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NOTE

Simultaneous analysis of nine components in patch preparations of Ru-Yi-Jin-Huang-San by high-performance liquid chromatography

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Abstract A facile high-performance liquid chromatography (HPLC) method for the resolution and quantitative measurement of nine marker substances, the active ingredients in patch preparations of Ru-Yi-Jin-Huang-San, was established using gradient elution in the reversed-phase mode. These marker substances included berberine (Phellodendri Cortex), curcumin (Curcumae Rhizoma), imperatorin (Angelicae Dahuricae Radix), magnolol (Magnoliae Cortex), hesperidin (Citri Leiocarpae Exocarpium), glycyrrhizin (Glycyrrhizae Radix), and emodin, sennoside A, sennoside B (Rhei Rhizoma). The ingredients in the water-based and oil-based patches of the formula from different manufactures were also analyzed for quality evaluation. Extracted samples were analyzed by HPLC using a reversed-phase column (Inertsil 5 ODS-2, 4.6-mm I.D. ×250 mm) at 30°C and eluted with a mixture of 20 and 70% acetonitrile aqueous solution in gradient manner at a flow rate of 1.0 ml/min. The detection wavelength varied with time as follows: 275 nm, 0–72 min; 250 nm, 72–105 min; 220 nm, 105–145 min. Relative coefficients of variations of intra- and interday analysis were less than 5%. All the recoveries were 93.30–113.63%. This method could be applied for the simultaneous determination of nine marker substances in Ru-Yi-Jin-Huang-San.

Keywords Ru-Yi-Jin-Huang-San · Berberine · Curcumin · Imperatorin · Magnolol · Hesperidin · Glycyrrhizin · Emodin · Sennoside A · Sennoside B

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Introduction

Since 2001 the Department of Health (DOH), Taiwan, has been promoting the use of high-performance liquid chromatography (HPLC) methods for quantitative analysis of ingredients in Chinese medicinal preparations. For example, when a manufacturer's product license expires, relevant analysis documentation is required to be included in the renewal application [1]. In Japan, since 1985 the Ministry of Health and Welfare has required that all concentrated herbal preparations submitted for inspection and registration should include a content analysis with at least two chemical components as markers [2]. Therefore, precise and reliable methods for marker substances analysis is an important factor in upgrading the qualities of Chinese medicinal preparations.

Recently, a number of analytical methods for the analysis of Chinese medicinal preparations have been established in our laboratory [3–11]. However, a method for analyzing the Ru-Yi-Jin-Huang-San, a very popular Chinese medicinal patch preparation, well known for its efficacy, e.g., in reducing swelling, removing poisons, and relieving pain, has, thus far, been unavailable due to the complexity of the product. The patch preparation contains active ingredients from a variety of Chinese crude drugs including Trichosanthis Radix, Phellodendri Cortex, Rhei Rhizoma, Curcumae Rhizoma, Angelicae Dahuricae Radix, Magnoliae Cortex, Citri Leiocarpae Exocarpium, Glycyrrhizae Radix, Atractylodis Lanceae Rhizoma, and Arisaematis Rhizoma. Analytical methods for these Chinese crude drugs were reported [11–23], but no analytical methods for Ru-Yi-Jin-Huang-San have been reported.

The goal of this study was to determine the nine marker substances in Ru-Yi-Jin-Huang-San simultaneously using an HPLC method. In this study, the nine marker substances berberine (Phellodendri Cortex), emodin, sennoside A, sennoside B (Rhei Rhizoma), curcumin (Curcumae Rhizoma), imperatorin (Angelicae Dahuricae Radix), magnolol (Magnoliae Cortex), hesperidin (Citri Leiocarpae Exocarpium), and glycyrrhizin (Glycyrrhizae Radix) were resolved and quantitatively measured using a reversed-phase HPLC approach. The method developed will be demonstrated to be facile in the routine analysis for quality control by quantitatively determining the active ingredients in water-based and oil-based patches of Ru-Yi-Jin-Huang-San from different manufactures.

Materials and methods

Materials

The crude drugs for the Ru-Yi-Jin-Huang-San preparation are Trichosanthis Radix, Phellodendri Cortex, Rhei Rhizoma, Curcumae Rhizoma, Angelicae Dahuricae Radix, Magnoliae Cortex, Citri Leiocarpae Exocarpium, Glycyrrhizae Radix, Atractylodis Lanceae Rhizoma, and Arisaematis Rhizoma. Each material was obtained from a local herbal market and pulverized through a #8 mesh sieve (2.36 mm). The origins of crude drugs were identified by microscopic and thin-layer chromatography (TLC) examinations. Voucher specimens were deposited in the department of Plant Industry, National Pingtung University Science and Technology.

Oil-based and water-based patches of Ru-Yi-Jin-Huang-San were obtained from Sheng Chun Tang Pharmaceutical Co., Ltd., Taiwan.

Chemicals and reagents

The structures of the nine marker substances are shown in Fig. 1. Curcumin and glycyrrhizin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Emodin, sennoside A, and sennoside B were purchased from Extrasynthese (Genay, France). Berberine, imperatorin, magnolol, hesperidin, and internal standard paeonol were purified and identified in our laboratory.

The 95% ethanol was purchased from Taiwan Tobacco and Wine Board (Taipei, ROC). Acetonitrile and methanol (HPLC grade) were obtained from Mallinckrodt, Inc. (NJ, USA), and phosphoric acid from Kanto Chemical (Tokyo, Japan). Ultra-pure distilled water with a resistivity greater than 18 MΩ was obtained from a Millipore mini-Q system (Bedford, MA, USA). Samples for HPLC were filtered through a 0.45-μm Millipore membrane filter (Bedford, MA, USA). All other reagents were analytical grade.

HPLC instruments and conditions

HPLC separation was conducted using an Hitachi system equipped with a degasser DG-2410, pump L-7100, UV/Vis detector L-7420, photodiode array detector L-4500, and autosampler L-7200. Peak areas were calculated with D-7000 HSM software.

A reversed-phase column, Inertsil 5 ODS-2 (Nacalai, 4.6-mm I.D. ×250 mm), was used. The column oven temperature was set at 30°C. The mobile phases consisting of 20 and 70% acetonitrile aqueous solutions in

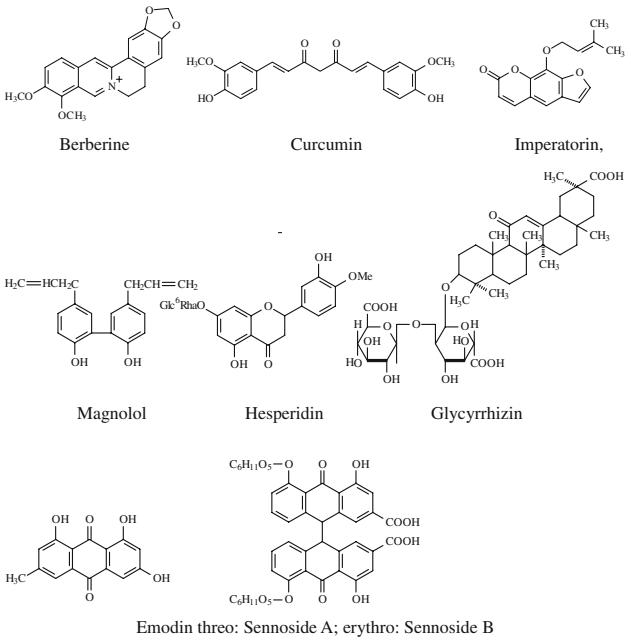

Fig. 1 Structures of the marker substances in Ru-Yi-Jin-Huang-San

gradient elution are shown in Table 1. The detection wavelength varied with time as follows: 275 nm, 0–72 min; 250 nm, 72–105 min; 220 nm, 105–145 min. The flow rate was set at 1.0 ml/min. The volume for each injection was 20 μl.

Extraction conditions

According to Ref. [24], Ru-Yi-Jin-Huang-San consists of 25.0 g of Trichosanthis Radix, 12.5 g of Phellodendri Cortex, 12.5 g of Rhei Rhizoma, 12.5 g of Curcumae Rhizoma, 12.5 g of Angelicae Dahuricae Radix, 5.0 g of Magnoliae Cortex, 5.0 g of Citri Leiocarpae Exocarpium, 5.0 g of Glycyrrhizae Radix, 5.0 g of Atractylodis Lanceae Rhizoma, and 5.0 g of Arisaematis Rhizoma. In order to obtain better extraction rates of these nine marker substances from Ru-Yi-Jin-Huang-San, solvents such as sesame oil, 50% ethanol, ethanol, and water were used. A 100-g batch of the above-mentioned Chinese crude drugs was extracted by using four different methods (A–D) as listed below. The purpose of this test was to optimize extraction conditions for Ru-Yi-Jin-Huang-San. And the extraction is able to be applied to the manufacture of both oil-based and water-based patches. The following extraction methods were used:

Table 1 Gradient elution program using mobile phase A and B

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	97	3
10	97	3
25	94	6
36	94	6
45	88	6
50	87	12
60	87	13
70	70	30
75	57	43
80	55	45
88	55	45
90	40	60
100	28	72
106	15	85
110	15	85
120	10	90
130	0	100
140	0	100
145	97	3

Flow rate 1.0 ml/min

A 20% acetonitrile (adjusted to pH 2.8 with phosphoric acid), *B* 70% acetonitrile (adjusted to pH 2.8 with phosphoric acid)

A. Addition of 1000 ml of sesame oil; the mixture was stored at room temperature (25°C) for 1 day and then heated at 150°C for 3 h in a reflux condenser
B. Addition of 1000 ml of 50% ethanol and then heated at 90°C for 3 h in a reflux condenser
C. Addition of 1000 ml of 95% ethanol and then heated at 80°C for 3 h in a reflux condenser
D. Addition of 1000 ml of water and then heated at boiling temperature for 3 h in a reflux condenser

The extract from method A was partitioned between *n*-hexane and methanol. The methanol layer was evaporated under vacuum and adjusted to 50.0 ml by adding 70% methanol; a suitable amount of internal standard paeonol was then added to the solution to give a concentration of 216.0 μg/ml. The extracts from method B, C, and D were evaporated under vacuum and adjusted to 50.0 ml by adding 70% methanol. A 1.0-ml aliquot of each solution was diluted to 5.0 ml with 70% methanol solution, and internal standard paeonol was added to give a concentration of 216.0 μg/ml. All solutions were then analyzed using HPLC.

Preparation of standard solution and internal standard solution

The standard solutions were prepared by dissolving the amount of each marker substance indicated in parenthesis in 70% methanol solution to obtain the desired concentration: berberine (400.0 μg/ml), emodin (120.0 μg/ml), sennoside A (320.0 μg/ml), sennoside B (40.0 μg/ml), curcumin (720.0 μg/ml), imperatorin (120.0 μg/ml), magnolol (80.0 μg/ml), hesperidin (560.0 μg/ml), and glycyrrhizin (480.0 μg/ml).

The internal standard solution (432.0 μg/ml) was prepared by dissolving 108.0 mg of paeonol in of 250.0 ml 70% methanol.

Preparation of sample solution from oil-based patch

Three pieces of oil-based patch of Ru-Yi-Jin-Huang-San were extracted with 500.0 ml of *n*-hexane by refluxing at 75°C for 3 h. The *n*-hexane extract was then partitioned with methanol, the methanol layer was evaporated under vacuum and adjusted to 25.0 ml by adding 80% methanol, and added with a suitable amount of internal standard paeonol to give a concentration of 216.0 μg/ml.

Preparation of sample solution from water-based patch

Three pieces of water-based patch of Ru-Yi-Jin-Huang-San were extracted with 500.0 ml of methanol by refluxing for 3 h. The extract was evaporated under vacuum and

adjusted to 25.0 ml by adding 80% methanol, and internal standard paeonol was added to the solution to give a concentration of 216.0 μg/ml.

Calibration method

The standard solutions of each marker substance were prepared from the stock solutions by diluting with 80% methanol to give concentrations of berberine: 6.25, 12.5.0, 25.0, 50.0, 100.0, and 200.0 μg/ml; sennoside B: 0.625, 1.25.0, 2.5.0, 5.0, 10.0, and 20.0 μg/ml; hesperidin: 8.75, 17.5, 35.0, 70.0, 140.0, and 280.0 μg/ml; sennoside A: 5.0, 10.0, 20.0, 40.0, 80.0, and 160.0 μg/ml; glycyrrhizin: 7.5, 15.0, 30.0, 60.0, 120.0, and 240.0 μg/ml; curcumin: 11.25, 22.5, 45.0, 90.0, 180.0, and 360.0 μg/ml; emodin and imperatorin: 1.875, 3.75, 7.5, 15.0, 30.0, and 60.0 μg/ml; magnolol: 1.25, 2.5, 5.0, 10.0, 20.0, and 40.0 μg/ml.

Each standard solution contained the internal standard solution (paeonol) at 216 μg/ml. All standard solutions were filtered and 20 μl of each was injected into the HPLC column for analysis. The calibration curve was plotted by using the ratio of the peak areas (standard solution/internal standard solution) as the *y*-axis, and concentrations as the *x*-axis. Linear regression was used to evaluate the equation of $y = ax + b$ and the correlation coefficient.

Validation

Precision

Standard stock solutions were diluted with 80% methanol to three different concentrations (Table 2). Intraday test (injecting each concentration three times within 24 h), and an interday test (injecting each concentration four times over 7 days with each injection separated by at least 24 h) were run to check reproducibility. The standard deviation (SD) and relative standard deviation (RSD) were calculated.

Accuracy

Each standard stock solution of a series of various concentrations was spiked into an ethanol solution of Ru-Yi-Jin-Huang-San, and then refluxed at 80°C for 3 h. Internal standard solution was added to each solution to afford a concentration of 216.0 μg/ml. Then the solution was filtered and subjected to HPLC analysis in triplicate. The recovery (%) was calculated by the equation of $((C3 - C2)/C1) \times 100\%$, in which C1 represents the amount of each standard spiked, C2 represents the amount of each marker in ethanol solution of Ru-Yi-Jin-Huang-San, and C3 represents the total amount of each marker in the solution.

Results

Separation of marker substances by HPLC

All marker substances and internal standard, paeonol, were successfully separated in a single HPLC run for the ethanol extracts of Ru-Yi-Jin-Huang-San. By using gradient elution, berberine, sennoside B, sennoside A, emodin, curcumin, imperatorin, magnolol, hesperidin, glycyrrhizin, and paeonol were resolved and eluted at 48.51, 55.51, 69.64, 108.92, 103.39, 111.14, 113.63, 68.96, 95.25, and 84.34 min, respectively (Fig. 2).

The peak purity of marker substances in the Ru-Yi-Jin-Huang-San was qualified by HPLC (Fig. 3) and was high for each marker substance. The ethanol extract of Ru-Yi-Jin-Huang-San was compared with seven solutions of incomplete Ru-Yi-Jin-Huang-San preparations, which were made by omitting one material in turn from the standard preparation, namely Phellodendri Cortex, Rhei Rhizoma, Citri Leiocarpae Exocarpiumx, Glycyrrhizae Radi, Curcumae Rhizoma, Angelicae Dahuricae Radix, and Magnoliae Cortex, respectively. As shown in Fig. 2b–h, no peak for

Table 2 Calibration curves of marker substances

Compound	Concentration range (μg/ml)	Regression equation	*r*
Berberine	6.25–200.0	$y = 0.0279x - 0.1469$	0.9996
Sennoside B	0.63–20.0	$y = 0.0108x + 0.00007$	0.9998
Hesperidin	8.75–280.0	$y = 0.0056x + 0.0219$	0.9996
Sennoside A	5.00–160.0	$y = 0.0169x + 0.0021$	0.9997
Glycyrrhizin	7.50–240.0	$y = 0.0044x - 0.0045$	0.9994
Curcumin	11.25–360.0	$y = 0.0155x + 0.0382$	0.9999
Emodin	1.88–60.0	$y = 0.0549x + 0.0522$	0.9997
Imperatorin	1.88–60.0	$y = 0.0527x + 0.0466$	0.9995
Magnolol	1.25–40.0	$y = 0.0668x + 0.0219$	0.9999

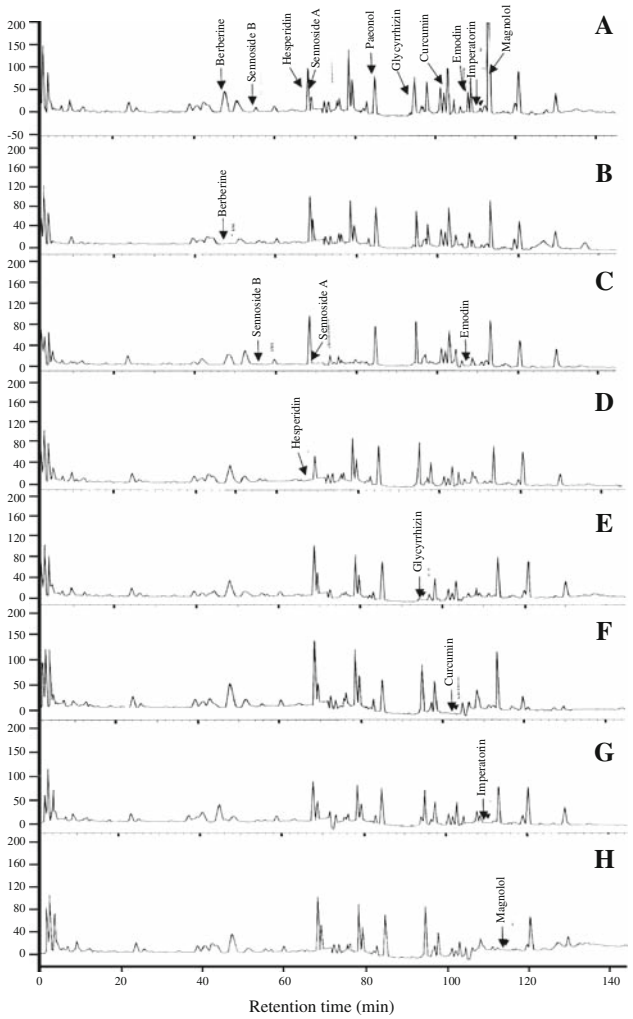

Fig. 2 Chromatograms of marker substances in ethanol extracts of "incomplete" Ru-Yi-Jin-Huang-San preparations. **a** Ethanol extraction of Ru-Yi-Jin-Huang-San containing internal standard, paeonol. **b** Ethanol extraction of Ru-Yi-Jin-Huang-San without Phellodendri Cortex. **c** Ethanol extraction of Ru-Yi-Jin-Huang-San without Rhei Rhizoma. **d** Ethanol extraction of Ru-Yi-Jin-Huang-San without Citri Leiocarpae Exocarpium. **e** Ethanol extraction of Ru-Yi-Jin-Huang-San without Glycyrrhizae Radix. **f** Ethanol extraction of Ru-Yi-Jin-Huang-San without Curcumae Rhizoma. **g** Ethanol extraction of Ru-Yi-Jin-Huang-San without Angelicae Dahuricae Radix. **h** Ethanol extraction of Ru-Yi-Jin-Huang-San without Magnoliae Cortex

the omitted material was observed at retention times corresponding to the respective marker substances. Apparently, there was no interaction between components of Ru-Yi-Jin-Huang-San. Therefore, the above conditions can be used for quantification of the marker substances.

Calibration line

The linear regression equations, correlation coefficients, and concentration range of calibration lines for the marker substances are listed in Table 2. All calibration curves

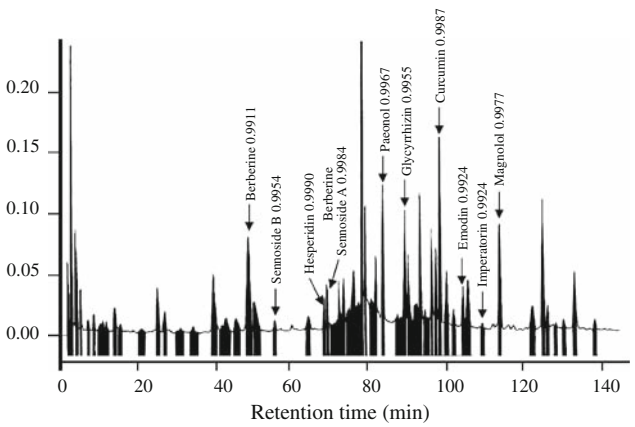

Fig. 3 Peak purities of each marker substance in Ru-Yi-Jin-Huang-San

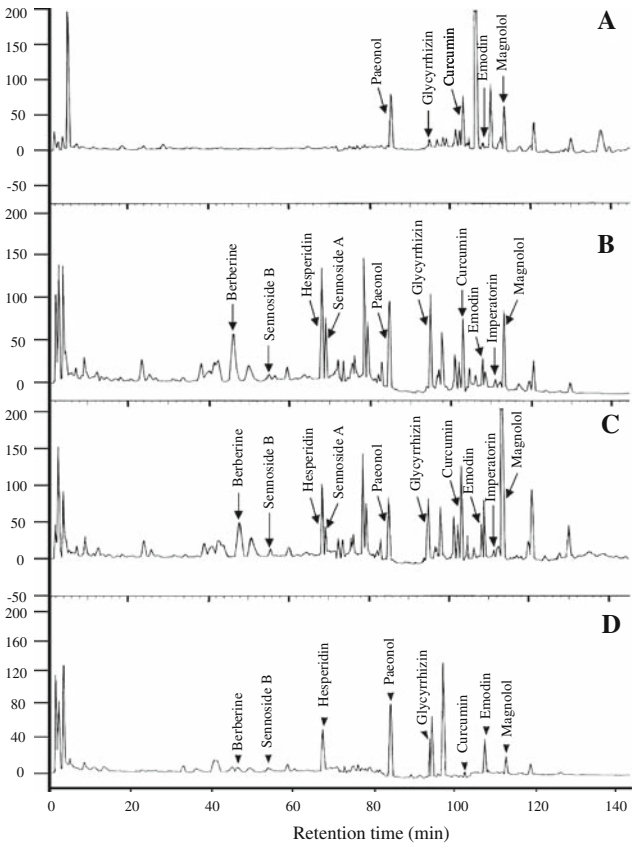

Fig. 4 HPLC chromatograms of Ru-Yi-Jin-Huang-San extracts obtained using the four extraction methods. **a** Addition of 1000 ml of sesame oil, storing the mixture at room temperature (25°C) for 1 day, and then heating in a reflux condenser at 150°C for 3 h. **b** Addition of 1000 ml of 50% ethanol and then heating in a reflux condenser at 90°C for 3 h. **c** Addition of 1000 ml of ethanol and then heating in a reflux condenser at 80°C for 3 h. **d** Addition of 1000 ml of water and then heating in a reflux condenser at boiling temperature for 3 h

Table 3 Relative extraction ratios of the nine marker substances of Ru-Yi-Jin-Huang-San

Compound	Extraction method[a]			
	A	B	C	D
Berberine	–	77.20 ± 0.45 (100.0)	75.11 ± 0.32 (97.3)	11.17 ± 0.10 (14.5)
Sennoside B	–	4.94 ± 0.02 (65.4)	7.55 ± 0.26 (100.0)	1.14 ± 1.08 (15.1)
Hesperidin	–	182.72 ± 1.33 (78.5)	232.91 ± 1.81 (100.0)	123.34 ± 0.74 (53.0)
Sennoside A	–	27.54 ± 2.21 (36.7)	79.38 ± 2.39 (100.0)	–
Glycyrrhizin	117.46 ± 1.68 (52.1)	139.85 ± 0.98 (62.1)	225.33 ± 0.89 (100.0)	128.38 ± 1.72 (57.0)
Curcumin	93.72 ± 1.48 (40.2)	233.32 ± 1.26 (100.0)	60.96 ± 2.65 (26.1)	4.53 ± 2.25 (1.9)
Emodin	11.41 ± 1.80 (100.0)	9.62 ± 3.20 (84.3)	9.36 ± 1.81 (82.0)	5.73 ± 2.25 (50.2)
Imperatorin	–	24.03 ± 1.05 (26.5)	6.36 ± 2.05 (100.0)	–
Magnolol	1.47 ± 0.14 (4.5)	32.37 ± 3.61 (100.0)	22.18 ± 2.14 (68.5)	4.42 ± 2.16 (13.7)

Data are presented as mean (mg/one dose) ± coefficient of variance (CV) value (%)

–, not detected

[a] See Extraction conditions for details of each method

Table 4 Reproducibilities of intraday and interday analysis of Ru-Yi-Jin-Huang-San

Compound	Concentration (μg/ml)	Mean ± SD (RSD %)	
		Intraday ($n = 5$)	Interday ($n = 4$)
Berberine	200.00	201.61 ± 0.24 (0.12)	201.33 ± 1.31 (0.65)
	50.00	48.38 ± 0.21 (0.44)	49.46 ± 0.50 (1.01)
	12.50	13.55 ± 0.03 (0.24)	12.11 ± 0.16 (1.33)
Sennoside B	20.00	19.85 ± 0.25 (1.26)	21.65 ± 0.28 (1.31)
	5.00	5.03 ± 0.06 (1.11)	6.47 ± 0.05 (0.81)
	1.25	1.22 ± 0.02 (1.36)	1.46 ± 0.02 (1.65)
Hesperidin	280.00	279.75 ± 3.80 (1.36)	281.22 ± 2.08 (0.74)
	70.00	70.27 ± 0.36 (0.56)	71.48 ± 0.22 (0.31)
	17.50	16.68 ± 0.42 (2.05)	17.87 ± 0.16 (0.89)
Sennoside A	160.00	160.41 ± 2.10 (1.31)	160.66 ± 1.69 (1.05)
	40.00	38.64 ± 0.23 (0.59)	39.82 ± 0.67 (1.68)
	10.00	9.80 ± 0.04 (0.41)	10.12 ± 0.13 (1.25)
Glycyrrhizin	240.00	242.10 ± 3.43 (1.42)	241.45 ± 3.31 (1.37)
	60.00	62.29 ± 0.74 (1.19)	61.21 ± 0.25 (0.41)
	15.00	14.53 ± 0.22 (1.52)	16.21 ± 0.30 (1.88)
Curcumin	360.00	358.66 ± 3.16 (0.88)	359.77 ± 1.98 (0.55)
	90.00	89.82 ± 1.10 (1.22)	91.65 ± 0.42 (0.46)
	22.50	21.75 ± 0.27 (1.25)	22.15 ± 0.12 (0.53)
Emodin	60.00	59.38 ± 0.30 (0.51)	60.48 ± 0.26 (0.43)
	15.00	14.99 ± 0.09 (0.59)	14.11 ± 0.06 (0.43)
	3.75	3.21 ± 0.01 (0.33)	3.58 ± 0.02 (0.54)
Imperatorin	60.00	59.59 ± 0.32 (0.54)	59.12 ± 0.34 (0.58)
	15.00	15.21 ± 0.04 (0.25)	15.83 ± 0.05 (0.33)
	3.75	3.37 ± 0.01 (0.33)	3.78 ± 0.02 (0.41)
Magnolol	40.00	40.40 ± 0.21 (0.52)	41.21 ± 0.22 (0.54)
	10.00	10.25 ± 0.02 (0.16)	10.54 ± 0.20 (1.89)
	2.50	2.68 ± 0.04 (1.64)	2.73 ± 0.01 (0.37)

exhibited good linear correlation with correlation coefficients of 0.9994–0.9999.

Extraction methods

The HPLC chromatograms and the contents of all marker substances extracted with the four extraction methods are shown in Fig. 4 and Table 3. The results indicated that method B (addition of 1000 ml of 50% ethanol, and then reflux at 90°C for 3 h) and method C (addition of 1000 ml of ethanol, and then reflux at 80°C for 3 h) afforded higher yields of the nine marker substances; in terms of safety regarding a large-scale manufacturing process, method B would be the better way to produce the oil-based and water-base patches with 50% ethanol extract.

Precision and accuracy

The relative standard deviations (RSD) of the intraday and interday analysis were 0.12–2.05 and 0.31–1.89%, suggesting that method had very good reproducibility (Table 4).

All of the recoveries for the analysis were greater than 93.30% (Table 5).

Table 5 Recoveries of the nine marker substances from Ru-Yi-Jin-Huang-San

Compound	Concentration (μg/ml)	Recovery (%) mean ± SD (RSD %)
Berberine	200.00	103.56 ± 0.46 (0.44)
	50.00	106.13 ± 0.52 (0.55)
	12.50	109.00 ± 1.01 (0.93)
Sennoside B	20.00	100.58 ± 0.54 (0.54)
	5.00	107.81 ± 0.24 (0.22)
	1.25	108.58 ± 0.50 (0.46)
Hesperidin	280.00	101.81 ± 3.83 (3.76)
	70.00	107.31 ± 2.76 (2.57)
	17.50	94.40 ± 3.19 (3.38)
Sennoside A	160.00	99.55 ± 3.48 (3.50)
	40.00	98.55 ± 4.34 (4.41)
	10.00	93.30 ± 2.14 (2.30)
Glycyrrhizin	240.00	106.54 ± 0.22 (0.21)
	60.00	108.65 ± 0.07 (0.06)
	15.00	113.63 ± 0.90 (0.79)
Curcumin	360.00	101.56 ± 0.65 (0.64)
	90.00	104.13 ± 0.36 (0.35)
	22.50	111.47 ± 1.04 (0.93)
Emodin	60.00	100.56 ± 0.54 (0.54)
	15.00	107.80 ± 0.24 (0.22)
	3.75	108.80 ± 0.50 (0.46)
Imperatorin	60.00	106.92 ± 4.02 (3.76)
	15.00	107.33 ± 2.76 (2.57)
	3.75	96.27 ± 3.25 (3.38)
Magnolol	40.00	99.65 ± 1.22 (1.23)
	10.00	102.00 ± 1.10 (1.08)
	2.50	109.36 ± 1.45 (1.33)

Analysis of water-based and oil-based patch preparations of Ru-Yi-Jin-Huang-San

The HPLC chromatograms and the contents of marker substances in water-based and oil-based patch preparations, as shown in Fig. 5 and Table 6, were quite different from

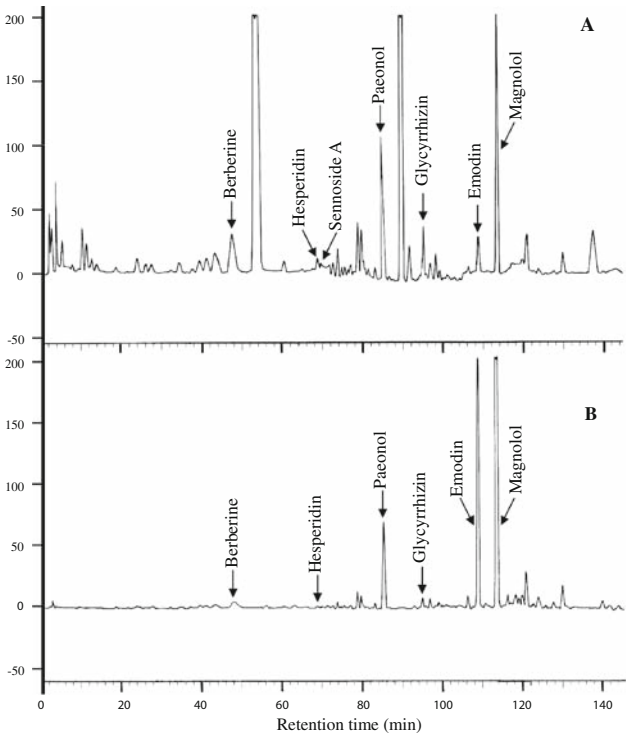

Fig. 5 HPLC chromatograms of **a** water-based and **b** oil-based patch preparations of Ru-Yi-Jin-Huang-San

Table 6 Contents of marker substances in water-based and oil-based patches of Ru-Yi-Jin-Huang-San

Compound	Water-based patch	Oil-based patch
Berberine	0.58 ± 1.25	0.12 ± 0.19
Sennoside B	–	0.02 ± 1.38
Hesperidin	0.25 ± 0.12	0.02 ± 1.20
Sennoside A	0.09 ± 1.06	–
Glycyrrhizin	0.80 ± 0.23	0.28 ± 0.89
Curcumin	–	–
Emodin	0.12 ± 2.22	0.45 ± 2.82
Imperatorin	–	–
Magnolol	0.46 ± 0.48	2.32 ± 0.78

Data represented as mean (mg/one piece) ± CV value (%)

–, not detected

each other. This is probably due to the different sources of the pharmaceutical ingredients and the different manufacturing processes used.

In this report, we established a precise and reliable quantification method for the simultaneous determination of nine marker substances in Ru-Yi-Jin-Huang-San. The method can be used for quality control of the manufacturing process of patches of Ru-Yi-Jin-Huang-San in the future.

Discussion

In the traditional manufacturing process of Chinese medicinal patch preparations, sesame oil is usually used for the extraction. In this study we compared the chemical constituents obtained by extraction with sesame oil, ethanol, 50% ethanol, and water, focusing on fingerprint regions of the HPLC chromatograms, and the amounts of their major marker compounds, namely berberine, sennoside B, sennoside A, emodin, curcumin, imperatorin, magnolol, hesperidin, glycyrrhizin, and paeonol in Ru-Yi-Jin-Huang-San. Sesame oil was the most ineffective solvent in terms of extraction yield; e.g., the low amounts of compounds obtained meant that only glycyrrhizin, emodin, curcumin, and magnolol were determined. On the other hand, ethanol afforded the highest number of peaks in the fingerprint region of the HPLC chromatograms, and amounts of the nine marker compounds, followed by 50% ethanol and water. In accordance with these results ethanol or 50% ethanol should be applied to the extraction process of patch preparations of Ru-Yi-Jin-Huang-San, when based on the toxicological safety of the corresponding manufacturing process.

We also analyzed the fingerprint region of HPLC chromatograms and the amounts of the nine marker compounds in water-based and oil-based patch preparations of Ru-Yi-Jin-Huang-San. As shown in Fig. 5 and Table 6, higher peak numbers and peak heights in the fingerprinting region of the HPLC chromatogram, and higher contents of berberine, hesperidin, sennoside A, and glycyrrhizin were found for the water-based patch; whereas, the amounts of emodin and magnolol were higher in the oil-based patch. These results suggest that the water-based patch was a more efficacious release component than the oil-based patch. There were also trace amounts of sennoside B, curcumin, and imperatorin in both the patch preparations. Two dramatic unknown peaks were obtained at retention times of 54 and 90 min in the HPLC chromatogram for the water-based patch, which might be caused by release of other pharmaceutical ingredients.

A multicomponent HPLC method was developed for the simultaneous quantification of nine marker substances in Ru-Yi-Jin-Huang-San. A matrix of 20% acetonitrile and 70% acetonitrile, which were both adjusted to pH 3.0 with phosphoric acid, was used at the mobile phase in a gradient elution program, with an ODS column for the stationary phase. The detection wavelength varied with time as follows: 275 nm, 0–72 min; 250 nm, 72–105 min; 220 nm, 105–145 min. The internal standard used to determine the calibration line resulted in a precise and reliable quantification method. The results of the quantitative analysis showed that the method can be used to establish the standards for quality control to ensure accuracy and efficiency in the manufacturing process of Ru-Yi-Jin-Huang-San in the future.

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