藥物食品檢驗局調查研究年報. 17: 236-243 1999
Ann. Rept. NLFD Taiwan R.O.C 17: 236-243 1999

Determination of parishin, parishin B and parishin C in traditional Chinese medicinal formulas by micellar electrokinetic capillary chromatography[1]

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Received 31 October 1997; received in revised form 2 February 1998; accepted 10 February 1998

Abstract

Micellar electrokinetic capillary chromatographic methods were established for the determination of parishin, parishin B and parishin C in two kinds of the traditional Chinese medicinal formulas including Gastrodiae Rhizoma: Tsan-Siang-Tian-Ma-Tang and Ban-Shia-Bai-Ju-Tian-Ma-Tang. The samples were analyzed using 60-cm of uncoated capillary with various concentrations of sodium cholate and methanol as buffer, and detected at UV 222 nm. Regression equations were derived showing linear relationships (correlation coefficients: 0.9991–0.9997) between the peak-area ratios of each marker (parishin, parishin B and parishin C) to internal standard and concentration. The recoveries of parishin, parishin B and parishin C from the Chinese medicinal preparations were 102.3–102.7%, 103.9–106.3% and 97.8–104.0%, respectively. The relative standard deviations of three marker constituents ranged between 1.46 and 3.08% (intra-day), and 2.30 and 4.81% (inter-day). The parishin, parishin B and parishin C contents measured 1.92–6.07 mg/g, 4.70–5.12 mg/g and 9.74–10.61 mg/g, respectively.

Keywords: Pharmaceutical analysis; Parishins

1. Introduction

Traditional Chinese medicines have been used for centuries, and have been widely adopted for clinical use. Whether their effectiveness and safety are comparable to conventional methods for clinical therapy still remains to be established, hence proper methods for quality control are needed. Currently, official importance is attached to TLC and HPLC analytical methods. For this reason, a method of standardization to assess traditional Chinese medicines is as important as the discovery of new active principles.

Gastrodiae Rhizoma (Tianma) is the dried rhizome of *Gastrodia elata Blume* (Orchidaceae) and is a commonly used Chinese herb. It has sedative and anticonvulsant actions and is used to treat vertigo, blackout, headache and hemiplegia [1]. Because traditional medicine is usually prepared by decoction, their active constituents may be polar substances. Therefore, we isolated three highly polar constituents: parishin {tris [4-(β-D-glucopyranosyloxy) benzyl] citrate}, parishin B and parishin C {1,2- and 1,3-bis [4-(β-D-glucopyranosyloxy) benzyl] citrate} [2] from the rhizomes and there structures are shown in Fig. 1. HPLC

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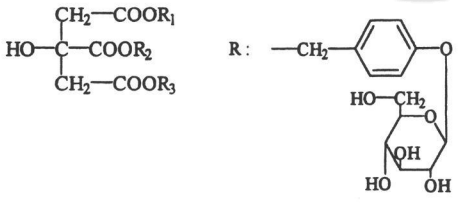
[1]Presented at the 21st International Symposium on Column Liquid Chromatography, Birmingham, 22–27 June 1997.

原載於Journal of Chromatography A. 1998.805:301-308



1. Parishin B: $R_1=R_2=R$, $R_3=H$
2. Parishin C: $R_1=R_3=R$, $R_2=H$
3. Parishin: $R_1=R_2=R_3=R$

Fig. 1. Structures of marker constituents.

methods were developed [3] for the determination of these constituents in traditional Chinese medicinal formulas including Gastrodiae Rhizoma. Capillary electrophoresis (CE) is a recently developed technique, which requires only a short run time, a small amount of sample, and the capillary column can be easily and thoroughly cleaned. In addition, combined with an autosampler apparatus it is convenient for the analysis of large sample numbers. Therefore, it is a useful method for quality control in pharmaceutical plants. Several papers have described the analysis of Chinese herb drugs by CE [4–6]. In our laboratory the determination of parishin, parishin B and parishin C in the crude drug of Gastrodiae Rhizoma by micellar electrokinetic capillary chromatography (MEKC) has also shown good results [7]. In this study, the determination of these three constituents, parishin, parishin B and parishin C in traditional Chinese medicinal formulas including Gastrodiae Rhizoma, Tsan-Siang-Tian-Ma-Tang (F1) and Ban-Shia-Bai-Ju-Tian-Ma-Tang (F2) by MEKC was developed.

2. Experimental

2.1. Materials

The materials used to prepare the traditional Chinese medicinal formulas were as follows [8]:

Tsan-Siang-Tian-Ma-Tang (hereafter abbreviated as F1): Gastrodiae Rhizoma, Amomi Amari Fructus, Angelicae Radix, Aquilariae Lignum, Pinelliae Rhizoma (2.0 g each), Angelicae Tuhuo Radix, Sileris Radix, Notopterygii Rhizoma (3.0 g each), Glycyrrhizae Radix, Aconiti Tuber (1.0 g each) and Bombyx Batryicatus (1.5 g).

Ban-Shia-Bai-Ju-Tian-Ma-Tang (F2): Gastrodiae Rhizoma, Hordei Germinatus Fructus, Zingiberis Recens Rhizoma, Messa Medicata Fermentat (2.0 g each), Pinelliae Rhizoma, Citri Reticulatae Pericarpium, Atractylodis Ovatae Rhizoma, Atractylodis Rhizoma, Hoelen (3.0 g each), Astragali Radix, Ginseng Radix, Alismatis Rhizoma (1.5 g each) and Phellodendri Cortex, Zingiberis Rhizoma (1.0 g each).

All materials were obtained from local markets in Taipei and cut into pieces. Two different commercial brands of concentrated herbal preparations of Ban-Shia-Bai-Ju-Tian-Ma-Tang were also purchased.

2.2. Chemicals and reagents

Parishin, parishin B and parishin C were isolated from the rhizome of *Gastrodia elata* Blume [2]. Sodium cholate, 2-(4-hydroxyphenyl)ethylammonium chloride (HPEA) and benzyltriethylammonium chloride (BTEA) were purchased from Sigma (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from BDH (Poole, UK). Ultrapure distilled water with a resistance greater than 18 MΩ was used.

2.3. Instruments

The analyses were carried out on a Beckman P/ACE 5500 capillary electrophoretic system equipped with a photodiode array detector set at 222 nm and a 67 cm×75 μm I.D. uncoated capillary (Beckman) with the detection window placed at 60 cm. The GOLD software for system controlling and data handling was used.

2.4. Capillary electrophoresis

The respective conditions used for formula F1 and F2 are given in Table 1. The electrolyte was filtered through a 0.45-μm syringe filter (Whatman) before use. After each run, the capillary was washed successively with 1% sodium hydroxide, 1 min; water, 1 min; 10 m*M* sodium dodecyl sulfate, 1 min and water, 2 min. The marker peaks were checked with photodiode array scan in each analysis.

Table 1
Conditions used for F1 and F2

Formula	Sampling time (s)	Running time (hydrostatic, min)	Voltage (constant, kV)	Temperature (°C)	Buffer	Internal standard
F1	4	35	13	25	140 m*M* sodium cholate–methanol (9:1, v/v)	HPEA, (71.4 μg/ml)
F2	4	40	15	25	200 m*M* sodium cholate–methanol (9.5:0.5, v/v)	BTEA (180.0 μg/ml)

HPEA, 2-(4-hydroxyphenyl)ethylammonium chloride.
BTEA, benzyltriethylammonium chloride.

2.5. Preparation of standard solutions

To prepare a standard solution containing parishin, parishin B and parishin C, an appropriate amount of internal standard (I.S.) solution was added to an accurately weighed amount of parishin, parishin B and parishin C standards dissolved in 70% methanol. The various concentrations were within the range 26.4–422.4, 27.36–437.76 and 77.14–1234.24 μg/ml for F1 and F2, respectively. Calibration curves were plotted based on linear regression analysis of the peak-areas versus concentrations.

2.6. Preparation of sample solutions

2.6.1. Standard decoction

Amounts of individual crude drugs equivalent to a daily dose of two kinds of Chinese medicinal formulas were weighed and pulverized, a twenty-fold weight of water was added and the mixture of each formulas was boiled respectively for more than 30 min to halve the original volume. After filtration while hot, 100 ml of the filtrate was concentrated under reduced pressure to dryness.

Each concentrated extract was dissolved in 10 ml of 70% methanol to yield a ten-fold diluted extract. Part of this solution (2.5 ml) and a suitable amount of I.S. (0.9 ml) were mixed to give a final concentration of 71.4 μg/ml of HPEA for F1 and 180.0 μg/ml of BTEA for F2.

2.6.2. Blank decoction

Amounts of individual crude drugs equivalent to daily doses of three kinds of Chinese medicinal formulas without Gastrodiae Rhizoma were treated according to the method described above for the preparation of standard decoction.

2.7. Concentrated herbal preparation Ban-Shia-Bai-Ju-Tian-Ma-Tang from market

An amount of the concentrated herbal preparation equivalent to a daily dose was weighted accurately and extracted with 30 ml of 70% methanol for 60 min in an ultrasonic bath. After extraction, the sample was filtered while hot. The filtrate was concentrated under reduced pressure to dryness. The residue was dissolved in 5 ml of 70% aqueous methanol to get a ten-fold extract and a suitable amount of I.S. was added. The solution was immediately filtered (0.45-μm, Whatman) and analysed by MEKC.

2.8. Solutions for recovery studies

Three marker solutions of different concentrations were added to each sample solution. To each solution a suitable amount of I.S. was added to yield a final concentration of 71.4 μg/ml of HPEA for F1 and 180 μg/ml of BTEA for F2. All sample solutions were filtered through a 0.45-μm syringe filter and injected for MEKC analysis to calculate the concentrations of parishin, parishin B and parishin C from their calibration curves.

3. Result and discussion

Traditional Chinese medicine is usually prepared by water decoction, the highly polar constituents are major part of decoction. Therefore, there are very

complex chemical compositions in decoction which may interfere in the analyses. MEKC is a highly efficient separation method for the determination of highly polar constituents including positively charged, negatively charged and neutral compounds. In this study, three neutral compounds (parishin, parishin B and parishin C) were used as marker constituents for controlling the quality of Gastrodiae Rhizoma in Chinese medicinal formulas. The present work describes for the first time the determination of the above constituents in traditional Chinese medicinal formulas using MEKC.

3.1. Analytical conditions

The detection wavelength was chosen at 222 nm because the absorbances of the three marker constituents are high at this wavelength. Because more complex peaks appeared beyond the electroosmotic flow, tetraammonium salts were used as I.S.s for two formulas, respectively.

The marker constituents, parishin, parishin B and parishin C, in two traditional Chinese medicinal formulas including Gastrodiae Rhizoma, were successfully analyzed by MEKC under suitable conditions. The separation was achieved by optimizing the concentration of sodium cholate, the temperature, the percentage of modifier (methanol) and the applied voltage.

Preliminary experiments were first conducted at various pH values of the buffer (composed of 20 m*M* sodium tetraborate and sodium dihydrogenphosphate). In all instances, the markers could not be separated from coexisting constituents. Therefore buffers composed of varied sodium cholate (surfactant) and methanol (organic solvent modifier) concentrations were developed. In order to study the effect of sodium cholate concentration, temperature, methanol concentration and voltage, these parameters were varied. The results on F1 are shown in Fig. 2. Four electrolyte systems containing different sodium cholate concentrations (100, 120, 140 and 160 m*M*) were used to study the effect of sodium cholate concentration on the selectivity of the separation. Although parishin, parishin B and parishin C were completely separated at all four concentrations, when the sample solution was determined parishin B and parishin C were still not free of interference from coexisting constituents at 100 and 120 m*M* sodium cholate. Concentrations of both 140 and 160 m*M* gave good separation, but the run time was shorter at 140 m*M* sodium cholate. A 140 m*M* sodium cholate buffer was used to study the effect of temperature (19, 22, 25 and 28°C) on the selectivity of the separation. The migration times of parishin, parishin B and parishin C were reduced when the temperature was increased. Once again, however, although separation was complete at all temperature, the parishin B and parishin C peaks in the sample solution were still subject to interference; only at 25°C were the sample solution parishin B and parishin C peaks interference-free. A 140 m*M* sodium cholate buffer was used with different methanol concentrations (0, 5, 10 and 15%) to study the effect of organic solvent on the selectivity of the separation. The 10% solution gave the best resolution; coexisting constituents interfered with all marker peaks. The results also indicated that the three markers were best resolved with no coeluting constituents at 13 kV. The results of the effect of sodium cholate concentration (160, 180, 200 and 220 m*M*), temperature (19, 22, 25 and 28°C), methanol concentration (0, 5, 10 and 15%) and voltage (11, 13, 15 and 17 kV) in F2 were similar for F1. Finally, the optimum conditions for F2 were obtained with a buffer containing 200 m*M* sodium cholate, 5% methanol and with the cartridge temperature and voltage of 25°C and 15 kV.

To ensure the specificity and selectivity of the method, we prepared a blank decoction of each formula for interference test. The electropherograms of F1 and F2 are shown in Figs. 3 and 4, respectively. Photodiode array detection was also used in this experiment, so that UV spectra of the marker constituents could be compared with those of the reference standards. The migration times of the marker constituents, parishin C, parishin B and parishin, in F1 and F2 were 27.0, 27.6 and 28.8 min; and 26.6, 27.2 and 32.5 min, respectively. No peak was detected at these migration times in two blank decoctions. The reproducibilities (relative standard deviation) of migration times of parishin C, parishin B and parishin in F1 and F2 were 0.53, 0.55 and 0.54%; and 0.15, 0.15 and 0.23%, respectively.

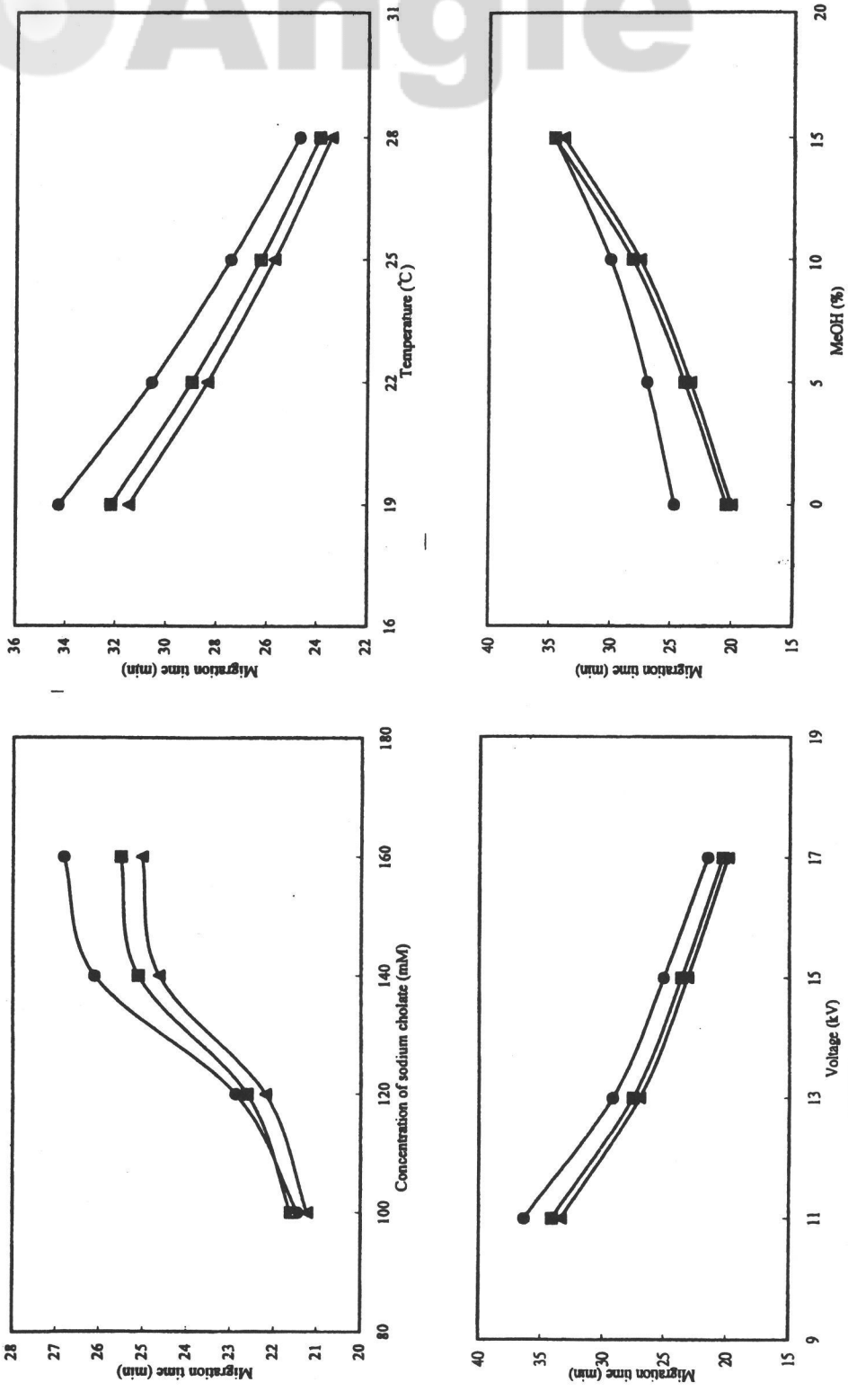


Fig. 2. Effect of analytical conditions of Tsan-Siang-Tian-Ma-Tang on migration time of the three markers. (●) Parishin; (■) parishin B; (▲) parishin C.

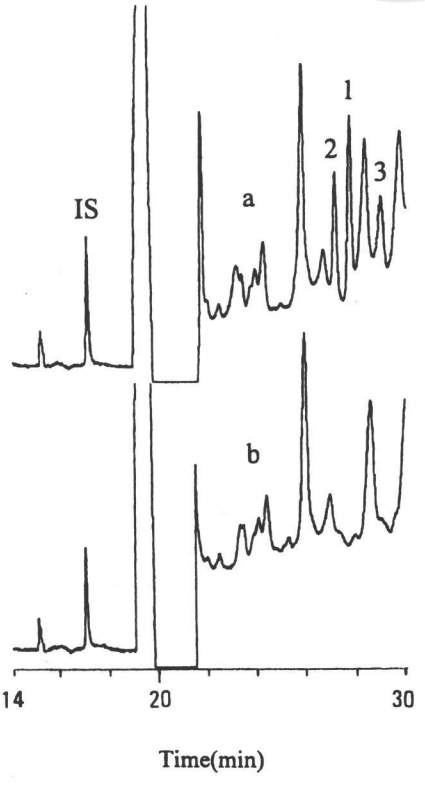


Fig. 3. Electropherograms of parishin, parishin B and parishin C in Tsang-Siang-Tian-Ma-Tang (F1): (a) standard decoction; (b) blank decoction. Peak numbers refer to compound numbers in Fig. 1. I.S.=internal standard [2-(4-hydroxyphenyl)ethylammonium chloride].

Table 2
Regression equations and their correlation coefficient (r) of marker constituents for Chinese medicinal formulas

Formulas	Marker constituent	Linear regression	r
F1	Parishin	$y=63.66x+7.48$	0.9997
	Parishin B	$y=85.52x+6.14$	0.9996
	Parishin C	$y=271.37x-6.82$	0.9991
F2	Parishin	$y=74.82x+9.23$	0.9996
	Parishin B	$y=102.17x+8.10$	0.9991
	Parishin C	$y=315.12x+1.91$	0.9993

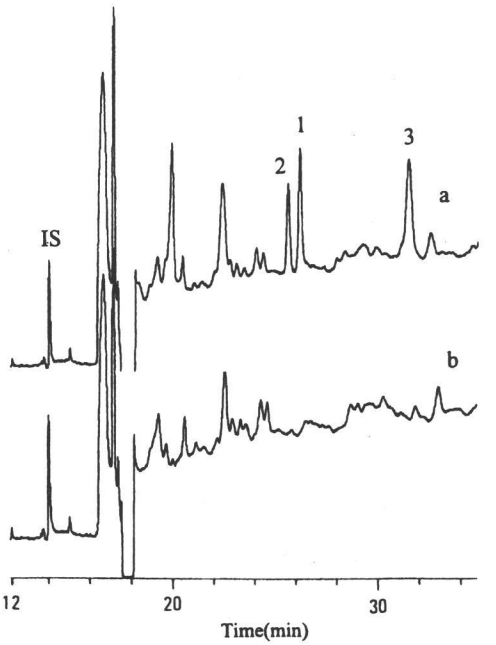


Fig. 4. Electropherograms of parishin, parishin B and parishin C in Ban-Shia-Bai-Ju-Tian-Ma-Tang (F2): (a) standard decoction; (b) blank decoction. Peak numbers refer to compound numbers in Fig. 1. I.S.=internal standard (benzyltriethylammonium chloride).

3.2. Calibration graphs for parishin, parishin B and parishin C

Calibration graphs were constructed in the range 26.40–422.40 μg/ml for parishin, 27.36–437.76 μg/ml for parishin B and 77.14–1234.24 μg/ml for parishin C. The regression equations of these curves and their correlation coefficients are shown in Table 2.

3.3. System suitability test

To assess the precision of these methods, we injected standard solutions of parishin, parishin B and parishin C, respectively, six times on the same day. The intra-day and inter-day variation studies found that the coefficients of variation were less than 2.9 and 4.8% for F1 and F2 and indicated that the

Table 3
Intra-day and inter-day R.S.D. values (n=6) for Chinese medicinal formulas

Formulas	Marker constituent	Concentration (μg/ml)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)
F1	Parishin	26.40	2.25	4.05
		105.60	3.08	4.47
		422.40	2.87	3.49
	Parishin B	27.36	1.98	2.30
		109.44	2.54	4.76
		437.76	1.98	3.75
	Parishin C	77.14	2.61	3.51
		308.56	2.64	3.87
		1234.24	2.21	4.18
F2	Parishin	26.40	1.93	2.84
		105.60	1.09	3.18
		422.40	2.36	3.08
	Parishin B	27.36	2.85	4.50
		109.44	1.46	4.17
		437.76	2.11	2.98
	Parishin C	77.14	1.49	3.69
		308.56	1.77	4.81
		1234.24	2.76	3.20

precision as well as accuracy of these assay were satisfactory (Table 3). The results of recovery studies of parishin, parishin B and parishin C indicated that recoveries ranged from 97.8 to 106.3% for F1 and from 102.7 to 104.0% for F2 (Table 4).

3.4. Determination of marker constituents in traditional Chinese medicinal formulas

When the sample solution was analyzed by MEKC under the selected conditions, the peaks were iden-

Table 4
Recoveries of parishin, parishin B and parishin C from Chinese medicinal formulas

Formulas	Marker constituent	Amount added (μg/ml)	Amount measured[a] (μg/ml)	Recovery (%)	Mean±S.D. (%)	R.S.D. (%)
F1	Parishin	22.0	22.9	104.2	102.3±3.1	3.07
		44.0	43.1	97.9		
		88.0	92.2	104.8		
	Parishin B	15.2	16.2	106.3	106.3±2.6	2.42
		30.4	33.3	109.4		
		60.8	62.7	103.1		
	Parishin C	20.3	19.3	95.0	97.8±2.6	2.65
		40.6	41.1	101.3		
		81.2	78.9	97.2		
F2	Parishin	22.0	23.7	107.8	102.7±3.7	3.58
		44.0	43.7	99.3		
		88.0	88.8	100.9		
	Parishin B	15.2	15.6	102.8	103.9±0.8	0.72
		30.4	31.7	104.4		
		60.8	63.5	104.4		
	Parishin C	20.3	20.9	102.8	104.0±0.9	0.86
		40.6	42.2	104.0		
		81.2	85.3	105.0		

[a] n=3.

tified by comparison of the migration time with those obtained from authentic samples of Gastrodiae Rhizoma. The contents of the markers mentioned above in standard decoction of Chinese medicinal formulas given in Table 5 were obtained by using the above regression equations. The results indicate that both of the proposed methods are relatively suitable for the determination of marker constituents. The contents of marker constituents in commercial preparations were directly calibrated by calibration curves and are shown in Table 6. The electropherograms are shown in Fig. 5. The contents were lower than those found in standard decoction. The difference might result from the manufacturing process and the variation of crude drugs. The commercial concentrated herbal preparation was large scale, and the standard decoction was laboratory scale.

In conclusion, the study demonstrates that MEKC can be successfully applied to separate glucosides of Gastrodiae Rhizoma in Chinese medicinal formulas. The technique offers high separation efficiencies, rapid analyses, low running costs and is aqueous, rather than organic solvent based. All of these are advantages exceeding the limitations of traditional chromatographic procedures.

Table 5
Content of marker constituents in standard decoction of Chinese medicinal formulas

Formulas	Content (mg/g) mean±S.D.[a]		
	Parishin	Parishin B	Parishin C
F1	1.92±0.04	5.12±0.12	9.74±0.14
F2	6.07±0.17	4.70±0.16	10.61±0.18

Table 6
Content of marker constituents in commercial concentrated herbal preparations of Ban-Shia-Bai-Ju-Tian-Ma-Tang

Commercial preparation	Content (mg/g) mean±S.D.[a]		
	Parishin	Parishin B	Parishin C
1	1.13±0.01	1.05±0.01	1.82±0.04
2	1.53±0.02	1.36±0.01	1.18±0.04

[a] $n=3$.

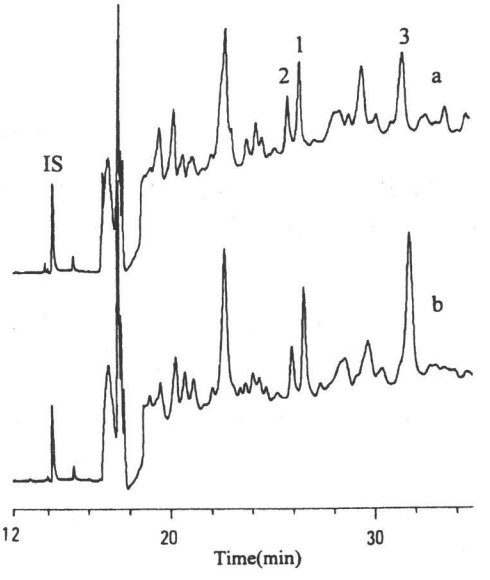


Fig. 5. Electropherograms of parishin, and parishin B and parishin C in commercial preparations (a and b) of Ban-Shia-Bai-Ju-Tian-Ma-Tang. Peak numbers refer to compound numbers in Fig. 1.

Acknowledgements

This work was supported by the National Health Research Institute, Republic of China (DOH 85-HR-307). The authors thank Miss Fehng-Chirn Chou for experimental assistance.

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