Journal of Food and Drug Analysis, Vol. 13, No. 4, 2005, Pages 324-330 藥物食品分析 第十三卷 第四期

Determination of Seven Pueraria Constituents by High Performance Liquid Chromatography and Capillary Electrophoresis

CHING-CHE LIN[1], MING-HSING HUANG[2] AND SHUENN-JYI SHEU[1]*

[1.] Department of Chemistry, National Taiwan Normal University, 88, Sec. 4, Tingchow Rd., Taipei City 116, Taiwan, R.O.C
[2.] Department of Applied Life Science and Health, Chia Nan University of Pharmacy and Science, 60, Erh-Jen Rd., Sec.1, Jan-Te Township, Tainan County 717, Taiwan, R.O.C

(Received: March 2, 2005; Accepted: August 9, 2005)

ABSTRACT

Puerariae Radix is a commonly used Chinese herbal medicine derived from the dried root of the legume plant, which contains a series of isoflavones as its chief pharmacologically active constituents. Using 7 pueraria constituents as marker substances, a high performance liquid chromatography (HPLC) and a capillary electrophoresis (CE) method were developed to assay the quality of Puerariae Radix within 65 and 50 min, respectively. Extracted samples were analyzed with a Cosmosil 5C18-MS column and eluted with a mixture of potassium dihydrogenphosphate (30 mM, adjusted to pH 3.0 with phosphoric acid) and aqueous acetonitrile solution, or an uncoated capillary (75 μm I.D.) and carried with an aqueous solution containing 10 mM sodium dihydrogenphosphate and 40 mM sodium dodecyl sulphate. The relative standard deviation of the marker substances, on the basis of peak-area ratios for 6 replicate injections, was 0.50~0.97% (intraday) and 0.48~0.97% (interday) for HPLC, and 0.88~1.25% (intraday) and 0.85~1.38% (interday) for CE. Both methods yielded accurate results differing by only 5% when applied to the analysis of practical samples of Puerariae Radix.

Key words: pueraria constituents, high performance liquid chromatography (HPLC), capillary electrophoresis (CE)

INTRODUCTION

Puerariae Radix is a commonly seen Chinese herbal medicine derived from the dried root of the legume plant *Pueraria thomsonii* Benth and *P. lobata* Ohwi[(1,2)]. Its biologically active compounds included isoflavones such as puerarin, daidzin, daidzein, 7-xylosidepuerarin, 3'-hydroxypuerarin, 3'-methoxypuerarin, formononetin and genistein[(1,3-8)]. In addition, pueraria also contains puerosides A and B, 7 saponin alcohols, allantoin, 6,7-dimethoxycou marin[(9)], 5-methylhydrantoin, tuberosin, choline chloride, acetylcholine chloride, D-mannitol, succinic acid, behenic acid, carnaubic acid and glycerol-1-monotetracosanoate[(1)], etc. This study examined 7 major constituents , puerarin **(1)**, daidzin **(2)**, 6,7-dimethoxycoumarin **(3)**, daidzein **(4)**, genistein **(5)**, formononetin **(6)** and biochanin A **(7)**, as shown in Figure 1.

HPLC and CE are presently most commonly used methods for assaying constituents in drug materials and herb formulas. Although several HPLC and CE methods[(10-17)] have been established for analyzing the constituents of pueraria, none of these methods is entirely adequate, as their resolution is limited to 5 isoflavones. Moreover, the peak width in some of the chromatograms or electrophergrams was very broad, and the theoretical plate numbers were very low.

We describe here the development of direct and rapid method for determining 1 coumarin and 6 isoflavone

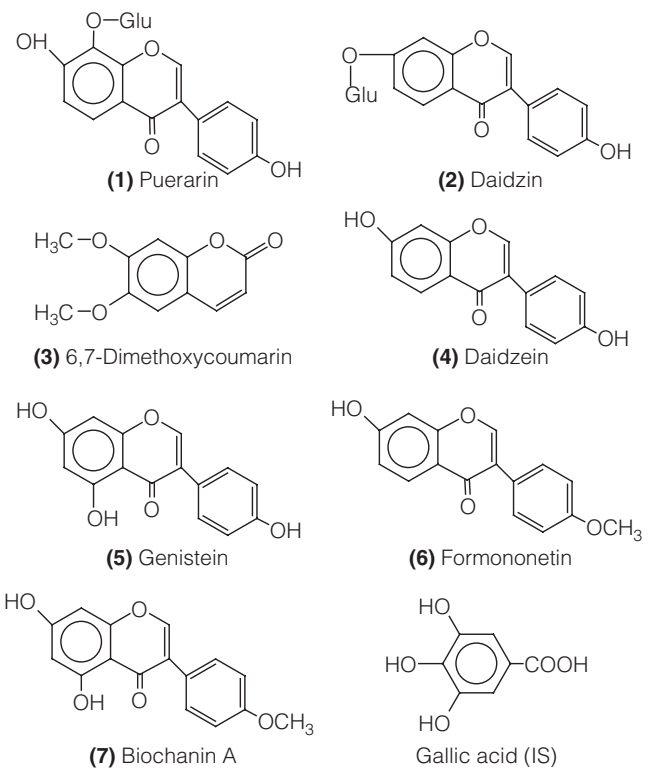

Figure 1. Structures of pueraria constituents.

* Author for correspondence. Tel: +886-2-29350749 ext. 405; Fax: +886-2-29324249; E-mail: chefv002@scc.ntnu.edu.tw

Journal of Food and Drug Analysis, Vol. 13, No. 4, 2005

constituents in pueraria extracts within 65 min by HPLC and within 50 min by CE. The suitabilities of these two methods were compared and discussed. Either method can be applied to assay the herbal drug extract fluids directly.

MATERIALS AND METHODS

I. *Reagents and Materials*

Daidzein, daidzin, puerarin, genistein, 6,7-dimethoxycoumarin and biochanin A were purchased from Sigma (St. Louis, MO, USA). Formononetin was from Extrasynthese (Genay, France). Potassium dihydrogenphosphate, sodium hydroxide and gallic acid were obtained from Acros (NJ, USA), where as tetra-*n*-heptylammonium bromide, phosphoric acid was from Merck (Dermstadt, Germany). Sodium dodecyl sulphate (SDS) was purchased from Aldrich (Milwaukee, WI, USA). Deionized water was produced through a Milli-Q system (Millipore, Bedford, MA, USA). A LC grade methanol and acetonitrile were from Fison (Loughborough, England).

Pueraria samples obtained from Brion Research Institute of Taiwan were identified by external appearance and pharmacognostic histological anatomy and were found to be *Pueraria thomsonii* Benth.

II. *Prepration of Puerariae Radix Extracts*

One gram sample of pulverized Puerariae Radix was extracted with 70% methanol (20 mL) by stirring at room temperature for 30 min and then centrifuged at 1500 ×g for 5 min. Extraction was repeated 3 times and the extracts were combined. After adding 10 mL of internal standard (IS, 100 mg gallic acid in 100 mL of 70% MeOH), the pueraria extract was diluted to 100 mL with 70% methanol. This solution was filtered through a 0.45 μm PVDF-filter before the filtrate was injected into the HPLC (10 μL) or CE system (8.5 nL).

III. *Calibration Curve*

For each of the 7 components, stock solutions were prepared by dissolving 5~50 mg of marker substances (puerarin 50 mg, daidzin 25 mg, 6,7-dimethoxycoumarin 5 mg, daidzein 25 mg, genistein 5 mg, formononetin 5 mg and biochanin A 5 mg) in 50 mL of 70% methanol. Stock solutions (0.1, 0.5, 1, 2.5, 5, 8 mL) and 1 mL of IS were added into flask and diluted to 10 mL (dilution factor: 1.25, 2, 4, 10, 20 and 100). The linearity of the plot of peak-area ratio (y) vs. concentration (x, μg/mL) for each of the marker substances in CE and HPLC was investigated.

IV. *Instruments and Analytical Conditions*

(I) *HPLC System*

The HPLC system was consisted of two Waters 510 pumps, a Waters 680 automated gradient controller (Waters, USA) and a SPD-M10AVP photodiode array detector set at 254 nm (Shimadzu, Japan). The separations were achieved with a 10-μL loop, a reversed phase column (Cosmosil 5C18-MS, 5 μm, 25 cm × 4.6 mm I.D; Nacalai Tesque, Kyoto, Japan) and by linear gradient elution using eluents A

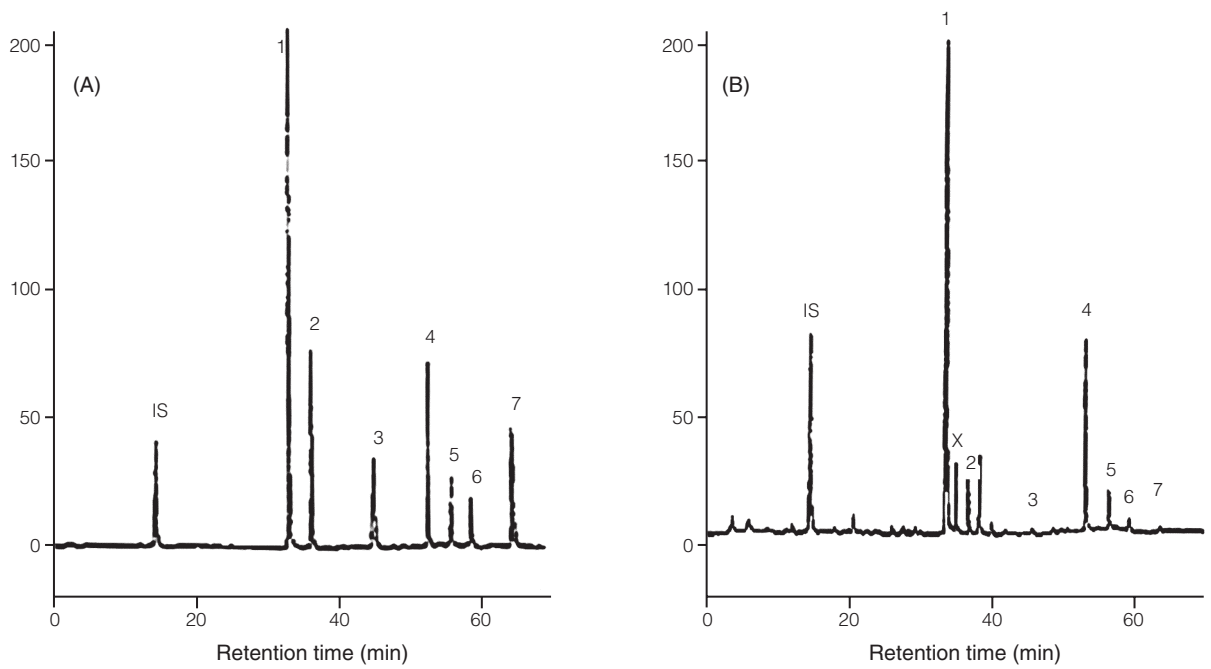

Figure 2. HPLC chromatograms of (A) 7 authentic standards, and (B) pueraria extract fluid. IS: internal standard (gallic acid); other symbols as in Figure 1.

and B [A = 30 mM KH_2PO_4 buffer solution (adjusted to pH 3.0 with H_3PO_4); B = CH_3CN/H_2O (65:35, v/v)] according to the following A-B profile: 0~5 min, 100~90% A; 5~10 min, 90~80% A; 10~20 min, 80~65% A; 20~25 min, 65~50% A; 25~30 min, 50~60% A; 30~40 min, 60~50% A; 40~50 min, 50~0% A; 50~70 min, 0% A. The flow-rate was kept at 1 mL/min. A precolumn of μ-Bondapak^{TM}C18 (Millipore, Milford, MA, USA) was attached to protect the analytical column.

(II) *CE System*

All analyses were carried out on a Waters Quanta 4000CE system equipped with an UV detector set at 254 nm and 90 cm, 75 μm I.D. uncoated capillary (Polymicro Technologies, Phoenix, AZ, USA) with the detection window placed at 82.4 cm. The conditions were as follows: sampling time, 5 sec hydrostatic (injection volume, 8.5 nL); running time, 50 min; voltage, 20 kV (constant voltage, positive to negative polarity); and temperature, 25.0~26.0°C. The electrolyte was a pH 6.0 buffer solution that contained 10 mM sodium dihydrogenphosphate and 40 mM SDS.

V. *Suitability Evaluation*

To prepare the test solution, the standard stock solution (2 mL) and IS (1 mL) were spiked into a 10-mL volumetric flask and 70% methanol was then added to the volume. Intraday and interday analyses were performed separately 6 times using the optimum HPLC or CE condition.

Series of dilutions were injected to HPLC or CE and the detection limit was determined based on the signal to noise ratio (S/N ratio) of 3.

RESULTS AND DISCUSSION

I. *HPLC Analysis*

Through a series of experiments and by using different ODS columns and adjusting the salts of eluents, the contents of organic solvents and the solution pH, we developed a method that can successfully assay 7 pueraria constituents within 65 min with chromatograms shown in Figure 2. The 7 peaks of marker substances have been confirmed by comparing their UV spectra with standards, and by spiking standards with sample.

With 10 mM phosphate and aqueous methanol/acetonitrile solution as mobile phase, 6 columns, Cosmosil 5C18, Cosmosil 5C18-AR, Cosmosil 5C18-ARII, Cosmosil 5C18-MS, Lichrosorb RP-18 and Intersil ODS-2 were tested. The calculated theoretical plate numbers of the peaks were found to differ slightly and not magnificently. Compound 1 showed partially overlapped with an adjacent unknown peak in all columns. Except column 5C18-MS, it presents a higher resolution for compound 1 and so was chosen in this study.

Table 1. Theoretical plate numbers (× 10^4) of the constituents at different phosphate concentrations

	Theoretical plate number						
(mM)	**1**[a]	**2**	**3**	**4**	**5**	**6**	**7**
0	1.41	0.26	0.28	0.22	0.35	0.38	0.38
10	1.81	0.92	0.30	0.25	0.38	0.38	0.42
20	4.04	1.56	0.34	0.24	0.40	0.43	0.44
30	6.24	3.98	0.43	0.25	0.45	0.45	0.48
40	6.35	3.88	0.42	0.26	0.47	0.48	0.48
50	6.27	3.65	0.44	0.25	0.50	0.49	0.46

[a]Compounds symbols see Figure 1.

The relationship between phosphate concentration and separation was that as in salt concentration increased, K' showed a tendency of slow but not marked increase, while the theoretical plate numbers differed drastically. Compounds 1 and 2 that contained glycoside groups were affected the most by salt concentration. At 30 mM, the theoretical plate number was 5 times than that of the salt-free solution, as shown in Table 1. All concentrations higher than 30 mM, could achieve very good separation. In view of the low solubility of salts in organic solvents and the column clogging or damaging effect caused by higher salt concentrations to reduce separation effect, we chose to use 30 mM as the best operating concentration.

With the KH_2PO_4 (pH 4.6) concentration fixed at 30 mM, different amounts of phosphoric acid were added to make the mobile at different pH within the range of 2.0~7.0. As the pH value is greater than 5.0, some unknown compounds with acidic moieties in the drug material may deprotonate and cause the baselines of pueraria chromatograms to drift upward in the front portion (in the first 25 min section of retention time), thereby interfering with the assay accuracy. Experimental results show good separation effect at pH 3.0 and 4.0, with pH 3.0 yielding higher theoretical plate numbers (N values for **4**: pH 3.0, 89587; pH 4.0, 67586). Therefore, the optimal result is to select 30 mM KH_2PO_4 for the mobile phase (A) and adjust it to pH 3.0 with 5% phosphoric acid.

Owing to the presence of salts in mobile phase A and to avoid salting out and baseline drift, mobile phase B was fixed at 40% water. As the organic portion in mobile phase B was purely methanol, the retention time was longer, resulting in severe overlapping of **1** with adjacent peak **X**. If it was purely acetonitrile, the retention could be shortened though. **1** and adjacent peak **X** were still found to have partial overlap. Hence, the volume ratios of methanol/acetonitrile/water were made as 0/60/40, 15/45/40, 30/30/40, 45/15/40 and 60/0/40. The relationship of acetonitrile in the organic portion with K' is shown in Figure 3. A general view indicates that as there is more acetonitrile, the various compounds will have shorter retention times in the column, and that compounds with higher K' values are more affected by acetonitrile. It is only at 30/30/40 (acetonitrile/methanol = 1/1) and 45/15/40 (acetonitrile/methanol = 3/1) that **1** and **X** can be completely separated. Taking into account the factor of

Journal of Food and Drug Analysis, Vol. 13, No. 4, 2005

retention time, the mobile phase (B) composition was made as methanol/acetonitrile/water = 30/30/40 (v/v).

II. *The CE Analysis*

Judging by the molecular structures of the various constituents in pueraria, there includes coumarin (6,7-dimethoxycoumarin), isoflavones (daidzein, genistein, formononetin, biochanin A), and isoflavone glycosides (puerarin and daidzin). By reference to Wang *et al.'s* method which uses the CZE[15] with an alkaline borate as the buffer, the 7 reference standards could also be separated within 10 min though. Compounds daidzin, daidzein and genistein were found to have serious or partial overlapping with adjacent compounds in the electropherogram, making assay difficult. In view of the excellent performance of MEKC in separating various substances of different polarities, we further adopted the MEKC mode using sodium dodecyl sulphate (SDS) and sodium dihydrogenphosphate instead of borate, and varying the pH value and organic solvents of the buffer. A completing separation of all compounds was finally achieved within 50 min, and the resulted electropherogram is shown in Figure 4. The 7 peaks of marker substances have been confirmed by spiking standards with sample.

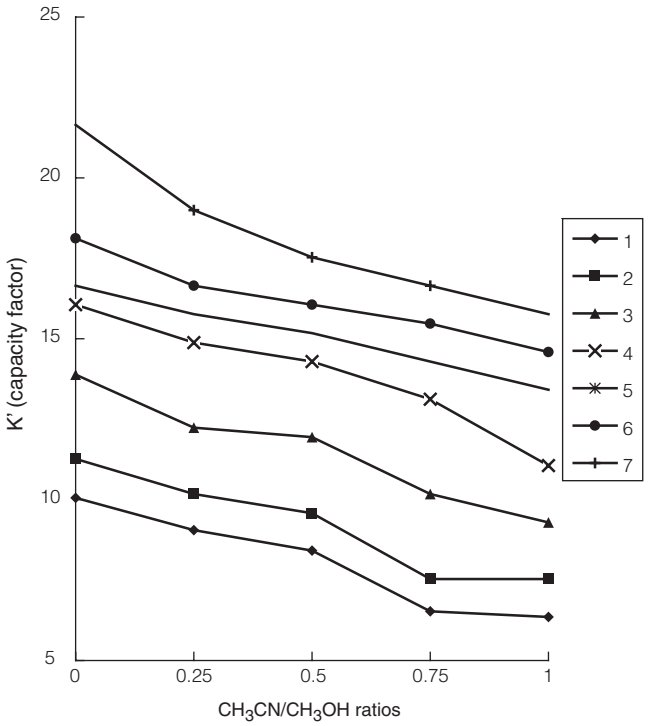

Figure 3. Effect of organic solvent content on capacity factor.

In order to investigate the effect of phosphate concentration on the separation of the various compounds, we fixed SDS concentration at 40 mM and prepared a series of phosphate buffers at different concentrations, namely 5, 10, 15, 20, 25 and 30 mM, as shown in Figure 5. As the phosphate concentration became higher, ion concentration and solution viscosity increased while zeta potency decreased. As the result, EOF became slow and migration times of the various compounds increased. Isoflavones and isoflavonoglycosides migration times were markedly slower than EOF. In addition, higher salt concentration makes migration time extended, the peaks of the various compounds become broadened and the theoretical plate numbers reduced. Phosphate concentrations at 10, 15

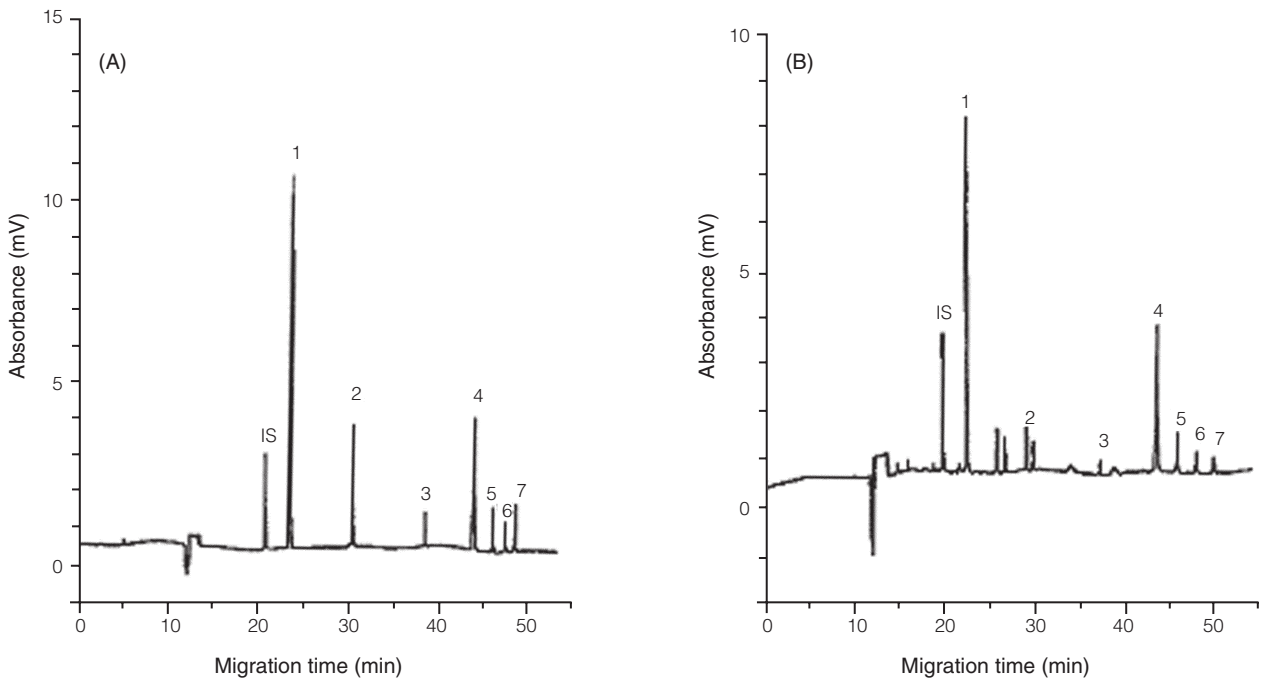

Figure 4. CE electropherograms of (A) 7 authentic standards, and (B) pueraria extract fluid. IS: internal standard (gallic acid); other symbols as in Figure 1.

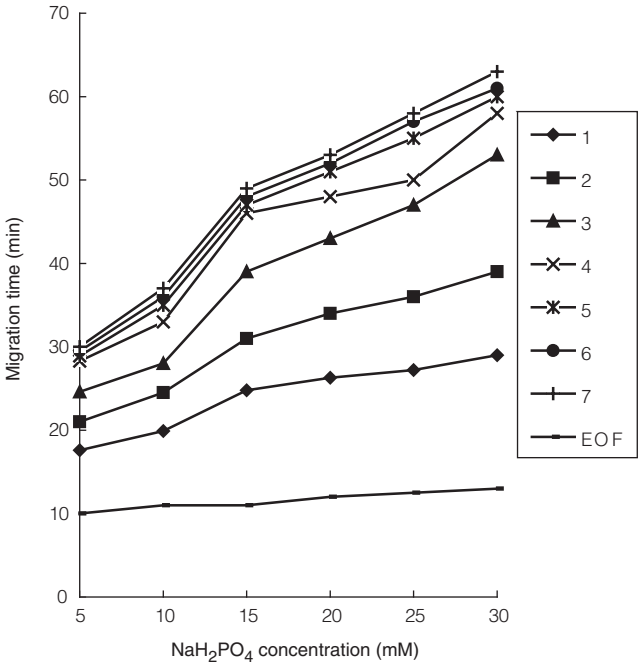

Figure 5. Effect of phosphate concentration on migration time.

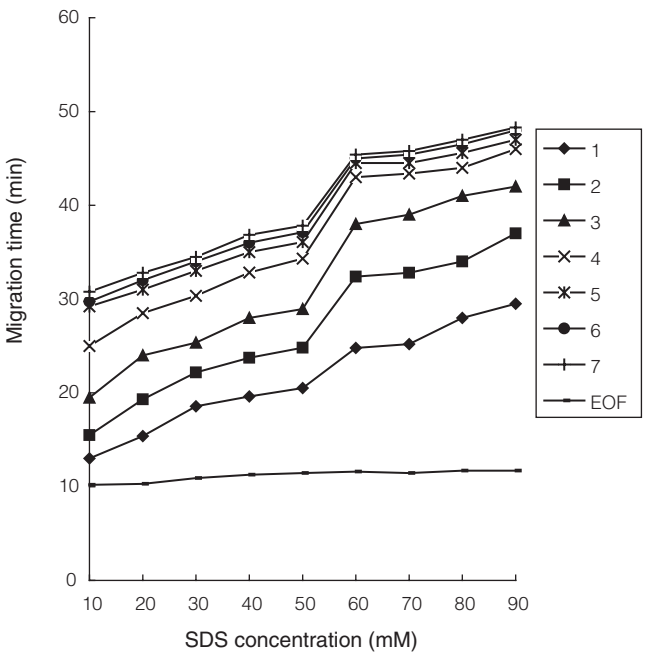

Figure 6. Effect of SDS concentration on migration time.

or 20 mM produced good separation, of which 10 mM concentration showed the advantages of a shorter analysis time and a higher theoretical plate number. Therefore, we chose to use 10 mM.

To investigate the effect of SDS concentration on compounds separation, we added different amounts of SDS to a phosphate solution fixed at 10 mM to create 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mM concentrations of SDS. Figure 6 shows the relationship between migration time and SDS concentration. When SDS concentration increases,

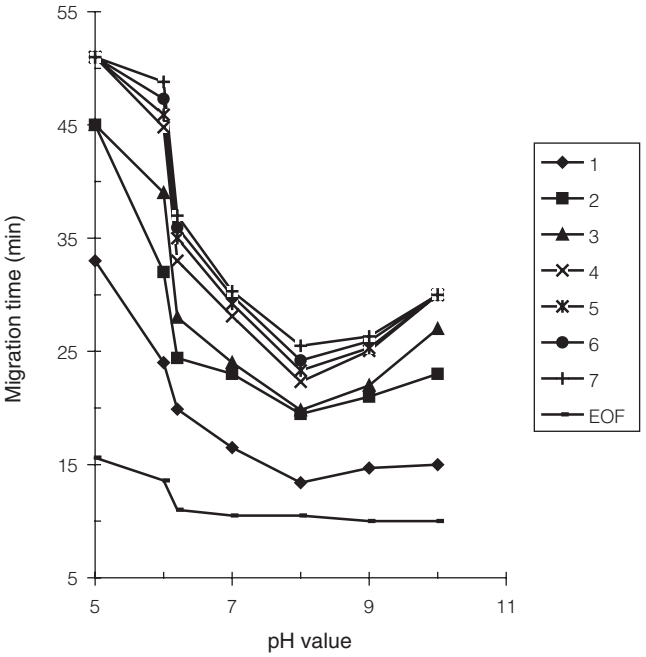

Figure 7. Effect of pH on migration time.

Table 2. Resolutions of pairs of compounds at different SDS concentrations

SDS concentration	Resolution		
(mM)	**4/5**	**5/6**	**6/7**
10	5.6	1.3	2.3
20	5.0	2.0	2.1
30	4.1	2.0	1.3
40	3.5	1.8	1.5
50	3.1	1.8	1.3
60	2.0	1.8	1.1
70	1.9	1.8	1.0
80	1.9	1.7	0.9
90	1.9	1.7	0.9
100	1.9	1.6	0.8

EOF retarded and caused the 7 compounds to retard in their migration times. At 20 mM, the separatory effect can be achieved, although peaks **4, 5, 6** and **7** appeared with tailing and the theoretical plate numbers were low. At 30 mM, **6** and **7** had partial overlap. At 40 and 50 mM, good separation was achieved, of which 40 mM yielded better resolution for the pair of **6/7** peaks (Table 2). Hence, we adopted 40 mM as the optimal SDS concentration.

It was understood that the pH of a buffer solution can affect the dissociation of carboxyl groups, thereby affecting the binding force between a compound and SDS, and causing differences in eluation activity, which is an important factor in promoting separation. Therefore, we added different amounts of sodium hydroxide and phosphoric acid to a mixture of 10 mM phosphate and 40 mM SDS solution to prepare 6 buffers with pH values ranging from 5.0 to 10.0. As shown in Figure 7, the migration is longer at pH 6.0 than at pH 6.2, but the resolution for **6/7** is higher (pH 6.0, 1.8; pH 6.2, 1.5). So we chose pH 6.0 for the analysis.

Following a series of investigation on analysis conditions, the optimal MEKC condition was found to be a mixture of 10 mM sodium dihydrogenphosphate solution and 40 mM SDS solution (pH adjusted to 6.0).

III. *The Calibration Curves*

According to the statements given in the experimental part, the ratio of the peak area to the internal standard peak area is taken as y, which is correlated to the constituent concentration (x, μg/mL) in making the calibration curves as shown in Table 3. The linearity of calibration curves is good over 2~3 orders, and the correlation coefficient (R^2) exceeds 0.999 in all compounds except **3** (0.9985) for HPLC, and **4, 5** (0.9986 and 0.9981) for CE.

IV. *Assessment of Analysis Optima*

From assays under the optimal analysis conditions for the various compounds by repetitive injections on the same day (intraday) or different days (interday) for a total of 6 injections, the relative standard deviation (RSD) of the peak-area ratios and migration times is calculated (See Tables 4). The HPLC method has intraday RSD at 0.02~0.03% and interday RSD at 0.02~0.03%, in terms of retention time; whereas the CE method has intraday RSD at 1.20~2.86%, and interday RSD, 1.28~2.93% in terms of migration time. In terms of the peak-area ratio, the HPLC method has intraday RSD at 0.50~0.97%, and interday RSD, 0.48~0.95%; on the other hand, the CE method has interday RSD at 0.88~1.25%, and interday RSD, 0.85~1.38%. The data showed good accuracy with both methods.

Suitable amounts of the 7 pueraria constituents were added to a sample of Puerariae Radix of known content. Mixture was analyzed using the proposed procedure. The recoveries of the constituents were 98.87~100.52% (n = 3) for HPLC and 97.34~102.00% (n = 3) for CE, as shown in Table 4. All tailing factors of the peaks are very close to unity.

By diluting the stock solutions of the reference standards step by step, 10 μL of what was infected for analysis until signal/noise ≦ 3/1. Concentration and injection volume at each step are recorded. The detection limit for HPLC method ranged from 0.16~0.61 μg/mL, and 0.88~7.35 μg/mL for CE. The results obtained are shown in Table 5.

V. *Analysis of Drug Material Samples*

Three samples of pueraria drug materials, 1.0 g each, were repetitively assayed for 3 times and the average assay value was taken. The assay results (average ± standard deviation, mg/g) are as follows. For the HPLC part: **1**, 2.70

Table 3. Calibration curves for HPLC and CE analysis

		y = ax + b			
		y = peak area ratio, x = conc. (mg/mL)			
		Linear range (μg/mL)	Slope a	Intercept b	R^2
	HPLC				
1	puerarin	5.0~5000.0	18.51	0.30	0.9992
2	daidzin	2.0~250.0	26.52	0.08	0.9994
3	6,7-dimethoxycoumarin	5.0~60.0	22.27	0.18	0.9985
4	daidzein	2.0~200.0	19.28	0.12	0.9994
5	genistein	0.5~60.0	33.59	0.06	0.9999
6	formononetin	0.5~40.0	20.78	0.03	0.9994
7	biochanin A	0.5~30.0	101.24	-0.02	0.9997
	CE				
1	puerarin	5.0~5000.0	11.23	0.13	0.9996
2	daidzin	2.0~50.0	23.06	-0.01	0.9992
3	6,7-dimethoxycoumarin	1.0~50.0	19.69	0.05	0.9993
4	daidzein	2.0~200.0	22.33	0.18	0.9986
5	genistein	0.5~60.0	21.11	0.06	0.9981
6	formononetin	0.5~40.0	20.78	0.03	0.9994
7	biochanin A	0.5~60.0	101.24	-0.02	0.9996

Table 4. Assessment of reproducibility and recovery under HPLC and CE analysis conditions

	Recovery[a] (%)	Intraday RSD (%)		Interday RSD (%)	
		Peak area ratio	Retention time	Peak area ratio	Retention time
HPLC					
1	100.30	0.50	0.02	0.48	0.02
2	99.75	0.82	0.03	0.82	0.02
3	98.87	0.88	0.03	0.89	0.03
4	100.52	0.69	0.03	0.62	0.03
5	100.24	0.87	0.03	0.90	0.03
6	100.11	0.92	0.03	0.88	0.02
7	100.45	0.97	0.03	0.95	0.03
CE					
1	100.12	0.88	1.20	0.85	1.28
2	98.46	0.97	1.28	0.94	1.27
3	100.51	0.98	1.83	1.38	1.98
4	97.34	1.03	2.68	1.00	2.45
5	102.00	1.08	2.81	0.98	2.50
6	100.21	1.10	2.86	0.95	2.87
7	100.52	1.25	3.01	1.23	2.93

[a]Concentration of each compound for recovery test:
Sample: **1**, 27.2; **2**, 10.2; **3**, 5.5; **4**, 13.0; **5**, 1.9; **6**, 0.6; **7**, 0.9 (μg/mL).
Added standard: **1**, 50.0; **2**, 25.0; **3**, 25.0; **4**, 25.0; **5**, 5.0; **6**, 5.0; **7**, 5.0 (μg/mL).

Table 5. Detection limits of pueraria marker substances (S/N = 3)

Method	Detection limit	1	2	3	4	5	6	7
HPLC	(μg/mL)	0.61	0.5	0.52	0.44	0.38	0.39	0.16
	(ng)	6.1	5.0	5.2	4.4	3.8	3.9	1.6
CE	(μg/mL)	0.74	0.39	0.42	0.28	0.27	0.36	0.09
	(ng)	0.06	0.03	0.04	0.02	0.02	0.03	0.01

± 0.33; **2**, 0.23 ± 0.01; **3**, 0.08 ± 0.02; **4**, 1.57 ± 0.50; **5**, 0.09 ± 0.01; **6**, 0.06 ± 0.02; **7**, 0.08 ± 0.02. For the CE part: **1**, 2.69 ± 0.32; **2**, 0.22 ± 0.01; **3**, 0.08 ± 0.01; **4**, 1.65 ± 0.56; **5**, 0.09 ± 0.01; **6**, 0.06 ± 0.02; **7**, 0.08 ± 0.02. Both methods give good results differing by only 5% when applied to the analysis of practical samples of Puerariae Radix.

CONCLUSIONS

The present study provides a HPLC and a CE analysis method for the separation of the reference standards of 1 coumarin and 6 isoflavones contained in pueraria. Both methods possess the advantages of facility, uniqueness, high sensitivity and good reproducibility. Such HPLC and CE analysis methods that can be applied directly to the analysis and assay of pueraria extract solution are excellent for analyzing the major constituent contents in pueraria, and can be further used as a tool for efficacy assessment and chemical identification of pueraria.

ACKNOWLEDGEMENTS

Financial support from the National Science Council, Republic of China (NSC92-2113-M-003-010), and the Hong-Yen and Lin-Run Hsu Charitable Foundation is gratefully acknowledged.

REFERENCES

1. Hsu, H. Y., Chen, P., Sheu, S. J., Hsu, C. H., Chen, C. C. and Chang, H. C. 1985. Chinese Materia Medica – a Concise Guide. p. 62. Modern Drug Weekly of Taiwan. Taipei, Taiwan, R. O. C.
2. Lou, Z. Q. and Qin, P. 1995. An arrangement of the species of commonly used Chinese herb drugs along with a study on their qualities and quantities. Vol. 1 – the Northern-area Part. pp. 379-419. Fujian Scientific Technologies. Fujian.
3. Shibata, S., Murakami, T., Nishikawa, Y. and Harada, M. 1959. The constituents of Puerariae root. Chem. Pharm. Bull. 7: 134-136.
4. Khan, R., Agrawal, P. and Kapil, R. 1996. Puetuberosanol, an epoxychalcanol from *Pueraria Tuberosa*. Phytochemistry 42: 243-244.
5. Kazuhiro, H., Makoto, M., Kaoru, N., Koh, S., Koji, T. and Minoru, O. 1997. Phenolic glucosides from the root of *Puerariae Lobata*. Phytochemistry 46: 923-928.
6. Hsu, H. Y. 1980 Studies on Chinese Herb Drugs. p. 124. Modern Drug Weekly of Taiwan. Taipei, Taiwan, R. O. C.
7. Lin, C. C. 1986. Chung Yao Hui Tung. p. 14. Tachun Traditional Chinese Medicine Magazine. Tainan, Taiwan, R. O. C.
8. Committee on Chinese Medicine and Pharmacy, Department of Health, Executive Yuan. 1999. Understanding Commonly Used Chinese Herb Drugs, II. p. 82. Taichai Cultural Enterprises, Ltd. Taipei, Taiwan, R. O. C.
9. Miahua, C. and Siju, Z. 1985. Studies on the chemical constituents of *Pueraria lobata*. Zhongyao Tongbao 10: 274-276.
10. Hayakawa, J., Noda, N., Yamada, S. and Uno, K. 1984. Studies on physical chemical quality evaluation of crude drug preparations. I. Analysis of Pueraria Radix and Species Puerariae. Yakugaku Zasshi 104: 50-56.
11. Zhao, S. and Zhang, Y. 1985. TLC-densitometry of isoflavones in *Pueraria lobata* (Wild.) Ohwi, Yaoxue Xuebao 20: 203-208.
12. Zhong, Y., Ding, X. B., Zho, C. and Lan, J. 1992. Quantitative determination of puerarin in lobed kudzuvine (*Pueraria lobata*) collected at different seasons by HPLC. Zhongcaoyao 23: 294-295.
13. Wen, K. C., Huang, C. Y. and Lu, F. L. 1993. Determination of baicalin and puerarin in traditional Chinese medicinal preparations by high-performance liquid chromatography. J. Chromatogr. 631: 241-247.
14. Yasuda, T., Momma, N. and Ohsawa, K. 1993. A simultaneous determination of daidzin and puerarin and determination of daidzein in oriental pharmaceutical decoction containing Puerariae Radix by ion-pair high-performance liquid chromatography. Yakugaku Zasshi 113: 881-889.
15. Wang, C. Y., Huang, H. Y., Kuo, K. L. and Hsieh, Y. Z. 1998. Analysis of Puerariae Radix and its medicinal preparations by capillary electrophoresis. J. Chromatogr. A 802: 225-231.
16. Chen, G., Zhang, J. and Ye, J. 2001. Determination of puerarin, daidzein and rutin in *Pueraria lobata* (Wild.) Ohwi by capillary electrophoresis with electrochemical detection. J. Chromatogr. A 923: 255-262.
17. Jiang, R. W., Lau, K. M., Lam, H. M., Yam, W. S., Leung, L. K., Choi, K. L., Waye, M. M. Y., Mak, T. C. W., Woo, K. S. and Fung, K. P. 2005. A comparative study on aqueous root extracts of *Pueraria thomsonii* and *Pueraria lobata* by antioxidant assay and HPLC fingerprint analysis. J. Ethnopharmacol. 96: 133-138.