ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
19 (1999) 603–612

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Simultaneous high-performance liquid chromatographic determination of puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin in Kampo medicines

Nobuyuki Okamura *, Hirotsugu Miki, Hiroshi Orii, Yasue Masaoka, Satie Yamashita, Hiroshi Kobayashi, Akira Yagi

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Hiroshima 729-0292, Japan

Received 30 March 1998; received in revised form 25 June 1998; accepted 2 July 1998

Abstract

We report a high-performance liquid chromatographic method to determine the quantities of puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin in Kampo medicine. All seven compounds were separated in less than 30 min with a Wakosil-II 5C18 AR column by linear gradient elution using 0.01% (v/v) phosphoric acid–acetonitrile (0 min 90:10, 10 min 88:12, 22 min 70:30, 30 min 30:70) as the mobile phase at a flow-rate of 1.0 ml min^{-1}, and detection at 250 nm. The detection limits of these compounds are 0.15–0.3 μM with response linearity. This method was applied to determine the quantities in eight Kampo decoctions; Mao-to, Makyo-yokukan-to, Makyo-kanseki-to, Yokuinin-to, Sho-seiryu-to, Keima-kakuhan-to, Kakkon-to and Kakkon-to-ka-senkyu-sin'i. Glycyrrhizin content was lower in both the decoction and the methanol-diluted decoction of Sho-seiryu-to compared with the others. Low pH due to organic acids of Schisandrae fructus in the decoction caused inhibition for glycyrrhizin dissolution in Sho-seiryu-to.

Keywords: Kampo medicine; Puerarin; Daidzin; Paeoniflorin; Liquiritin; Cinnamic acid; Cinnamaldehyde; Glycyrrhizin

1. Introduction

Many kinds of traditional oriental medicines (Kampo medicine in Japan) have been used for treatment of a variety of diseases. Generally Kampo medicines containing Ephedrae herba are called Mao-drugs in Japan, including Mao-to, Makyo-yokukan-to, Makyo-kanseki-to, Yokuinin-to, Sho-seiryu-to, Keima-kakuhan-to, Kakkon-to and Kakkon-to-ka-senkyu-sin'i. These drugs are in strong demand. Mao-to, Kakkon-to and Keima-kakuhan-to are used to improve some syndromes that accompany cold virus infection. Makyo-kanseki-to and Sho-seiryu-to are used clinically as cough remedies. Recently, the effectiveness of Sho-seiryu-to for allergic nasal inflam-

* Corresponding author. Fax: +81-849-362024.

0731-7085/99/$ - see front matter
PII: S0731-7085(98)00262-3

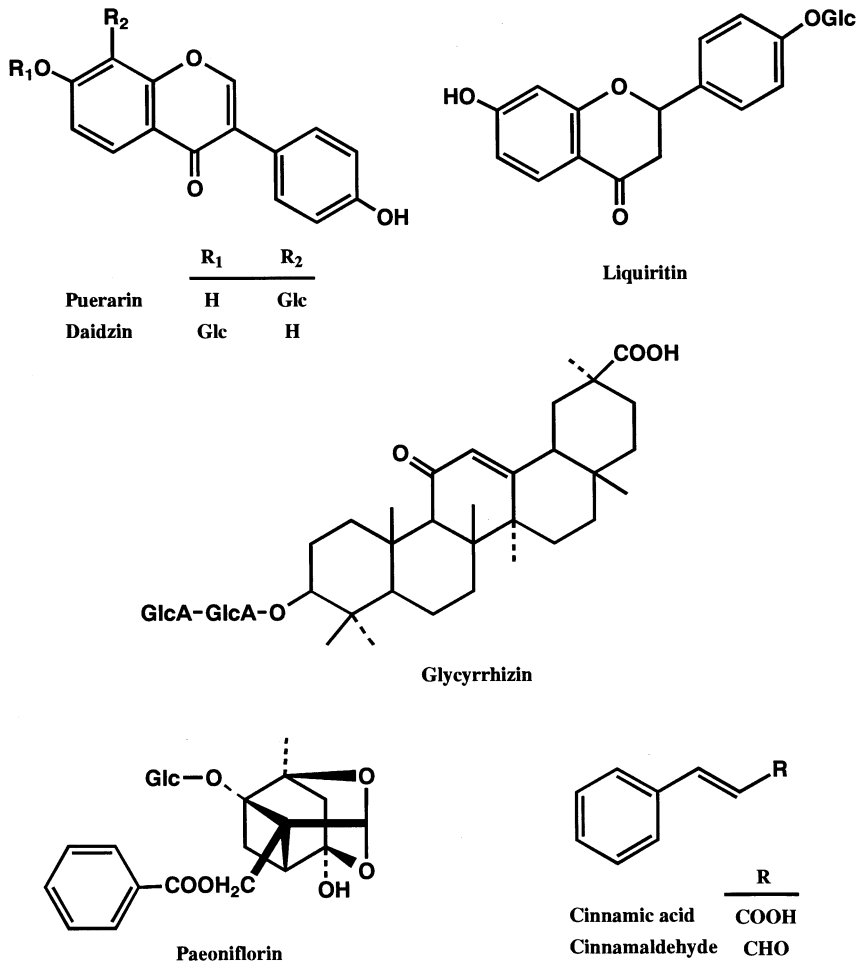

Fig. 1. Structures of the investigated compounds.

mation and bronchial asthma treatment has been recognized by double-blind trial. Kakkon-to-ka-senkyu-sin'i has been used as a sinusitis remedy. Yokuinin-to and Makyo-yokukan-to are often used to treat arthralgia. Table 1 shows the combination of crude drug to establish Mao-drug in which both Ephedrae herba and Glycyrrhizae radix are included at least.

Ephedrine, liquiritin and glycyrrhizin, cinnamic acid and cinnamaldehyde, amygdarin and paeoniflorin are marker components of Ephedrae herba, Glycyrrhizae radix, Cinnamomi cortex, Armeniacae semen and Paeoniae radix, respectively. Puerariae radix, containing puerarin and daidzin, is the main constituent in Kakkon-to and Kakkon-to-ka-senkyu-sin'i. It is necessary to analyze the percentages of the marker components in a decoction in order to evaluate Kampo medicine. High-performance liquid chromatography (HPLC) has been employed to analyze several components in a medicinal preparation composed of several crude drugs [1–3]. Ion-pair HPLC only separates ephedrine alkaloids, i.e. ephedrine, pseudoephedrine, norephedrine and methylephedrine, in Mao-drugs [4]. However, a simultaneous analysis for the other marker components in Mao-drug has not been performed. Amygdarin had not been quantitatively estimated, because it suffers from enzyme hydrolysis in the decoctions [5].

Table 1
Combinations of crude drugs in Mao-drug

Crude drug	Compounded crude drugs (g)							
	Makyo-to	Makyo-yokukan-to	Makyo-kanseki-to	Yokuinin-to	Sho-seiryu-to	Keima-kakuhan-to	Kakkon-to	Kakkon-to-ka-senkyu-sin'i
Ephedrae herba	4	4	4	4	3	2	4	4
Glycyrrhizae radix	1.5	2	2	2	3	2	2	2
Cinnamomi cortex	3			3	3	3.5	3	2
Paeoniae radix				3	3	2	3	2
Armeniacae semen	4	3	4			2.5		
Zingiberis rhizoma						2	1	1
Zingiberis siccatum rhizoma					3			
Zizyphi fructus						2	4	3
Puerariae radix							8	4
Coicis semen		10		8				
Asiasari radix					3			
Schisandrae fructus					3			
Pinelliae tuber					6			
Angelicae radix				4				
Atractylodis rhizoma				4				
Cnidii rhizoma								3
Magnoliae flos								3
Gypsum fibrosum			10					

Table 2
Retention times, capacity factors, linear response ranges, regression equations, correlation coefficient for puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin

Maker substance	t_R (min)	k'	Linear response range (μM–mM)	Regression equation[a]	Correlation coefficient[b]	Detection limit[c] (μM)
Puerarin	4.52	2.12	0.29–1.18	$y = 7.00x - 0.08$	1.0000	0.15
Daidzin	7.34	4.06	0.30–1.19	$y = 8.04x - 1.97$	1.0000	0.15
Paeoniflorin	8.81	5.08	1.56–6.25	$y = 0.60x + 0.23$	1.0000	0.39
Liquiritin	11.94	7.23	1.50–5.98	$y = 1.08x + 1.14$	1.0000	0.38
Cinnamic acid	22.57	14.57	0.42–1.68	$y = 2.28x - 0.84$	1.0000	0.21
Cinnamaldehyde	23.68	15.33	0.45–1.82	$y = 0.87x - 0.17$	1.0000	0.23
Glycyrrhizin	27.05	17.62	0.60–2.41	$y = 3.40x - 3.27$	1.0000	0.15

[a] y = peak-area, x = μM.
[b] Triplicate assay about the different concentration ($n = 10$).
[c] Signal-to-noise ratio = 3.

The aims of this research were to develop a HPLC method to quantify seven marker substances, puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin (Fig. 1) simultaneously in Mao-drug, and to apply the chromatographic mean for the analysis of combination in Kampo medicines.

2. Experimental

2.1. Materials

Kampo medicines were prepared according to the literature [6] (Table 1). Crude drugs were purchased from Tochimototenkaido (Osaka, Japan). Reagent-grade chemicals and high-purity solvents were used except when specified otherwise. Ultrapure distilled water with a resistivity greater than 18 MΩ was prepared with deionized-distilled water. Acetonitrile was of HPLC grade and other solvents and chemicals were purchased from Wako (Osaka, Japan). Millipore syringe filters (Millex-GP, 0.22 μm pore size) were purchased from Nihon Millipore (Tokyo, Japan). Puerarin and daidzin were purchased from Funakoshi (Tokyo, Japan), paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin from Wako.

2.2. Instrumentation and HPLC analysis

The HPLC apparatus was a Hewlett-Packard (Waldbronn, Germany) system composed of a HP1100 series binary pump, a HP1100 series photodiode array detector and a HP1100 series autosampler (set at 5 μl). A HP1100 series ChemStation (Hewlett-Packard) was used for data acquisition and integration. Separations were carried out with a Wako Wakosil-II 5C18 AR reversed-phase column (particle size of the packing 5 μm, 150 × 4.6 mm i.d.). Five microliters were injected onto the HPLC system. The column eluent was monitored at UV 250 nm. The mobile phase was a linear gradient of 0.01% (v/v) phosphoric acid-acetonitrile (0 min 90:10, 10 min 88:12, 22 min 70:30, 30 min 30:70) and degassed with an ultrasonic bath prior to use. A re-equilibration period of 13 min was used between individual runs. Chromatography was performed at 45°C with a flow-rate of 1.0 ml min^{-1}. The identification and the purity of the chromatographic peaks were estimated with a HP1100 series photodiode array detector.

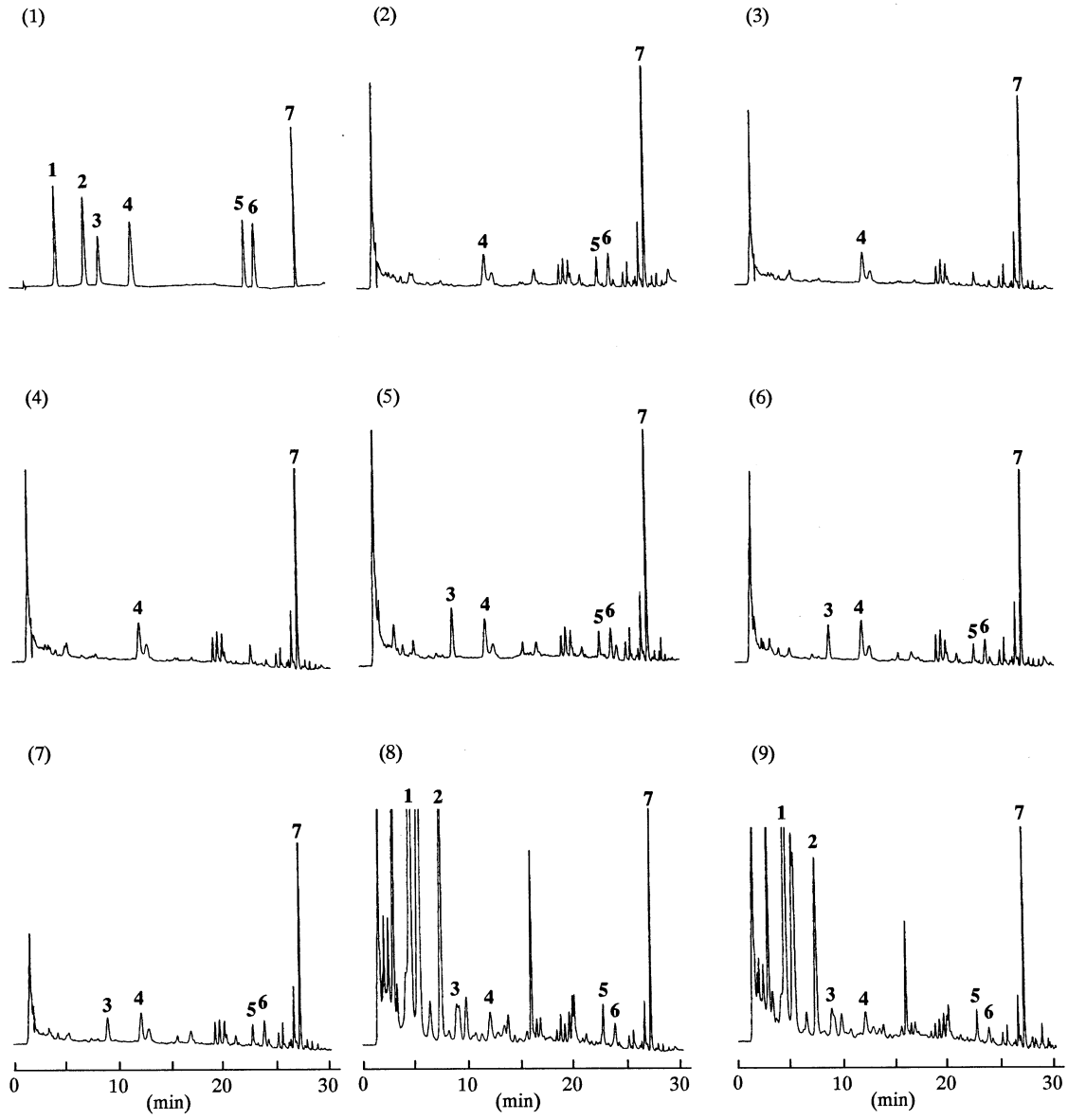

Fig. 2. Chromatograms of standard mixture (1) and Kampo decoctions (2–9). Chromatograms: (2) Mao-to; (3) Makyo-yokukan-to; (4) Makyo-kanseki-to; (5) Yokuinin-to; (6) Sho-seiryu-to; (7) Keima-kakuhan-to; (8) Kakkon-to; (9) Kakkon-to-ka-senkyu-sin'i. Peaks: 1 = puerarin; 2 = daidzin; 3 = paeoniflorin; 4 = liquiritin; 5 = cinnamic acid; 6 = cinnamaldehyde; 7 = glycyrrhizin.

2.3. Standard curve preparation

Each marker substance, puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin, was accurately weighed and dissolved in methanol to give serial concentrations within the ranges 0.121–900, 0.125–900, 0.749–4700, 0.627–4800, 0.062–870, 0.059–650 and 0.493–2600 μg ml^{-1}, respectively. The standard curve was analyzed using the linear least-squares regression equation derived from the peak area. Concentrations of these marker sub-

stances in samples were calculated from this regression analysis.

2.4. Preparation of sample solutions

A daily dosage of crude drugs compounded according to each Kampo medicine was placed in a 1-l beaker and boiled with 500 ml water on an electric heater for more than 35 min halving the original volume. The decoction was filtered through a colander while hot, and the volume adjusted to 250 ml with water after cooling. The adjusted decoction was centrifuged ($1500 \times g$ for 10 min) and the supernatant was filtered through a Millipore syringe filter unit and analyzed as the decoction. In addition, the adjusted decoction (10 ml) was made up to 50 ml with methanol and the mixture was filtered through a Millipore syringe filter unit and analyzed as the methanol-diluted decoction.

Table 3
Within-day and day-to-day relative standard deviations (RSD) for marker substances in Mao-to, Kakkon-to and Sho-seiryu-to[a]

Marker substance	RSD (%)	
	Within-day[b]	Day-to-day[c]
Mao-to		
Liquiritin	0.80	1.39
Cinnamic acid	0.42	0.66
Cinnamaldehyde	0.43	1.39
Glycyrrhizin	0.37	0.75
Kakkon-to		
Puerarin	0.35	1.74
Daidzin	0.48	4.40
Paeoniflorin	1.85	1.68
Liquiritin	0.95	1.69
Cinnamic acid	1.02	2.56
Cinnamaldehyde	0.57	1.31
Glycyrrhizin	0.45	1.39
Sho-seiryu-to		
Paeoniflorin	1.56	2.23
Liquiritin	1.66	1.47
Cinnamic acid	2.94	3.48
Cinnamaldehyde	1.59	1.39
Glycyrrhizin	0.48	1.63

[a] Methanol-diluted decoction was used.
[b] Within-day precision test at ten times in 1 day.
[c] Day-to-day precision on 5 different days.

2.5. Interference trial

Amounts of crude drugs equivalent to a daily dose of each Kampo medicine without, one at a time, Puerariae radix, Paeoniae radix, Glycyrrhizae radix and Cinnamomi cortex were weighed, 500 ml water was added and each mixture was boiled for more than 35 min halving the original volume. The decoction was filtered through a colander while hot, and the volume adjusted to 250 ml with water after cooling and centrifugation. The supernatant was diluted with methanol to give a five-fold dilute decoction and the mixture was filtered through a Millipore syringe filter unit and used for analysis of blank decoction.

2.6. Solutions for recovery study

Recoveries of puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin added to decoctions of Mao-to, Kakkon-to and Sho-seiryu-to were calculated from comparing three sets of chromatograms: standard solution, decoctions and spiked decoctions. Standard solutions were prepared by methanol extract of freeze-dried decoctions. The purity of marker substances in the standard solutions were estimated with a photodiode array detector. Each decoction was extracted as previously described. Decoctions were divided into eight portions, three controls and five spiked solutions. The control decoctions (20 ml) were made up to a final volume (100 ml) with methanol. The spiked decoctions (20 ml) were prepared by adding the standard solutions to the decoctions, and brought to the final volume. All samples were filtered through a Millipore syringe filter unit and injected for HPLC analysis to calculate recovery.

3. Results and discussion

Absorption maxima of puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin were observed around 215–290 nm on the UV spectra with three dimensional chromatograms and a monitoring wavelength for

Table 4
Recovery of puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin from Mao-to, Kakkon-to and Sh-seiryu-to

Marker substance	Initial amount (μg ml^{-1})	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery[a] (%)
Mao-to				
Liquiritin	22.92	10.03	33.29	101.0
		19.93	43.52	101.6
Cinnamic acid	1.62	0.83	2.44	99.7
		1.68	3.30	99.9
Cinnamaldehyde	9.21	1.32	10.24	97.2
		2.83	11.39	94.8
Glycyrrhizin	46.83	17.70	64.16	99.4
		34.65	81.49	100.0
Kakkon-to				
Puerarin	138.67	68.61	207.24	100.0
		134.96	273.80	100.1
Daidzin	21.64	12.59	34.60	101.1
		24.53	46.43	100.6
Paeoniflorin	55.11	32.82	87.17	99.1
		64.06	119.00	99.9
Liquiritin	26.78	13.06	40.05	100.5
		26.45	53.13	99.8
Cinnamic acid	1.45	0.68	2.09	97.9
		1.33	2.75	98.8
Cinnamaldehyde	7.32	1.53	8.37	94.6
		3.85	9.57	85.7
Glycyrrhizin	46.64	12.78	58.76	98.9
		24.51	70.13	98.6
Sho-seiryu-to				
Paeoniflorin	49.77	20.55	70.40	100.1
		40.95	91.02	100.3
Liquiritin	28.71	15.11	43.92	100.2
		28.86	58.13	101.0
Cinnamic acid	0.89	0.42	1.25	95.7
		0.78	1.61	96.5
Cinnamaldehyde	5.64	0.93	6.37	97.0
		2.00	7.11	93.0
Glycyrrhizin	41.57	17.83	59.07	99.4
		34.71	76.01	99.7

[a] Values are means of five experiments.

quantitative determination at 250 nm was used. Table 2 gives the retention times (t_R), capacity factors (k'), linear response range, regression equation, correlation coefficient and detection limit of puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin. These results, through linear regression analysis, showed a good linear relationship between the peak-area (y) and the concentration (x: μM).

The chromatograms of standard mixture, Mao-to, Makyo-yokukan-to, Makyo-kanseki-to, Yokuinin-to, Sho-seiryu-to, Keima-kakuhan-to, Kakkon-to and Kakkon-to-ka-senkyu-sin'i are shown in Fig. 2. These peaks were shown to be satisfactorily separated and were identified with standards by inspection of retention times and UV spectra in three-dimensional chromatograms. There were no overlapping peaks within marker

substances in the decoctions which were made by excluding Puerariae radix, Paeoniae radix, Glycyrrhizae radix and Cinnamomi cortex from Mao-to, Makyo-yokukan-to, Makyo-kanseki-to, Yokuinin-to, Sho-seiryu-to, Keima-kakuhan-to, Kakkon-to and Kakkon-to-ka-senkyu-sin'i, respectively. Separation of marker substances in these Kampo decoctions was successful with a gradient elution of 0.01% (v/v) phosphoric acid-acetonitrile in less than 30 min.

The within-day and the day-to-day precision of this method for each marker substance were evaluated using decoctions of Mao-to, Kakkon-to and Sho-seiryu-to, by ten same-day replicate assays and assays on five different days. As Table 3

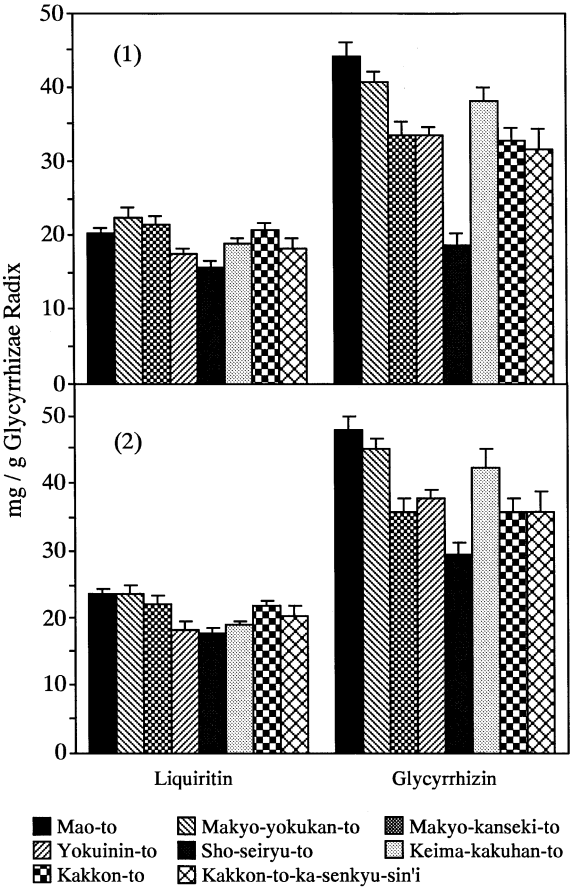

Fig. 3. Relative amounts of liquiritin and glycyrrhizin in Kampo decoctions. (1) were decoctions, (2) were methanol-diluted decoctions.

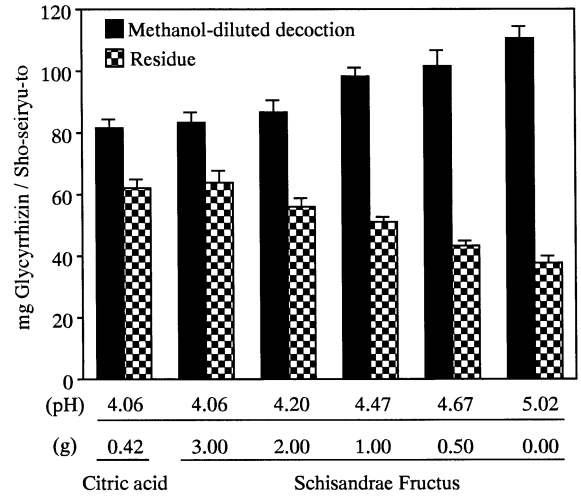

Fig. 4. Effect of Schisandrae fructus and citric acid in the methanol-diluted decoction of Sho-seiryu-to on the extraction of glycyrrhizin.

shows, the within-day and day-to-day relative standard deviations (RSDs) were 0.35–2.94% and 0.66–4.40%, respectively. The average recoveries of standards spiked into decoctions of Mao-to, Kakkon-to and Sho-seiryu-to were 100.0–100.1% for puerarin, 100.6–101.1% for daidzin, 99.1–100.3% for paeoniflorin, 99.8–101.6% for liquiritin, 95.7–99.9% for cinnamic acid, 85.7–97.2% for cinnamaldehyde and 98.6–100.0% for glycyrrhizin, as shown in Table 4.

The quantitative results in decoctions and methanol-diluted decoctions of Mao-to, Makyo-yokukan-to, Makyo-kanseki-to, Yokuinin-to, Sho-seiryu-to, Keima-kakuhan-to, Kakkon-to and Kakkon-to-ka-senkyu-sin'i are shown in Table 5. Glycyrrhizin in both the decoction and the methanol-diluted decoction of Sho-seiryu-to showed a relatively lower content than that of other decoction, whereas liquiritin had no significant difference among these Kampo decoctions (Fig. 3). Previously, it has been shown that the analysis value of glycyrrhizin in Sho-seiryu-to is low, but the cause has not been clearly explained [7]. By comparison of the decoction and the methanol-diluted decoction, the content of glycyrrhizin in the methanol-diluted decoction increased by 1.58 times. Therefore, the solubility was deeply related to the content of glycyrrhizin

Table 5
Contents of puerarin, daidzin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde and glycyrrhizin in Kampo decoctions

Marker substance		Contents (mg/g crude drug)[a]							
		Makyo-to	Makyo-yokukan-to	Makyo-kanseki-to	Yokuinin-to	Sho-seiryu-to	Keima-kakuhan-to	Kakkon-to	Kakkon-to-ka-senkyu-sin'i
Puerarin	W[b]							28.9 ± 0.37	28.8 ± 1.54
	M[c]							28.7 ± 1.02	28.6 ± 1.41
Daidzin	W							4.6 ± 0.10	4.1 ± 0.30
	M							4.9 ± 0.17	4.5 ± 0.36
Paeoniflorin	W				23.7 ± 1.18	23.6 ± 0.91	26.7 ± 1.81	29.9 ± 1.71	33.0 ± 3.16
	M				24.8 ± 1.37	25.2 ± 1.18	27.9 ± 1.99	27.8 ± 1.41	28.3 ± 1.94
Liquiritin	W	20.3 ± 0.70	22.3 ± 1.38	21.4 ± 1.30	17.5 ± 0.73	15.6 ± 1.03	18.9 ± 0.75	20.7 ± 0.88	18.3 ± 1.18
	M	23.5 ± 0.86	23.7 ± 1.26	22.0 ± 1.39	18.3 ± 1.13	17.6 ± 0.78	18.9 ± 0.72	21.7 ± 0.98	20.4 ± 1.32
Cinnamic acid	W	0.8 ± 0.03			0.8 ± 0.03	0.7 ± 0.03	0.7 ± 0.05	1.3 ± 0.04	1.4 ± 0.10
	M	0.9 ± 0.03			0.8 ± 0.05	0.7 ± 0.02	0.7 ± 0.04	1.3 ± 0.04	1.6 ± 0.10
Cinnamaldehyde	W	2.5 ± 0.16			2.2 ± 0.20	2.7 ± 0.25	2.6 ± 0.25	2.4 ± 0.23	2.6 ± 0.18
	M	2.9 ± 0.17			2.6 ± 0.19	3.1 ± 0.26	2.8 ± 0.22	2.7 ± 0.19	2.8 ± 0.23
Glycyrrhizin	W	44.0 ± 1.98	40.5 ± 1.54	33.5 ± 1.87	33.4 ± 1.28	18.6 ± 1.64	38.0 ± 1.96	32.7 ± 1.80	31.7 ± 2.61
	M	48.1 ± 1.92	45.1 ± 1.54	35.8 ± 2.12	37.9 ± 1.15	29.3 ± 1.80	42.4 ± 2.71	35.8 ± 1.90	35.8 ± 3.05

[a] See Table 1, $n = 5$.
[b] W, decoction.
[c] M, methanol-diluted decoction.

in the decoction of Sho-seiryu-to. The crude drug residue after extraction with boiling water was extracted again with boiling methanol (150 ml) for 1 h, and glycyrrhizin in each extract that was adjusted to 200 ml after cooling was analyzed. The ratio of glycyrrhizin in the extract from each crude drug residue, were 0.13, 0.14, 0.23, 0.41, 0.76, 0.25, 0.44 and 0.46, when compared with the methanol-diluted decoctions of Mao-to, Makyo-yokukan-to, Makyo-kanseki-to, Yokuinin-to, Sho-seiryu-to, Keima-kakuhan-to, Kakkon-to and Kakkon-to-ka-senkyu-sin'i, respectively. Thus, the extraction of glycyrrhizin was proved to be inefficient in the original decoction of Sho-seiryu-to, as 76% of glycyrrhizin in the decoction remained in the crude drug residue. The pH in the decoctions of Mao-to, Makyo-yokukan-to, Makyo-kanseki-to, Yokuinin-to, Keima-kakuhan-to, Kakkon-to and Kakkon-to-ka-senkyu-sin'i was 5.17, 5.27, 5.10, 5.17, 5.02, 5.05 and 5.08, respectively. However, the decoction of Sho-seiryu-to showed pH 4.06. A correlation between glycyrrhizin extraction efficiency and pH in the decoction of Kampo medicines containing Glycyrrhizae radix has not been previously investigated [8]. We therefore examined whether the pH of the decoction could influence the solubility and the extraction efficiency of glycyrrhizin, which has two glucuronic acid moieties bound as a glycoside. Some coexistent components prevented the dissolution of specific components in both the crude drug and Kampo medicine by precipitation [9,10] and adsorption [8,11]. Schisandrae fructus contains a number of organic acids as citric acid, and the organic acids may play an important role for low pH in Sho-seiryu-to. A daily dose of Sho-seiryu-to without Schisandrae fructus was added 0.0, 0.5, 1.0, 2.0 or 3.0 g (original amount in Sho-seiryu-to) of Schisandrae fructus and each decoction was extracted as previously described. As shown in Fig. 4, the content of glycyrrhizin in each methanol-diluted decoction compared to that in the residue was inversely proportional. Citric acid (0.42 g) was added to Sho-seiryu-to from which only Schisandrae fructus was excluded, to make pH at 4.06 (after extraction). Glycyrrhizin content in the methanol-diluted decoction was equivalent to that of Sho-seiryu-to methanol-diluted decoction. Similar experimental findings to methanol-diluted decoction were obtained in the decoction. It is therefore reasonable to conclude that the lower glycyrrhizin content in the Sho-seiryu-to decoction comes from low pH from the organic acids in Schisandrae fructus. Quantification of marker substance in prescriptions by simultaneous HPLC method is essential for clinical evaluation of Kampo medicines.

Acknowledgements

The authors are grateful to Miss Takako Harada, Miss Yoko Ono and Mr Tomonori Maki for their technical assistance.

References

[1] K.-C. Wen, C.-Y. Huang, F.-S. Liu, J. Chromatogr. 593 (1992) 191–199.
[2] Y.-C. Lee, C.-Y. Huang, K.-C. Wen, T.-T. Suen, J. Chromatogr. A 660 (1994) 299–306.
[3] Y.-C. Lee, C.-Y. Huang, K.-C. Wen, T.-T. Suen, J. Chromatogr. A 692 (1995) 137–145.
[4] K. Sagara, T. Oshima, T. Misaki, Chem. Pharm. Bull. (Tokyo) 31 (1983) 2359–2365.
[5] N. Hitomi, K. Shimizu, K. Yamamoto, O. Miura, E. Yumioka, Bunseki Kagaku 35 (1986) 202–206.
[6] Supervision by the Pharmaceutical and Supply Bureau of the Welfare Ministry of Japan (Ed.), Ippanyo Kampo Syoho No Tebiki, Yakugyo Jihosya, Tokyo, 1976.
[7] S. Ogawa, A. Yoshida, Y. Mitani, Yakugaku Zasshi 96 (1976) 1488–1491.
[8] T. Tomimori, M. Yoshimoto, Shoyakugaku Zasshi 34 (1980) 138–144.
[9] N. Noguchi, M. Kubo, T. Hayashi, M. Ono, Chem. Pharm. Bull. (Tokyo) 26 (1978) 3652–3657.
[10] T. Okuda, K. Mori, M. Shiota, Yakugaku Zasshi 102 (1982) 854–858.
[11] N. Noguchi, M. Kubo, Y. Naka, Yakugaku Zasshi 98 (1978) 923–928.