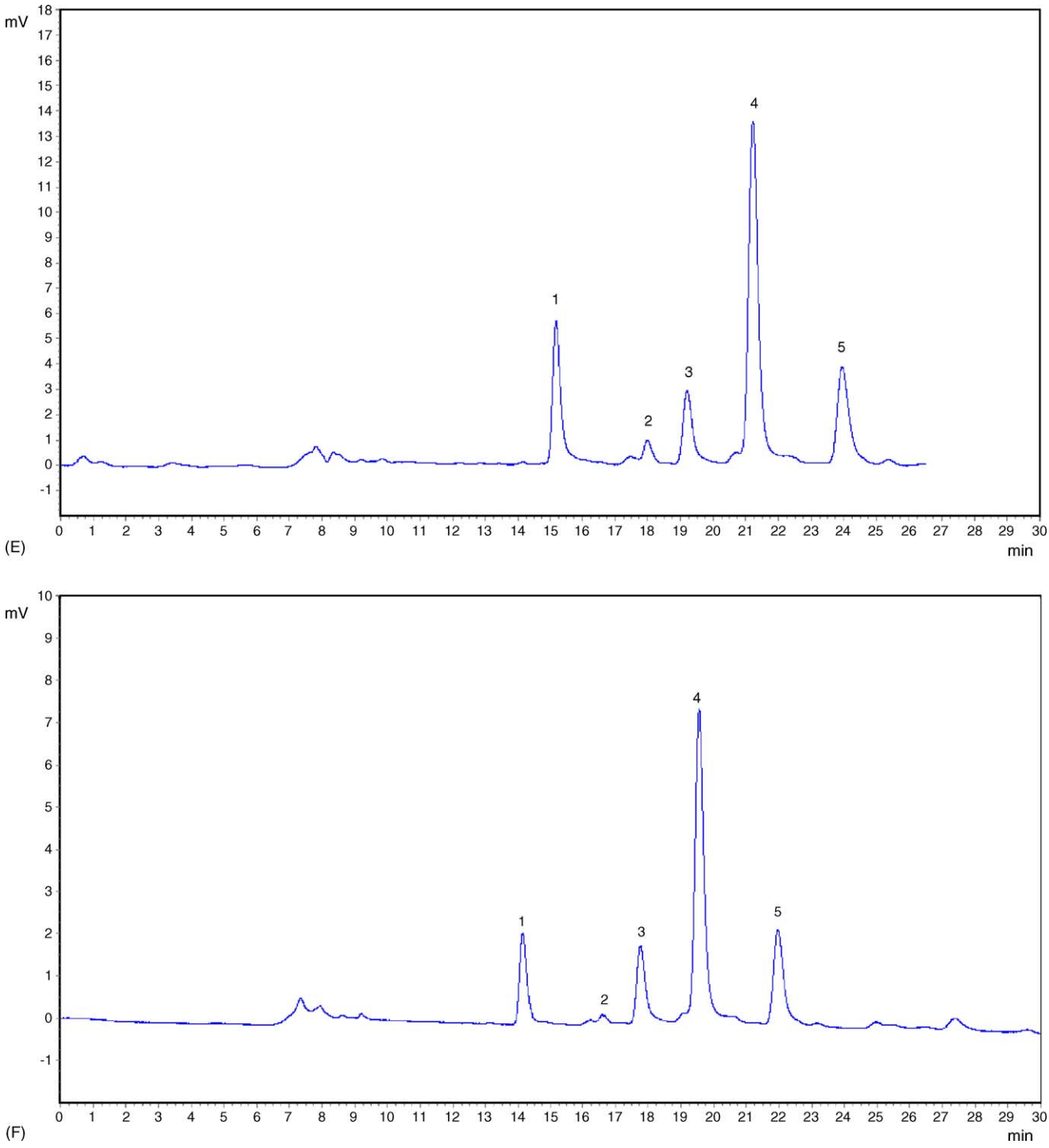

Fig. 8. (*Continued*).

in the purification process of macroporous resin eluting from *A. dahurica*.

4. Conclusions

Pressurized capillary electrochromatography can be applied successfully to analyze and quantify the coumarin compounds in *A. dahurica*. Baseline separation of five coumarins, without the use of gradient elution, was achieved only with inorganic ion additives and higher column efficiency and shorter analysis time were also obtained in pCEC when compared to both capillary HPLC and HPLC. The method also showed satisfactory retention time and peak area repeatability with the first use of post-column detection cell in the pCEC instrument. The pCEC method developed in this project has the potential for a rapid separation and, furthermore, specific determination of coumarins

from *A. dahurica* as well as the quality control of dried herb, intermediate products in steps of plant extraction and active fraction of plant.

Acknowledgments

The authors would like to acknowledge Unimicro Technologies for providing the reconstruction of the pCEC system and guide of technology. This work was supported by Fundamental Research Key Project founded by Science & Technology department of Shanghai, PR China, Grant No. 03JC14005.

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