

Determination of Polar Constituents of Scrophulariae Radix in Bai-He-Gu-Jin-Tang by Micellar Electrokinetic Capillary Chromatography

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ABSTRACT

A micellar electrokinetic capillary chromatographic method was established for the determination of 2-(3-hydroxy-4-methoxyphenyl)ethyl 1- O -[α -L-arabinopyranosyl(1 $\rightarrow$ 6)]-feruloyl(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl(1 $\rightarrow$ 3)- β -D-glucopyranoside (SN-A), harpagoside (SN-B) and cinnamic acid (SN-C) in the traditional Chinese medicinal prescription, Bai-He-Gu-Jin-Tang, which contains *Scrophulariae Radix*. This chromatographic technique was carried out using a buffer composed of 20 mM sodium tetraborate and sodium dihydrogen phosphate containing 120 mM sodium cholate and 12 % methanol at pH 7.5. 2-(4-Hydroxyphenyl)-ethylammonium chloride was used as an internal standard and detected at 280 nm. The linear calibration ranges for SN-A, B and C were 11.76-188.16 μ g/mL ($r = 0.9998$), 8.16-130.56 μ g/mL ($r = 0.9992$) and 4.48-71.68 μ g/mL ($r = 0.9991$), respectively. The recoveries of the markers listed above were SN-A: 104.3 ± 2.8 %, SN-B: 98.1 ± 2.2 % and SN-C: 98.2 ± 1.4 %. The relative standard deviations of the three marker substances for intraday and interday analyses ranged from 0.85 to 2.87 % and from 1.42 to 4.14 %, respectively.

Key words: Pharmaceutical analysis; *Scrophulariae Radix*; Bai-He-Gu-Jin-Tang; Harpagoside; MEKC.

INTRODUCTION

Traditional Chinese medicines have been used for centuries, and have been widely adopted for clinical use. Quality assessment of traditional Chinese medicinal prescriptions

requires that it be carried out by using known chemical constituents as reference standards. For the drug analysis phase, chromatographic methods can be effectively applied including the current methods of thin layer chromatography and high-performance liquid chromatography.

Capillary electrophoresis (CE) is a recently developed technique with the advantages that it requires a short analysis time, uses a small amount of sample, and can be used for autosampling. In addition, the capillary can easily be thoroughly cleaned, an important advantage when large numbers of samples are involved and for quality control in pharmaceutical plants.

Scrophulariae Radix (Chinese name: Xuanshen) is the dried root of *Scrophularia ningpoensis* and *S. buergeriana* (Scrophulariaceae) and is a commonly used Chinese herb. It is administered to allay thirst in febrile disease, in macula, pharyngolaryngitis and constipation.¹ In our previous paper, we described a method for the isolation and identification of three polar constituents, 2-(3-hydroxy-4-methoxyphenyl)ethyl 1-*O*-[α -L-arabinopyranosyl(1 $\rightarrow$ 6)]-feruloyl(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl(1 $\rightarrow$ 3)- β -D-glucopyranoside (SN-A), harpagoside (SN-B) and cinnamic acid (SN-C) from the roots of *Scrophularia ningpoensis*.² Furthermore, HPLC methods were also developed for the determination of the above constituents in *Scrophulariae Radix* and in *Scrophulariae Radix* containing prescriptions.^{2,3}

In our laboratory the determination of markers of *Gastrodiae Rhizoma*, including parishin, parishin B and parishin C, and markers of *Scrophulariae Radix*, including SN-A, SN-B and SN-C, in crude drug samples by micellar electrokinetic capillary chromatography (MEKC) also showed good results.^{4,5} The determination of these three constituents, SN-A, SN-B and SN-C, in Bai-He-Gu-Jin Tang by MEKC was developed in the present study.

EXPERIMENTAL

Materials

The materials used to prepare Bai-He-Gu-Jin-Tang were as follows:⁶ *Scrophulariae Radix*, *Fritillariae Bulbus*, *Paeoniae Radix* (3.0 g each), *Lilii Bulbus*, *Angelicae Radix*, *Rehmanniae Radix* (4.0 g each), *Ophiopogonis Radix* (6.0 g), *Glycyrrhizae Radix* (1.5 g) and *Platycodi Radix* (2.0 g).

All materials were obtained from retail outlets in Taipei and cut into pieces. Two different commercial brands of concentrated preparations of Bai-He-Gu-Jin-Tang were also purchased from retail outlets and included in our analysis.

Reagents and Chemicals

2-(3-Hydroxy-4-methoxyphenyl)ethyl 1-*O*-[α -L-arabinopyranosyl(1 $\rightarrow$ 6)]-feruloyl-

(1→4)- α -L-rhamnopyranosyl(1→3)- β -D-glucopyranoside (SN-A, purity 96.3 %) and harpagoside (SN-B, purity 97.1 %) were isolated from the roots of *Scrophularia ningpoensis*.² The structures of the marker constituents are shown in Chart 1. Sodium cholate was purchased from Sigma (St. Louis, MO, USA). 2-(4-Hydroxyphenyl)-ethylammonium chloride (HPEA) was purchased from Merck (Darmstadt, Germany). Cinnamic acid, sodium tetraborate and sodium dihydrogenphosphate were purchased from Nacalai (Kyoto, Japan). Methanol (HPLC grade) was purchased from Labscan (Dublin, Ireland). Ultrapure distilled water with a resistivity greater than 18 M Ω was used.

Apparatus and Conditions

The analysis was carried out on a Beckman P/ACE 5500 capillary electrophoresis system equipped with a photodiode array detector set at 280 nm and a 67 cm $\times$ 75 μ m I.D. uncoated capillary (Beckman) with the detection window placed at 60 cm. The conditions were as follows: sampling time, 4 sec, hydrostatic; run time, 60 min; applied voltage, 15 kV (constant voltage, positive to negative polarity); and temperature, 25 °C. The running buffer was a solution containing 120 mM sodium cholate, 12 % methanol and 20 mM sodium dihydrogenphosphate which was adjusted to pH 7.5 with 20 mM sodium tetraborate. The electrolyte was filtered through a 0.45 μ m syringe filter (Gelman, Ann Arbor, MI, USA) be-

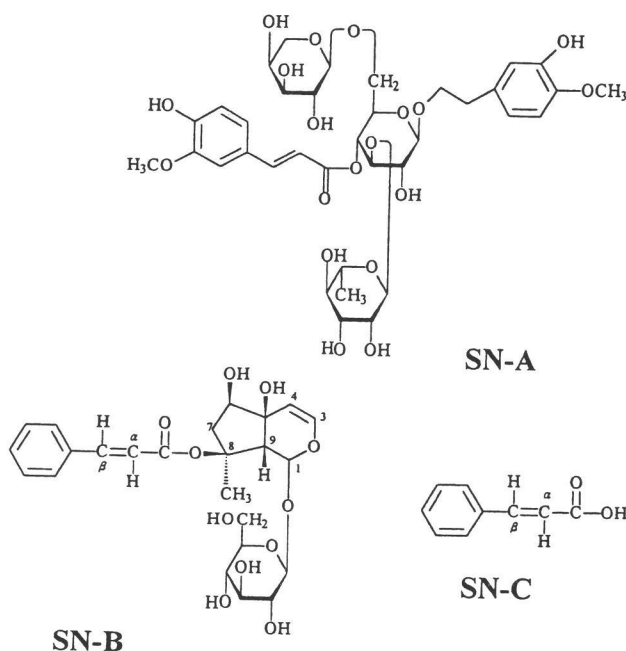


Chart 1. Structures of the marker constituents

fore use. The capillary was cleaned after all experiments by the following steps: 1 % sodium hydroxide, 1 min; water, 1 min; 10 mM sodium dodecyl sulfate, 1 min; and water, 2 min, continuously. Gold software (Beckman) was used for system control and data handling.

Preparation of Sample Solution

Standard decoction

The individual crude drugs of Bai-He-Gu-Jin-Tang in amounts equivalent to a daily dose were weighed and pulverized separately. A twentyfold weight of water was added and the mixture was boiled for more than 30 min to half the original volume. The extract was filtered while hot. One hundred milliliter of this solution was concentrated under vacuum to dryness. The residue was dissolved in 10 mL of 70 % methanol. The sample solution was prepared by mixing 1 mL of the above solution and 0.16 mL of HPEA (0.74 mg/mL) in a 2 mL volumetric flask made up to 2 mL with 70 % methanol. The final concentration of the internal standard (HPEA) was 59.2 µg/mL.

Blank decoction

Amounts of the individual crude drugs equivalent to a daily dose of Bai-He-Gu-Jin-Tang but without *Scrophulariae Radix* were processed as above for the preparation of the standard decoction.

Preparation of Standard Solutions

To prepare a standard solution containing SN-A, SN-B and SN-C, an appropriate amount of internal standard solution was added to accurately weighed amounts of SN-A, SN-B and SN-C standard dissolved in 70 % methanol. The various concentrations were within the ranges 11.76~188.16, 8.16~130.56 and 4.48~71.68 µg/mL, respectively. Calibration graphs were plotted to perform linear regression analysis of the peak area ratios versus concentration (µg/mL).

Concentrated Preparations of Bai-He-Gu-Jin-Tang from Retail Outlets

An amount of the concentrated preparations equivalent to a daily dose was weighed and extracted with 35 mL of 70 % methanol for 30 min in an ultrasonic bath. After filtration, the filtrate was diluted to 50 mL with 70 % methanol, and a suitable amount of internal standard was added to the solution to give a final concentration of 59.2 µg/mL of HPEA. The solutions were filtered through a 0.45 µm filter (Gelman) and analysed by MEKC.

Solution for Recovery Studies

Three different concentrations of markers; 9.8, 19.6 and 39.2 $\mu\text{g/mL}$ for SN-A, 10.2, 20.4 and 40.8 $\mu\text{g/mL}$ for SN-B, 5.6, 11.2 and 22.4 $\mu\text{g/mL}$ for SN-C, were added to each sample solution. A suitable amount of internal standard was added to give a final concentration of 59.2 $\mu\text{g/mL}$ of HPEA. All samples were filtered through a 0.45 μm syringe filter (Gelman) and applied for MEKC analysis to calculate the concentrations of SN-A, SN-B and SN-C from their calibration graphs.

RESULTS AND DISCUSSION

Traditional Chinese medicine is usually prepared by water decoction, with highly polar constituents making of the major part of the decoction. The chemical constituents in the decoction are very complex, and thus analysis is difficult. MEKC has proven to be a highly efficient separation method for the determination of highly polar compounds regardless of whether they are positive, negative or neutral. In this study, two neutral compounds (SN-A and SN-B) and one acidic compound (SN-C) were used as marker constituents for the *Scrophulariae Radix* containing prescription, Bai-He-Gu-Jin-Tang, and for analysis by the MEKC method.

Analytical Conditions

The detection wavelength of 280 nm was chosen because the absorption maxima of SN-B, SN-A and SN-C are at 280, 287 and 267 nm. In a previous study,⁵ we reported that the marker constituents SN-A, SN-B and SN-C in a methanol extract of *Scrophulariae Radix* were successfully separated in a single run by MEKC using a buffer composed of 20 mM NaH_2PO_4 and $\text{Na}_2\text{B}_4\text{O}_7$ containing 100 mM sodium cholate and 10 % acetonitrile at pH 7.5. The cartridge temperature and the voltage were set at 25 °C and 25 kV. This condition was utilized for the preliminary experiments but interference was caused by co-existing components in the prescriptions. Thus, the surfactant concentration, voltage and organic solvent were modified to obtain an improved condition.

The best resolution was obtained with an electrolyte containing 120 mM sodium cholate, 12 % methanol, 20 mM NaH_2PO_4 and $\text{Na}_2\text{B}_4\text{O}_7$ at pH 7.5 and with the cartridge temperature and voltage set at 25 °C and 15 kV. Because complex peaks appeared beyond electroosmotic flow, a tetraammonium salt, HPEA, was used as the internal standard. Fig. 1 shows an electropherogram of the separation of the marker constituents with the migration times of 15.1 min for HPEA, 27.1 min for SN-A, 33.3 min for SN-B and 57.4 min for SN-C.

Calibration Graphs for SN-A, SN-B and SN-C

Calibration graphs were constructed in the range of 11.76-188.16 $\mu\text{g/mL}$ for SN-A, 8.16-130.56 $\mu\text{g/mL}$ for SN-B and 4.48-71.68 $\mu\text{g/mL}$ for SN-C. The regression equations of the data and the correlation coefficients were calculated as follows: SN-A, $Y = 33.13X + 0.86$ ($r = 0.9998$); SN-B, $Y = 5.97X - 1.32$ ($r = 0.9992$); SN-C, $Y = 1.11X + 2.29$ ($r = 0.9991$).

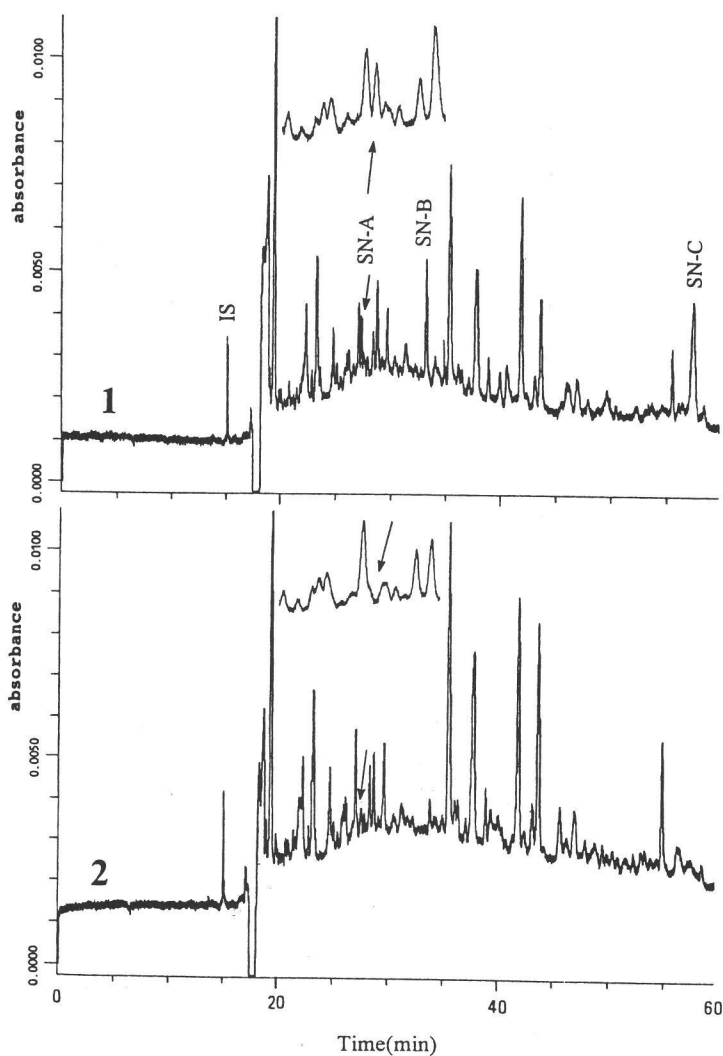


Fig. 1. Electropherograms of SN-A, SN-B and SN-C in Bai-He-Gu-Jin-Tang: (1) standard decoction; (2) blank decoction, IS = internal standard (2-(4-hydroxyphenyl)ethylammonium chloride).

Table 1. Intraday and Interday Relative Standard Deviations (n = 6) For Bai-He-Gu-Jin-Tang

Marker constituents	Concentration ($\mu\text{g/mL}$)	Intraday* R.S.D. (%)	Interday* R.S.D. (%)
SN-A	11.76	2.87	3.16
	47.04	1.90	4.14
	188.16	1.12	3.78
SN-B	8.16	1.85	1.73
	32.64	1.65	3.32
	130.56	1.42	1.71
SN-C	4.48	0.85	1.42
	17.92	2.25	3.46
	71.68	1.35	2.33

* n = 6

Table 2. Recoveries of SN-A, SN-B and SN-C from Bai-He-Gu-Jin-Tang

Marker constituents	Amount added ($\mu\text{g/mL}$)	Amount measured* ($\mu\text{g/mL}$)	Recovery (%)	Mean $\pm$ S.D. (%)	R.S.D. (%)
SN-A	9.8	10.5	107.1	104.3 $\pm$ 2.8	2.68
	19.6	20.6	105.3		
	39.2	39.4	100.5		
SN-B	10.2	9.7	95.0	98.1 $\pm$ 2.2	2.26
	20.4	20.3	99.7		
	40.8	40.7	99.7		
SN-C	5.6	5.4	96.6	98.2 $\pm$ 1.4	1.37
	11.2	11.2	99.9		
	22.4	22.0	98.1		

* n = 3

System Suitability Test

The intra- and inter-assay precisions of the method were examined with standard solutions of SN-A, SN-B and SN-C six times on the first day and then once a day for a 6-day period (Table 1). The intraday and interday variation studies at three concentrations found that the coefficients of variation were less than 2.87 and 4.14 %. This indicates that the precision as well as the accuracy of this assay were satisfactory. Recovery studies of SN-A, SN-B and SN-C demonstrated recoveries ranging from 98.1 to 104.3 % as shown in Table 2.

Table 3. The Contents of Marker Constituents in the Standard Decoction and Commercial Concentrated Herbal Preparation Samples of Bai-He-Gu-Jin-Tang

	SN-A	SN-B	SN-C
	Mean $\pm$ S.D.* (mg/g)	Mean $\pm$ S.D.* (mg/g)	Mean $\pm$ S.D.* (mg/g)
Standard decoction	83.6 $\pm$ 3.0	38.9 $\pm$ 0.7	19.4 $\pm$ 0.03
Commercial sample 1	45.1 $\pm$ 0.9	78.5 $\pm$ 1.2	12.4 $\pm$ 0.1
Commercial sample 2	93.7 $\pm$ 2.0	75.2 $\pm$ 1.6	9.9 $\pm$ 0.3

* n = 3

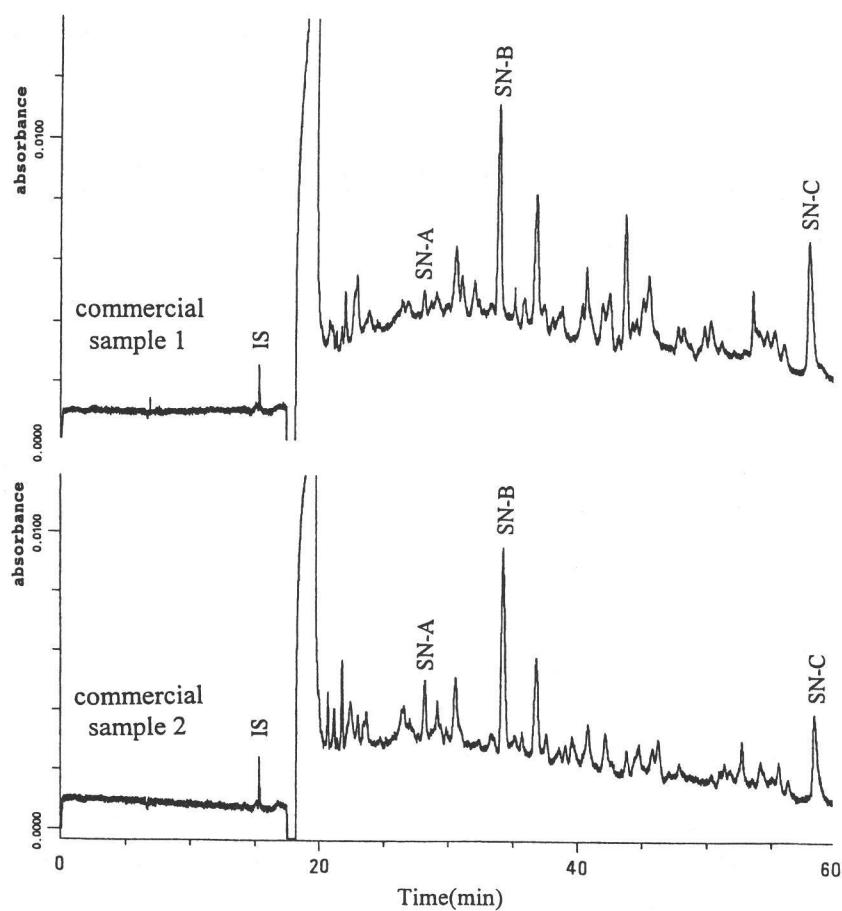


Fig. 2. Electropherograms of SN-A, SN-B and SN-C in commercial preparation samples (1-2) of Bai-He-Gu-Jin-Tang.

Determination of Marker Constituents in Bai-He-Gu-Jin-Tang

The contents of the markers in the standard decoction and in commercial concentrated herbal preparations of Bai-He-Gu-Jin-Tang are given in Table 3. Fig. 2 shows electropherograms of the marker constituents in two brands of commercial concentrated herbal preparations of Bai-He-Gu-Jin-Tang.

In conclusion, this study demonstrates that MEKC can successfully separate SN-A, SN-B and SN-C in Bai-He-Gu-Jin-Tang. This technique offers high separation efficiencies and low running costs. Instead of organic solvent, aqueous solvent was adapted throughout the CE analysis. All these are advantages over traditional chromatographic procedures.

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