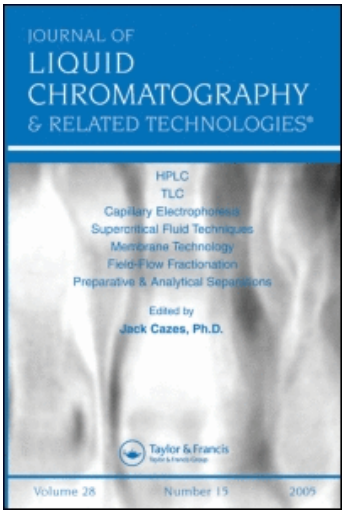

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DETERMINATION OF POLAR CONSTITUENTS OF *SCROPHULARIAE RADIX* IN TRADITIONAL CHINESE MEDICINAL PRESCRIPTIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

High performance liquid chromatographic methods were established for the determination of 2-(3-hydroxy-4-methoxyphenyl) ethyl 1-O-[α-L-arabinopyranosyl (1→6)]-feruloyl (1→4)-α-L-rhamnopyranosyl (1→3)-β-D-gluco pyranoside (SN-A), harpagoside (SN-B) and cinnamic acid (SN-C) in four kinds of traditional Chinese medicinal prescriptions which contain Scrophulariae Radix: Tian-Uang-Bu-Shin-Dan, Bai-He-Gu-Jin-Tang, Bay-Du-San and Ching-Reh-Bu-Shiee-Tang. The samples were processed with a Cosmosil ODS reversed phase column by gradient elution with various ratios of 1 % (v/v) acetic acid, acetonitrile and methanol as the mobile phases and detected at UV 278 nm. Regression equations were derived showing linear relationships (correlation coefficients: 0.9918-0.9999) between the peak area ratios of each marker to an internal

standard (sulfadimethoxine, chlorzoxazone or dexamethasone) and concentration. The recoveries of SN-A, B, and C from the Chinese medicinal prescriptions were 99.3-103.0 %, 94.0-102.2 % and 97.0-104.7 %, respectively. The relative standard deviations of markers ranged between 0.96-5.91 % (intraday) and 0.34-4.83 % (interday). The contents of SN-A, B, and C from Chinese medicinal prescriptions were 2.35-3.30 mg/g, 0.19-1.72 mg/g and 0.21-0.99 mg/g, respectively.

INTRODUCTION

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, the isolation and identification of three polar constituents, 2-(3-hydroxy-4-methoxyphenyl) ethyl 1-O-[α-L-arabinopyranosyl (1→6)]-feruloyl (1→4)-α-L-rhamnopyranosyl (1→3)-β-D-glucopyranoside (SN-A), harpagoside (SN-B) and cinnamic acid (SN-C) from the root of *Scrophularia ningpoensis* was reported.[2] For the determination of the above constituents in Scrophulariae Radix, an HPLC method using acetonitrile and 1.0% (v/v) acetic acid as mobile phase on an ODS column was also developed.[2]

Guillerault *et al.* reported the determination of harpagide, 8-para-coumaroyl harpagide, and harpagoside by HPLC in *Harpagophytum procumbens* using methanol and water as mobile phase on an RP18 column.[3] However, the assay for the markers from Chinese medicinal prescriptions containing Scrophulariae Radix by HPLC has not yet been described. For the purpose of quality control of traditional Chinese medicinal prescriptions, we used the three polar constituents mentioned above as marker constituents and studied HPLC methods for the assay of these markers in prescriptions containing Scrophulariae Radix .

In this study, we selected four kinds of Chinese medicinal prescriptions: Tian-Uang-Bu-Shin-Dan (hereafter abbreviated as P1), Bai-He-Gu-Jin-Tang (P2), Bay-Du-San (P3) and Ching-Reh-Bu-Shiee-Tang (P4) all of which contain Scrophulariae Radix. This paper deals with the HPLC methods for determining the contents of the marker constituents, SN-A, B and C, in each prescription. Validation methods are also reported.

EXPERIMENTAL

Materials

The materials used to prepare the traditional Chinese medicinal prescriptions were as follows:[4]

Tian-Uang-Bu-Shin-Dan (P1): Scrophulariae Radix, Polygalae Radix, Platycodi Radix, Ophiopogonis Tuber, Coptidis Rhizoma, Ginseng Radix, Biotae Semen, Zizyphi Spinosi Semen, Salviae Miltiorrhizae Radix, Hoelen, Asparagi Cochinchinensis Tuber, Angelicae Radix, Acori Graminei Rhizoma (1.2 g each) and Schizandrae Fructus (1.5 g).

Bai-He-Gu-Jin-Tang (P2): 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 (2.0g).

Bay-Du-San (P3): Scrophulariae Radix, Trichosanthis Radix, Platycodi Radix, Arctii Fructus, Forsythiae Fructus, Lonicerae Flos (2.5 g each), Moutan Cortex, Bupleuri Radix (2.0 g each), Phellodendri Cortex, Paeoniae Radix, Menthae Folium (1.5 g each), Rehmanniae Radix, Gypsum Fibrosum (4.5 g each) and Glycyrrhizae Radix (1.0 g).

Ching-Reh-Bu-Shiee-Tang (P4): Scrophulariae Radix, Phellodendri Cortex, Bupleuri Radix, Moutan Cortex (1.5 g each), Angelicae Radix, Rehmanniae Radix, Schizandrae Fructus, Paeoniae Radix, Ophiopogonis Radix and Ligustici Wallichii Rhizoma (3.0 g each).

All materials were obtained from retail outlets in Taipei and pulverized. Two different commercial brands of concentrated preparations of Tian-Uang-Bu-Shin-Dan and three of Bai-He-Gu-Jin-Tang were also purchased from retail outlets.

Chemicals and Reagents

2-(3-Hydroxy-4-methoxyphenyl) ethyl 1-O-[α-L-arabinopyranosyl (1→6)]-feruloyl (1→4)-α-L-rhamnopyranosyl (1→3)-β-D-glucopyranoside (SN-A) and harpagoside (SN-B) were isolated from the root of *Scrophularia ningpoensis*.[2] Chlorzoxazone and sulfadimethoxine were obtained from Sigma

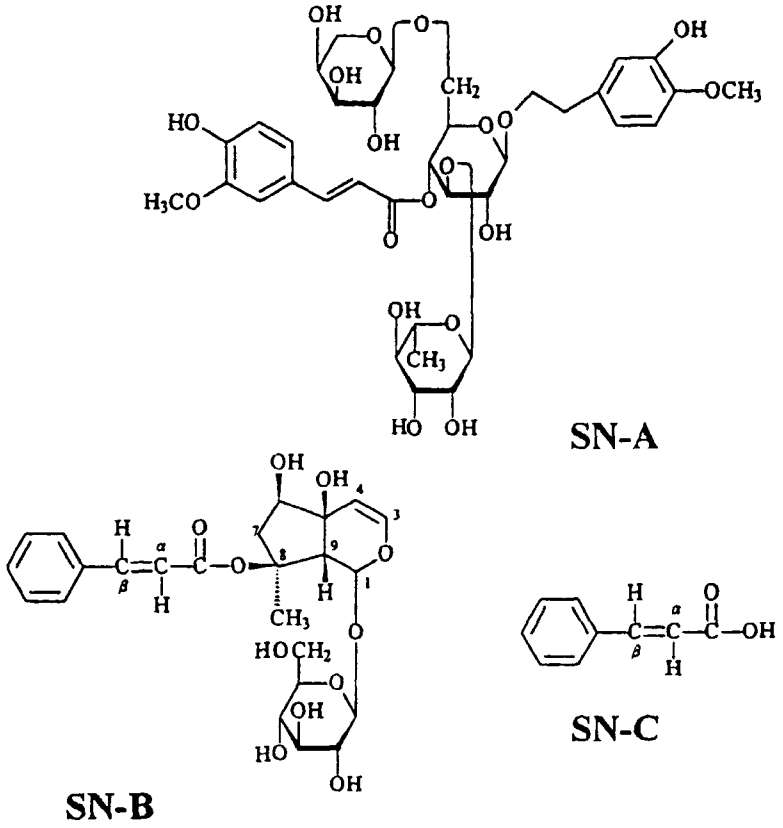

Figure 1. Structures of marker constituents.

Chemical Co. (St. Louis, U.S.A.). Cinnamic acid (SN-C) and dexamethasone were obtained from Nacalai Tesque (Kyoto, Japan). Acetonitrile and methanol were HPLC grade (purchased from Labscan, Dublin, Ireland) and acetic acid was analytical reagent grade. Ultrapure distilled water with a resistivity greater than 18 MΩ was used. The structures of the marker constituents are shown in Fig. 1.

Instruments

HPLC was conducted with a Waters 600E HPLC pump with a Waters 486 UV Detector and a Shimadzu SIL-9A autoinjector. Cosmosil 5C18-AR (5, 15 and 25 cm x 4.6 mm I.D.) reversed phase columns were used. Peak areas were calculated with a Shiunn Haw computing integrator.

Table 1

Gradient Conditions

Prescriptions	Internal Standard	Column Length (cm)	Gradient Conditions							
P1	SDM	5[a] + 25	**Time (Min)**:	0	25	26	36	42	50	53	55
			A/B/C:	12/88/0	25/75/0	15/75/10	18/72/10	20/70/10	19/71/10	18/72/10	17/73/10
P2	CZX	5[a] + 25	**Time (Min)**:	0	15	30	35				
			A/B/C:	20/80/0	20/80/0	30/70/0	20/80/0				
P3	DM	15	**Time (Min)**:	0	15	20	30	40			
			A/B/C:	15/85/0	19/81/0	19/76/5	22/73/5	28/67/5			
P4	CZX	15	**Time (Min)**:	0	40						
			A/B/C:	10/85/5	25/70/5						

[a] Connected as a guard column

Liquid Chromatography

The mobile phases which were used in these four prescriptions were mixtures of acetonitrile (A), 1.0 % (v/v) acetic acid (B) and methanol (C). Each mobile phase was filtered through a 0.45 μm Millipore filter and degassed prior to use. The flow rate was 1.0 mL/min and the detecting wavelength was 278 nm for each prescription. A constant operating temperature (room temperature) was maintained. The internal standards, sulfadimethoxine (SDM, 5.4 mg for P1), chlorzoxazone (CZX, 5.3 mg for P2 and P4), dexamethasone (DM, 5.3 mg for P3), were dissolved in 25mL of 70 % methanol respectively to give the internal standard solutions. The mobile phases, column and internal standard for each prescription are given in Table 1.

Preparation of Standard Solution

To prepare a standard solution containing SN-A, B and C, accurately weighed amounts of SN-A, B and C standard were dissolved in 70 % methanol after which an appropriate amount of internal standard solution was added to give various concentrations within the ranges 5.2-21.8, 1.04-15.60 and 1.0-20.0 μg/mL, respectively. Calibration graphs were plotted after linear regression analysis of the peak area ratios with concentrations.

Preparation of Sample Solution

Standard decoction

The individual crude drugs of each of the four kinds of Chinese medicinal prescriptions in amounts equivalent to a daily dose were weighed and pulverized separately, a twentyfold weight of water was added and the mixture of each prescription was boiled respectively for more than 30 min to half the original volume. The extract was filtered while hot. Finally it was diluted with 70 % methanol stock solution and then a suitable amount of internal standard was added to the solution to give a concentration of 10.6 μg/mL of CZX, 10.6 μg/mL of DM and 10.8 μg/mL of SDM.

Blank decoction

Amounts of individual crude drugs equivalent to a daily dose of the four kinds of Chinese medicinal prescriptions but without Scrophulariae Radix were processed as above.

Table 2

Inter-Day and Intra-Day Relative Standard Deviations (n = 5) of Marker Constituents for Chinese Medicinal Prescriptions

Prescriptions	Marker Constituents	Concentration (μg/mL)	Intraday R.S.D. (%)	Interday R.S.D. (%)
P1	SN-A	21.6	2.61	1.86
	SN-B	10.4	4.44	2.83
	SN-C	5.0	2.10	2.03
P2	SN-A	15.6	4.65	4.83
	SN-B	10.4	1.36	1.03
	SN-C	3.75	1.06	0.40
P3	SN-A	10.8	2.34	2.75
	SN-B	2.08	5.91	2.16
	SN-C	10.0	2.81	2.03
P4	SN-A	15.6	2.26	1.75
	SN-B	10.4	0.96	0.34
	SN-C	3.75	1.27	0.94

Concentrated Preparations (Tian-Uang-Bu-Shin-Dan and Bai-He-Gu-Jin-Tang) from Retail Outlets

An amount of the concentrated preparation equivalent to a daily dose was weighed accurately and extracted with 35 mL of 70 % methanol for 30 min in an ultrasonic bath. After filtration, the filtrate was diluted to 50.0 mL with 70 % methanol, and a suitable amount of internal standard was added to the solution to give a concentration of 10.8 μg/mL of SDM for Tian-Uang-Bu-Shin-Dan and 10.6 μg/mL of CZX for Bai-He-Gu-Jin-Tang. The solutions were filtered by 0.45 μm Millipore and analysed by HPLC.

Solutions for Recovery Study

Three different concentrations of markers were used for each prescription. For P1 and P3: 32.4, 54.0 and 108.0 μg/mL for SN-A; 31.2, 52.0 and 104.0 μg/mL for SN-B and 30.0, 50.0 and 100.0 μg/mL for SN-C, were added. For

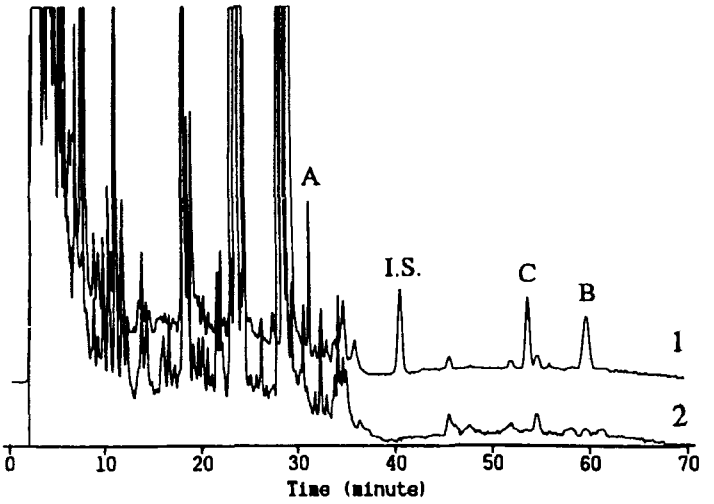

Figure 2. Chromatograms of SN-A, B and C in Tian-Uang-Bu-Shin-Dan (P1): (1) standard decoction; (2) blank decoction. Peaks: A = SN-A; B = SN-B; C = SN-C; I.S. = internal standard (sulfadimethoxine).

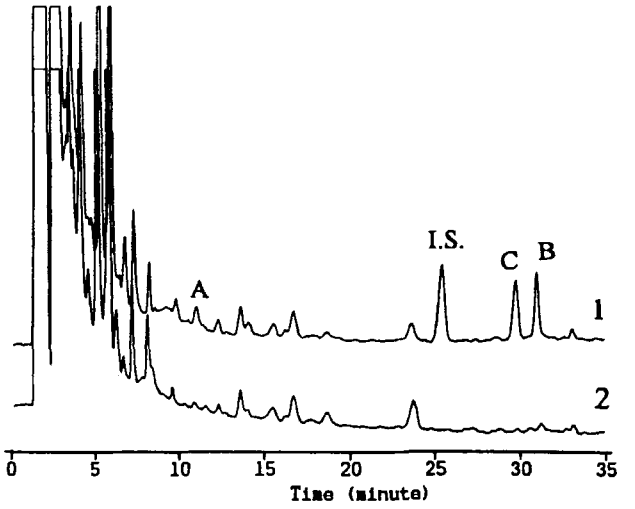

Figure 3. Chromatograms of SN-A, B and C in Bai-He-Gu-Jin-Tang (P2): (1) standard decoction; (2)blank decoction, I.S. = Internal Standard (chlorozoxazone). Marker peaks as in Figure 2.

Table 3

Recovery of SN-A, B, and C from Chinese Medicinal Prescriptions

Sample	Marker Constituents	Amount Added (μg/mL)	Amount Measured (μg/mL)	Recovery (%)	Mean ±S.D. (%)	R.S.D. (%)
		32.4	31.6	97.6		
	SN-A	54.0	56.9	105.3	100.9±3.2	3.2
		108.0	107.8	99.8		
		31.2	31.5	101.1		
P1	SN-B	52.0	46.8	90.0	98.5±0.2	0.2
		104.0	108.7	104.5		
		30.0	29.4	97.9		
	SN-C	50.0	51.9	103.8	102.8±3.6	3.5
		100.0	106.7	106.7		
		31.2	32.1	102.9		
	SN-A	52.0	48.8	93.9	99.3±3.9	3.9
		104.0	105.0	100.9		
		31.2	33.8	108.3		
P2	SN-B	52.0	50.3	96.7	101.9±4.8	4.7
		104.0	104.7	100.7		
		9.5	6.9	92.6		
	SN-C	12.5	12.0	96.1	97.0±4.0	4.1
		25.0	25.6	102.3		
		32.4	32.4	100.1		
	SN-A	54.0	56.2	104.1	101.1±2.2	2.2
		108.0	106.9	99.0		
		31.2	28.2	90.3		
P3	SN-B	52.0	47.8	92.0	94.0±4.1	4.4
		104.0	103.7	99.7		
		30.0	29.2	97.5		
	SN-C	50.0	49.8	99.6	102.1±5.2	5.1
		100.0	109.3	109.3		

(continued)

Table 3 (Continued)

Sample	Marker Constituents	Amount Added (μg/mL)	Amount Measured (μg/mL)	Recovery (%)	Mean ±S.D. (%)	R.S.D. (%)
		31.2	31.4	100.7		
	SN-A	52.0	57.3	110.2	103.0±5.1	5.0
		104.0	102.2	98.3		
		31.2	28.5	91.4		
P4	SN-B	52.0	54.9	105.5	102.2±7.8	7.6
		104.0	114.1	109.7		
		9.5	8.2	108.7		
	SN-C	12.5	12.7	101.2	104.7±3.1	3.0
		25.0	26.1	104.4		

for SN-B and 9.5, 12.5 and 25.0 μg/mL for SN-C, were added. To each solution a suitable amount (the peak height was similar to the marker peaks) of internal standard was added. All samples were filtered through a 0.45 μm Millipore filter and injected for HPLC analysis. The recoveries of SN-A, B and C were calculated from their calibration graphs.

RESULTS AND DISCUSSION

The detection wavelength of 278 nm was chosen because the absorbance of SN-B is the highest at this wavelength. The other constituents SN-A (UV $\lambda_{max}^{70\%MeOH}$: 287 nm) and C (267 nm) also show high absorbance.

The intra- and inter-assay precisions of these methods were examined with standard solutions of SN-A, B and C, five times on the first day and then once a day for a 5-day period (Table 2). The intra- and inter-assay relative standard deviations (R.S.D.s) indicate that the precision is acceptable.

To ensure the specificity and selectivity of the methods, we prepared four blank decoctions of these prescriptions for comparison. The chromatograms of P1-P4 are shown in Figs. 2-5. The retention times of the marker constituents, SN-A, B and C in P1, P2, P3 and P4, were 31.2, 59.2 and 53.6 min; 11.3, 31.1 and 29.9 min; 23.8, 45.6 and 37.6 min and 27.5, 40.8 and 34.1 min, respectively. No peaks were detected at these retention times in the four blank

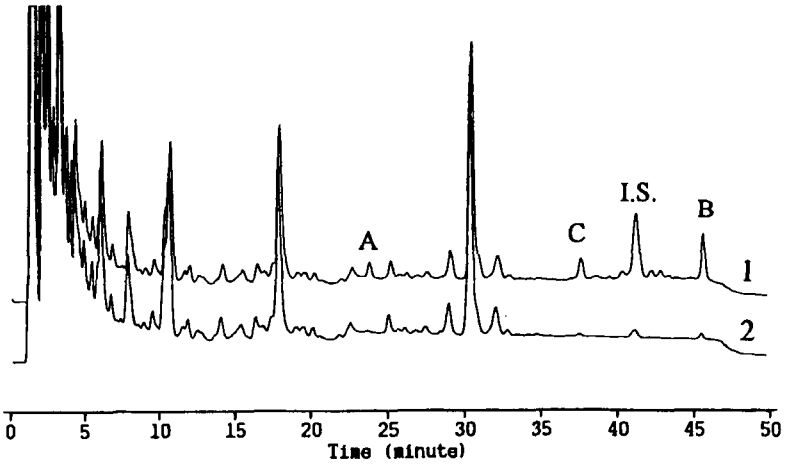

Figure 4. Chromatograms of SN-A, B and C in Bay-Du-San (P3) : (1) standard decoction; (2) blank decoction. I.S. = internal standard (dexamethasone). Marker peaks as in Figure 2.

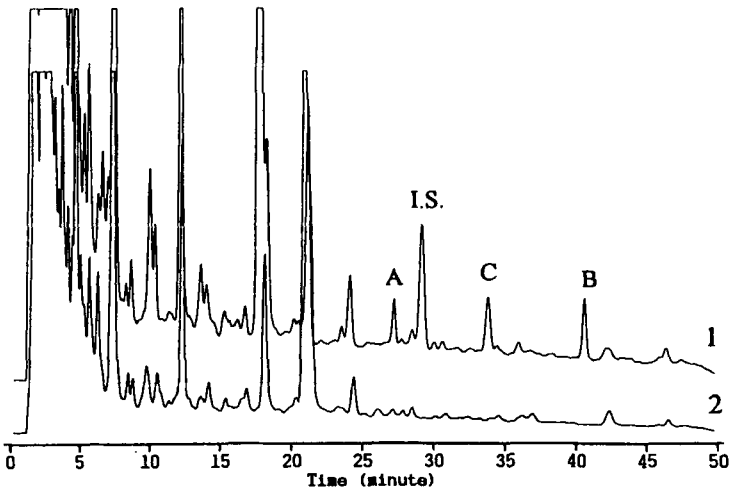

Figure 5. Chromatograms of SN-A, B and C in Ching-Reh-Bu-Shiee-Tang (P4): (1) standard decoction; (2) blank decoction. I.S. = internal standard (chlorzoxazone). Marker peaks as in Figure 2.

Table 4

The Regression Equations and Their Correlation Coefficients (r) of Marker Constituents for Chinese Medicinals Prescriptions

Prescriptions	Marker Constituents	Concentration Range (μg/mL)	Slope	Intercept	r
	SN-A	5.4-21.6	30.45	-1.28	0.9942
P1	SN-B	1.04-15.60	4.45	0.23	0.9997
	SN-C	1.0-10.0	1.61	0.01	0.9998
	SN-A	5.2-20.8	56.64	1.03	0.9943
P2	SN-B	2.08-15.60	7.65	-0.12	0.9998
	SN-C	1.25-5.00	2.78	-0.01	0.9996
	SN-A	5.4-21.6	14.09	-1.14	0.9950
P3	SN-B	1.04-15.6	2.48	0.01	0.9999
	SN-C	0.5-15.0	0.95	-0.18	0.9997
	SN-A	5.2-20.8	70.91	-1.34	0.9918
P4	SN-B	2.08-15.60	9.41	-0.70	0.9997
	SN-C	1.25-5.00	3.68	0.400	0.9992

decoctions. The recoveries of SN-A, B and C were 100.9, 98.5 and 102.8 % for P1; 99.3, 101.9 and 97.0 % for P2; 101.1, 94.0 and 102.1 % for P3; 103.0, 102.2 and 104.7 % for P4, respectively (Table 3). The R.S.D.s of SN-A, B and C ranged from 2.2 to 5.0 %, 0.2 to 7.6 % and 3.0 to 5.1 %, respectively. The regression equations and their coefficients of the marker constituents for the four Chinese medicinal prescriptions are shown in Table 4. Linear regression analysis showed good linear relationships between the peak area ratio and the concentration of marker constituents in the four prescriptions.

The contents of marker constituents in the standard decoctions are shown in Table 5. The contents of the three marker constituents, SN-A, B and C, corresponded to the contents in the crude drug (Scrophulariae Radix), 0.53-17.10, nondetected-1.75 and 0.58-5.35 mg/g, respectively.[2] The contents of marker constituents in commercial concentrated preparations of Tian-Uang-Bu-Shin-Dan (P1) and Bai-He-Gu-Jin-Tang (P2) are shown in Tables 6 and 7.

Table 5

The Content of Marker Constituents in Chinese Medicinal Prescriptions

Prescriptions	Marker Constituents		
	SN-A Mean±S.D.[a] (mg/g)	SN-B Mean±S.D.[a] (mg/g)	SN-C Mean±S.D.[a] (mg/g)
P1	2.58±0.03	0.19±0.01	0.68 ±0.02
P2	3.30±0.01	1.72±0.04	0.99±0.02
P3	2.35±0.01	0.54±0.01	0.21±0.04
P4	2.47±0.01	0.47±0.01	0.22±0.02

[a] n = 3.

Table 6

The Contents of Marker Constituents in Commercial Concentrated Herbal Preparation of Tain-Uang-Bu-Shin-Dan (P1)

Sample	Marker Constituents		
	SN-A Mean±S.D.[a] (mg/g)	SN-B Mean±S.D.[a] (mg/g)	SN-C Mean±S.D.[a] (mg/g)
1	0.86±0.002	0.15±0.01	none detected
2	0.66±0.02	0.19±0.01	0.07±0.02

[a] n = 3.

Their chromatograms are shown in Figs. 6 and 7. The contents of SN-A and C were lower than in the standard decoctions (0.30-0.86 mg/g versus 2.35-3.30mg/g and nondetected-0.12 mg/g versus 0.21-0.99 mg/g). These differences might have resulted from the manufacturing process.

Although the constituents of crude drugs are complex, and these four prescriptions were composed of at least nine kinds of crude drugs, in this study we developed HPLC methods for the determination of three markers, SN-A, B and C, in all four prescriptions. The methods diffrered only in the ratio of the

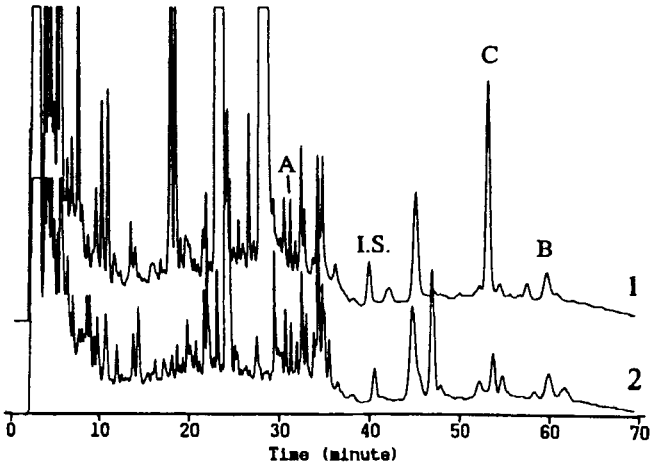

Figure 6. Chromatograms of SN-A, B and C in commercial preparations (1, 2) of Tian-Uang-Bu-Shin-Dan. Marker peaks as in Figure 2.

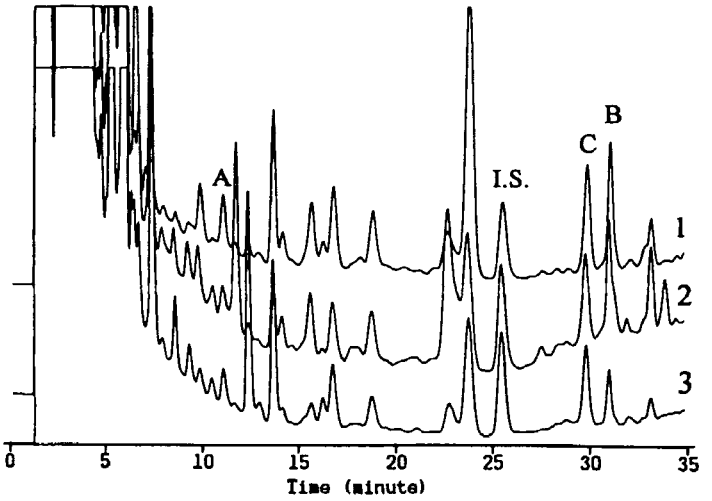

Figure 7. Chromatograms of SN-A, B and C in commercial preparations (1-3) of Bai-He-Gu-Jin-Tang. Marker peaks as in Figure 2.

Table 7

The Contents of Marker Constituents in Commercial Concentrated Herbal Preparation of Bai-He-Gu-Jin-Tang (P2)

Sample	Marker Constituents		
	SN-A Mean±S.D.[a] (mg/g)	SN-B Mean±S.D.[a] (mg/g)	SN-C Mean±S.D.[a] (mg/g)
1	0.43 ± 0.01	0.10 ± 0.01	0.08 ± 0.03
2	0.30 ± 0.01	0.21 ± 0.02	0.07 ± 0.03
3	0.84 ± 0.01	0.33 ± 0.01	0.12 ± 0.02

[a] n = 3.

solvents of the mobile phases and the internal standards. Therefore, these methods are economical and suitable for quality control of the Chinese medicinal prescriptions containing Scrophulariae Radix. For instance, Tian-Uang-Bu-Shin-Dan (P1) is composed of 14 kinds of crude drugs. The weight ratio of Scrophulariae Radix was 7.0 % in this prescription.

A 25 cm column with 5 cm of guard column was used and three solvents, acetonitrile (A), 1.0% (v/v) acetic acid (B) and methanol (C), were used as the mobile phase. Acetonitrile and acetic acid were used initially (12/88) and at 25 min (25/75) for eluting more interference peaks, then at 26 min methanol was added to the mobile phase (15/75/10) for separating SN-A. The ratio of the mixture was then changed by linear gradient: 36 min (18/72/10), 42 min (20/70/10), 50 min (19/71/10), 53 min (18/72/10) and 55 min (17/73/10) for separating SN-B and C. Bay-Du-San (P3) was complicated too, like Tian-Uang-Bu-Shin-Dan (P1). It was also separated by adding the third solvent. Nonetheless, the intensity of some of the marker peaks was weaker than that of the interference (Fig. 6).

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REFERENCES

1. H. M. Chang, P. H. But, **Pharmacology and Applications of Chinese Materia Medica**, Vol. I, World Scientific Publishing Co. Ltd., Singapore, 1986, pp. 424-427.

2. J.-H Lin, Y.-R Ku, Y.-S. Huang, J.-P. Hau,F.-M. Yarn, K.-C. Wen, W.-F. Huang, J. of Food and Drug Analysis, **4**, 131-140 (1996).

3. L. Guillerault, E. Ollivier, R. Elias, G. Balansard. J. Liq. Chromatogr, **17**, 2951-60 (1994).

4. H.-Y. Hsu, C.-S. Hsu, **Commonly Used Chinese Herb Formulas with Illustrations,** Oriental Health Arts Institute, Taiwan, 1980, pp. 145, 190, 195, and 433.

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