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Analysis of Flavonoids in Propolis and *Ginkgo biloba* by Micellar Electrokinetic Capillary Chromatography

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A micellar electrokinetic capillary chromatography (MEKC) method has been developed for simultaneous determination of 10 bioactive flavonoids: rutin, apigenin, luteolin, eriodictyol, kaempferol, chrysin, acacetin, flavanone, flavone, and fisetin. The effect of several parameters, such as UV detection wavelength, buffer pH, buffer concentration, sodium dodecyl sulfate (SDS) concentration, β-cyclodextrin (β-CD) concentration, separation voltage, and injection time on the separation of these flavonoids were systematically investigated. The 10 flavonoids were successfully separated within 18 min in 20 mM $Na_2B_4O_7$−10 mM NaH_2PO_4 buffer (pH 9.7) containing 100 mM SDS and 16 mM β-CD at a separation voltage of 19 kV, with UV detection at 254 nm. Regression analysis revealed a good linear relationship between the peak area of each analyte and its concentration with detection limits (S/N = 3), ranging from 0.15 to 1.36 μg mL^{-1}. This method could simultaneously quantify the 10 flavonoids and be used in the quality control of functional foods containing propolis and *Ginkgo biloba*.

KEYWORDS: Flavonoids; MEKC; propolis; *Ginkgo biloba*

INTRODUCTION

Flavonoids exist abundantly in various vegetables, fruits, and medicinal plants and possess a wide range of biological activities, such as anti-inflammatory, anti-tumor, anti-allergic, anti-virus, anti-bacteria, and anti-oxidation (*1*). Qualitative and quantitative analyses of flavonoids are of considerable interest because these flavonoids constitute an important part of our daily diet.

Propolis and *Ginkgo biloba* have been used as ingredients in many functional foods. Propolis, also called bee glue, is a resinous hive product collected by honeybees from leaf buds and cracks in the bark of various plant sources; it is composed of 50% resin (flavonoids and related phenolic acids), 30% wax, 10% essential oils, 5% pollen, and 5% various organic compounds (*2*). Propolis has been reported to possess many beneficial biological activities, including being anti-bacterial, anti-oxidative, anti-inflammatory, anti-ulcer, anti-tumor, and hepatoprotective (*3*−*5*). For this reason, it is used extensively as a popular remedy in folk medicine and apitherapy as a constituent of "biocosmetics" and "health foods" and for numerous other purposes. *G. biloba* is a valuable tree, regarded as a "living fossil", which belongs to the gymnosperm family. Numerous research has shown that *G. biloba* extracts can reduce blood pressure, dilate peripheral blood vessels (*6, 7*), improve the viscoelasticity of blood vessels (*8*), and reduce oxidative stress in brain neurons (*9*). At present, propolis and *G. biloba* are popular health food products and available commercially all over the world. To ensure the reliability of pharmacological and clinical research, to understand their bioactivities, and to enhance product quality control, it is necessary to develop a simple, rapid, sensitive, and reliable method to quantify the active ingredients in propolis and *G. biloba* health foods.

Many methods, such as gas chromatography (GC) (*10*−*12*), high-performance liquid chromatography (HPLC) (*13*−*16*), and thin-layer chromatography (TLC) (*17, 18*), have been applied into analysis of propolis and *G. biloba* components. Among these methods, HPLC is still the most popular analytical technique. However, HPLC has some shortcomings, such as long analysis time, low resolution, and short life span of columns, owing to easy contamination. Capillary electrophoresis (CE) enjoys the advantage of rapidity, high separation efficiency, low cost, and the most important, long longevity and ease to elute from the capillary column. Therefore, CE is considered as an alternative technique in the analysis of propolis and *G. biloba*. The application of CE in propolis and *G. biloba* component analyses is rarely reported (*19*−*24*). Micellar electrokinetic capillary chromatography (MEKC) is the combination of capillary electrophoresis and chromatography. In MEKC, a surfactant is added into a buffer at a concentration above its critical micelle concentration to improve the selectivity of the separation. Micelles provide both ionic and hydrophobic sites

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Table 1. Comparison of Analytical Parameters for the Determination of Flavonoids

analytes	method	linear range (mg L^{-1})	detection limit (mg L^{-1})	separation time (min)	sample	reference
1 flavonoids:	MEKC			20	propolis oral liquids	*25*
hesperetin;		20−600	6			
2 phenolic acids:						
cinnamic acid		50−1200	17			
and nicotinic acid		100−1400	36			
4 flavonoids:	CZE			14	raw propolis and propolis capsules	*20*
rutin,		5−100	1.1			
apigenin,		2−500	1.1			
luteolin, and		5−100	0.37			
quercetin;		1−100	0.9			
2 phenolic acids:						
caffeic acid,		2−200	1.8			
and ferulic acid		5−200	0.9			
10 flavonoids:	mixed MEKC			20	raw propolis, *G. biloba* leaves, red wine, orange, peel, and pulp	*26*
catechin,		5−200	1.38			
epicatechin,		5−200	1.24			
naringenin,		5−200	2.25			
morin,		5−200	3.96			
fisetin,		5−200	2.54			
quercetin,		5−200	2.54			
kaempferol,		5−200	2.78			
galangin,		5−200	3.30			
apigenin, and		5−200	3.30			
chrysin		5−200	2.47			
6 flavonoids:	CZE			22	*G. biloba* leaves, *G. biloba* capsules, and *G. biloba* tablets	*22*
epicatechin,		1−100	0.2			
catechin,		1−200	0.17			
rutin,		1−100	0.26			
apigenin,		1−100	0.14			
luteolin, and		1−200	0.19			
quercetin		5−200	0.50			
10 flavonoids:	MEKC			18	raw propolis, five brands of propolis soft gels, *G. biloba* leaves, *G. biloba* tea, *G. biloba* tablets, and three brands of *G. biloba* soft gels	this paper
rutin,		4.5−100	1.36			
apigenin,		1.5−80	0.47			
luteolin,		0.6−80	0.15			
eriodictyol,		2.0−100	0.56			
kaempferol,		2.3−100	0.74			
chrysin,		1.0−80	0.33			
acacetin,		1.5−100	0.47			
flavanone,		3.5−80	1.05			
flavone, and		3.5−100	0.90			
fisetin		3.5−100	0.85			

of interaction; therefore, the separation of ionic and uncharged solutes can be obtained. Although some methods, such as CZE and MEKC (including mixed MEKC), have been developed on the characterization of flavonoids from propolis and *G. biloba* (**Table 1**), they either need long separation time (*22, 25*) or have a high limit of detection (*25, 26*), with a small number of flavonoids separated (*20, 22, 25*). Besides, none of these methods has focused on the simultaneous determination of flavonoids in different brands of functional foods made of propolis and *G. biloba* soft gels by CE.

The present study was to develop a simple, rapid, sensitive, and reliable MEKC method for the analysis of selected flavonoids in propolis and *G. biloba* health foods, including five brands of propolis soft gels, three brands of *G. biloba* soft gels, *G. biloba* tablets and tea.

MATERIALS AND METHODS

Reagents and Materials. Acacetin, luteolin, flavone, eriodictyol, fisetin, and kaempferol were purchased from Fluka (Buchs, Switzerland), whereas chrysin, flavanone, rutin, and apigenin were obtained from Sigma (St. Louis, MO). The structures of the flavonoids were presented in **Figure 1**. Sodium dodecyl sulfate (SDS) was obtained from Beijing Xizhong Chemical Factory (Beijing, China). β-Cyclodextrin (β-CD) was purchased from Beijing AoBoXing Biotech Co. (Beijing, China). Sodium hydroxide, disodium tetraborate, and sodium dihydrogen phosphate were obtained from Tianjing Chemical Reagent Factory (Tianjing, China). Raw propolis and *G. biloba* leaves were purchased from Xinhua Durgstore in Tianjin (Tianjin, China). Five brands of propolis soft gels were Beijian propolis (Dongguan GuangFa Pharmaceutical Co., Ltd., Guangzhou, China), Heshi propolis (Shantou Sirio Pharmacy Co., Ltd., Shantou, China), Jin'aoli propolis (Unisplendour Group Technology Development Co., Ltd., Weihai, China), Wanshiji propolis (Hangzhou Tianchu Miyuan Health Food Co., Ltd., Hangzhou, China), and Tongrentang propolis (Beijing Tongrentang Group Co., Ltd., Beijing, China). *G. biloba* tablets and Tianfang *G. biloba* tea were obtained from Jiangsu Yangzi River Pharmaceutical Co. Ltd. (Taizhou, China) and Anhui Tianfang Tea Industry (Group) Company (Anhui, China), respectively. Three brands of *G. biloba* soft gels were Jiankangzaixian *G. biloba* (Guangdong B&H Health-care Products Co. Ltd., Guandong, China), Heshi *G. biloba* (Shantou Sirio Pharmacy Co., Ltd., Shantou, China), and Huana *G. biloba* (Beijing Wanbang Huana Health Food Co., Ltd., Beijing, China). All reagents used were of at least analytical grade. Deionized water was used throughout. All solutions and samples were filtered through a 0.22 μm syringe filter.

Standard stock solutions of 10 flavonoids at a concentration of 1000 μg mL^{-1} were prepared in methanol, and various concentrations of the work solutions were prepared by appropriate dilution from the stock solution when needed. The pH of the running buffer was adjusted by the addition of HCl or NaOH solution.

Sample Preparation. Propolis soft gels (0.38 g for Beijian, 0.41 g for Heshi, 0.46 g for Jin'aoli, 0.26 g for Wanshiji, and 0.40 g for Tongrentang; one soft gel without the soft gel coat) or 4 g of raw propolis were accurately weighed and extracted with 15 mL of methanol

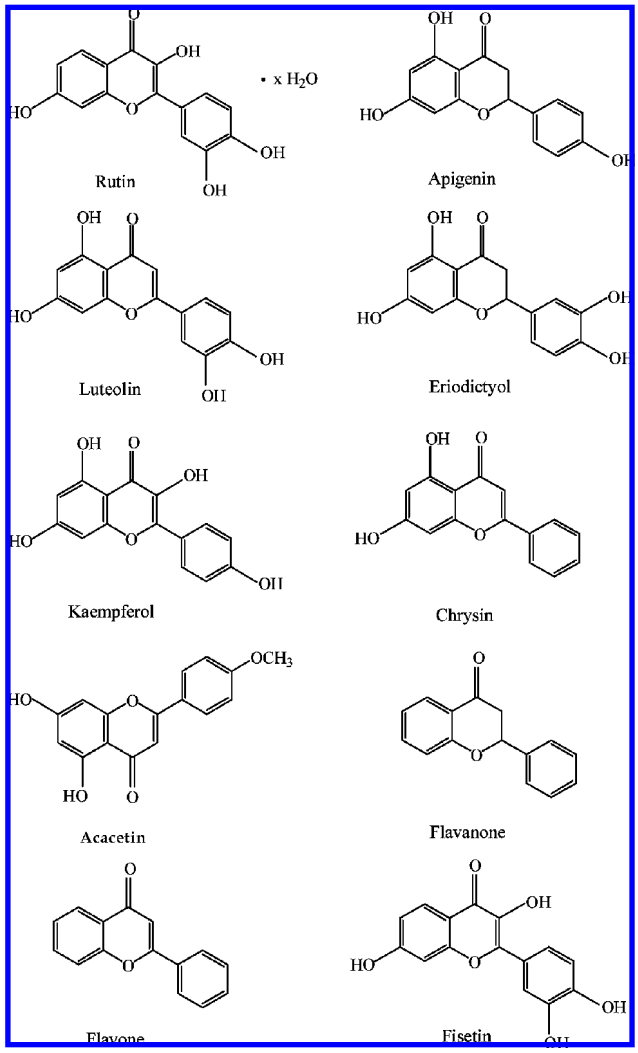

Figure 1. Molecular structures of 10 bioactive flavonoids.

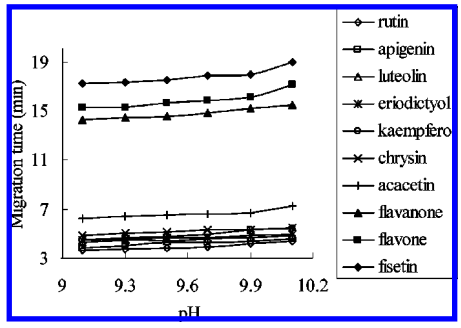

Figure 2. Effect of buffer pH on the migration time of the analytes (5 mg L^{-1} each). Conditions: fused capillary, 75 μm inner diameter × 37 cm length (30 cm effective); running buffer, 20 mM $Na_2B_4O_7$−10 mM NaH_2PO_4 containing 100 mM SDS and 16 mM β-CD; injection time, 10 s at 0.5 psi; separation voltage, 19 kV; wavelength of UV detector, 254 nm.

for 10 min in an ultrasonic bath. After centrifugation, the resultant supernatant solution was moved into a volumetric flask of 50 mL. The extraction procedure was repeated 3 times. The combined extracts were diluted with methanol to 50 mL and then diluted with the running buffer (3-fold dilution for Beijian, Heshi, and Tongrentang, 7-fold dilution for Jin'aoli, and 6-fold dilution for Wanshiji), with the exception of raw propolis extract.

G. biloba soft gels (0.40 g for Jiankangzaixian, 0.36 g for Heshi, and 0.76 g for Huana; one soft gel without the soft gel coat) were sonicated with 10 mL of methanol for 30 min and then diluted with the running buffer (5-fold dilution).

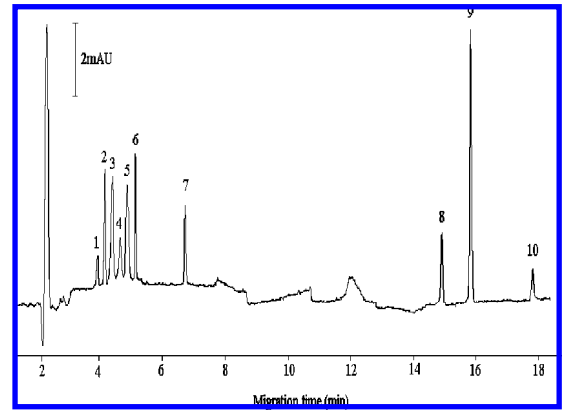

Figure 3. Typical electropherogram of a standard mixture of flavonoids under the optimized conditions. Peak identification: 1, rutin; 2, apigenin; 3, luteolin; 4, eriodictyol; 5, kaempferol; 6, chrysin; 7, acacetin; 8, flavanone; 9, flavone; and 10, fisetin.

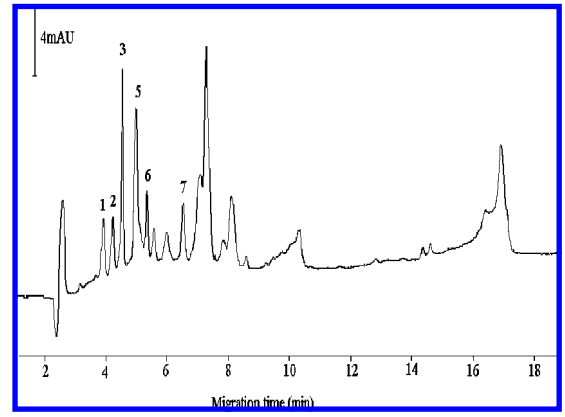

Figure 4. Typical electropherogram of raw propolis under the optimized conditions. Peak identification is the same as in **Figure 3**.

Dried *G. biloba* leaves (2 g), *G. biloba* tea (approximately 2 g), or *G. biloba* tablets (0.50 g; three tablets) were first ground to a powder in a mortar, then sonicated in 5 mL of methanol for 30 min, and diluted with the running buffer (5 mL). Sample solutions were initially passed through a filter paper and then through a 0.22 μm syringe filter prior to injection.

MEKC Analysis of Flavonoids. MEKC analysis of flavonoids was carried out on a Beckman P/ACE MDQ capillary electrophoresis system from Beckman (Beckman, Fullerton, CA), equipped with a diode array detector. The scan range was 190−400 nm, and all of the electropherograms were recorded at 254 nm. A software for data acquisition and treatment (32 Karat Software, version 7.0) was used for peak integration and data analysis. The temperature of the capillary cartridge was maintained at 25 °C. The running buffer was 20 mM $Na_2B_4O_7$−10 mM NaH_2PO_4 (pH 9.7) containing 100 mM SDS and 16 mM β-CD. Samples were introduced by pressurized injection at 0.5 psi for 10 s, and the separation voltage was 19 kV. The column was an untreated fused-silica capillary, with a total length of 37 cm and an effective length of 30 cm (75 μm inner diameter) (Yongnian Optical Fiber Factory, Hebei, China). Before use, the capillary was conditioned by flushing with 0.1 M NaOH for 5 min, water for 2 min, and finally, with the running buffer for 5 min. A good reproducibility of the migration time was obtained by flushing the capillary between runs as follows: 2 min with 0.1 M NaOH, 2 min with water, and 2 min with running buffer.

RESULTS AND DISCUSSION

Selection of UV Detection Wavelength. Using a diode array detector, UV spectra of the flavonoids were obtained. On the basis of the spectra, the UV detection was investigated at 254, 280, and 335 nm for the flavonoids. The measurement at 254

Table 2. Analytical Characteristic Data of the Developed MEKC for the Determination of 10 Flavonoids

analytes	migration time (min)	RSD (%) ($n = 3$) migration time	RSD (%) ($n = 3$) peak area	regression equation ($n = 3$) $y = a + bx$[a]	correlation coefficient	linear range (mg L^{-1})	detection limit (mg L^{-1})
rutin	3.94	1.27	1.96	$y = (1270.1 \pm 3.2)x - (1151.6 \pm 415.8)$	0.9994	4.5−100	1.36
apigenin	4.26	0.73	0.97	$y = (2925.5 \pm 6.1)x - (1342.4 \pm 426.1)$	0.9991	1.5−80	0.47
luteolin	4.53	0.96	1.54	$y = (3980.6 \pm 4.8)x + (1156 \pm 271.0)$	0.9985	0.6−80	0.15
eriodictyol	4.71	1.38	2.21	$y = (1802.3 \pm 16.6)x - (472.6 \pm 163.7)$	0.9964	2.0−100	0.56
kaempferol	4.92	0.65	2.79	$y = (5631.8 \pm 54.1)x - (3950.1 \pm 2409.6)$	0.9954	2.3−100	0.74
chrysin	5.27	1.13	0.93	$y = (3626.6 \pm 5.1)x - (1011.7 \pm 236.3)$	0.9994	1.0−80	0.33
acacetin	6.59	0.90	3.30	$y = (1894.7 \pm 4.4)x + (354.2 \pm 380.8)$	0.9990	1.5−100	0.47
flavanone	14.82	1.79	2.82	$y = (5302.2 \pm 12.7)x - (5502.8 \pm 3803.0)$	0.9989	3.5−80	1.05
flavone	15.81	1.88	2.65	$y = (12194 \pm 100.0)x - (10918 \pm 565.6)$	0.9976	3.5−100	0.90
fisetin	17.85	1.56	3.58	$y = (1614.8 \pm 4.7)x - (1320.6 \pm 734.8)$	0.9992	3.5−100	0.85

[a] y and x stand for the peak area and the concentration (mg L^{-1}) of the analytes, respectively. $\bar{x} \pm$ SD ($n = 3$).

Table 3. Analytical Results for the Determination of Flavonoids in Raw Propolis and Five Brands of Propolis Soft Gels ($n = 3$)

	content (μg g^{-1})[a]					
analytes	raw propolis	Bejian	Heshi	Jin'aoli	Wanshiji	Tongrentang
rutin	177 ± 7	1859 ± 46	2620 ± 66	6558 ± 197	5749 ± 161	3677 ± 114
apigenin	52.2 ± 2.3	1364 ± 27	2461 ± 81	7674 ± 199	5574 ± 167	4737 ± 137
luteolin	198 ± 7	2864 ± 80	2811 ± 82	5093 ± 178	1391 ± 38	581 ± 15
eriodictyol	nd[b]					
kaempferol	128 ± 4	1604 ± 40	1980 ± 55		2940 ± 94	
chrysin	78.1 ± 1.8	1646 ± 61	1398 ± 34	4407 ± 123	4669 ± 163	2313 ± 79
acacetin	167 ± 7					3541 ± 99
flavanone						
flavone						
fisetin						

[a] $\bar{x} \pm$ SD ($n = 3$). [b] Not detected.

Table 4. Recoveries of Flavonoids in Beijian Propolis Soft Gels ($n = 3$)

analytes	original amount (μg g^{-1})	added amount (μg g^{-1})	found (μg g^{-1})	recovery (%)	RSD (%)
rutin	1859	2000	3715	92.8	2.6
apigenin	1364	2000	3424	103.0	2.1
luteolin	2864	2000	4832	98.4	2.1
eriodictyol	nd[a]	2000	1896	94.8	3.7
kaempferol	1604	2000	3468	93.2	2.3
chrysin	1646	2000	3517	93.6	2.9
acacetin	nd	2000	1860	93.0	2.1
flavanone	nd	2000	1940	97.0	3.5
flavone	nd	2000	2040	102.0	3.2
fisetin	nd	2000	2024	101.2	3.8

[a] Not detected.

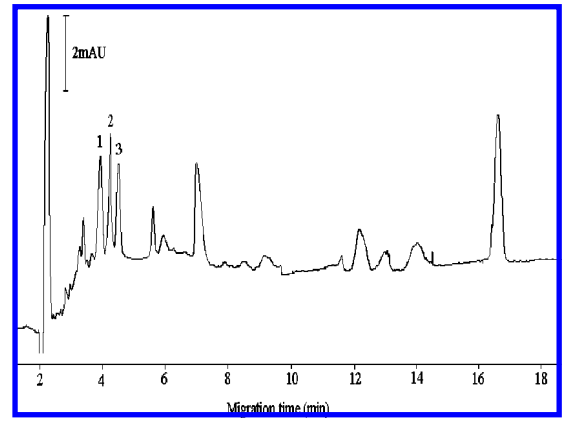

Figure 5. Typical electropherogram of *G. biloba* leaves under the optimized conditions. Peak identification is the same as in **Figure 3**.

nm yielded the best signal and a more stable background, as well as the best separation of the flavonoids. Thus, 254 nm was chosen as the optimum UV detection wavelength throughout the experiment.

Effect of Buffer pH. The buffer pH can affect the ζ potential, electro-osmotic flow (EOF), and the charge status of the analytes, which influence the separation selectivity and resolution. Under the conditions of 20 mM $Na_2B_4O_7$−10 mM NaH_2PO_4 with 100 mM SDS and 16 mM β-CD, with a series of buffer solutions at pH from 9.1 to 10.1, the effect of buffer pH on the separation was explored. As shown in **Figure 2**, the migration times were increasing with the increase of buffer pH; therefore, the apparent mobilities (μ_{app}) of flavonoids were decreasing, which were calculated from the following equation:

$$\mu_{app} = \frac{LL_{tot}}{V_t} \quad (1)$$

where L_{tot} is the total length of capillary, L is the length from the inlet end to the detector, V is the applied voltage, and t is the migration time of the analyte. As it is well-known, the mobility of the EOF (μ_{eof}) is increasing with the increase of buffer pH. In accordance with eq 2, negative effective mobility ($-\mu_{eff}$) was increasing with buffer pH. It was mainly caused by the enhancement of the ionization of flavonoids with increasing buffer pH. We chose pH 9.7 as the optimum pH because good resolution and short analysis time were obtained.

$$\mu_{eff} = \mu_{app} - \mu_{eof} \quad (2)$$

Effect of the Buffer Concentration. Besides the pH value, the buffer concentration is also an important parameter. The experiments indicated that the migration time and resolution of the analytes increased with an increasing concentration of the buffer, because the increase in ionic strength reduced the EOF. In addition, with an increasing buffer concentration, the complex formation reaction between borate and the natural products became stronger and stronger. However, the high buffer

Table 5. Analytical Results for the Determination of Flavonoids in the *G. biloba* Leaves, Soft Gels, Tablets, and Tea ($n = 3$)

sample	analytes	content (μg g^{-1})[a]
G. biloba leaves	rutin	113 ± 2
	apigenin	68.3 ± 1.2
	luteolin	22.1 ± 0.9
Jiankangzaixian *G. biloba* soft gels	rutin	1177 ± 29
Heshi *G. biloba* soft gels	rutin	927 ± 23
	apigenin	1423 ± 38
	luteolin	1188 ± 27
Huana *G. biloba* soft gels	rutin	1056 ± 23
	apigenin	1395 ± 36
	luteolin	402 ± 8
G. biloba tablets	rutin	2137 ± 55
	apigenin	101 ± 3
	luteolin	27.5 ± 1.2
Tianfang *G. biloba* tea	rutin	205 ± 6
	apigenin	126 ± 3
	luteolin	29.3 ± 0.9

[a] $\bar{x} \pm$ SD ($n = 3$).

Table 6. Recoveries of Flavonoids in *G. biloba* Leaves ($n = 3$)

analytes	original amount (μg g^{-1})	added amount (μg g^{-1})	found (μg g^{-1})	recovery (%)	RSD (%)
rutin	113	100	206	93.0	2.8
apigenin	68.3	50	119	101.4	3.5
luteolin	22.1	25	46.7	98.4	1.9
eriodictyol	nd[a]	25	23.5	94.0	2.2
kaempferol		25	22.8	91.2	2.9
chrysin		25	23.2	92.8	3.4
acacetin		25	23.8	95.2	3.0
flavanone		25	25.4	101.6	2.5
flavone		25	25.5	102.0	2.2
fisetin		25	25.2	100.8	2.4

[a] Not detected.

concentration made the Joule heating effect obvious, which had a negative effect on the detection limits. Consequently, 20 mM $Na_2B_4O_7$−10 mM NaH_2PO_4 buffer was employed for subsequent optimization, considering the resolution, analysis time, and sensitivity.

Effect of the SDS Concentration. On the basis of the linear solvation energy relationship (LSER), the surfactant concentration could influence the retention behavior of uncharged solutes by changing the phase ratio without affecting selectivity significantly (*27*−*29*). Generally, the increase of the surfactant concentration would lead to an increased migration time. An increase in the migration time and resolution of the 10 analytes was observed when the SDS concentration in the electrophoretic solution increased. The reason for the increase may be that at higher SDS concentrations the phase ratio of the micelle to the aqueous phase would be larger and the probability of the solubilization of the constitutents by the micelles would be higher, resulting in an increase in the migration time for these compounds. Good separation for the 10 flavonoids was found when the SDS concentration was higher than 100 mM, whereas a further increase of the SDS concentration resulted in a longer analysis time. Therefore, 100 mM was chosen as the optimum SDS concentration.

Effect of the β-CD Concentration. Adding SDS into the 20 mM $Na_2B_4O_7$−10 mM NaH_2PO_4 buffer at pH 9.7 improved the resolution, but it was still not satisfactory. Therefore, β-CD was investigated as "hosts" for separation of the analytes by the "guest−host" principle and could meet the demand. The effect of the β-CD concentration was evaluated over the range 0−22 mM. The results suggest that, as the β-CD concentration increased from 0 to 16 mM, the migration times of the analytes decreased slightly and the resolution was improved, which was usually attributable to the ability of β-CD to include selectively a wide variety of guest molecules in its hydrophobic cavity. When the concentration was higher than 16 mM, the migration times were hardly changeable and the resolution decreased slightly. Therefore, 16 mM β-CD was selected for the next experiments.

Effects of the Separation Voltage and Injection Time. The separation voltage affects not only the electric field strength, which in turn affects the EOF, but also the migration velocity of charged particles, which determine the migration time of analytes. Moreover, a higher separation voltage may result in a higher Joule heating. The effect of the separation voltage on the migration time of the analytes was investigated, and the results illustrated that the increase of the separation voltage gave shorter migration times for all compounds but also increased the baseline noise, resulting in poorer detection limits. It was found that higher separation voltages were not beneficial to the resolution of all analytes. However, separation voltages that were too low would increase the analysis time considerably, which in turn would cause peak broadening. Therefore, 19 kV was chosen for further optimization, at which good separation can be obtained for all analytes within 18 min.

Injection time determines the amount of sample and affects both the peak current and peak shape. The effect of injection time was explored by changing the injection time from 2 to 12 s at 0.5 psi. It was found that both the peak current and peak width increased with the injection time ranging from 2 to 10 s. When the injection time was longer than 10 s, the peak current almost leveled off but peak broadening became more severe. In this experiment, 10 s was selected as the optimum injection time.

From the above results, the optimum conditions were 20 mM $Na_2B_4O_7$−10 mM NaH_2PO_4 containing 100 mM SDS and 16 mM β-CD (pH 9.7) as buffer solution, 19 kV as separation voltage, and 10 s (at 0.5 psi) as injection time. Under the optimum conditions, 10 analytes could be well-separated, and the typical electropherogram of a standard mixture solution was shown in **Figure 3**.

Analytical Performance Characteristics. A series of standard mixture solutions of the 10 analytes were tested to determine the linear relationships between the concentrations of the 10 analytes and the corresponding peak areas. The regression equations, linear ranges, and detection limits were summarized in **Table 2**. The calibration curves exhibited excellent linear behavior between the peak area of each compound and its concentration, with detection limits (S/N = 3) ranging from 0.15 to 1.36 μg mL^{-1} for all analytes.

The reproducibility of the MEKC analysis was determined by performing repetitive analysis of a standard mixture solution under the optimum conditions. The relative standard deviations (RSDs) of the migration times and the peak areas were calculated, and the results were listed in **Table 2**. All of the RSDs of the migration times and the peak areas were <2 and <4%, respectively, which demonstrated that this method was of good repeatability.

Analysis of Propolis Samples. The established MEKC method was used to analyze the flavonoids in raw propolis and five brands of propolis soft gels (Beijian, Heshi, Jin'aoli,

Tongrentang, and Wanshiji). All of the common flavonoids were successfully separated on top of the complex sample matrix, and peaks were identified by spiking standard substances of the analytes (**Figure 4**). Contents of flavonoids in the analyzed propolis samples were given in **Table 3**. The results revealed no significant differences with the distribution of flavonoids between raw propolis and the five brands of propolis soft gels. It was found that rutin, apigenin, luteolin, and chrysin co-existed in the raw propolis and the five brands of propolis soft gels, and the contents of rutin, apigenin, and luteolin in the five brands were overall highest in Jin'aoli propolis soft gels. It was also observed that eriodictyol, flavanone, flavone, and fisetin were not found in raw propolis and five brands of propolis soft gels. Meanwhile, kaempferol could not be detected in Jin'aoli and Tongrentang under the current conditions, while acacetin was only detected in Tongrentang, which may be a deficiency of the extraction processing or because of different raw propolis. Recovery was determined by the standard addition method, and the results obtained from Bejian propolis soft gels sample ranged from 92.8 to 103.0% (**Table 4**). The assay results indicated that this method was accurate, sensitive, and reproducible, providing a useful quantitative method for the analysis of flavonoids in raw propolis and its health foods.

Analysis of *G. biloba* Samples. A total of 10 flavonoids in *G. biloba* leaves, tablets, and tea and three brands of *G. biloba* soft gels (Jiankangzaixian, Heshi, and Huana) were also determined by MEKC under the optimum conditions. The typical electropherogram obtained from *G. biloba* leaves was shown in **Figure 5**. Rutin, apigenin, and luteolin were simultaneously found in the *G. biloba* leaves, tea, and tablets and Heshi and Huana *G. biloba* soft gels. Only rutin was found in Jiankangzaixian *G. biloba* soft gels, and we wondered whether the compositions of *G. biloba* leaves used to produce soft gels varied or rutin was added in soft gels during the production process. The assay results were listed in **Table 5**. Recovery was also determined by standard addition, and the results were satisfactory (**Table 6**).

In conclusion, we developed a simple, rapid, sensitive, and reliable method to quantify the flavonoids present in propolis, *G. biloba*, and their related health foods. The present MEKC method can provide simultaneously a detailed analysis of 10 flavonoids and may have an important application in the quality control of these health foods.

ABBREVIATIONS USED

β-CD, β-cyclodextrin; CE, capillary electrophoresis; EOF, electro-osmotic flow; GC, gas chromatography; HPLC, high-performance liquid chromatography; LSER, linear solvation energy relationship; MEKC, micellar electrokinetic capillary chromatography; RSD, relative standard deviation; SDS, sodium dodecyl sulfate; TLC, thin-layer chromatography.

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