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Original Article

Simultaneous quantification of eight organic acid components in *Artemisia capillaris* Thunb (Yinchen) extract using high-performance liquid chromatography coupled with diode array detection and high-resolution mass spectrometry

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ABSTRACT

We aim to determine the chemical constituents of Yinchen extract and Yinchen herbs using high-performance liquid chromatography coupled with diode array detection and high-resolution mass spectrometry. The method was developed to analyze of eight organic acid components of Yinchen extract (including neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, 1,3-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid). The separation was conducted using an Agilent TC-C18 column with acetonitrile – 0.2% formic acid solution as the mobile phases under gradient elution. The analytical method was fully validated in terms of linearity, sensitivity, precision, repeatability as well as recovery, and subsequently the method was performed for the quantitative assessment of Yinchen extracts and Yinchen herbs. In addition, the changes of selected markers were studied when Yinchen herbs decocting in water and isomerization occurred between the chlorogenic acids. The proposed method enables both qualitative and quantitative analyses and could be developed as a new tool for the quality evaluation of Yinchen extract and Yinchen herbs. The changes of selected markers in water decoction process could give us some novel idea when studying the link between substances and drug efficacy.

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1. Introduction

Yin-zhi-huang (YZH) preparations (granule, capsule, injection and oral liquid et al.) are widely used in clinical. YZH is a classical multi-herbal prescription derived from the famous traditional Chinese medicine formula of "Yin-chen-hao-tang", which has been widely used in many oriental countries for more than 1000 years to treat jaundice and chronic liver diseases [1–3]. According to the Chinese Pharmacopoeia (2015) [4], theoral polyherbal formulation of YZH is comprised of four Chinese herbal medicine extracts, i.e., *Artemisia capillaris* Thunb (Yinchen) extract, *Ganiema jasminoides* Ellis extract, *Scutellaria baicalensis* Georgi extract, *Lonicera japonica* Thunb extract [5]. Yinchen extract as a monarch drug in YZH prescription, is obtained by the methods of water extracting-alcohol precipitating of Yinchen herbs [4]. Yinchen (young shoot of *Autemisia capillaries*), belonging to Asteracea family, has the effects of clearing heat and eliminating damp. Biological and pharmacological studies have shown that it can be used clinically to treat cholestasis, hepatitis C, primary biliary cirrhosis, liver fibrosis, and cholestatic diseases [2,3]. Therefore, it is necessary to quality control the Yinchen extract and Yinchen herbs.

So far, Several analytical techniques have been reported for the analysis of the herbal components [6] and the measurement of one or several active ingredients in YZH preparations [7] or herbal formulas containing Yinchen [2,3,8]. Although those studies have a certain role in quality control of Yinchen herbs or its preparations, Yinchen extract as a direct and main feeding drug, there is no method available. Meanwhile, the preparation process of Yinchen extract is complex and there has been no reports on its active ingredients until now. Lack of qualitative and quantitative study of Yinchen extract would lead to the imperfection of quality control. In the recent years, YZH preparations, especially YZH injection, has increasing clinical adverse reaction reports [9], so the establishment of a quality control method on the main ingredients in Yinchen extract is essential to ensure YZH preparations efficacy and safety. Chemical investigations have shown that organic acids, coumarins, flavonoids and 4-hydroxyacetophenone are the major constituents in this herb [6]. Organic acids have been regarded as the material basis of heat clearing and detoxification, such as chlorogenic acid, caffeic acid, 3, 5-dicaffeoylquinic acid. Regarding this, chlorogenic acid is one of the most abundant organic acids, it is an ester formed between caffeic acid and quinic acid. Chlorogenic acid exhibits many biological properties, including antibacterial, antioxidant and anti-inflammatory activities, particularly hypoglycemic and hypolipidemic effects [10–12]. Caffeic acid is also an intriguing compound because it possesses various pharmacological activities, including antioxidants [13,14], antiviral [15,16], anti-cancer [17], anti-inflammatory [18,19] and antidiabetic [20,21] properties. The dicaffeoylquinic acids have potent activity against HIV integrase in vitro and prevent HIV replication in tissue culture [22]. Therefore, we aim to determine organic acids of Yinchen extract.

The objective of the present study was to develop and validate an analytical method for quality control of Yinchen extract using multiple markers, namely neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, 1,3-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid. For this purpose, a hyphened method of HPLC coupled with diode array detection and high-resolution mass spectrometry (HPLC-DAD-HRMS) was established to explore the chemical profiles of Yinchen extract and herbs. In addition, the changes of selected markers were studied in water decoction process and isomerization occurred between the chlorogenic acids. The proposed method enables both qualitative and quantitative analyses and could be developed as a new tool for the quality evaluation of Yinchen extract and Yinchen herbs. The changes of selected markers in water decoction process that we found could give us some novel idea when studying the link between substances and drug efficacy.

2. Experimental

2.1. Chemicals, reagents and materials

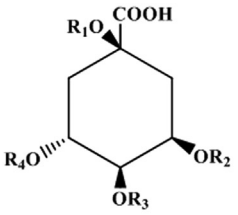
The reference standards of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, 1,3-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid were purchased from Bio-purify Phytochemicals Ltd.(Chengdu, China). Their structures are shown in Fig. 1. The purity of all reference compounds was determined to be >98% by normalization of the peak areas detected by HPLC-DAD.

Acetonitrile and formic acid of LC-MS grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other analytical chemical reagents of analytical grade were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Deionized water was purified using a Milli-Q system (Millipore, Billerica, MA, USA).

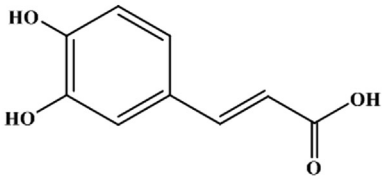
2.2. Plant material and sample pretreatment

The materials of Yinchen were collected from different areas of China. All the identities of Yinchen samples were respectively authenticated to be dried aboveground part of *Artemisia capillaris* Thunb by morphological and histological methods by Dr. Ying Zhang. Voucher specimens were deposited at College of Pharmacy, Jinan University. After collection, the samples were dried at 50 °C.

Preparation of Yinchen extracts: Yinchen (500 g) were immersed in 6000 mL distilled water (1:12, w/v) for 30 min and then decocted by boiling for 1 h. The extract was filtered. This procedure was then repeated twice by adding 4000 mL water at every turn. The three extracts were then combined and concentrated in vacuum to obtain a concentration of 2 g crude drug/mL, and then the water-extract was slowly added with ethanol to achieve a ratio of 7:3 (v/v), cold storage (4 °C) for 24 h and filtered. Then concentrated in vacuum to obtain a concentration of 5 g crude drug/mL. This procedure was then repeated by adding ethanol to achieve a ratio of 9:1 (v/v), cold storage (4 °C) for 24 h and filtered. Next, concentrated in vacuum to obtain a concentration of 10 g crude drug/ml. Then, 250 mL water was added, cold storage (4 °C) for 48 h and filtered to concentrate in vacuum to obtain Yin Chen extract (75 mg/g crude drug). Samples of individual herb were prepared with the same procedure to make extracts.



Quinic acid



Caffeic acid

No.	Compound name	R1	R2	R3	R4
1	neochlorogenic acid	H	H	H	caffeoyl
2	chlorogenic acid	H	caffeoyl	H	H
3	cryptochlorogenic acid	H	H	caffeoyl	H
4	caffeic acid	/	/	/	/
5	1,3-dicaffeoylquinic acid	caffeoyl	caffeoyl	H	H
6	3,4-dicaffeoylquinic acid	H	caffeoyl	caffeoyl	H
7	3,5-dicaffeoylquinic acid	H	caffeoyl	H	caffeoyl
8	4,5-dicaffeoylquinic acid	H	H	caffeoyl	caffeoyl

Fig. 1 – Structures of eight organic acid components.

2.3. *Preparation of standard solutions*

A mixed standard stock solution containing neochlorogenic acid (1), chlorogenic acid (2), cryptochlorogenic acid (3), caffeic acid (4), 1,3-dicaffeoylquinic acid (5), 3,4-dicaffeoylquinic acid (6), 3,5-dicaffeoylquinic acid (7) and 4,5-dicaffeoylquinic acid (8) was prepared in 50 mL methanol–water (50:50, v/v). The working-standard solutions were prepared by diluting the mixed standard solution with 50 mL methanol–water (50:50, v/v) to a series of proper concentrations within the ranges: (i) 0.88–444.00 μg/mL; (ii) 1.06–546.00 μg/mL; (iii) 2.04–153.00 μg/mL; (iv) 1.66–241.00 μg/mL; (v) 1.12–234.00 μg/mL; (vi) 1.66–819.00 μg/mL; (vii) 1.32–558.00 μg/mL and (viii) 0.70–318.00 μg/mL. All standard solutions were stored at 4 °C until use and filtered through a 0.45 μm nylon membrane, prior to injection.

2.4. *Preparation of sample solutions*

The Yinchen extract (0.4 g) and dried powders of Yinchen herb samples (1.0 g, 65 mesh) were weighed accurately into a 50 mL conical flask with stopper, and 50 mL methanol–water (50:50, v/v) was added. Yinchen extract was shaked and diluted at room temperature. Yinchen herb samples were followed by ultrasonic-assisted extraction (20 KHz) for 30 min. After the extraction, the extraction solvent was used to adjust the resultant mixture to its original weight, and the aliquots of supernatant were filtered through 0.45 μm membranes for HPLC injection.

2.5. *Apparatus and chromatographic conditions*

High-resolution mass spectral analysis was conducted using an HPLC-LTQ/Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). This hybrid system consists of a linear ion trap coupled to an Orbitrap Fourier transform mass spectrometer for accurate mass measurements. Samples were analyzed in negative ion mode with a tune method set as follows: sheath gas flow rate (N_2) of 30 arb; aux gas flow rate (N_2) of 5 arb; capillary temperature of 350 °C; ion spray voltage of 3.0 kV; capillary voltage of 25 V, and tube lens voltage of 110 V. Accurate mass analyses were calibrated according to the manufacturer's guidelines using a standard-solution mix of SDS, sodium taurocholate, the tetrapeptide MRFA acetate salt, and Ultramark (all from Sigma–Aldrich). Centroid mass spectra were acquired in the mass range of *m*/z 100–1200. In the full scan mode, resolution of the Orbitrap mass analyzer was set at 30,000 (full width at half maximum as defined at *m*/z 400).

An Agilent TC-C18 column (5 μm, 4.6 × 250 mm) was used and maintained at 30 °C. The mobile phase was 0.2% formic acid aqueous solution (A) and acetonitrile (B) with a gradient program as follows: 0–20 min, linear gradient 7–15% B; 20–30 min, linear gradient 15–20% B; 30–40 min, isocratic elution 20% B; 40–50 min, linear gradient 20–30% B, and the postrun (10 min) at a flow rate of 1.0 mL/min. The injection volume was 10 μL.

Quantitative analysis was conducted using an Agilent 1260 LC system (Santa Clara, CA, USA), equipped with a DAD (190–400 nm), a quaternary solvent delivery system, a column temperature controller, and an autosampler. Chromatographic data were recorded and processed with Agilent chromatographic workstation software. The HPLC conditions were identical to those used for HPLC-MS analyses mentioned above. Signal monitoring was performed at 330 nm.

2.6. *Validation of the HPLC method*

2.6.1. *Calibration curves, limits of detection, and quantification*

The calibration curve for each compound was established by plotting the peak area (y) versus concentration (x) of each analyte. The LOD and LOQ for eight analytes were estimated at S/N of 3 and 10, respectively, by injecting a series of dilute solutions with known concentration.

2.6.2. Precision, repeatability, and accuracy

The intra- and inter-day precisions were determined by analyzing known concentrations of the 8 analytes in 6 replicates during a single day and by duplicating the experiments on three successive days. In order to confirm the repeatability, 6 different working solutions prepared from the same sample (No. 1) were analyzed. Variations of the peak area were taken as the measures of precision and repeatability expressed as percentage RSD values. The results are shown in Table 3, indicating that the intra-, inter-day and repeatability RSD values of the eight compounds were all less than 2.98%, which showed good reproducibility of the developed method. To confirm the stability, sample 1 was tested at room temperature and analyzed at 0, 3, 6, 12, 18, 24 h, respectively. The RSD values of peak areas of 8 target compounds were taken as a measurement of stability.

Recovery tests were carried out to further investigate the accuracy of the method by adding three concentration levels (150%, 100% and 50%) of the mixed standard solutions to known amounts of Yinchen extract samples. Then the samples were extracted and analyzed with the proposed method, and triplicate experiments were performed at each level. The mean recoveries were estimated according to the following formula: recovery (%) = [(amount found − original amount)/amount spiked] × 100%. The recovery of the method was in the range of 94.88–103.8%, with RSD less than 2.96% as shown in Table 4. Considering the results, the method was deemed to be accurate.

3. Results and discussion

3.1. Optimization of the HPLC conditions

Different mobile phases and columns were investigated to optimize the HPLC analysis. Compared with methanol, acetonitrile was more suitable for the separation as mobile phase. Formic acid was used as a mobile phase modifier to suppress the peak tailing. An Agilent TC-C18 column (5 μm, 4.6 × 250 mm) and an Agilent eclipse XDB-C18 column (5 μm, 4.6 × 250 mm) were tested. The analytical results showed that the former one was more suitable, exhibiting better peak separation. According to the analytical results of 3D plots for 8 target organic acid components, the maximum absorption wavelength was approximately at 330 nm. Hence, we choose 330 nm as the detection wavelengths. Representative chromatograms for the standard analytes and the samples are shown in Fig. 2.

3.2. Identification of constituents in the samples by HPLC-HRMS

MS spectra were collected in both positive and negative modes. The negative mode was used in our studies because it provided better sensitivity compared with the positive mode. Under the present chromatographic and MS conditions, eight peaks detected from these samples were identified as neochlorogenic acid (peak 1), chlorogenic acid (peak 2), cryptochlorogenic acid

Table 1 – Components identified from Yinchen extract and Yinchen herbs by HR-ESI-MS.

Peak no.	Formula	Calculated (m/z)	Measured (m/z)	Error (ppm)	LC-MS^n	Proposed compound
1	$C_{16}H_{18}O_9$	353.0867	353.0833	−9.540	MS^2[353]191(100), 179(46), 135(10), 173(10) MS^3[191] 127(100), 173(62), 93(60)	Neochlorogenic acid
2	$C_{16}H_{18}O_9$	353.0867	353.0839	−8.068	MS^2[353] 191(100), 179(4), 135(1) MS^3[191] 127(100), 173(76), 93(57)	Chlorogenic acid
3	$C_{16}H_{18}O_9$	353.0867	353.0835	−9.087	MS^2[353]173(100), 179(60), 191(17), 135(9) MS^3[173] 93(100), 111(57), 155(19)	Cryptochlorogenic acid
4	$C_9H_8O_4$	179.0339	179.0345	3.601	MS^2[179] 135(100)	Caffeic acid
5	$C_{25}H_{24}O_{12}$	515.1184	515.1163	−2.072	MS^2[515] 353(100), 335(31), 179(22), 191(7) MS^3[353]191(100), 179(49), 135(10), 173(3)	1,3-dicaffeoylquinic acid
6	$C_{25}H_{24}O_{12}$	515.1184	515.1168	−3.042	MS^2[515]353(100), 173(15), 335(12), 179(11), 191(5) MS^3[353]173(100), 179(66), 191(47), 135(11)	3,4-dicaffeoylquinic acid
7	$C_{25}H_{24}O_{12}$	515. 1184	515.1160	−2.237	MS^2[515]353(100), 191(2), 179(1), 335(1) MS^3[353]191(100), 179(45), 135(9), 173(4)	3,5-dicaffeoylquinic acid
8	$C_{25}H_{24}O_{12}$	515.1184	515.1162	−2.252	MS^2[515] 353(100), 203(11), 299(9), 173(7), 255(6) MS^3[353]173(100), 179(61), 191(27), 135(9)	4,5-dicaffeoylquinic acid

Table 2 – Regression Equations, Correlation Coefficients, Linear Ranges and LOD and LOQ of eight Target Compounds.

No.	Compound name	Liner (μg/mL)	Regression equation	Correlation coefficient (r)	LOD (μg/mL)	LOQ (μg/mL)
1	Neochlorogenic acid	0.88–444	y = 30.09x + 1.8574	0.9999	0.30	0.88
2	Chlorogenic acid	1.06–546	y = 28.08x + 8.77	0.9999	0.37	1.06
3	Cryptochlorogenic acid	2.04–153	y = 28.88x + 26.352	0.9997	0.66	2.04
4	Caffeic acid	1.66–241	y = 44.29x + 128.11	0.9999	0.54	1.66
5	1,3-dicaffeoylquinic acid	1.12–234	y = 110.27x − 388.37	0.9996	0.37	1.12
6	3,4-dicaffeoylquinic acid	1.66–819	y = 32.17x + 16.66	0.9999	0.52	1.66
7	3,5-dicaffeoylquinic acid	1.32–558	y = 38.82x + 17.29	0.9999	0.41	1.32
8	4,5-dicaffeoylquinic acid	0.70–318	y = 38.31x + 8.08	0.9999	0.21	0.70

Table 3 – Precision, repeatability, stability, and recovery of eight organic acids in Yinchen extract.

Analytes	Precision (RSD%)[a]		Repeatability (RSD%, n = 6)	Stability[a] (RSD%, n = 6)	Recovery[b] (%,n = 3)	
	Intraday (n = 6)	Interday (n = 3)			Mean	RSD%
1. Neochlorogenic acid	0.01	2.73	0.60	1.53	103.8	1.46
					96.77	0.66
					94.88	1.84
2. Chlorogenic acid	0.01	2.98	0.46	0.88	98.00	2.88
					94.98	1.34
					98.90	2.71
3. Cryptochlorogenic acid	0.01	0.49	0.53	2.29	97.78	1.47
					97.77	1.33
					97.56	1.66
4. Caffeic acid	0.01	2.90	1.20	2.17	99.03	2.56
					99.05	2.78
					96.32	2.26
5. 1,3-dicaffeoylquinic acid	0.03	2.51	0.36	2.20	99.23	2.72
					101.1	1.74
					99.34	2.96
6. 3,4-dicaffeoylquinic acid	0.01	2.98	1.01	2.59	100.4	1.25
					103.4	2.37
					99.20	1.49
7. 3,5-dicaffeoylquinic acid	0.02	2.82	2.88	2.78	99.47	0.31
					101.9	1.83
					98.87	2.68
8. 4,5-dicaffeoylquinic acid	0.01	2.95	1.52	1.52	95.92	2.35
					98.23	2.38
					95.54	1.34

[a] RSD (%) = (SD/mean) × 100.
[b] Recovery (%) = 100 × (amount found − original amount)/amount spiked.

Table 4 – The changes of selected markers in water decoction process at different point.

Time (h)	Concentration (mg/mL, n = 3)							
	1[a]	2	3	4	5	6	7	8
0	0.0328 ± 0.003	0.2769 ± 0.001	0.0481 ± 0.005	0.0139 ± 0.001	0.0123 ± 0.001	0.0267 ± 0.002	0.1018 ± 0.001	0.0507 ± 0.001
0.5	0.0466 ± 0.001	0.2472 ± 0.001	0.0619 ± 0.006	0.0145 ± 0.007	0.0203 ± 0.003	0.0311 ± 0.001	0.0893 ± 0.002	0.0474 ± 0.002
1.0	0.0656 ± 0.005	0.2206 ± 0.004	0.0753 ± 0.001	0.0156 ± 0.003	0.0296 ± 0.001	0.0368 ± 0.002	0.0776 ± 0.009	0.0440 ± 0.001
1.5	0.0760 ± 0.009	0.1959 ± 0.003	0.0790 ± 0.010	0.0157 ± 0.001	0.0338 ± 0.004	0.0382 ± 0.004	0.0615 ± 0.001	0.0407 ± 0.004
2.0	0.0868 ± 0.001	0.1853 ± 0.002	0.0888 ± 0.004	0.0164 ± 0.005	0.0412 ± 0.001	0.0425 ± 0.001	0.0603 ± 0.003	0.0402 ± 0.001
2.5	0.0955 ± 0.003	0.1683 ± 0.002	0.0952 ± 0.003	0.0168 ± 0.003	0.0463 ± 0.001	0.0431 ± 0.002	0.0546 ± 0.007	0.0385 ± 0.002
3.0	0.1008 ± 0.002	0.1572 ± 0.001	0.0977 ± 0.001	0.0168 ± 0.005	0.0497 ± 0.009	0.0456 ± 0.005	0.0484 ± 0.001	0.0384 ± 0.003

[a] The analysts are the same as in Fig. 1 and Table 1.

(peak 3), caffeic acid (peak 4), 1,3-dicaffeoylquinic acid (peak 5), 3,4-dicaffeoylquinic acid (peak 6), 3,5-dicaffeoylquinic acid (peak 7) and 4,5-dicaffeoylquinic acid (peak 8) by comparison of their HPLC retention times and HRMS spectrometric data with those of reference compounds. Table 1 summarizes the related data of the identified compounds, including measured and calculated *m/z* for the molecular formulas provided, error value and the main fragments obtained by MS^n, as well as the proposed compound for each peak.

3.3. *HPLC method validation*

The proposed HPLC-DAD method for quantitative analysis was validated by determining the linearity, LOD, LOQ, intra- and interday precisions, stability, and accuracy. As shown in Table 2, all calibration curves showed good linearity ($R > 0.9997$) within the test ranges, and the overall LODs and LOQs were in the ranges of 0.21–0.66 and 0.70–2.04 μg/mL, respectively. The RSD values of intra- and interday variations, repeatability, and stability of the eight analytes were <2.98%, and the overall recoveries lay between 94.88 and 103.80% with RSD < 2.96% (Table 3). In addition, the peak purity was investigated by analyzing the DAD and MS data; no indications of impurities could be found. Taken together, the results indicated that the established method was accurate for the determination of eight chemical markers in the samples. Considering the results, the method was deemed to be accurate.

3.4. *Quantitative analysis of different areas of Yinchen extract*

The developed HPLC method was applied to simultaneous determination of the chemical markers including

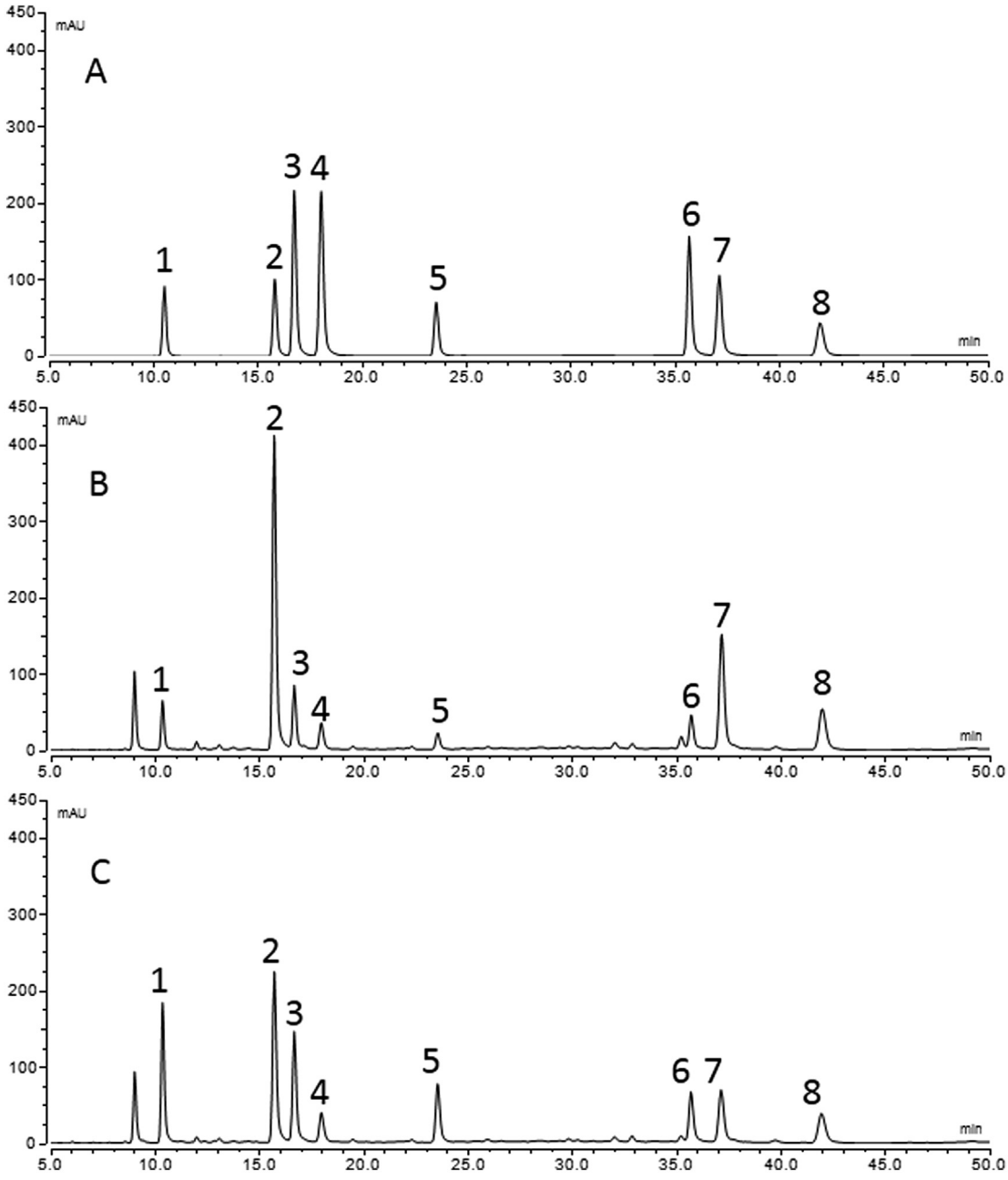


Fig. 2 – Representative HPLC chromatograms of mixed standards (A), Yinchen herb (B), Yinchen extract (C). The peak numbers are stand for neochlorogenic acid(1), chlorogenic acid (2), cryptochlorogenic acid (3), caffeic acid (4), 1,3-dicaffeoylquinic acid (5), 3,4-dicaffeoylquinic acid (6), 3,5-dicaffeoylquinic acid (7) and 4,5-dicaffeoylquinic acid (8).

neochlorogenic acid (1), chlorogenic acid (2), cryptochlorogenic acid (3), caffeic acid (4), 1,3-dicaffeoylquinic acid (5), 3,4-dicaffeoylquinic acid (6), 3,5-dicaffeoylquinic acid (7) and 4,5-dicaffeoylquinic acid (8) in samples. The results are shown in Supplementary Tables 1 and 2 as the mean values of the three replicate injections.

There were remarkable differences among the contents analyzed in different samples as shown in Supplementary Tables 1 and 2. For example, although chlorogenic acid was abundant in Yinchen herbs among the analyzed compounds, other ingredients like caffeic acid, 1,3-dicaffeoylquinic acid were lower than LOQ in some batches. These differences in chemical composition might be attributable to multiple factors, such as differences in climate, growing region, harvest conditions. While, Yinchen extract quantitative results show that the content differences decreases by a water extracting-alcohol precipitating method. Yinchen extract as a monarch drug in YZH prescription, should reflect the quality of medicinal preparations more directly. While, there has been no quantitative analysis for multiple compounds belonging to one group in Yinchen extract until now. Simple quantitative analysis of one or two active components in herb does not represent its integral quality. The method enables both qualitative and quantitative analyses, this will contribute to the

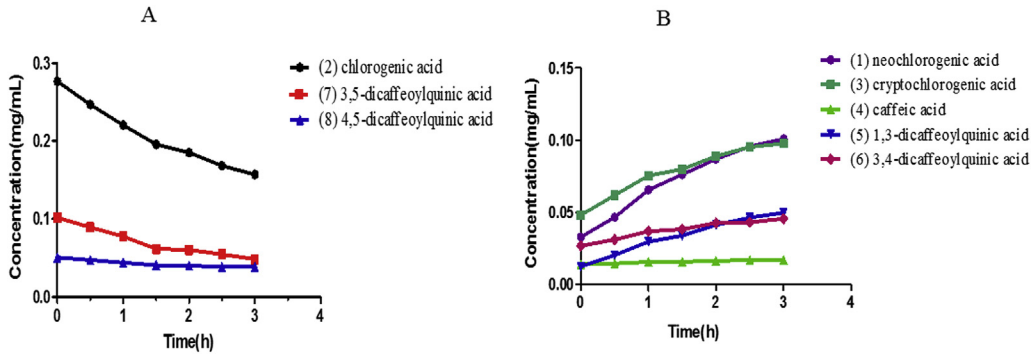


Fig. 3 – The change curves of selected markers in water decoction process (A, B).

comprehensive development of Yinchen. Thus, the establishment of a quality control method is essential to ensure the product efficacy and safety.

3.5. *The changes of selected markers in water decoction process*

Yinchen extract quantitative results were compared with that of Yinchen herbs, we found through a water extracting-alcohol precipitating procedure, chlorogenic acid reduced by a large margin and the content differences decreases. In order to figure out how the selected markers change in procedure, Yinchen herb (50 g) were immersed in 600 mL distilled water and decocted by boiling for 1 h. Then 500 mL Yinchen decoction was filtered and transferred for boiling and refluxing for different time. Samples were obtained at every half an hour. Then, the samples were centrifuged at 12,000 g for 10 min at 4 °C and the supernatant was injected into the HPLC system for analysis. The results are shown in Table 4 and Fig. 3. Hydrolysis of the esters may occur during decocting process with the caffeic acid in a small increase. However, the content of caffeic acid and 4,5-dicaffeoylquinic acid are very few, and they both have little change in water decoction process. Hence, they are not included in the subsequent discussion. The chlorogenic acids have the same structure as the parent nucleus, as the heating time increased, chlorogenic acid reduced by a large margin, with the concentration from 0.2769 to 0.1572 mg/mL, reduced by 43.2%, while neochlorogenic acid and cryptochlorogenic acid significantly increased, from 0.0328 to 0.1008, 0.0481 to 0.0977 mg/mL, respectively. In addition, the proportion of the double caffeic acid components changed greatly, 3,5-dicaffeoylquinic acid declined significantly with time, with the concentration from 0.1018 to 0.0484 mg/mL, the other two, 1,3-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid increased from 0.0123 to 0.0497, 0.0267 to 0.0456 mg/mL, respectively. It is speculated that the reason may be the occurrence of isomerization. The possible transformation pathways are shown in Supplementary Fig. 1.

4. Conclusion

This study developed and validated an HPLC-DAD-HRMS method for qualitative and quantitative analyses of eight organic acids, namely, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, 1,3-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid in Yinchen extract and Yinchen herbs. The proposed method enables both qualitative and quantitative analyses and could be developed as a new tool for the quality evaluation of Yinchen extract and Yinchen herbs. The changes of selected markers in water decoction process that we found could give us some novel idea when studying the link between substances and drug efficacy.

Conflicts of interest

All authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfda.2017.04.003.

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