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Short communication

Simultaneous determination of gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid in Si–Wu decoction by high-performance liquid chromatography DAD method

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Abstract

A high-performance liquid chromatographic method was applied to the determination of gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid in Si–Wu decoction and other 13 combinations of the formula. These five compounds were analyzed simultaneously with a Zorbox SB C-18 column by gradient elution using 0.01% (v/v) phosphoric acid–acetonitrile as the mobile phase. The flow rate was 1 ml min^{-1}, and detection was set at 230 nm. The recovery of the method was in the range of 94.8–103.1%, and all the compounds showed good linearity ($r > 0.9995$) in a relatively wide concentration range. The result indicated that the content of these five compounds changed after decocting process. The contents of paeoniflorin, albiflorin, ferulic acid and gallic acid increased and that of benzoic acid decreased significantly.

Keywords: Paeoniflorin; Albiflorin; Ferulic acid; Gallic acid; Benzoic acid; Si–Wu decoction

1. Introduction

A composite traditional Chinese medicine, Si–Wu decoction, is a basic prescription consisting of four herbs: Radix Paeoniae Alba, Radix Angelicae Sinensis, Rhizoma Chuanxiong and Radix Rehmanniae. Si–Wu decoction is a representative tonic formula in traditional Chinese medicine, possessing the ability to improve a deficiency of blood, promote blood circulation, regulate menstruation and relieve pains. Recent studies showed that Si–Wu decoction was useful for the inhibition of cutaneous inflammatory diseases [1] and it could improve the working memory performance impaired by scopolamine in the eight-arm radial maze task and in the T-maze delayed alternation task in rats [2]. Si–Wu decoction was the major contribution in Ba-Zhen decoction responsible for the prevention of endometrial carcinogenesis in mice [3].

Gallic acid, albiflorin, paeoniflorin and benzoic acid are the main constituents in the roots of *Paeonia alba*; ferulic acid is the marker compound in the roots of *Angelica sinensis* and *Ligusticum chuanxiong*. It was reported that paeoniflorin and ferulic acid are the two active compounds of Si–Wu decoction [4]. It is, therefore, necessary to analyze the percentage of the marker constituents in a decoction in order to evaluate Si–Wu decoction and to preliminarily elaborate the drug interactions after decocting process. Several high-performance liquid chromatographic (HPLC) methods and capillary electrophoretic methods have been developed for the determination of these constituents in herbs and Chinese medicinal preparations [5–17]. However, none of these methods is able to simultaneously separate these five compounds of Si–Wu decoction.

The aim of the current study was to develop a direct and rapid HPLC method to simultaneously quantify the five marker constituents, gallic acid, albiforin, paeoniflorin, ferulic acid and benzoic acid (Fig. 1) in Si–Wu decoction and its

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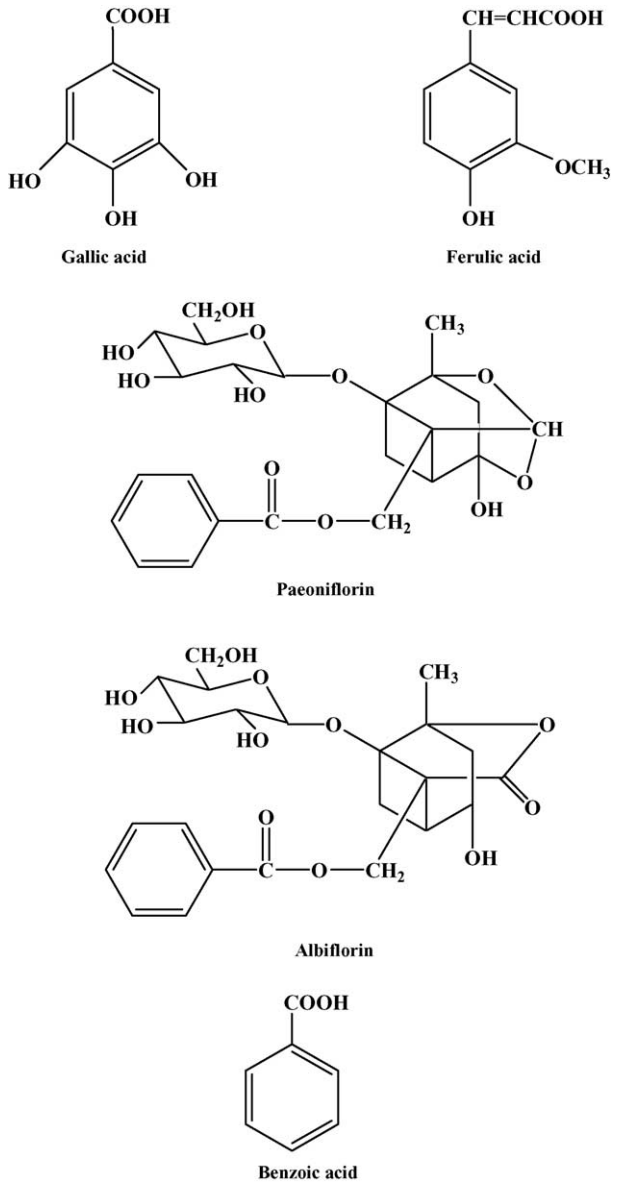

Fig. 1. The chemical structures of gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid.

related 13 combinations, and to illustrate the drug interactions after decocting process according to the quantity changes of these marker compounds.

2. Experimental

2.1. Materials

Crude drugs were purchased from TongRenTang Pharmaceutical Group (Beijing, PR China). Authentic standards were purchased from the National Institute for the Control of Pharmaceutical and Biologocal Products (Beijing, PR China). Acetonitrile was of HPLC grade from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA) and other solvents and chemicals were purchased from Beijing Chemical Engineering Factory (Beijing, China). The deionized water was prepared from Millipore water purification system (Millipore, Milford, MA, USA) and was filtered with 0.45 μm membranes.

2.2. HPLC apparatus and conditions

An Agilent 1100 liquid chromatograph system (Agilent Technologies, Palo Alto, CA, USA) consisting of a quaternary pump, an autosampler and photodiode array detector coupled with an analytical workstation was used.

Separations were carried out with a Zorbax SB C-18 reversed-phase column (5 μm, 250 mm × 4.6 mm) (Agilent Technologies, USA). The mobile phase was gradient of aqueous 0.01% (v/v) phosphoric acid–acetonitrile (0 min, 95:5; 7 min, 95:5; 12 min, 83:17; 25 min, 83:17; 27 min, 80:20). The flow rate was 1 ml min^{-1}. The detection was set at UV 230 nm, and absorption spectra of compounds were recorded between 200 nm and 400 nm. The column temperature was 25 °C, and the sample injection volume was 10 μl. The compounds were identified by comparing their retention time values and UV spectra with those of the standards.

2.3. Calibration curve

Each marker compound, gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid, was accurately weighed and dissolved in 50% methanol to give serial concentrations within the ranges of 16.75–50.25 μg ml^{-1}, 36.40–93.60 μg ml^{-1}, 63.60–143.10 μg ml^{-1}, 10.90–76.30 μg ml^{-1} and 5.00–11.25 μg ml^{-1}, respectively. All calibration curves were obtained from peak areas of the standard solutions over the concentrations. Concentrations of these compounds in samples were calculated from this regression analysis.

2.4. Sample preparations

Powders of crude drugs compounded according to Table 1 were boiled with water on an electric heater for 30 min. The decoction was filtered under vacuum. The residue was re-extracted in the same way. The filtrates were evaporated to dryness at 40 °C in vacuo. The evaporated residue was dissolved with 50% methanol into a volumetric flask. The final volume of the extracting solution was set to 50 ml.

For determination of ferulic acid and benzoic acid, the solutions were filtered through a membrane (0.45 μm) and then injected into HPLC directly. But for gallic acid, albiflorin and paeoniflorin, the solutions were diluted with 50% methanol to give a five-fold dilute decoction and then filtered.

Table 1
Combinations of crude drugs in Si–Wu decoction

Combinations	Crude drug (g)			
	Paeoniae radix	Rehmanniae radix	Angelicae radix	Chuanxiong rhizoma
1	1.2	1.2	1	0.8
2	1.2			
3	1.2	1.2	1	
4	1.2	1.2		0.8
5	1.2		1	0.8
6	1.2	1.2		
7	1.2			0.8
8	1.2		1	
9		1.2		0.8
10		1.2	1	0.8
11		1.2	1	
12			1	0.8
13				0.8
14			1	

Table 2
Linear relation between peak area and concentration ($n = 6$)

Marker compounds	Regression equation	r	Linear range ($\mu g\,ml^{-1}$)	LOD ($ng\,ml^{-1}$)
Gallic acid	$y = 15.8904x - 2.1119$	0.9999	16.75–0.25	17
Albiflorin	$y = 10.2144x - 5.7998$	0.9998	36.40–3.60	13
Paeoniflorin	$y = 12.9261x - 22.517$	0.9996	63.60–43.1	20
Ferulic acid	$y = 33.5675x - 5.9571$	0.9998	10.90–6.30	14
Benzoic acid	$y = 77.2503x - 8.8086$	0.9997	5.00–1.25	10

y = peak area, x = concentration ($\mu g\,ml^{-1}$). Triplicate assay about the different concentration ($n = 6$).

2.5. Recovery

An appropriate amount of Si–Wu decoction to be extracted was divided into four portions (one as control group) and each portion (except the control group) was spiked with standard gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid. All samples were filtered through a 0.45 μm membrane filter and assayed by HPLC to calculate recoveries.

Table 3
Within-day and day-to-day relative standard deviation (R.S.D.) for the HPLC method for the determination of five marker substances

Marker compounds	Nominal concentration ($\mu g\,ml^{-1}$)	Observed concentration ($\mu g\,ml^{-1}$) ±S.D.		R.S.D. (%)		Accuracy (%)	
		Within-day[a]	Day-to-day[b]	Within-day[a]	Day-to-day[b]	Within-day[a]	Day-to-day[b]
Gallic acid	16.75	16.44 ± 0.01	16.78 ± 0.81	0.04	4.86	98.15	100.2
	30.15	30.59 ± 0.26	29.55 ± 0.98	0.85	3.31	101.5	98.01
	50.25	50.20 ± 0.09	50.62 ± 0.33	0.19	0.64	99.90	100.7
Albiflorin	36.40	37.30 ± 0.06	36.95 ± 1.71	1.53	4.63	102.5	101.5
	57.20	57.08 ± 1.74	56.34 ± 2.73	3.05	4.85	99.79	98.50
	93.60	92.11 ± 3.86	95.74 ± 4.04	0.77	4.22	98.41	102.3
Paeoniflorin	63.60	63.19 ± 0.05	62.72 ± 2.68	0.08	4.28	99.36	98.62
	95.40	93.38 ± 0.04	93.76 ± 4.60	0.04	4.90	97.88	98.28
	143.1	141.5 ± 1.02	140.5 ± 6.30	0.72	4.48	98.85	98.18
Ferulic acid	10.90	10.96 ± 0.03	10.47 ± 0.58	0.25	5.54	100.6	96.06
	43.60	42.47 ± 0.05	42.71 ± 1.67	0.11	3.92	97.41	97.96
	65.40	64.15 ± 0.41	63.68 ± 2.69	0.65	4.23	98.09	97.37
Benzoic acid	5.00	5.15 ± 0.01	5.16 ± 0.11	0.21	2.13	103.0	100.2
	8.75	8.71 ± 0.05	8.83 ± 0.21	0.53	2.34	99.54	100.9
	11.25	11.28 ± 0.03	11.29 ± 0.16	0.29	1.44	100.3	100.4

[a] Within-day precision test at six times in 1 day.
[b] Day-to-day precision on four different days.

Table 4
Recovery of gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid from Si–Wu decoction ($n = 5$)

Marker compounds	Initial amount (mg)	Added amount (mg)	Detected amount (mg)	Recovery (%)	R.S.D. (%)
Gallic acid	0.1662	0.0165	0.1812	94.8	5.12
Albiflorin	0.3172	0.026	0.3427	98.3	3.64
Paeoniflorin	0.5324	0.03975	0.5734	103.0	4.88
Ferulic acid	0.1162	0.0275	0.1446	103.1	1.60
Benzoic acid	0.01163	0.00625	0.01806	102.9	1.77

3. Results

3.1. Optimization of separation conditions

Absorption maxima of gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid were observed to be in the range of 200–400 nm on the UV spectra with three dimensional chromatograms and a monitoring wavelength for quantitative determination at 230 nm was altered to obtain the baseline separation of marker compounds.

Since the polarity and other properties of the marker substances differ greatly from each other, gradient elution was carried out to successfully separate the compounds in Si–Wu decoction using 0.01% (v/v) phosphoric acid–acetonitrile as

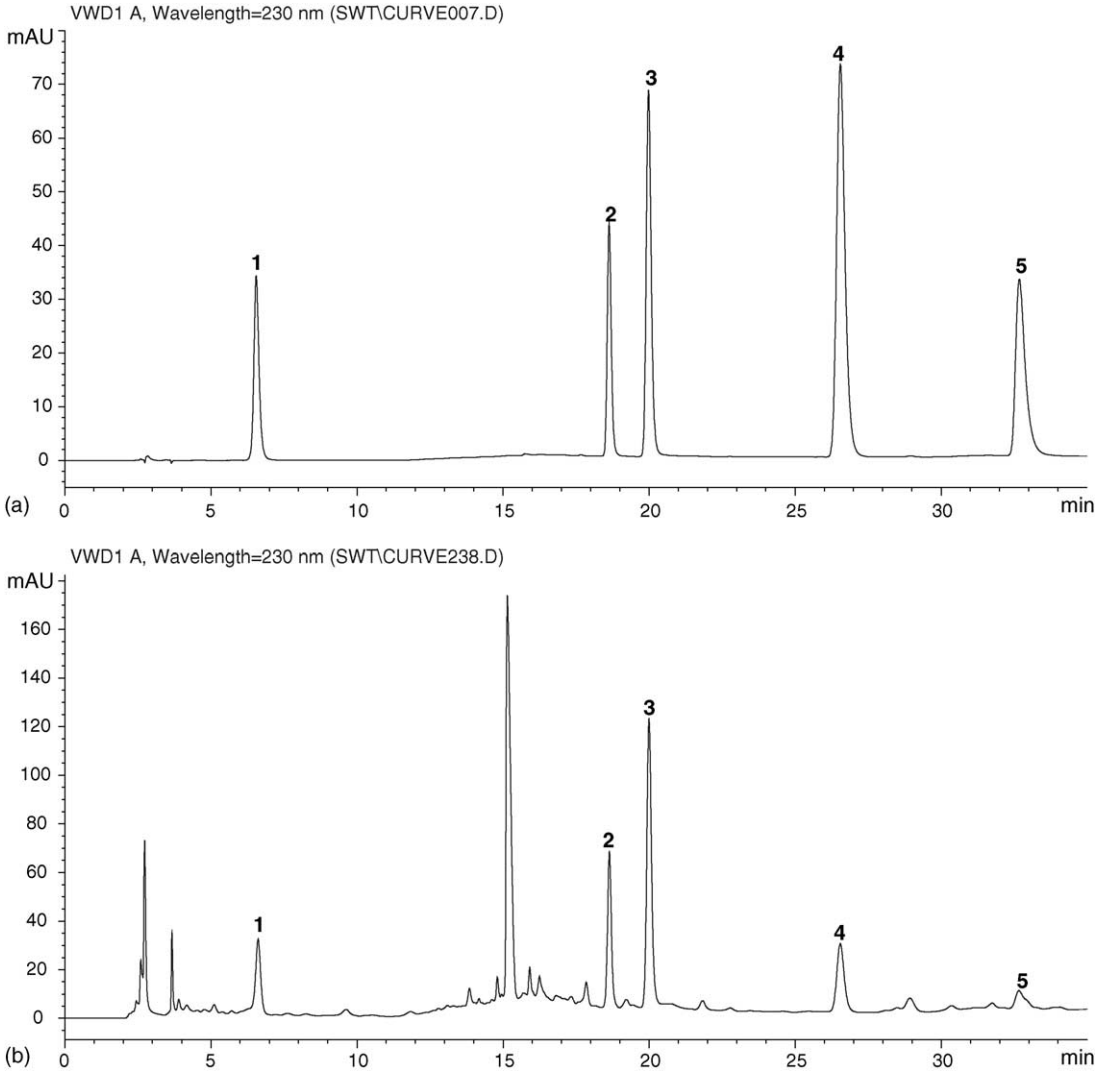

Fig. 2. Typical chromatograms of (a) standard mixture, 23.45 μg ml^{-1} gallic acid (1), 46.80 μg ml^{-1} albiflorin (2), 79.50 μg ml^{-1} paeoniflorin (3), 43.60 μg ml^{-1} ferulic acid (4) and 8.75 μg ml^{-1} benzoic acid (5). (b) Si–Wu decoction. Conditions: mobile phase, 0.01% phosphoric acid–acetonitrile; flow rate, 1 ml min^{-1}; detection wavelength, 230 nm; column temperature, 25 °C injection volume, 10 μl. Peaks: 1 = gallic acid; 2 = albiflorin; 3 = paeoniflorin; 4 = ferulic acid; 5 = benzoic acid.

mobile phase in less than 35 min. There were no interfered peaks within the time frame in which five compounds in the decoction were detected.

3.2. Regression equations

Linear regression analysis for each of the five compounds was performed by the external standard method. The calculated results were given in Table 2, where *a*, *b* and *r* were the coefficients of the regression equation $y = ax + b$, *x* referred to the concentration of the marker compounds ($\mu g\,ml^{-1}$), *y* the peak area, and *r* the correlation coefficient of the equation. All the marker substances showed good linearity ($r > 0.9995$) in a relatively wide concentration range. The limits of detection (LOD) ranged from 10 ng ml^{-1} to 20 ng ml^{-1}, detected at 230 nm.

3.3. Precision and accuracy

The within-day and the day-to-day accuracy data for each marker substance are listed in Table 3, relative standard deviations (R.S.D.s) of the within-day and day-to-day were 0.04–3.05% and 0.64–5.54%, respectively.

3.4. Recovery

The average recoveries of standards spiked into Si–Wu decoction were 94.8% for gallic acid, 98.3% for albiflorin, 103% for paeoniflorin, 103.1% for ferulic acid and 102.9% for benzoic acid, as shown in Table 4.

3.5. Determination of gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid

Fig. 2 shows the chromatogram obtained from RP-HPLC separation of a mixture of these five compound standards and the typical chromatogram of Si–Wu decoction, respectively.

The retention times of the five compounds were 6.55 min for gallic acid, 18.63 min for albiflorin, 19.98 min for paeoniflorin, 26.55 min for ferulic acid and 32.68 min for benzoic acid, respectively.

The quantitative results of all the analyzed samples are shown in Table 5.

4. Discussion

The five marker compounds in Si–Wu decoction and its related 13 combinations showed significantly different contents (Table 5). For gallic acid, albiflorin and paeoniflorin, their contents in the decoction of single Radix Paeoniae Alba were 1.85 mg/g, 3.51 mg/g and 6.49 mg/g, respectively. However, they are in the range of 1.73–3.23 mg/g, 3.51–6.36 mg/g and 6.49–11.38 mg/g among the decoctions composed of Radix Paeoniae Alba and other ingredients in Si–Wu decoction. The content of benzoic acid in the decoction of single Radix Paeoniae Alba was 0.21 mg/g, while it varied from 0.09 mg/g to 0.15 mg/g after combination. It was shown that the contents of gallic acid, albiflorin and paeoniflorin increased and benzoic acid decreased significantly after Radix Paeoniae Alba combining with other ingredients in Si–Wu decoction.

The content of ferulic acid in Radix Angelicae Sinensis decoction and Rhizoma Chuanxiong decoction were 0.27 and 0.7 mg/g, respectively. For the decoction composed of Radix Angelicae Sinensis and Rhizoma Chuanxiong, the ferulic acid content was approximately the sum of that in single Radix Angelicae Sinensis and single Rhizoma Chuanxiong, and it increased after either Radix Angelicae Sinensis or Rhizoma Chuanxiong combining with Radix Rehmanniae.

Traditional Chinese herbal preparations are usually prepared by boiling the mixed crude drugs in water. In the decocting process, crude drugs may interact with each other so that the components of the formula could change quantitatively and qualitatively. Consequently, the process may lead

Table 5
Contents of gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid in Si–Wu decoction and 13 combinations

Combinations[a]	Contents[b] (mg/g crude drug)				
	Gallic acid	Albiflorin	Paeoniflorin	Ferulic acid	Benzoic acid
1	2.77 ± 0.04	5.29 ± 0.25	8.87 ± 0.15	1.16 ± 0.09	0.10 ± 0.0017
2	1.85 ± 0.06	3.51 ± 0.22	6.49 ± 0.09		0.21 ± 0.0064
3	3.27 ± 0.45	6.36 ± 0.28	11.38 ± 0.47	0.47 ± 0.03	0.16 ± 0.0085
4	2.41 ± 0.03	5.42 ± 0.28	9.75 ± 0.29	0.84 ± 0.05	0.11 ± 0.0045
5	2.77 ± 0.28	5.47 ± 0.16	9.85 ± 0.49	0.88 ± 0.01	0.15 ± 0.0032
6	1.99 ± 0.13	4.88 ± 0.09	8.92 ± 0.29		0.09 ± 0.0062
7	2.27 ± 0.03	5.23 ± 0.15	9.34 ± 3.66	0.56 ± 0.01	0.12 ± 0.0054
8	3.23 ± 0.01	5.65 ± 0.12	9.98 ± 0.47	0.35 ± 0.01	0.10 ± 0.0017
9				0.83 ± 0.01	
10				1.17 ± 0.02	
11				0.35 ± 0.03	
12				0.95 ± 0.03	
13				0.70 ± 0.03	
14				0.27 ± 0.02	

[a] See Table 1.
[b] Data expressed mean ± S.D., $n = 3$.

to enhance solubility, facilitate absorption, increase pharmacological action, reduce toxic side-effects, remove odours, etc. As for Si–Wu decoction, paeoniflorin and ferulic acid were two effective constituents [4], but benzoic acid was toxic if its content reached certain concentration. According to the results, it could be deduced that the curative effects might be improved and the toxicity or side-effects be reduced by compatibility. In addition, the crude drugs of Si–Wu decoction had some interaction, Radix Rehmanniae could enhance the solubility of ferulic acid from Radix Angelicae Sinensis and Rhizoma Chuanxiong during processing.

5. Conclusion

This was the first report of simultaneous determination of the major compounds in Si–Wu decoction. A simple, rapid and accurate assay approach was presented. According to the quantification results, the change of marker substances contents in prescription and drug interactions during processing were analyzed. The HPLC method is essential for clinical evaluation of traditional Chinese medicine prescription.

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