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Simultaneous Determination of 16 Chemical Constituents in the Traditional Chinese Medicinal Prescription Si-Ni-San and Chaihu-Shugan-San by Ultra Performance Liquid Chromatography Coupled with Photodiode Array Detection

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A simple, rapid and sensitive ultra performance liquid chromatography (UPLC) coupled with photodiode array (PDA) detection method was established for the simultaneous determination of 16 components in two traditional Chinese medicines *viz.*, Si-Ni-San (SNS) and Chaihu-Shugan-San (CSS) *i.e.*, gallic acid, oxypaeoniflora, albiflorin, paeoniflorin, liquiritin, benzoic acid, narirutin, naringin, hesperidin, neohesperidin, meranzin hydrate, liquiritigenin, quercimelin, benzoylpaeoniflorin, isoliquiritigenin and formononetin. These were separated in less than 18 min using an Acquity BEH C_{18} column (100 mm × 2.1 mm i.d. 1.7 μM). The mobile phase comprising acetonitrile (A) and 0.5 % acetic acid (B) was used to elute the targets in gradient elution mode. All calibration curves showed good linear regression ($R^2 \leq$ 0.9992) within the test ranges. Inter- and intra-day precisions for all investigated components expressed as relative standard deviation (RSD) ranged from 1.86-6.02 %. Recoveries ranged from 90.31-104.05 % with RSD ≤ 4.5 %. The proposed method was validated for specificity, accuracy, precision and limits of detection. The method was successfully applied to determine the contents of 16 components in SNS and CSS.

Key Words: Ultra performance liquid chromatography, Traditional Chinese medicines, Chemical constituents, Chaihu-Shugan-San.

INTRODUCTION

Si-Ni-San (SNS) and Chaihu-Shugan-San (CSS) are traditional Chinese medicines (TCM), which have been used successfully to manage functional dyspepsia and depression in clinical practice[1-3]. Functional dyspepsia and depression are two illnesses which reduce patient's quality of life and enhance their economic burden[4-6]. Relationships between functional dyspepsia and depression have been described in several studies. It has been found that depression is prevalent in patients with functional dyspepsia[7-9]. Si-Ni-San and Chaihu-Shugan-San can improve some symptoms of functional dyspepsia including abdominal distention, hiccup and poor appetite[10,11] and also can release the disease status of depression such as: lassitude, anxiety, sleep disorders, hypochondriac pain and chest distress[12,13].

Si-Ni-San decoction is composed of four medicinal herbs: Radix Bupleuri, Raidix Paeoniae Alba, Radix Glycyrrhizae and Fructus Aurantii Immaturus. Chaihu-Shugan-San contains the following seven herbs: Radix Bupleuri, Raidix Paeoniae Alba, Radix Glycyrrhizae, Fructus Aurantii, Rhizoma Chuanxiong, Rhizoma Atractylodis Macrocephalae and Pericarpium Citri Reticulatae. Radix Bupleuri, Raidix Paeoniae Alba and Radix Glycyrrhizae mentioned above are the same herbs in SNS and CSS. In addition, Fructus Aurantii Immaturus of SNS and Fructus Aurantii of CSS are the young and mature fruit of *Citrus aurantium* L, respectively and they have the similar drug property[14]. No studies are reported on the quality control of the two TCMs in the literature.

It is widely accepted that the combined action of multiple constituents is considered to be crucial for the therapeutic effect of TCM[15]. What are the main medicinally effective constituents of SNS and CSS after boiling the herbs are unclear. The constituents in SNS and CSS are: gallic acid, oxypaeoniflorin, albiflorin, peoniflorin and benzoylpaeoniflorin from Raidix Paeoniae Alba. Liquiritin, liquiritigenin, isoliquiritigenin and formononetin from radix glycyrrhizae. Narirutin, naringin, hesperidin, neohesperidin, meranzin hydrate and quercetin from fructus aurantii immaturus and fructus Aurantii. The 15 compounds are similar to the SNS and CSS in some properties including antidepressant-like effect, improving gastrointestinal

motility, antiulcer effect on gastric lesions, antiinflammatory, antioxidant and alleviating pain[16-33]. Benzoic acid (present in Raidix Paeoniae Alba) at higher than permitted safety levels can do harm to human health, therefore its quality has to be controlled[34]. As a result, the 16 compounds should be considered as markers for quality control of SNS and CSS.

In recent years, many analytical techniques have been developed for evaluating the quality of herbs or herbal preparations. Technologies such as high-performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), liquid chromatography-mass spectrometry (LC-MS) and Fingerprint analysis are often used. Fingerprint analysis can evaluate the quality consistency and stability of herbal products, but cannot enable accurate quantification of analytes[35,36]. LC-MS methods are highly sensitive and reliable. But this instrument is expensive, which limits its availability[37,38]. HPLC and UPLC methods are simple and inexpensive and have been widely used in the pharmaceutical field. Compared with HPLC, UPLC has more advantages such as faster speed, better sensitivity and resolution. UPLC has been considered as one of the most promising developments in the area of fact chromatographic separations, which utilizes solid phase particles of 1.7 μM diameter to achieve high resolution separations in a shorter analysis time with little solvent consumption[39-41].

A simple, rapid, reliable and sensitive UPLC- PDA method for simultaneous determination of 16 active components in two well-known TCMs, SNS and CSS, was developed. This is the first experiment to determinate the 16 compounds in SNS and CSS simultaneously.

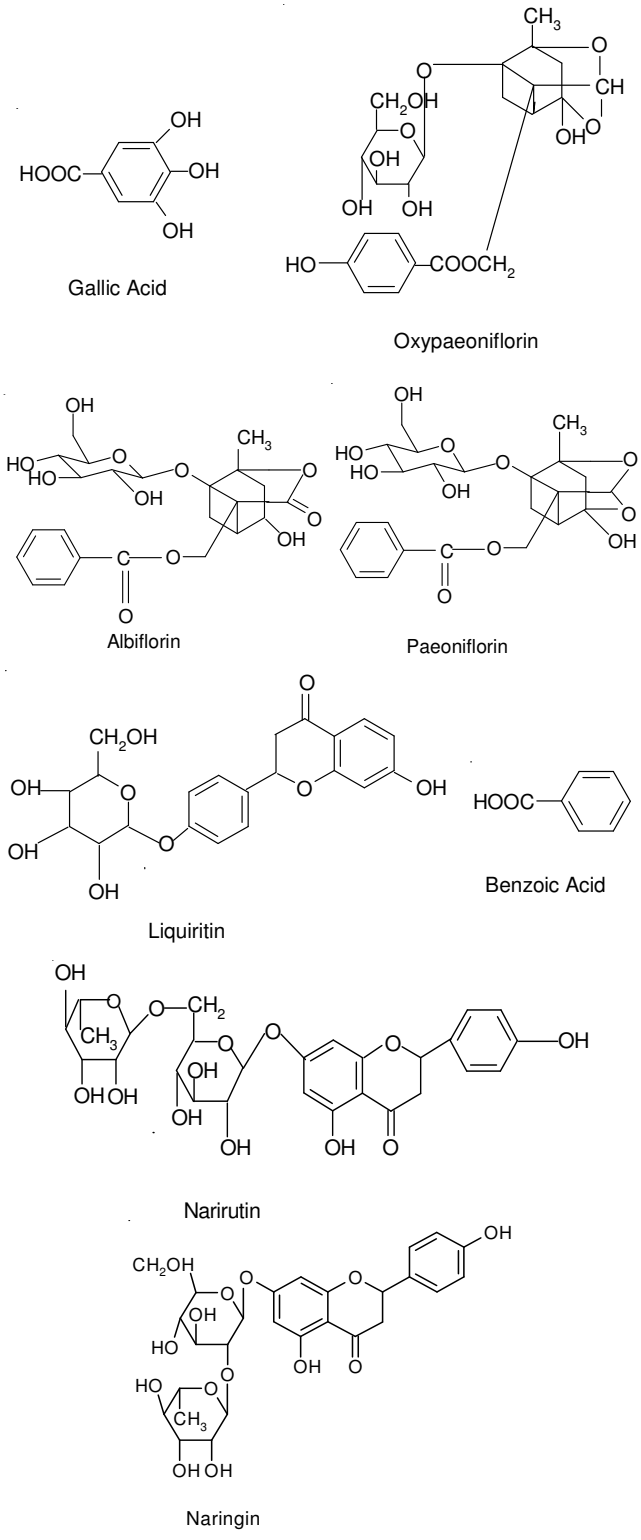
EXPERIMENTAL

The herbs of SNS and CSS mentioned above were purchased from a traditional Chinese medicine dispensary store in the Xiangya Hospital (Changsha, China) and identified. They were deposited at the Laboratory of Ethnopharmacology in Xiangya Hospital. The information about the drug of natural origin and voucher specimens is listed in Table-1. They were collected from six different provinces of China, respectively.

TABLE-1
PLACE OF PRODUCTION AND VOUCHER SPECIMENS OF THE DRUGS IN THIS EXPERIMENT

Drug name	Decoction name	Location	Voucher specimens No.
Radix Bupleuri	SNS and CSS	Hebei	20090803
Raidix Paeoniae Alba	SNS and CSS	Zhejiang	20090107
Radix Glycyrrhizae	SNS and CSS	Gansu	20090724
Fructus Aurantii Immaturus	SNS	Jiangxi	20090315
Fructus Aurantii	CSS	Jiangxi	20091112
Rhizoma Chuanxiong	CSS	Sichuan	20090609
Rhizoma Atractylodis Macrocephalae	CSS	Jiangsu	20090501
Pericarpium Citri Reticulatae	CSS	Sichuan	20091223

Authentic standards of gallic acid, oxypaeoniflorin, albiflorin, paeoniflorin, liquiritin, benzoic acid, narirutin, naringin, hesperidin, neohesperidin, liquiritigenin, quercetin, benzoylpaeoniflorin, isoliquiritigenin and formononetin (purity > 98 %) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Meranzin hydrate (purity > 98 %) was obtained from DIAO Company (Chengdu, China). The chemical structures of them were shown in Fig. 1. HPLC grade methanol and acetonitrile were provided by Tedia (Fairfield, Ohio, USA). Acetic acid was purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Water was purified using a Milli-Q purification system (Millipore Bedford Corp., Bedford, MA, USA) and used to prepare all buffer and sample solutions.



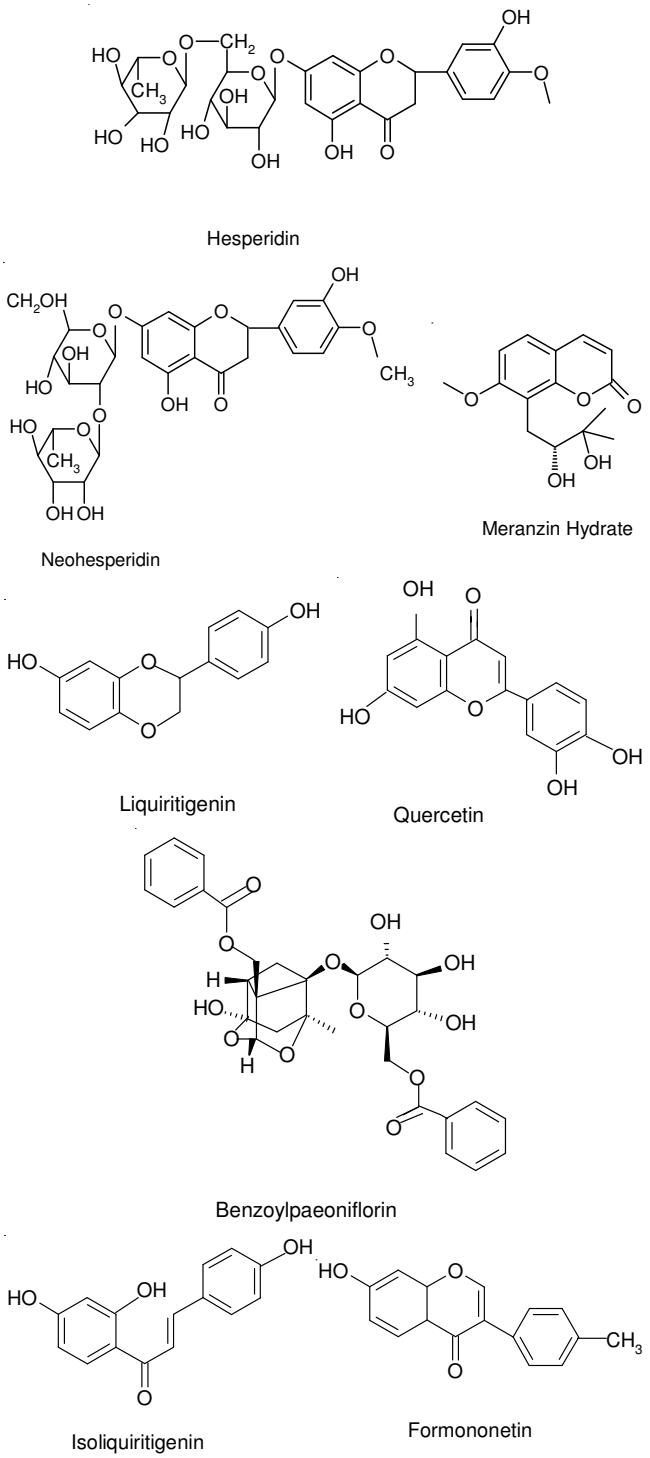


Fig. 1. Structures of 16 constituents in SNS and CSS

Chromatographic conditions: Analyses were performed on a Waters Acquity ultra performance liquid chromatography system and an Acquity photodiode array detector (Waters, Milford, MA, USA). The chromatographic analysis was carried out on an Acquity UPLC BEH 2.1 mm × 100 mm, 1.7 μm C_{18} column (Waters, Milford, MA, USA). The mobile phase comprised acetonitrile (A) and 0.5 % acetic acid water (B) was used to elute the targets with a gradient mode (0-2 min, 3-3 % A; 2-6 min, 3-15 % A; 6-11 min, 15-20 % A; 11-17.5 min, 20-45 % A). The column temperature was maintained at 40 ºC and the autosampler was conditioned at 25 ºC. The flow rate was 0.3 mL/min and injection volume was 3 μL. The components were quantified based on peak areas at their maximum wavelength in their UV spectrum.

Preparation of standard solutions: Standard stock solutions of the 16 reference standards (gallic acid, oxypaeoniflorin, albiflorin, paeoniflorin, liquiritin, benzoic acid, narirutin, naringin, hesperidin, neohesperidin, meranzin hydrate, liquiritigenin, quercetin, benzoylpaeoniflorin, isoliquiritigenin and formononetin) were prepared by dissolving them in methanol. Working standard solutions containing the 16 compounds were prepared and diluted with methanol to appropriate concentrations for establishment of calibration curves. The stock solutions and working solutions were all prepared in dark brown and stored at 4 ºC. The linearity of the responses was determined for six concentrations. Empower software was used to prepare the standard curves from the peak area of each compound. The contents of these constituents were calculated using the regression parameters obtained from the standard curves.

Preparation of sample solutions: SNS (Radix bupleuri 10 g, Radix Paeoniae Alba 10 g, Radix Glycyrrhizae 10 g and Fructus Aurantii Immaturus 10 g) and CSS (Radix Bupleuri 10 g, Raidix Paeoniae Alba 10 g, Radix Glycyrrhizae 10 g, Fructus Aurantii 10 g, Rhizoma Chuanxiong 10 g, Rhizoma Atractylodis Macrocephalae 10 g and Pericarpium Citri Reticulatae 10 g) were immersed in distilled water (1:8, w/v) for 1 h, respectively and then were boiled for 0.5 h. The boiling procedure was repeated twice. The filtrates from each decoction were mixed and lyophilized to obtain the powder form of SNS and CSS. The dried powder was stored at 4 ºC until used. For UPLC analysis, the lyophilized powders were dissolved in distilled water (200 mL) at a final concentration of SNS (200 mg/mL) and CSS (350 mg/mL). An aliquot (1.0 mL) of the solution was extracted by methanol (9.0 mL). The mixture solution was vortexed for 3 min by ultrasound and subsequently centrifuged for 10 min at 12000 × g. The supernatant solution was filtered through a 0.22 μM filter before UPLC analysis. The sample injection volume was 3 μL.

Recovery test: The accuracy of the analytical method was evaluated using the recovery test. The recovery of 16 compounds was investigated by spiking with the authentic standards to the samples of SNS and CSS before extraction. The percentage of recovery was calculated according to the formula: recovery (%) = (total amount after spiking – original amount in sample)/spiked amount × 100 %.

RESULTS AND DISCUSSION

Optimization of UPLC conditions: In general, in order to obtain a good separation of 16 compounds in the chromatogram, a suitable chromatographic column, mobile phase and elution mode are critically important. In this study, different columns packed with different mobile phases and elution modes were tested. The Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μM) and Acquity UPLC BEH shield PR_{18} column (2.1 mm × 100 mm, 1.7 μM) were used. Different mobile phases consisting of acetonitrile-water, methanol-water, acetonitrile-0.5 % acetic acid water and methanol-0.5 % acetic acid water were explored under different isocratic elution

and gradient elution modes. The flow rates of 0.3 mL/min and 0.5 mL/min were also attempted. After many efforts, the Acquity UPLC BEH C_{18} column, mobile phases consisting of acetonitrile-0.5 % acetic acid water, gradient elution modes and flow rates of 0.3 mL/min were choice as the more suitable chromatogram conditions. Under the optimized conditions, all the 16 marker constituents were sufficiently resolved and separated successfully in less than 18 min without interference peaks in the vicinity of the target components. Typical chromatograms of the blank solvent (90 % acetonitrile), authentic standards, SNS and CSS recorded at 284 nm were showed in Fig. 2.

With the PDA, UV spectra of the bioactive constituents could be compared with those of the authentic standards. The desired compounds in SNS and CSS were identified by comparing both the retention times and UV spectra with those of the authentic standards. These analytes were further confirmed by spiking the actual samples with the standards.

Optimization of extraction conditions: Boiling is the main method to extract the targets from the Chinese herbs in china. The extract method and theory of traditional Chinese herbs has been organically integrated with traditional Chinese medicine in clinical practice for thousands of years. The boiling of many herbs can achieve the purpose of enhancing efficacy

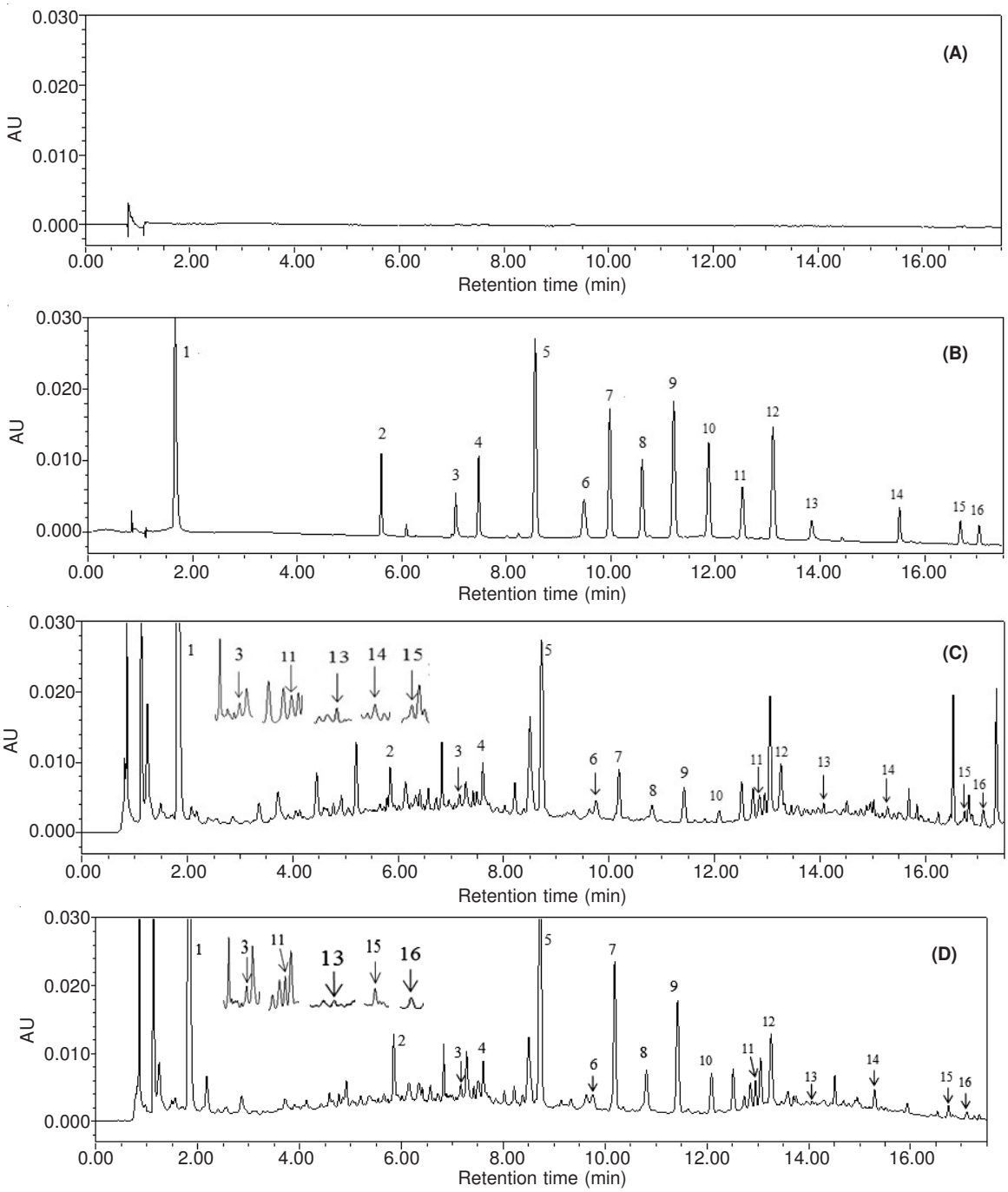


Fig. 2. Typical chromatograms for determination of 16 active compounds in SNS and CSS decoctions at 284 nm. (A) Sample solvent (90 % aqueous methanol); (B) mixed standards; (C) SNS decoction; (D) CSS decoction. Peak (1) gallic acid; (2) oxypaeoniflorin; (3) albiflorin; (4) paeoniflorin; (5) liquiritin; (6) benzoic acid; (7) narirutin; (8) naringin; (9) hesperidin; (10) neohesperidin; (11) meranzin hydrate; (12) liquiritigenin; (13) quercetin; (14) benzoylpaeoniflorin; (15) isoliquiritigenin; (16) formononetin

of decoction, easing the herb property and coinciding with the theory of traditional Chinese medicine[42]. In the extraction process, boiling time, extraction solvent, sample-solvent ratio are critical for high extraction efficiency. Different boiling times (15, 30, 45 and 60 min) were also optimized and the boiling time controlled at 0.5 h was sufficient for the extraction. (Fig. 3A-B). In the present study, methanol, acetonitrile, acetidin and ethanol solutions were used as the extraction solvents. The extraction effect by methanol was better than others for SNS and CSS decoction (Fig. 3C-D).

Method validation

Calibration curves: The calibration curves were plotted with six different concentrations of the standard solutions. All calibration curves showed good linear regression ($R^2 > 0.9992$) within test ranges. Detailed information regarding the regression data, linear ranges, LOD (S/N = 3) and LOQ (S/N = 10) were listed in Table-2.

Precision and accuracy: Instrument precision was evaluated by carrying out intra- and inter-day assays. Intra- and inter-day precisions were validated with three concentrations

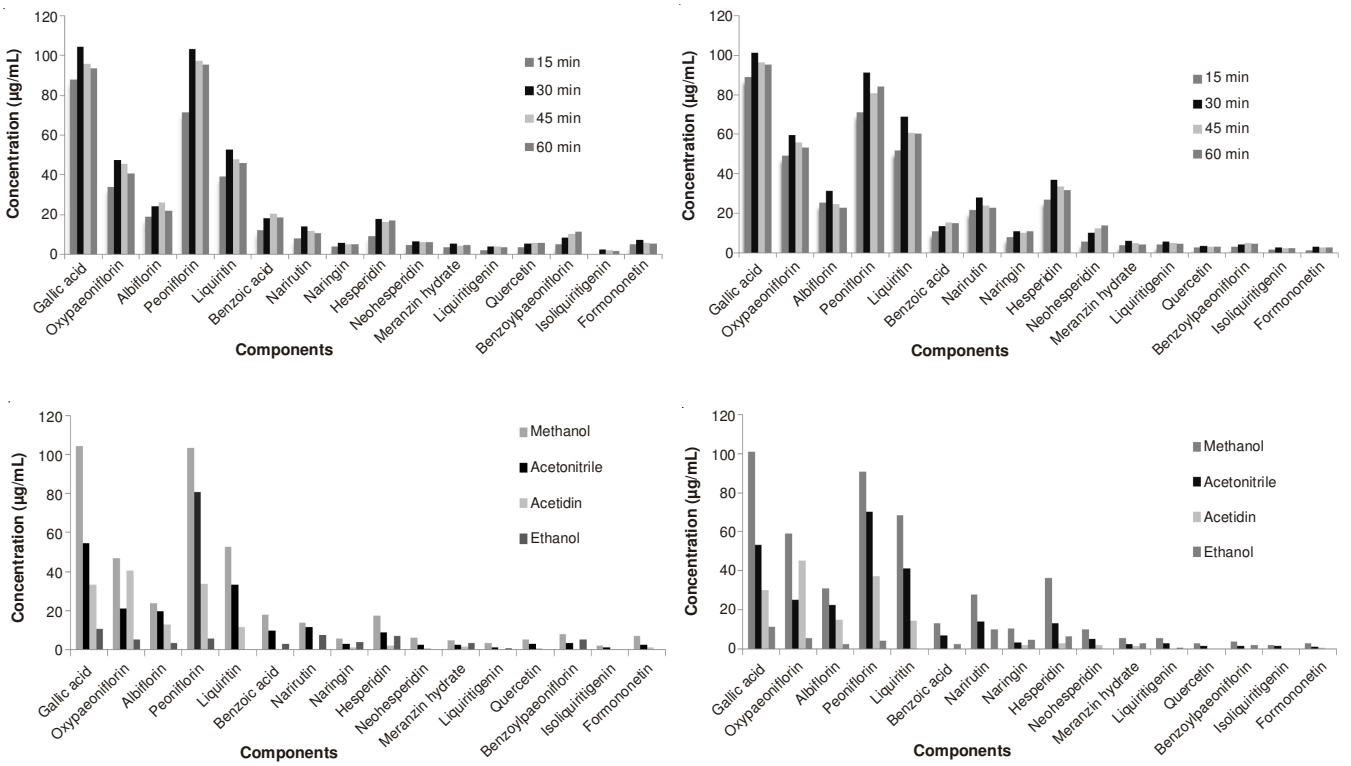


Fig. 3. Results of optimization suitable extraction conditions. (A) the influence of different boiling time to the compounds in SNS (extraction solvent: methanol); (B) the influence of different boiling time to the compounds in CSS (extraction solvent: methanol); (C) the influence of different extraction solvent to the compounds in SNS (boiling time: 0.5 h); (D) trhe influence of different extraction solvent to the compounds in CSS (boiling time: 0.5 h)

TABLE-2
LINEAR RELATIONSHIPS BETWEEN PEAK AREA AND SAMPLE CONCENTRATION

Components	Regression equation	R^2	Linear range (μg/mL)	LOD* (μg/mL)	LOQ** (μg/mL)
Gallic acid	y = 2E-05 x + 2.842	0.9997	2.05-65.60	0.631	1.565
Oxypaeoniflorin	y = 4E-04 x + 0.229	0.9998	0.53-16.96	0.117	0.239
Albiflorin	y = 4.00E-05x + 0.521	0.9997	0.64-20.32	0.091	0.199
Paeoniflorin	y = 3E-05 x + 0.135	0.9999	2.41-77.12	0.352	0.878
Liquiritin	y = 3E-05 x −0.059	0.9996	0.66-20.96	0.154	0.317
Benzoic acid	y = 9E-06 x + 0.007	0.9997	0.47-15.04	0.171	0.368
Narirutin	y = 2E-05 x −0.019	0.9998	0.20-6.40	0.052	0.136
Naringin	y = 3E-05 x −0.016	0.9999	0.20-6.40	0.053	0.145
Hesperidin	y = 3E-05 x −0.035	0.9992	0.36-11.52	0.061	0.176
Neohesperidin	y = 3E-05 x −0.008	0.9999	0.11-3.36	0.033	0.094
Meranzin hydrate	y = 1E-05 x −0.019	0.9996	0.19-5.92	0.027	0.081
Liquiritigenin	y = 1E-05 x −0.009	0.9997	0.21-6.56	0.092	0.176
Quercetin	y = 4E-05 x + 0.223	0.9992	0.15-4.80	0.032	0.108
Benzoylpaeoniflorin	y = 4E-05 x + 0.095	0.9995	0.29-9.28	0.057	0.169
Isoliquiritigenin	y = 6E-06 x + 0.066	0.9998	0.08-2.56	0.019	0.061
Formononetin	y = 7E-05 x + 0.021	0.9998	0.07-2.40	0.014	0.056

*LOD refers to the limits of detection, S/N = 3. **LOQ refers to the limits of quantity, S/N = 10.

of mixed standard solutions under the optimized conditions for five times in 1 day and for once a day on 5 sequential days. The relative standard deviation (RSD) was considered to be a measurement of precision. As shown in Table-3, All RSD values of intra- and inter-day precision were from 1.86-6.02 %, indicating good precision. The accuracy tests were carried out by a recovery test. In the present study, the average recoveries of investigated targets ranged from 91.46.0 to 104.05 % (SNS) and from 90.31 to 101.43 % (CSS), with RSD values ≤ 4.5 % (Tables 4 and 5). It was clear that the developed method was reliable and accurate for the measurement.

TABLE-3
PRECISION DATA OF THE PROPOSED UPLC-PDA METHOD

Components	Nominal conc. (μg/mL)	Precision			
		Intra-day (n = 5)		Inter-day (n = 3)	
		Mean±SD (μg/mL)	RSD (%)	Mean±SD (μg/mL)	RSD (%)
Gallic acid	4.10	3.95±0.66	3.91	4.03±0.54	4.03
	16.40	17.46±0.89	2.23	16.75±1.15	1.98
	65.60	66.34±2.19	2.71	65.17±3.15	2.09
Oxypaeoniflorin	1.06	1.27±0.27	4.06	1.13±0.45	3.87
	4.24	4.08±0.51	3.37	4.21±0.29	3.01
	16.96	16.54±1.63	3.02	16.37±1.85	2.36
Albiflorin	1.27	1.31±0.35	4.64	1.28±0.26	3.07
	5.08	5.19±0.67	2.60	5.01±0.53	2.08
	20.32	21.66±1.88	3.51	20.59±0.98	2.61
Peoniflorin	4.82	4.61±0.73	3.91	4.75±0.69	3.87
	19.28	20.16±0.91	2.37	19.88±0.74	2.12
	77.12	76.51±1.56	1.87	77.66±2.17	1.86
Liquiritin	1.31	1.32±0.31	5.53	1.35±0.29	4.96
	5.24	5.37±0.46	2.65	5.08±0.37	2.18
	20.96	21.21±0.93	3.12	20.57±1.06	2.54
Benzoic acid	0.94	0.88±0.16	6.02	0.91±0.23	5.57
	3.76	3.81±0.33	3.81	3.68±0.19	4.09
	15.04	15.52±0.67	2.47	14.96±0.81	2.60
Narirutin	0.40	0.41±0.09	4.67	0.40±0.11	3.09
	1.60	1.56±0.16	3.16	1.59±0.12	2.87
	6.40	6.51±0.42	2.78	6.47±0.29	2.41
Naringin	0.40	0.38±0.09	5.17	0.37±0.07	4.46
	1.60	1.56±0.16	2.81	1.61±0.22	2.29
	6.40	6.35±0.97	3.15	6.39±0.87	1.97
Hesperidin	0.72	0.73±0.12	4.91	0.73±0.09	4.45
	2.88	2.71±0.25	2.18	2.81±0.18	1.96
	11.52	11.18±0.41	2.75	11.38±0.29	2.18
Neohesperidin	0.21	0.21±0.07	5.12	0.21±0.07	5.12
	0.84	0.83±0.12	3.25	0.84±0.09	3.01
	3.36	3.34±0.23	2.98	3.35±0.17	2.37
Meranzin hydrate	0.37	0.36±0.06	4.67	0.37±0.09	4.31
	1.48	1.49±0.23	4.15	1.48±0.11	3.16
	5.92	5.95±0.98	3.66	5.93±0.76	3.29
Liquiritigenin	0.41	0.40±0.12	3.97	0.41±0.10	3.17
	1.64	1.65±0.21	2.17	1.64±0.26	3.04
	6.56	6.55±0.97	2.49	6.55±0.88	2.73
Quercetin	0.30	0.29±0.08	5.51	0.29±0.06	4.61
	1.20	1.21±0.13	3.49	1.20±0.18	3.09
	4.80	4.80±0.65	3.01	4.81±0.69	2.88
Benzoyl-paeoniflorin	0.58	0.57±0.13	3.61	0.57±0.15	3.41
	2.32	2.30±0.26	3.24	2.32±0.33	3.07
	9.28	9.32±0.83	2.19	9.30±0.71	2.26
Isoliquiritigenin	0.16	0.16±0.06	3.97	0.16±0.05	3.61
	0.64	0.65±0.15	3.08	0.65±0.19	2.90
	2.56	2.57±0.55	2.62	2.56±0.39	2.07
Formononetin	0.15	0.15±0.03	4.19	0.15±0.03	3.82
	0.60	0.61±0.09	3.07	0.60±0.08	3.21
	2.40	2.41±0.37	3.11	2.40±0.29	2.91

TABLE-4
RECOVERIES FOR THE ASSAY OF 16 COMPOUNDS IN THE SNS (n = 5)

Components	Original (ng)	Spiked (ng)	Found (ng)	Recovery (%)	RSD (%)
Gallic acid	31.31	16.40	46.73	94.02	3.3
Oxypaeoniflorin	14.14	4.24	18.21	95.99	2.7
Albiflorin	7.25	5.08	12.09	95.28	3.1
Paeoniflorin	30.97	19.28	50.17	99.59	1.9
Liquiritin	15.80	5.24	20.63	92.18	1.6
Benzoic acid	5.38	3.76	8.97	95.49	2.4
Narirutin	4.19	1.60	5.83	102.5	1.3
Naringin	1.75	1.60	3.352	100.13	2.9
Hesperidin	5.29	2.88	8.03	95.14	4.1
Neohesperidin	1.88	0.84	2.68	95.24	3.5
Meranzin hydrate	1.53	1.48	3.07	104.05	2.2
Liquiritigenin	1.16	1.64	2.66	91.46	3.6
Quercetin	1.58	1.20	2.73	95.83	2.1
Benzoylpaeoniflorin	2.47	2.32	4.65	93.96	1.3
Isoliquiritigenin	0.68	0.64	1.27	92.18	1.6
Formononetin	2.14	0.60	2.69	91.67	3.2

TABLE-5
RECOVERIES FOR THE ASSAY OF 16 COMPOUNDS IN THE CSS (n = 5)

Components	Original (ng)	Spiked (ng)	Found (ng)	Recovery (%)	RSD (%)
Gallic acid	30.351	16.40	46.69	99.63	1.1
Oxypaeoniflorin	17.853	4.24	22.11	100.40	3.7
Albiflorin	9.369	5.08	14.27	96.47	3.3
Paeoniflorin	27.309	19.28	46.39	98.97	2.6
Liquiritin	20.667	5.24	25.73	96.62	1.8
Benzoic acid	3.996	3.76	7.81	101.43	2.1
Narirutin	8.382	1.60	9.83	90.5	4.5
Naringin	3.195	1.60	4.64	90.31	3.7
Hesperidin	11.028	2.88	13.75	94.51	2.6
Neohesperidin	3.054	0.84	3.87	97.14	1.3
Meranzin hydrate	1.737	1.48	3.19	98.18	1.5
Liquiritigenin	1.707	1.64	3.26	94.70	2.2
Quercetin	1.005	1.20	2.09	90.42	2.9
Benzoylpaeoniflorin	1.251	2.32	3.48	96.08	2.6
Isoliquiritigenin	0.735	0.64	1.36	97.66	3.1
Formononetin	0.927	0.60	1.49	93.83	1.8

Stability: The stability was evaluated by analyzing the sample solutions placed under 4 ºC and room temperature (about 25 ºC) at different time points (0, 24 and 48 h). The RSD values of retention times and peak areas for the 16 compounds were not more than 0.71 and 1.93 %, respectively. These data confirmed that the 16 compounds were stable within 48 h at 4 ºC and 25 ºC.

Sample analysis: The developed assay was subsequently applied to the simultaneous determination of the16 major compounds in SNS and CSS. A representative chromatogram of the extracts is shown in Fig. 2(C-D). The comparison of the 16 components between SNS and CSS was listed in Table-6. Of these, gallic acid, paeoniflorin, liquiritin and oxypaeoniflorin were the main components (> 47 μg/mL) in SNS and CSS and their contents are different between them. The lowest contents of the targets were isoliquiritigenin in SNS (2.25 ± 0.03 μg/mL) and in CSS (2.45 ± 0.05 μg/mL).The concentrations of narirutin, naringin, hesperidin and neohesperidin in CSS were significantly higher than these in SNS. These dates indicated that the contents of the compounds were different in the

TABLE-6
CONTENTS OF 16 COMPONENTS IN SNS (200 mg/mL) AND CSS (350 mg/mL) (n = 5)

Components	Contents/SNS (μg/mL)	RSD (%)	Contents/CSS (μg/mL)	RSD (%)
Gallic acid	104.36 ± 1.31	2.17	101.17 ± 1.09	3.06
Oxypaeoniflorin	47.13 ± 0.73	0.66	59.51 ± 0.77	1.02
Albiflorin	24.16 ± 0.39	1.88	31.23 ± 0.43	1.16
Paeoniflorin	103.22 ± 0.97	3.07	91.03 ± 1.01	2.73
Liquiritin	52.67 ± 0.52	1.18	68.89 ± 0.86	1.39
Benzoic acid	17.92 ± 0.39	0.63	13.32 ± 0.15	0.71
Narirutin	13.97 ± 0.56	1.76	27.94 ± 0.31	2.46
Naringin	5.84 ± 0.08	3.38	10.65 ± 0.23	2.81
Hesperidin	17.63 ± 0.26	0.93	36.76 ± 0.43	1.33
Neohesperidin	6.25 ± 0.13	1.74	10.18 ± 0.19	1.53
Meranzin hydrate	5.11 ± 0.07	1.97	5.79 ± 0.08	1.28
Liquiritigenin	3.85 ± 0.09	0.83	5.69 ± 0.11	1.07
Quercetin	5.26 ± 0.07	0.79	3.35 ± 0.13	0.89
Benzoylpaeoniflorin	8.23 ± 0.11	1.36	4.17 ± 0.07	1.09
Isoliquiritigenin	2.25 ± 0.03	1.57	2.45 ± 0.05	2.08
Formononetin	7.14 ± 0.05	1.29	3.09 ± 0.06	1.73

decoctions with different compatibility of herbs. The different compatibility principle of traditional Chinese medicine can affect the contents in SNS and CSS when they have the same herbs and maybe led to the difference of SNS and CSS in therapeutic effect.

Conclusion

A simple, sensitive, rapid and reliable UPLC-PDA method for the simultaneous determination of 16 compounds in SNS and CSS was successfully developed for the first time. The results of present study showed that this method could be suitable to control the quality of the two famous medicinal decoctions. The contents of the 16 components were different in SNS and CSS with the same three herbs (Radix Bupleuri, Raidix Paeoniae Alba and Radix Glycyrrhizae). The information obtained from this study indicates that different compatibility of herbs can affect the contents of targets in the two clinical decoctions.

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