Journal of Chromatography A, 1216 (2009) 7329–7335

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Simultaneous determination of five flavonoids in licorice using pressurized liquid extraction and capillary electrochromatography coupled with peak suppression diode array detection

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ARTICLE INFO

Article history:
Available online 18 August 2009

Keywords:
Licorice
Flavonoids
Pressurized liquid extraction
Capillary electrochromatography
Peak suppression

ABSTRACT

Pressurized liquid extraction (PLE) and capillary electrochromatography (CEC) methods were developed for the simultaneous determination of five flavonoids, namely liquiritin, isoliquiritin, ononin, liquiritigenin and isoliquiritigenin, in licorice using baicalein as internal standard (IS). Peak suppression technique was used for the quantification of ononin because of its poor resolution with isoliquiritin. The analysis was performed on a Hypersil C_{18} capillary (3 μm, 100 μm/25 cm) with a mixture of 10 mM phosphate buffer (pH 3.0)/ACN (65:35, v/v) as mobile phase running at 25 kV and 30 °C. The detection wavelengths were set at 275 nm (without reference wavelength for liquiritin and liquiritigenin), 360 nm (without reference wavelength for isoliquiritin and isoliquiritigenin) and 254 nm (with reference wavelength of 405 nm for ononin). All calibration curves showed good linearity ($R^2 > 0.9993$) within the test ranges. The LOD and LOQ were lower than 2.1 and 8.3 μg/mL, respectively. The RSDs of intra- and interday for relative peak areas of five analytes to IS were less than 3.8 and 4.7%, respectively, and the recoveries were 98.2–103.8%. The validated method was successfully applied to the quantitative analysis of five flavonoids in licorice, which is helpful to its quality control.

1. Introduction

Licorice (or liquorice), the dried roots and rhizomes of *Glycyrrhiza* species (family Leguminosae), has been used as a natural sweetener, as well as a flavorant for a long time [1]. On the other hand, licorice is also one of the oldest and most popular herbal medicines in the world, and is recorded in the pharmacopoeias of many Asian and European countries, such as China, Japan and UK [2]. Numerous bioactive compounds, mainly including triterpene saponins and flavonoids, have been isolated from licorice. Among them, flavonoids, such as liquiritin, liquiritigenin, isoliquiritin and isoliquiritigenin, are gaining popularities because of their significant pharmacological activities including antiulcer, antioxidation, antiinflammatory, antimicrobial, antitumor effects, etc. [1–5]. Although licorice is considered as Generally Recognized as Safe (GRAS) for use in food by FDA (21 CFR 184.1408), large amount of licorice may result in severe hypertension, hypokalemia and other signs of mineralocorticoid excess [6,7]. Therefore, quality control is critical to ensure the efficacy and safety of licorice, and saponins and flavonoids are usually considered as the markers [3,8–18].

So far, a series of methods, including thin layer chromatography (TLC) [8], high performance liquid chromatography (HPLC) [9–12] and capillary electrophoresis (CE) [13–18] have been developed for the determination of saponins and/or flavonoids in licorice. However, these methods suffered from large consumption of organic solvents (HPLC), low resolution (TLC), bad repeatability (CE) and/or low sensitivity (TLC).

Capillary electrochromatography (CEC) is a hybrid technique of LC and CE, which uses an electric field to drive liquid through a packed capillary column. In this technique, the best properties of HPLC and CE operate in synergy. Due to its high selectivity and efficiency, CEC has attracted wide attention of pharmaceutical and biochemical analysts [19–21], although conventional gradient elution is difficult to perform. It has also been used for the analysis of natural products [22–24] except licorice.

On the other hand, sample preparation is crucial for quality control of herbal medicines. Traditional extraction methods such as ultrasonication, Soxhlet and reflux were usually time-consuming and solvent-intensive. As an alternative, pressurized liquid extraction (PLE), an extraction technique under elevated temperature and

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doi:10.1016/j.chroma.2009.08.034

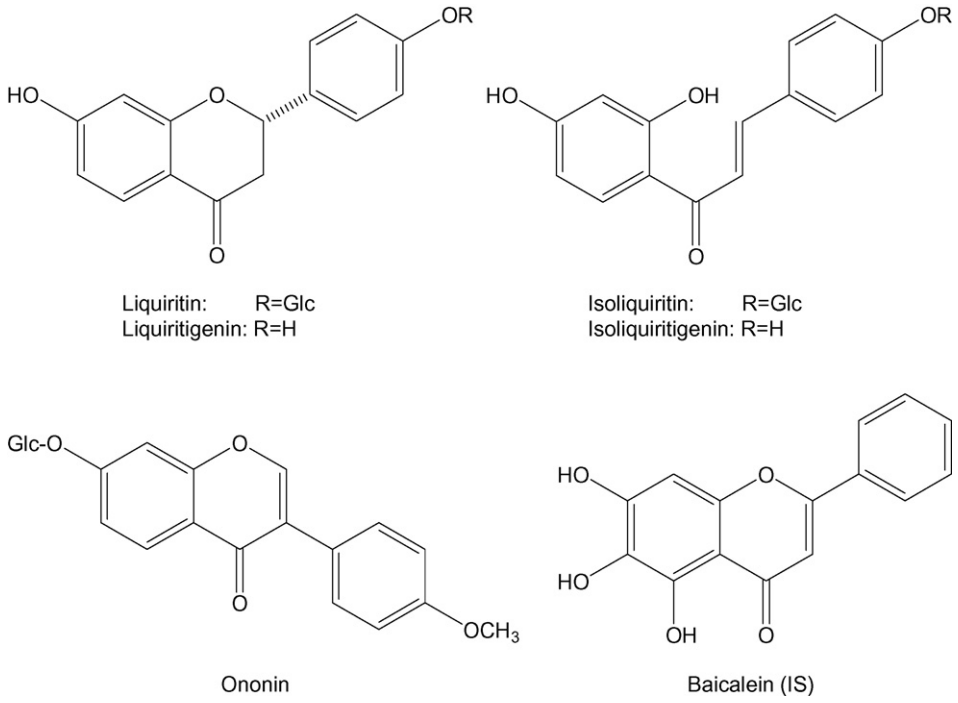

Fig. 1. Chemical structures of 5 investigated flavonoids and baicalein (IS).

pressures, could drastically improve the speed and efficiency of extraction. PLE has been applied to the extraction of glycyrrhizin in licorice using methanol [25], hot water [26] or water with surfactants [27] as solvent. However, these studies focused on glycyrrhizin, which may be unsuitable for flavonoids from licorice, although PLE has also been used for extraction of flavonoids from some other medicinal plants [28–30].

In this study, PLE and CEC coupled with peak suppression technique were developed for the simultaneous determination of five flavonoids, including liquiritin, isoliquiritin, ononin, liquiritigenin and isoliquiritigenin, in licorice. The validated method was applied to the assay of 25 samples from different species and/or parts of licorice.

2. Experimental

2.1. Chemicals and reagents and materials

Liquiritin was purchased from Mansite Pharmaceutical Co., Ltd. (Chengdu, Sichuan, China). Ononin, isoliquiritin, liquiritigenin and isoliquiritigenin were bought from Winherb Medical S&T Development Co. Ltd. (Shanghai, China). Baicalein was purchased from

Table 1
Summary for the tested samples of licorice.

Species	Sampling parts	Sources	Code
Glycyrrhiza uralensis Fisch.	Underground part	Inner Mongolia, China	GU-1
		Inner Mongolia, China	GU-2
		Inner Mongolia, China	GU-3
		Elion Resources Group Company, Inner Mongolia, China	GU-4
		Elion Resources Group Company, Inner Mongolia, China	GU-5
		Jiuquan, Gansu, China	GU-6
		Longxi, Gansu, China	GU-7
		Longxi, Gansu, China	GU-8
		Jiuquan, Gansu, China	GU-9
		Lanzhou, Gansu, China	GU-10
		Lintiao, Gansu, China	GU-11
		Jiuquan, Gansu, China	GU-12
		Yaxian, Ningxia, China	GU-13
		Ningxia, China	GU-14
		Yinchuan, Ningxia, China	GU-15
		Tongxin, Ningxia, China	GU-16
		Purchased from Heibei, China	GU-17
		Gansu, China	GU-18
	Aerial part	Gansu, China	GU-18A
		Inner Mongolia, China	GU-19A
Glycyrrhiza inflata Bat.	Underground part	Tianshan Pharmaceutical Industry Co. Ltd., Xinjiang, China	GI-1
		Tianshan Pharmaceutical Industry Co. Ltd., Xinjiang, China	GI-2
		Xinjiang, China	GI-3
		Jiuquan, Gansu, China	GI-4
Glycyrrhiza glabra L.	Underground part	Gansu, China	GG-1

ChromaDex (Irvine, CA, USA). The purity of all compounds is more than 95% (determined by HPLC) and their structures were shown in Fig. 1.

Absolute ethanol used for the extraction was bought from Uni-Chem (Belgrade, Serbia and Montenegro). Sodium dihydrogen phosphate was purchased from Riedel-de Haën (Seelze, Germany). Sodium hydroxide of analytical grade was bought from Labscan (Bangkok, Thailand). Orthophosphoric acid and acetonitrile (ACN) for liquid chromatography were products of Merck (Darmstadt, Germany). Deionized water was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA). Reagents not mentioned here were from standard sources.

Licorice samples were collected from Gansu, Xinjiang, and Inner Mongolia, China, respectively (Table 1). The voucher specimens of these samples were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China.

2.2. CEC analysis

All analyses were performed on an Agilent 3D CE instrument (Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector and an Agilent ChemStation software. A Hypersil C_{18} capillary (3 μm, 100 μm/25 cm; Agilent Technologies, Waldbronn, Germany) was used throughout this study. The buffer containing sodium dihydrogen phosphate was adjusted to the desired pH with same concentration of phosphoric acid or sodium hydroxide, then the mixture of the phosphate buffer and a certain ratio of ACN was used as mobile phase. The mobile phase was filtered through a 0.22 μm filter, and degassed by ultrasonication under room temperature for 10 min before it was transferred to the inlet/outlet vials. The new capillary is equilibrated by applying 10 bar pressure on both inlet and outlet vials and stepping up the voltage from 5 to 25 kV in 5 kV, 10–15 min steps. Then the capillary was conditioned with renewal buffer for 30 min at 25 kV until the current and detector baselines were stable. Between two consecutive injections of the same sample, the capillary was electroconditioned with mobile phase for 20 min. Both inlet and outlet were pressurized with 10 bars during conditioning and analysis. The mobile phase was refreshed every run. Electrokinetic injection was employed (10 kV for 10 s).

2.3. Standard and sample preparation

The stock solution of five flavonoids were prepared in mobile phase at the concentration of 1 mg/mL, and then diluted to appropriate concentration with mobile phase before use. Baicalein dissolved in mobile phase (300 μg/mL) was used as internal standard (IS). The standard solutions were mixed with IS solution in the proportion of 1:1, and then ultrasonically degassed for 10 min prior to injection into the CEC system.

Sample preparation was performed using PLE on a Dionex ASE 200 system (Dionex, Sunnyvale, CA, USA). In brief, dried powder of licorice (0.25 g) was mixed with diatomaceous earth in a proportion of 1:1 and placed into an 11 mL stainless steel extraction cell, and extracted under the optimized conditions. Then the extract was dried at 50 °C using a rotary evaporator (BÜCHI, Switzerland), and the residue was vortexed with 5 mL of the mobile phase. After centrifugation (Eppendorf, Hamburg, Germany) at 13000 rpm for 5 min, the supernatant was filtered through a 0.22 μm filter (Millipore, Ireland), and then mixed with IS solution in the proportion of 1:1. The CEC analysis was performed after the solution was degassed with ultrasonication for 10 min. For the samples containing high contents of analytes, appropriate dilution was made to avoid the concentration beyond the linear range.

2.4. Calibration curves, limits of detection and quantification

A series solutions containing appropriate concentrations of five reference compounds and 150 μg/mL IS were used for the construction of calibration curves. At least six concentrations of the solution were analyzed in duplicates, and then the calibration curves were constructed by plotting the peak area ratio of individual standard to IS *versus* the concentration of each analyte. Limits of detection (LOD) and quantification (LOQ) for each analyte were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively.

2.5. Precision, repeatability and accuracy

Intra- and interday variations were chosen to determine the precision of the developed assay. For intra-day variability test, the mixed standards solutions were analyzed for six replicates within one day, while for inter-day variability test, the solutions were examined in duplicates for consecutive three days. Variations were expressed by RSD.

The repeatability of the developed method was evaluated at three levels (0.20, 0.25 and 0.30 g) of the sample GU-17, which were extracted and analyzed in triplicate as mentioned above. The repeatability was present as RSD.

The recovery was used to evaluate the accuracy of the method. Known amounts of individual standards were added into a certain amount (0.13 g) of sample GU-17. The mixture was extracted and analyzed using the developed method. Three replicates were performed for the test.

3. Results and discussion

3.1. Optimization of CEC conditions

First, the optimization of CEC conditions was performed using the mixed standards solution. Several parameters including pH (3.0, 4.0, 5.0 and 6.0), buffer concentration (5 mM, 10 mM, 15 mM and 20 mM), and proportion of ACN (40%, 45%, 50% and 55%) were optimized using a univariate approach. 20 kV of voltage and 25 °C of temperature were used and the detection wavelength was set at the maximum absorbance wavelength of each analyte without reference wavelength, i.e. 275 nm for liquiritin and liquiritigenin, 360 nm for isoliquiritin and isoliquiritigenin, 254 nm for ononin. Based on preliminary investigation, the resolution (R_S) between isoliquiritin and ononin at 254 nm was poor, so it was chosen as the response for optimization of CEC conditions. The best R_S was

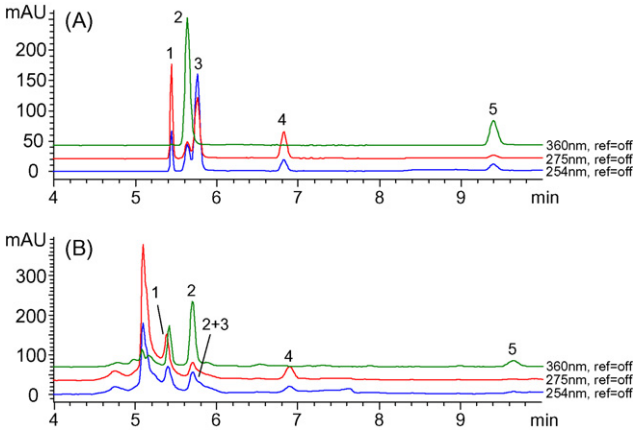

Fig. 2. Capillary electrochromatograms of (A) mixed standards and (B) licorice sample GU-17 using 10 mM phosphate buffer (pH 3.0)/ACN (50:50, v/v) as mobile phase at 20 kV of voltage and 25 °C of temperature. 1, liquiritin; 2, isoliquiritin; 3, ononin; 4, liquiritigenin; 5, isoliquiritigenin.

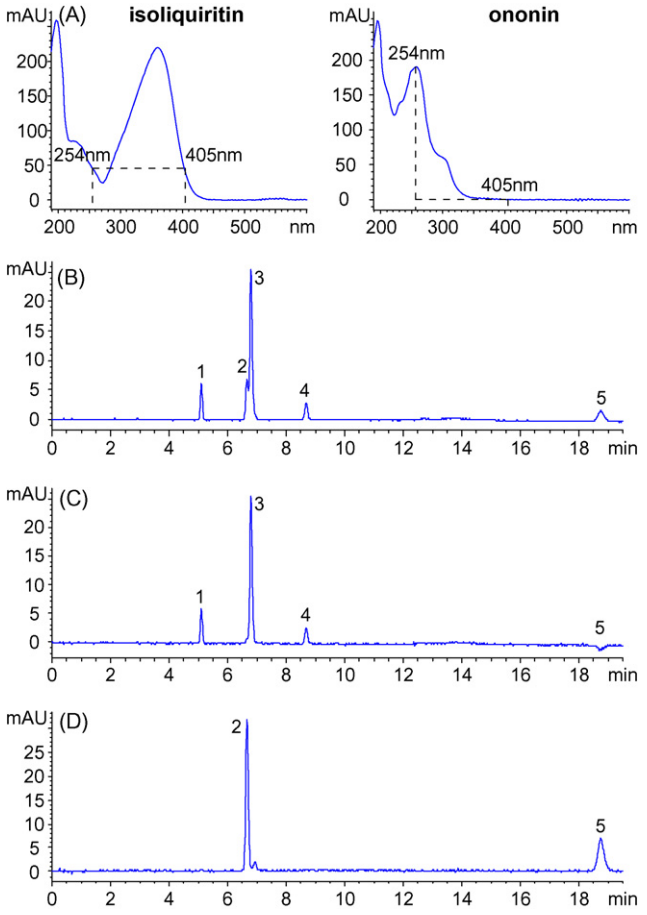

Fig. 3. The (A) UV spectra of isoliquiritin and ononin, and capillary electrochromatograms of mixed standards detected at 254 nm (B) without and (C) with reference wavelength of 405 nm, as well as (D) at 360 nm.
1, liquiritin; 2, isoliquiritin; 3, ononin; 4, liquiritigenin; 5, isoliquiritigenin. CEC conditions: Hypersil C_{18} column (3 μm, 100 μm/25 cm; Agilent Technologies, Waldbronn, Germany); electrokinetic injection (10 kV for 10 s); mobile phase: 10 mM phosphate buffer (pH 3.0)/ACN (65:35, v/v); temperature, 30 °C; voltage, 25 kV.

only 1.2 when using 10 mM phosphate buffer (pH 3.0)/ACN (50:50, v/v) as mobile phase. Especially, liquiritin was interfered by other component in licorice under the conditions (Fig. 2). Therefore, the proportion of ACN was decreased to 35% to improve liquiritin resolution. Moreover, the temperature was increased to 30 °C and the voltage was set at 25 kV so as to improve R_S and shorten the analysis time, respectively.

Unfortunately, isoliquiritin and ononin still could not be well separated. In this study, peak suppression technique was used in order to determine these two compounds with poor resolution. Briefly, based on the differences between the UV spectra of isoliquiritin and ononin, a reference wavelength of 405 nm was set to suppress the peak of isoliquiritin at 254 nm, while ononin still had strong absorbability. But ononin had no absorbance at 360 nm which could be used for detection of isoliquiritin (Fig. 3). Finally, the peaks of five investigated flavonoids in licorice could be well separated in 20 min (Fig. 4).

3.2. Selection of IS

In order to increase the repeatability of assay, IS is usually necessary for CEC analysis. Eleven compounds were tested for the selection of IS. Among them, puerarin, morin, daidzein, icariin, caffeic acid, and ferulic acid coeluted with the compounds in licorice samples, while wogonin, irisflorentin, genkwanin and chrysin had a long migration time. Finally, baicalein was selected as IS because it had good resolution and an acceptable analytical time (Fig. 4A).

3.3. Optimization of PLE procedure

The optimization of PLE procedure for extraction of five investigated flavonoids from licorice was performed using the sample GU-17. The parameters included the type of solvent (water, 70% and 95% ethanol aqueous solution), temperature (100, 120 and 140 °C), static extraction time (5, 10 and 15 min) and particle size (40–60 mesh, 60–80 mesh and 80–100 mesh), which were studied by using univariate approach while other conditions were kept constant (pressure, 1.034×10^7 Pa; flush volume, 40% and one extraction cycle). The amount of five investigated components was used as the response for evaluation of extraction efficiency. The result (Fig. 5)

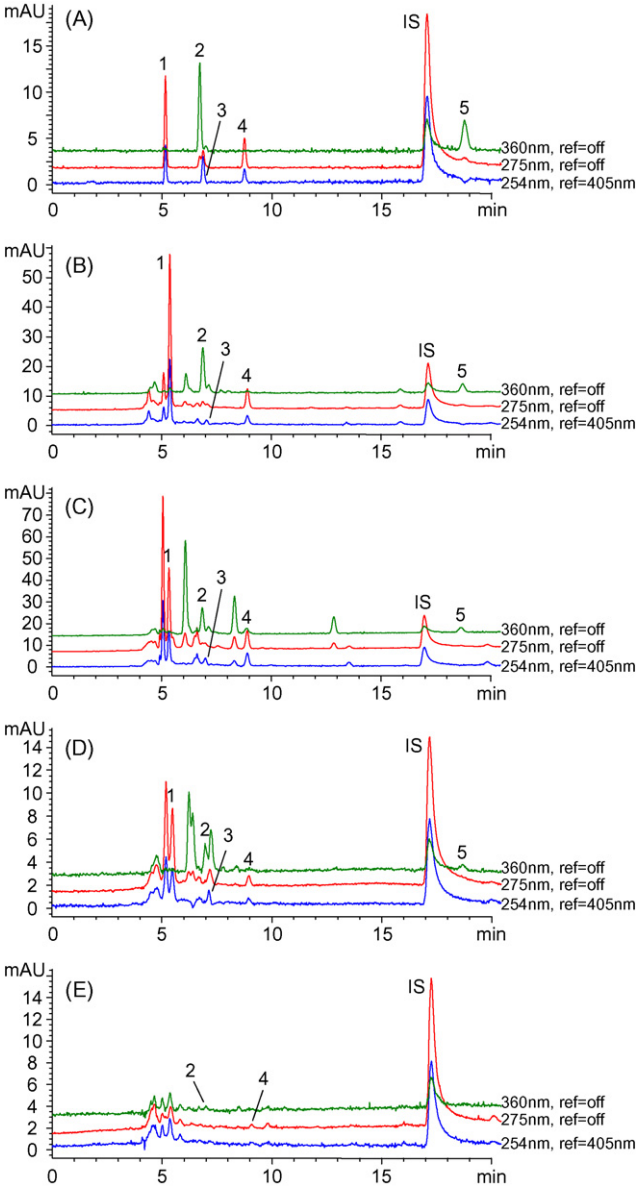

Fig. 4. Capillary electrochromatograms of (A) mixed standards and PLE extracts of underground part of (B) *Glycyrrhiza uralensis*; (C) *G. inflata*; (D) *G. glabra*; and (E) aerial part of *G. uralensis*. 1, liquiritin; 2, isoliquiritin; 3, ononin; 4, liquiritigenin; 5, isoliquiritigenin; IS, baicalein. CEC conditions: Detection, 275 nm (without reference wavelength for liquiritin and liquiritigenin), 360 nm (without reference wavelength for isoliquiritin and isoliquiritigenin) and 254 nm (with reference wavelength of 405 nm for ononin). Other conditions were the same as in Fig. 3.

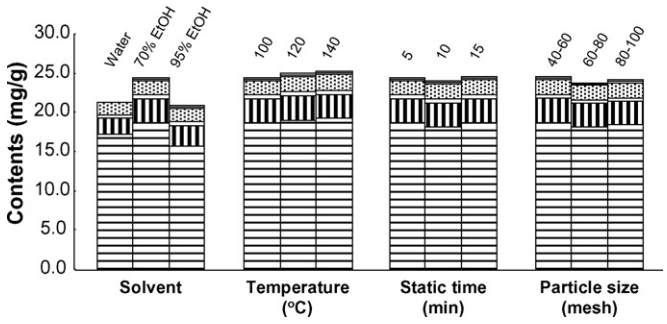

Fig. 5. Influences of solvent, temperature, static extraction time and particle size on pressurized liquid extraction of liquiritin (▤), isoliquiritin (▥), ononin (□), liquiritigenin (▦) and isoliquiritigenin (■) from licorice. To determine one of the parameters, the others were set at the definite values (temperature, 100 °C; static extraction time, 5 min; solvent, 70% ethanol; particle size, 40–60 mesh). CEC conditions were the same as in Fig. 4.

showed that solvent was the most important parameter affecting the extraction efficiency. The exhausted extraction for the PLE procedure was determined by performing consecutive pressurized liquid extractions on the same sample under the optimized PLE conditions, until no investigated compounds were detected by the analysis. The exhausted extraction was calculated based on the total extracted amount of the investigated components during the consecutive extractions, and the rate of the first time extraction was 99.6%. Considering the results mentioned above, the conditions of the PLE method proposed were: solvent, 70% ethanol; particle size, 40–60 mesh; temperature, 100 °C; static extraction time, 5 min; pressure, 1.034×10^7 Pa; flush volume, 40%; cycle, 1; and number of extraction, 1.

3.4. Validation of method

The linearity, regression, and linear ranges of five analytes were determined using the developed CEC method (Table 2). The data indicated good relationship between the investigated compound concentrations and their peak area ratios within the test ranges ($R^2 > 0.9993$). Their LODs and LOQs were less than 2.1 μg/mL and 8.3 μg/mL (Table 2), and the overall intra- and interday variations (RSDs) of the five analytes were less than 3.8% and 4.7%, respectively (Table 3). The repeatability was less than 3.3%, 3.2% and 2.6% for the three levels of test sample, respectively. The developed method also had good accuracy with overall recovery of 98.2–103.8% for the analytes (Table 4). The results showed that the developed CEC method was sensitive, precise and accurate for quantitative determination of five components including liquiritin, isoliquiritin, ononin, liquiritigenin and isoliquiritigenin in licorice.

3.5. Quantification of the investigated compounds in licorice

Typical electrochromatograms of the PLE extracts from different species and/or parts of licorice are shown in Fig. 4. The identification of investigated compounds was carried out by comparison of their migration time and UV spectra with those obtained by injecting standards in the same conditions, or by spiking the samples with stock standard solutions. The contents of five flavonoids in 25 samples of licorice were summarized in Table 5. The amounts of the investigated components obviously varied among different species of licorice, which may be derived from their different species, sampling parts, locations, collection times, and/or storage conditions. In general, the most abundant compound in underground parts of licorice was liquiritin, one of the markers for its quality control in Chinese Pharmacopoeia (2005 Edition). Moreover, the results showed that the aerial parts contained little or none of these flavonoids.

Table 3
Intra- and interday precision of the investigated compounds.

Analytes	Concentration (μg/mL)	Intra-day RSD (%, $n=6$)	Inter-day RSD (%, $n=6$)
Liquiritin	66.3	3.8	4.7
	265.0	1.8	2.1
	530.0	1.3	1.8
Isoliquiritin	28.3	2.7	4.7
	113.0	1.3	1.9
	226.0	1.2	1.9
Ononin	25.5	3.6	4.5
	102.0	1.0	2.4
	204.0	0.9	1.5
Liquiritigenin	26.8	3.5	4.2
	107.0	1.2	1.7
	214.0	1.6	0.9
Isoliquiritigenin	28.5	3.1	3.6
	114.0	1.3	2.0
	228.0	1.9	1.3

Table 4
Recoveries for the assay of five compounds in licorice.

Analytes	Original (μg)	Spiked (μg)	Found[a] (μg)	Recovery[b] (%)	RSD (%)
Liquiritin	2453.4	617.3	3071.7	100.2	2.1
Isoliquiritin	386.3	296.2	683.7	100.4	3.3
Ononin	69.9	103.4	171.5	98.2	2.1
Liquiritigenin	248.0	185.9	432.8	99.4	2.8
Isoliquiritigenin	51.0	130.5	186.5	103.8	3.1

[a] The data was present as average of three determinations.
[b] Recovery (%) = 100 × (amount found-original amount)/amount spiked.

3.6. Comparison of CEC with HPLC and CE for the analysis of flavonoids in licorice

HPLC and CE have been used for the analysis of flavonoids in licorice [10–14,16,17], but these five flavonoids were not determined simultaneously. In addition, the procedure of analysis covered sample preparation and separation, both should be considered carefully. Table 6 summarized the methods for analysis of flavonoids in licorice, which suggested that PLE combined with CEC

Table 2
Linear regression data, LOD and LOQ of the investigated compounds.

Analytes	Linear regression data			LOD (μg/mL)	LOQ (μg/mL)
	Regression equation	Test range (μg/mL)	R^2		
Liquiritin	$y = 3.34x - 0.01$	16.6–662.5	0.9998	2.1	8.3
Isoliquiritin	$y = 7.83x - 0.00$	7.1–282.5	0.9997	1.8	7.1
Ononin	$y = 7.17x - 0.01$	6.4–255.0	0.9997	1.6	6.4
Liquiritigenin	$y = 5.05x - 0.01$	6.7–267.5	0.9994	1.7	6.7
Isoliquiritigenin	$y = 12.87x - 0.01$	7.1–285.0	0.9993	1.8	7.1

Table 5
Contents (mg/g) of investigated compounds in licorice.

Samples	Analytes [mean (RD%[a], $n=2$)]				
	Liquiritin	Isoliquiritin	Ononin	Liquiritigenin	Isoliquiritigenin
GU-1	10.7 (0.8)	1.0 (0.6)	+[b]	1.8 (1.3)	+
GU-2	7.5 (1.8)	0.6 (1.8)	+	1.4 (2.5)	+
GU-3	13.0 (0.3)	1.5 (2.0)	0.4 (2.3)	2.6 (0.8)	0.3 (1.8)
GU-4	20.1 (0.4)	3.3 (0.1)	0.7 (2.5)	2.6 (0.2)	0.6 (4.1)
GU-5	18.2 (1.7)	4.6 (0.2)	0.9 (0.4)	3.5 (1.4)	1.2 (0.2)
GU-6	18.3 (2.5)	2.6 (0.5)	0.5 (0.4)	2.2 (2.7)	0.3 (0.6)
GU-7	35.1[c] (1.1)	8.2 (1.6)	1.2 (4.4)	1.9 (0.5)	0.6 (1.1)
GU-8	26.6 (1.2)	4.3 (0.6)	1.6 (1.5)	2.4 (3.4)	0.5 (3.1)
GU-9	5.2 (1.9)	0.9 (2.6)	+	1.6 (1.0)	+
GU-10	5.3 (4.4)	0.9 (2.8)	+	1.1 (3.2)	+
GU-11	5.1 (2.2)	0.8 (4.1)	–[d]	0.7 (1.0)	+
GU-12	4.6 (0.4)	0.5 (4.6)	0.4 (2.0)	1.1 (1.4)	+
GU-13	14.1 (1.5)	1.7 (0.7)	0.5 (0.8)	0.7 (3.9)	+
GU-14	5.3 (2.1)	0.6 (1.6)	0.3 (2.1)	1.0 (1.2)	0.3 (3.7)
GU-15	10.3 (2.1)	1.2 (0.7)	0.6 (3.6)	0.9 (1.2)	+
GU-16	4.1 (0.3)	0.7 (3.3)	0.3 (0.4)	1.0 (1.7)	+
GU-17	18.8 (1.3)	3.0 (3.0)	0.5 (1.5)	1.9 (1.5)	0.4 (3.1)
GU-18	14.9 (4.5)	2.5 (2.8)	0.2 (1.9)	1.8 (0.4)	0.4 (0.4)
GU-18A	–	+	–	+	–
GU-19A	–	–	–	–	–
GI-1	12.5 (2.3)	2.1 (3.1)	0.7 (2.9)	2.6 (0.4)	0.4 (2.7)
GI-2	2.1 (1.9)	0.3 (0.1)	+	1.0 (2.6)	0.4 (1.8)
GI-3	3.7 (2.5)	0.4 (0.9)	0.4 (0.7)	0.8 (0.8)	0.1 (3.9)
GI-4	6.4 (0.2)	0.9 (2.6)	0.5 (2.0)	1.0 (1.0)	+
GG-1	2.6 (0.1)	0.4 (1.7)	+	+	+

[a] RD, relative deviation (%) = (|measured value − mean|/mean) × 100.
[b] Under the limit of quantification.
[c] Determined after double dilution.
[d] Not detected.

Table 6
Summarized methods for the analysis of flavonoids in licorice.

Analytes	Sample preparation			Sample analysis			Total time (min)	Reference
	Method[a]	Time (min)	Solvent consumption (mL/g sample)	Method	Time[b] (min)	Solvent consumption (mL/run)		
Liquiritin, isoliquiritin, ononin, liquiritigenin, isoliquiritigenin	PLE	5	80	CEC	20	<3[c]	25	Current
Liquiritin, liquiritigenin, isoliquiritigenin and 4 triterpenoids	HE	360	25	HPLC	140	168	500	[10]
Liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin and 2 triterpenoids	IE, UE	60	53	HPLC	50	50	110	[11]
Rutin, liquiritin, ononin, calycosin, quercetin, isoliquiritigenin, formononetin, 2 triterpenoids and glycyrol	RE	180	200	HPLC	60	60	240	[12]
Liquiritigenin, isoliquiritigenin	UE	45	25	CE	8	<3	53	[13]
Isoliquiritigenin and 3 triterpenoids	RE	120	10	CE	15	<3	135	[14]
Glabridin, liquiritin, licochalcone A, liquiritin apioside and 2 triterpenoids	ND[d]	ND	200	CE	15	<3	ND	[16]
3,4′-Dimethoxy-5-hydroxychalone, formononetin, isoliquiritigenin and 2 triterpenoids	RE, HE	60	20	CE	9	<3	69	[17]

[a] HE, heat extraction; IE, immerse extraction; UE, ultrasonic extraction; RE, reflux extraction.
[b] According to the time of chromatogram in references.
[c] Estimated based on the buffer refreshed every two runs.
[d] No data.

analysis could be used as an alternative approach for determination of flavonoids in licorice.

4. Conclusions

A simple and reliable PLE and CEC method coupled with peak suppression technique was developed for the simultaneous quantitative determination of five flavonoids in licorice, which is helpful to control its quality.

Acknowledgements

This research was supported by grants from Macao Science and Technology Development Fund (049/2005/A-R1 to Y.T. Wang) and University of Macau (UL015/09-Y1 to S.P. Li).

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