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Short communication

Analysis of parishin, parishin B and parishin C in Gastrodiae Rhizoma by micellar electrokinetic capillary chromatography

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Abstract

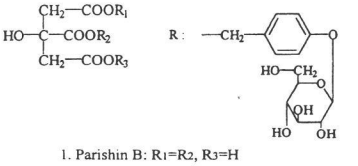
To evaluate the quality of Gastrodiae Rhizoma, a simple, rapid and accurate micellar electrokinetic capillary chromatographic method was developed for the assay of three marker constituents — parishin, parishin B and parishin C. The electrolyte was a buffer solution containing 100 m*M* sodium cholate and 20 m*M* sodium dihydrogenphosphate adjusted to pH 6.5 with 20 m*M* sodium tetraborate. Methylparaben was used as an internal standard and detected at 222 nm. The effects of buffer pH, electrolyte, temperature and voltage on separation are discussed. Regression equations revealed linear relationships (correlation coefficients: 0.9992–0.9998) between the peak-area rations of each marker to methylparaben. The relative standard deviations of three marker constituents ranged between 1.23–2.84% (intra-day) and 1.72–3.24% (inter-day). © 1998 Elsevier Science B.V.

Keywords: Pharmaceutical analysis; Parishins

1. Introduction

Traditional Chinese medicines are usually administered in the form of multicomponent prescriptions and each component may show a complicated profile of their constituents. Currently, the official quality control component is limited to formulated reference specifications. A method of standardization to approach traditional Chinese medicines is as important as the discovery of new active principles.

Gastrodiae Rhizoma (Chinese name: Tianma) is the dried rhizomes of *Gastrodia elata* Blume (Orchidaceae) and is a commonly used Chinese herb. It exerts sedative and anticonvulsant actions and is used to treat vertigo, blackout, headache and hemiplegia [1]. Because traditional Chinese medicine is usually prepared by decoction, their active constituents may be contained in the polar fraction. We have 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 their structures are shown in Fig. 1. For the determination of these constituents in Gas-



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

Fig. 1. Structures of marker constituents.

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trodiae Rhizoma, an HPLC method was developed [3]. Capillary electrophoresis (CE) is a recently developed technique that requires a short analysis time, a small amount of sample, and the capillary column can be thoroughly cleaned with ease. In addition, when combined with an autosampler apparatus, the analysis of large numbers of samples is convenient. Therefore it is a useful technique for quality control assessment in medicinal plants. CE also has given good results [4–6] in the analysis of Chinese herbs. In this study we report a micellar electrokinetic capillary chromatography (MEKC) for the determination of three Gastrodiae Rhizoma constituents: parishin, parishin B and parishin C. The precision of MEKC and HPLC method are compared and discussed.

2. Experimental

2.1. Reagents and materials

Parishin, parishin B and parishin C were isolated from the rhizomes of *Gastrodia elata* Blume [1]. Sulfamethoxypyridazine, sodium cholate and methylparaben were purchased from Sigma (St. Louis, MO, USA), sodium tetraborate and sodium dihydrogenphosphate from Nacalai Tesque (Kyoto, Japan). Methanol (HPLC grade) was purchased from BDH (Poole, UK). Phosphoric acid was of analytical reagent grade. Ultrapure distilled water with a resistivity greater than 18 MΩ was used. Four crude drugs of Gastrodiae Rhizoma were obtained from markets in Taipei, Taiwan, and verified by Dr H.-C. Chang, Brion Research Institute of Taiwan.

2.2. Preparation of sample solution for MECK

Gastrodiae Rhizoma (2 g) was extracted three times with 70% methanol (10, 10 and 4 ml, successively) by reflux at 80°C, each 1 h [3]. The extracts were combined and filtered into a volumetric flask, 70% methanol was added to 25 ml and used as stock solution. A 10-ml volume of this stock solution was concentrated under vacuum to dryness. The residue was dissolved in 10 ml of electrolyte buffer. The sample solution was prepared by 2.5 ml of above solution and 0.25 ml of methylparaben solution (1 mg/ml) into a 5-ml volumetric flask and is adjusted to 5 ml exactly with electrolyte buffer. This solution was filtered through a 0.45-μm syringe filter (Gelman Sciences, Ann Arbor, MI, USA) before use.

2.3. Preparation of sample solution for HPLC

In order to prepare sample solution for HPLC, 2.5 ml of stock solution and 0.5 ml of sulfamethoxypyridazine solution (0.76 mg/ml) was added to a volumetric flask and 70% methanol was added to bring the volume to 5 ml exactly. This solution was filtered through a 0.45-μm syringe filter (Gelman) before use.

2.4. Apparatus and conditions

2.4.1. CE system

The analysis was carried out on a Beckman P/ACE 5500 CE system equipped with a photodiode array detector. The detector was set at 222 nm and a 67 cm×75 μm I.D. uncoated capillary (Beckman) with the detection window placed at 60 cm. The conditions were as following: sampling time, 4 s, hydrostatic; run time, 20 min; applied voltage, 15 kV (constant voltage, positive to negative polarity); and temperature, 30°C. The electrolyte buffer was a solution containing 100 m*M* sodium cholate and 20 m*M* sodium dihydrogenphosphate solution which was adjusted to pH 6.5 with 20 m*M* sodium tetraborate. The electrolyte was filtered through a 0.45-μm syringe filter (Gelman) before use. Throughout the experiment, columns were cleaned with 1% sodium hydroxide, 1 min; water, 1 min; 10 m*M* sodium dodecyl sulfate, 1 min and water, 2 min, successively. The GOLD software (Beckman) for system control and data processing was used.

2.4.2. HPLC system

HPLC was performed on a Hitachi Model L-6200 Intelligent pump system equipped with a Hitachi Model L-3000 photodiode array detector and a Shimadzu SIL-9A autoinjector. Detector was set at 222 nm. Satisfactory separation of the marker substance was obtained with a reversed-phase column (Mightsil RP-18, 5 μm, 15 cm×4.6 mm I.D., Kanto, Tokyo, Japan) eluted at a rate of 1 ml/min with a linear solvent gradient of A–B [A=methanol; B=

0.1% (v/v) phosphoric acid] varying as follows: 0 min, 15:85; 15 min, 25:75; and 30 min, 30:70.

2.4.3. Preparation of standard solution

To prepare a standard solution (containing parishin, parishin B and parishin C), an appropriate amount of internal standard solution was added to an accurately weighed amount of parishin, parishin B and parishin C standard which was dissolved in electrolyle buffer for MECK and in 70% methanol for HPLC. The various concentrations of parishin, parishin B and parishin C were within the range 30.0–480.0, 30.6–489.6 and 40.2–643.2 μg/ml for MEKC; 32.00–512.00, 24.24–387.84 and 12.72–203.52 μg/ml for HPLC, respectively. Calibration graphs were plotted subsequent to linear regression analysis of the peak area with concentrations.

2.5. Preparation of recovery studies

Three different concentration of markers; 70.0, 140.0 and 280.0 μg/ml for parishin, 76.0, 152.0 and 304.0 μg/ml for parishin B, 72.0, 144.0 and 288.0 μg/ml for parishin C were added to each sample solution, respectively. To each solution, a suitable amount of internal standard was added to yield a final concentration of 50 μg/ml of methylparaben. All samples were filtered through a 0.45-μm syringe filter (Gelman) and injected for MEKC analysis to calculate the concentration of parishin, parishin B and parishin C from their calibration graphs.

3. Results and discussion

Traditional Chinese medicine is conventionally prepared by water decoction and the highly polar constituents form a major part of the decoction. Therefore, in analysis, the complicated chemical compositions in decoction often interfere with each other. Previously, determination of the marker constituents by HPLC method has been reported. The contents of parishin, parishin B and parishin C in 23 crude drugs of Gastrodiae Rhizoma, extracted by 70% methanol, were 3.66–15.01, 1.21–6.46 and 0.83–2.68 mg/g, respectively [3]. Recently MEKC methods show a highly efficient separation for highly polar constituents including positive, negative and neutral compounds. In this study, the effect of analytical conditions on separation were examined and three neutral compounds (parishin, parishin B and parishin C) were used as marker constituents to investigate the quality of Gastrodiae Rhizoma. It is the first time for determination of above constituents by MEKC.

3.1. Analytical conditions

In this study, the marker constituents parishin, parishin B and parishin C in Gastrodiae Rhizoma were successfully determined from a single run by MEKC under suitable conditions. The separation was achieved by optimizing the pH of the buffer, the sodium cholate concentration, the cartridge temperature and the voltage.

Preliminary experiments were first conducted at pH 6, 7, 8 and 9 (20 m*M* NaH_2PO_4 and 20 m*M* $Na_2B_4O_7$) without sodium cholate in the electrophoretic medium. In all instances, parishin and internal standard were successfully separated, but parishin B and parishin C overlapped, indicating that these two glucoside possess similar charges under these pH conditions. The electrophoretic medium in the absence of the micelles does not provide sufficient selectivity to separate the constituents. However, in the presence of sodium cholate, the components in the mixture sample can be separated on the basis of the relative affinity for the micellar environment or the bulk aqueous phase. Therefore, a buffer system was chosen with suitable amounts of sodium cholate, NaH_2PO_4 and $Na_2B_4O_7$.

In order to study the effect of pH, sodium cholate concentration, temperature and voltage, serial and different studies were tested. In Fig. 2, the migration times of the parishin, parishin B and parishin C increased with decreased of the pH of the buffer. The migration timed at pH 6.5 was prolonged, however it showed better separation than other. In Fig. 3, the results indicated that three constituents can be completely separated at concentrations of 60, 80, 100 and 120 m*M* sodium cholate. However, in the determination of the sample solution, the separation of three constituents was interfered with coexisting components at 60 and 80 m*M* sodium cholate. While both the concentrations of 100 and 120 m*M* yielded good separations, 100 m*M* sodium cholate has a shorter

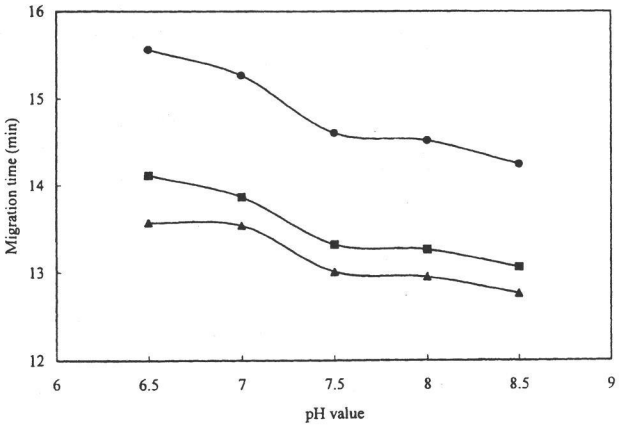

Fig. 2. Effect of pH on migration time. (●) Parishin; (■) parishin B; (▲) parishin C.

run time. The results also indicated that the three constituents were the best resolution and no coexisting components at 15 kV of voltage and 30°C of temperature.

According to the factors mentioned above, the best resolution was obtained with an electrolyte containing 100 m*M* sodium cholate, 20 m*M* NaH_2PO_4 and $Na_2B_4O_7$ at pH 6.5 and with the cartridge temperature and voltage setting at 30°C and 15 kV. Fig. 4 presents an electropherogram showing the separation of the marker constituents with the migration times of 16.1 min for the internal standard (methylparaben), 13.8 min for parishin C, 14.1 min for parishin B and 15.6 min for parishin. When the sample solution was injected directly and analyzed, the determination was carried out within 17 min. It required only a half of the separation time necessary for an HPLC method (chromatogram shown in Fig. 5). The run buffer was used as both the solvent of standard and sample diluent in all MEKC experi-

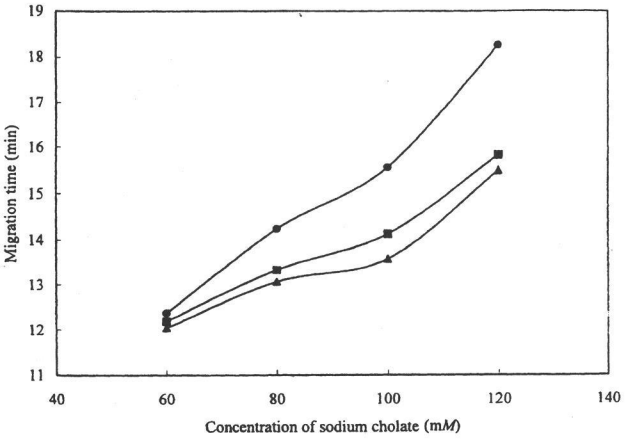

Fig. 3. Effect of sodium cholate concentration on migration time. Symbols as in Fig. 2.

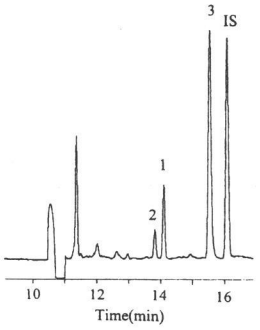

Fig. 4. Electropherogram of 70% methanol extract of Gastrodiae Rhizoma. Peak identity: same as Fig. 1. I.S.=internal standard (methylparaben).

ments. The use of organic solvents to dissolve the sample can significantly influence the separation as reported by Ackermans et al. [7]. In this experiment, all samples were dissolved in electrolyte buffer rather than 70% methanol which improved the peak shape, migration time and baseline.

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

Calibration graphs were constructed in the range 20.0–480.0 μg/ml for parishin, 30.6–489.6 μg/ml for parishin B and 40.2–643.2 μg/ml for parishin C. The regression equations of these curves and their correlation coefficients were calculated as follows: parishin, $y=150.16x-6.97$ ($r=0.9998$); parishin B, $y=174.67x-11.03$ ($r=0.9994$); parishin C, $y=218.07x-10.70$ ($r=0.9992$).

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 and a 6-day period analysis. The relative standard deviations (R.S.D.s) of intra-day and inter-day studies were less than 3.0 and 3.5%, respectively. The precision as well as accuracy of this assay was satisfactory (Table 1). The results for the recoveries of parishin, parishin B and parishin C ranged from 94.8 to 100.5% (Table 2).

3.4. Precision of MEKC and HPLC

The reproducibility (R.S.D.) of the proposed methods, on the basis of peak-area ratios for three replications, was 0.48–1.75% for HPLC and 1.18–2.01% for MEKC. The R.S.D. values of the retention time or migration time of each peak for three replicate injections were 1.14–1.24% for HPLC and

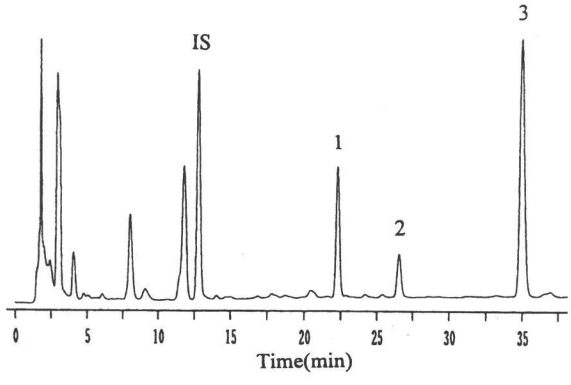

Fig. 5. Chromatogram of 70% methanol extract of Gastrodiae rhizoma. Peak identity: same as Fig. 1. I.S.=internal standard (sulfamethoxypyridazine).

Table 1
Intra-day and inter-day assay variations of parishin, parishin B and parishin C

Marker constituents	Concentration (μg/ml)	Intra-day[a] R.S.D. (%)	Inter-day[a] R.S.D. (%)
Parishin	30.0	1.75	2.99
	120.0	1.48	1.72
	480.0	2.60	3.24
Parishin B	30.6	1.23	3.10
	122.4	1.23	2.39
	489.6	2.22	3.03
Parishin C	40.2	2.84	2.98
	160.8	1.51	2.23
	643.2	2.35	2.56

[a] $n=6$.

1.26–1.54% for MEKC. The data for individual constituents are shown in Table 3.

3.5. Determination of marker constituents in Gastrodiae Rhizoma

When the sample solution was analyzed by MEKC and HPLC under the optimized conditions, the peaks were identified by comparison of the migration time with those obtained from authentic samples of Gastrodiae Rhizoma. The contents of the markers mentioned above in four crude drugs of Gastrodiae Rhizoma are given in Table 4. MEKC analyses showed that the contents of parishin, parishins B and C, (6.45–14.55, 2.46–4.88 and 1.25–2.17 mg/g,

Table 2
Recoveries of parishin, parishin B and parishin C in Gastrodiae Rhizoma

Marker constituents	Amount added (μg/ml)	Amount measured[a] (μg/ml)	Recovery (%)	Mean±S.D. (%)	R.S.D. (%)
Parishin	70.0	70.4	100.6	100.5±0.7	0.7
	140.0	139.4	99.6		
	280.0	283.2	101.2		
Parishin B	76.0	77.8	102.4	100.1±1.8	1.8
	152.0	151.4	99.6		
	304.0	298.4	98.2		
Parishin C	72.0	68.2	94.8	94.8±1.3	1.4
	144.0	134.3	93.3		
	288.0	283.2	96.8		

[a] $n=3$.

Table 3
Reproducibility of separation of marker constituents

Marker constituent	HPLC		MEKC	
	R.S.D. (%) ($n=3$)		R.S.D. (%) ($n=3$)	
	Retention time	Amount measured	Migration time	Amount measured
Parishin	1.24	0.48	1.54	1.18
Parishin B	1.14	0.20	1.31	2.01
Parishin C	1.22	1.75	1.26	1.59

Table 4
Contents of parishin, parishin B and parishin C in commercial Gastrodiae Rhizoma by MEKC and HPLC

Marker constituent	Commercial sample	MEKC (mg/g)	HPLC (mg/g)
Parishin	1	12.56	13.51
	2	14.55	15.15
	3	6.86	6.99
	4	6.45	6.42
Parishin B	1	4.83	4.64
	2	4.88	4.53
	3	3.31	3.06
	4	2.46	2.32
Parishin C	1	2.17	2.25
	2	2.17	2.08
	3	1.38	1.37
	4	1.25	1.30

respectively) were similar to those measured by an HPLC method. Therefore, both of the proposed methods are suitable for the determination of marker constituents.

In conclusion, this study demonstrated that MEKC can be successfully applied to separate glucosides of Gastrodiae Rhizoma. The technique offers high separation efficiencies, rapid analyses, low running costs. Instead of organic solvent, aqueous solvent was adapted throughout the CE analysis. All of these are advantages over traditional chromatographic procedures.

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References

[1] H.M. Chang, P.H. But, Pharmacology and Applications of Chinese Materia Medica, Vol. I, World Scientific Publishing, Singapore, 1986, p. 185.
[2] J.-H. Lin, Y.-C. Liu, J.-P. Hau, K.-C. Wen, Phytochemistry 42 (1996) 549.
[3] Y.-R. Ku, Y.-C. Lin, J.-P. Hau, J.-H. Lin, K.-C. Wen, W.-F. Huang, J. Food Drug Anal. 3 (1995) 233.
[4] Y.M. Lin, S.J. Sheu, J. Chromatogr. 634 (1993) 329.
[5] Y.M. Lin, S.J. Sheu, J. Chromatogr. 639 (1993) 323.
[6] Y.M. Lin, S.J. Sheu, Anal. Chim. Acta 288 (1994) 211.
[7] M.T. Ackermans, F.M. Everaerts, J.L. Beckers, J. Chromatogr. 585 (1991) 123.