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Simultaneous Determination of Seven Bioactive Compounds in Wuji Pill by HPLC

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Abstract

A high performance liquid chromatography coupled with photodiode-array detection method was developed for simultaneous determination of gallic acid, chlorogenic acid, paeoniflorin, jatrorrhizine hydrochloride, dehydroevodiamine, palmatine chloride and berberine hydrochloride in the Chinese proprietary medicine "Wuji Pill" (WJP). The analysis was performed by reversed phase gradient elution, using an aqueous mobile phase (containing 0.05 mol/L monopotassium phosphate and 0.1% aqueous phosphosnic acid) modified by acetonitrile and detection made at 340 nm. The method was validated for limits of detection and quantification, precision, repeatability, stability, accuracy and robustness. Five batches of self-manufactured WJP were analyzed and found to contain different amounts of the seven bioactive markers. The method could be used for quality assessment of this Chinese materia medica preparation.

Keywords: Simultaneous determination; Seven bioactive compounds; Wuji pill; High performance liquid chromatography

Introduction

Traditional Chinese Medicines (TCMs) are often prescribed by Chinese physicians and have been extensively used to prevent and cure human disease for thousands of years, which attract increasing attention in many fields up to the present time. They are usually composed of several herbs containing multiple chemical constituents. Hence, the analysis of such a complex mixture presents a great challenge to the pharmaceutical analysis. In recent years, chemical fingerprinting has gained increasing attention to be used for the quality control [1-8], which has been introduced and accepted by WHO as a strategy for the assessment of TCMs [9] and also required by Drug Administration Bureau of China to standardize injections made from raw materials [10]. Fingerprinting analysis offers integral characterization of a complex system with a quantitative degree of reliability and focuses on the identification and assessment of stability of the compounds. The identity, consistency and authenticity of samples could be determined by comparison of their chromatographic fingerprints using similarity analysis and chemometrics methods. However, if we want to realize the differences between the amounts of chemical compounds in different samples, quantification of the chemical constituents is one of the must means. WJP, a well-known ancient Chinese herbal formula, is included in Chinese Pharmacopoeia [11] for stomaching of gas, controlling nausea and vomiting. Modern pharmacological researches indicate that WJP could inhibit the colon contraction in guinea-pig [12], suppress enzymatic activity of CYP1A2 [13] and reduce the content of NO in mouse infected by Hp obviously [14]. The Chinese Pharmacopoeia records the formula of WJP as follow: 300 g of *Coptis chinensis* Franch., 50 g of *Evodia rutaecarpa* Juss. Benth and 300 g of *Paeonia lactiflora* Pall. Currently, there are a few analytical methods available for evaluating the quality of WJP [15-17], which are only able to determine one or two active constituents, even though there are several biomarkers in WJP, which couldn't evaluate its quality comprehensively to ensure the safety and efficacy in clinical applications effectively. In the present study, therefore, a simple, precise, accurate and reliable HPLC method for simultaneous determination of the seven bioactive chemical compounds, i.e. gallic acid, chlorogenic acid, paeoniflorin, jatrorrhizine hydrochloride, dehydroevodiamine, palmatine chloride and berberine hydrochloride (structures shown in Figure 1), in WJP was developed. Compared with the reported methods [15-17], to improve the sensitivity and selectivity of the separation, the optimization of the mobile phase system (including the composition of solvents, pH value and concentration of buffer), detection wavelength and extraction method was undertaken for quantitative analysis. To the best of our knowledge, the seven chemical compounds were firstly quantified as bioactive markers in this Chinese proprietary medicine and no study was carried out to simultaneously determine the seven compounds in one run. In addition, the method was fully validated and also successfully applied for quantification of the 5 batches of WJP. More importantly, five batches of WJP in our study were self-manufactured in our lab with the three herbs that were collected from different regions, which showed that the method established in the present study was durable.

Experimental

Chemicals and reagents

Acetonitrile, HPLC grade, was purchased from TEDIA Technology Corporation Inc. (Product of Tedia, USA). Ultrapure water was prepared with the Sartorius Arium611UF water purification system (18.2 MΩ, Sartorius, Germany). Phosphoric acid, analytical grade, was purchased from Sichuan Reagent Company (Sichuan, China). Other reagent solutions were of analytical grade. The chemical references of gallic acid, chlorogenic acid, paeoniflorin, jatrorrhizine hydrochloride, palmatine chloride and berberine hydrochloride were from

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National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Dehydroevodiamine was offered by the Research Center for Quality Control of Natural Medicine, Guizhou Normal University, and the structure was confirmed on the basis of spectroscopic analysis (1H-NMR, 13C-NMR, ESI-MS, UV). The purity was 98.13%.

Plant materials

Two batches of *Evodia Rutaecarpa* (Juss.) Benth. were collected from Guizhou (A-1) and Hunan (A-2). One batche of *Paeonia lactiflora* Pall. was collected from Anhui (B-1). Three batches of the *Coptidis chinensis* Franch were collected from Shichuan (C-1), Yunnan (C-2) and Guizhou (C-3). All of them were purchased from Ji-Ren-Tang Pharmaceutical Corporation (Guiyang, China) and were authenticated by Professor Zhuying He, who is the professor from the first affiliated hospital of Guiyang College of Traditional Chinese Medicine. The medicinal parts of them were fruits, roots and rhizomes, respectively.

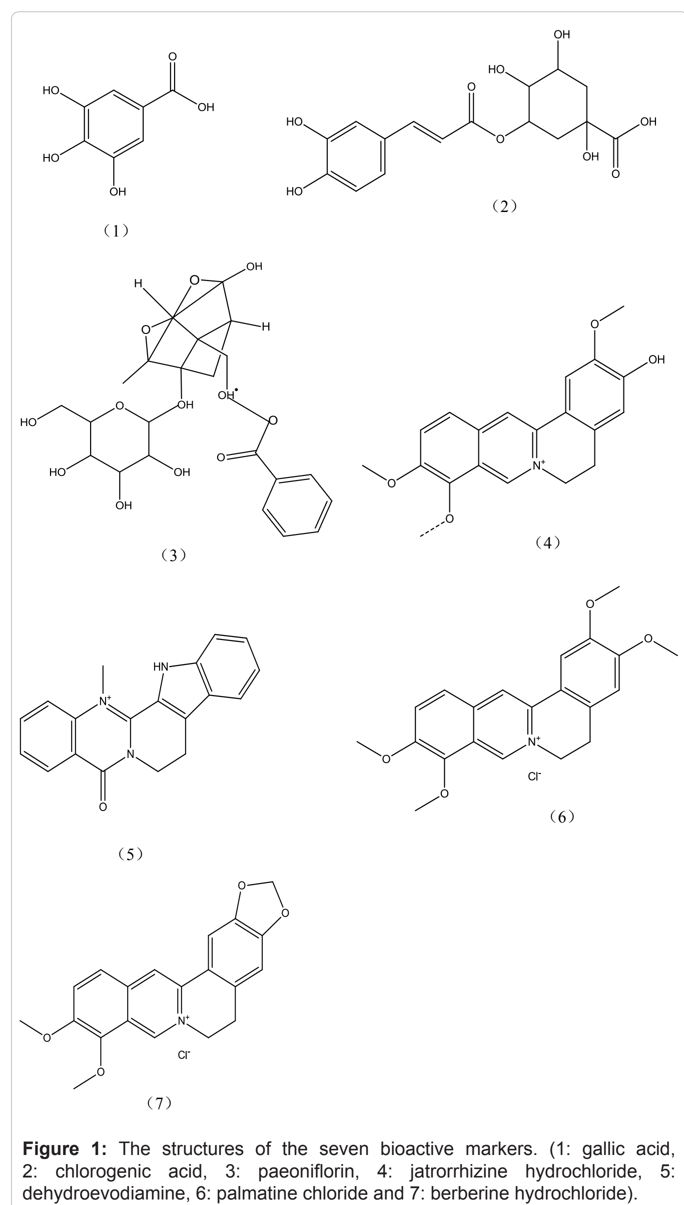

Figure 1: The structures of the seven bioactive markers. (1: gallic acid, 2: chlorogenic acid, 3: paeoniflorin, 4: jatrorrhizine hydrochloride, 5: dehydroevodiamine, 6: palmatine chloride and 7: berberine hydrochloride).

Sample preparation

Firstly, different proportions of different batches of *E. rutaecarpa*, *P. lactiflora* and *C. chinensis* were used to self-manufactured WJP. The proportions of the components is 1:6:6.

Secondly, the self-manufactured WJP was ground into powder. The finely pulverized powder was weighed (1.5 g), and 100 mL of methanol was added, and the mixture was extracted for 30 min, using ultrasonic extraction device. The sample solution was filtered through a 0.45 μm membrane filter (Millipore, Nylon, 170 mm) prior to analysis.

Standard solution preparation

The reference compounds were weighed accurately and dissolved in methanol in a 25 ml volumetric flask to make a stock solution (0.4020, 0.3872, 0.8420, 0.3988, 0.2068, 0.4888 and 0.4722 mg/ml for gallic acid, chlorogenic acid, paeoniflorin, jatrorrhizine hydrochloride, dehydroevodiamine, palmatine chloride and berberine hydrochloride, respectively). Working standard solutions were prepared from the stock solution by further dilution with the appropriate volume of methanol. These solutions were stored away from light at 4°C.

Instrumentation and chromatographic conditions

An Agilent 1100 series HPLC instrument equipped with a quaternary pump, a diode-array detector, an autosampler, a column compartment and a Chem Station for LC 3D software was used. Chromatographic separations were carried out on an Agela promosil C18 column (250 mm × 4.6 mm I.D., 5 μm). The mobile phase consisted of acetonitrile (A) and 0.05 mol/L monopotassium phosphate and 0.1% aqueous phosphosnic acid (B). The flow rate was set at 0.8 mL/min. The flow rate program was as follow: 0 ~ 15 min, linear gradient 5 ~ 15% A; 15 ~ 70 min, linear gradient 15 ~ 28%A. The volume injected was 5 μL. The column temperature was maintained at 25°C. Detection was made simultaneously at 340 nm.

Results and Discussion

Optimization of extraction method

Comparison of two common extraction methods: Two frequently-used extraction methods were compared with contents of gallic acid, paeoniflorin and berberine hydrochloride as indicators: 1) Ultrasonic extraction: 1.5 g WJP was dissolved in 100 mL water (80~100°C) and then sonicated for 20 min; 2) Shaking method: 1.5 g WJP was dissolved in 100 mL water (80~100°C) and then shaked out till the solid was totally dissolved. The solutions obtained were filtered through a 0.45 μm micropore membrane, and 10 μL of each treated solution was injected into the HPC instrument for analysis.

The results indicated that the contents of gallic acid, paeoniflorin and berberine hydrochloride in the solution with ultrasonic extraction (1.74 ± 0.21, 6.73 ± 0.18, and 4.68 ± 0.09 mg/g, respectively) were significantly higher (P<0.01) than those in the solution with shaking method (1.47 ± 0.23, 6.17 ± 0.22, 4.08 ± 0.12 mg/g, respectively). So, ultrasonic extraction was selected as extraction method.

Chosen of extraction solvents and extraction time: On the basis of the ultrasonic extraction, different solvents and extraction times were further optimized.

Water, absolute ethanol and methanol were firstly tested to obtain an efficient extraction of the target compounds and avoid the co-extraction of undesired compounds, the extract obtained from each

test was quantitatively analyzed by HPLC-DAD to determine the contents of the three representative bioactive compounds and then the extraction yields of the extract and each compound were calculated. The results of experiments indicated that the highest contents of paeoniflorin and berberine hydrochloride (7.68 ± 0.26 and 6.71 ± 0.31 mg/g) were obtained by using methanol as solvent (P<0.01). The highest content of gallic acid was obtained by absolute ethanol (2.04 ± 0.08 mg/g), but the result was not significant different (P<0.05) from that obtained by using methanol as extraction solvents (Figure 2a). So, methanol was selected as extraction solvent.

Different times (5, 15, 25, 30, 60 min) were further studied respectively in order to find out the optimum extraction time. All the experiments were repeated for three times (n=3). Figure 2b showed that, for the three chemical compounds, the extraction efficiency increased when extraction time increased from 5 min to 60 min, reaching the highest value at 2.28 ± 0.13, 7.92 ± 0.21, and 6.92 ± 0.22 mg/g for gallic acid, paeoniflorin and berberine hydrochloride, respectively. But, the values (2.15 ± 0.22, 7.71 ± 0.38, 6.75 ± 0.42 mg/g for gallic acid, paeoniflorin and berberine hydrochloride, respectively) at 30 min were not significant different (P<0.05) from those obtained by extracting for 40 min and for 60 min. Hence, 30 min was the most appropriate time for extraction.

The results demonstrated that the established extraction method was adequate and appropriate for the analysis. (Figure 2)

Optimization of the chromatographic conditions

In the present study, the seven bioactive compounds could not be separated effectively by using the isocratic mobile phases. In order to find an easy way to analyze the compounds, we employed a gradient solvent system (acetonitrile, monopotassium phosphate and phosphosnic acid solution), which could effectively separate the seven bioactive compounds simultaneously. Optimized chromatographic conditions were achieved after several trials with methanol, acetonitrile, different concentrations of monopotassium phossphate and phosphosnic acid in different proportions as mobiles phases. The solution obtained from each sample was injected into the instrument and the peaks of the seven compounds in the chromatograms obtained were identified by comparison of retention times and online UV spectra with those of the standards. Retention times for gallic acid, chlorogenic acid, paeoniflorin, jatrorrhizine hydrochloride, dehydroevodiamine, palmatine chloride and berberine hydrochloride were 8.16, 18.00, 26.62, 52.24, 54.84, 63.39 and 65.95 min, respectively. The representative chromatograms are shown in Figure 3 and it could be concluded that all reference compounds were eluted with highly symmetrical peaks under the condition.

Full UV spectra (190-400 nm) of mixed standards were obtained

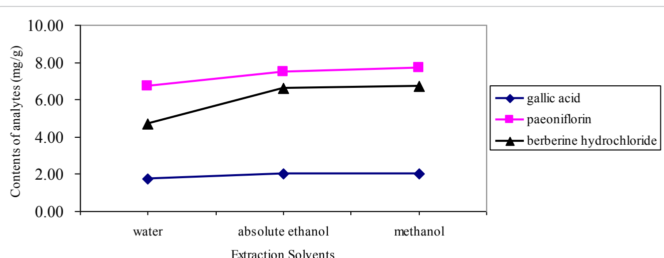

Figure 2a: The influence of different solvents on extraction of gallic acid, paeoniflorin and berberine hydrochloride.

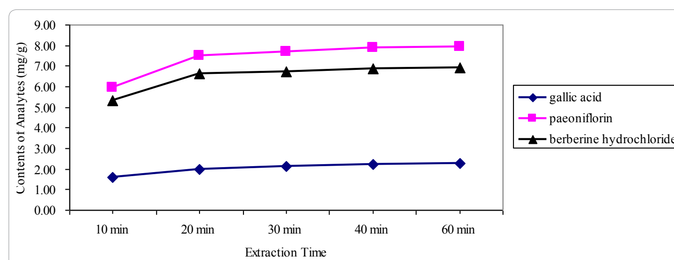

Figure 2b: The influence of different times on extraction of gallic acid, paeoniflorin and berberine hydrochloride.

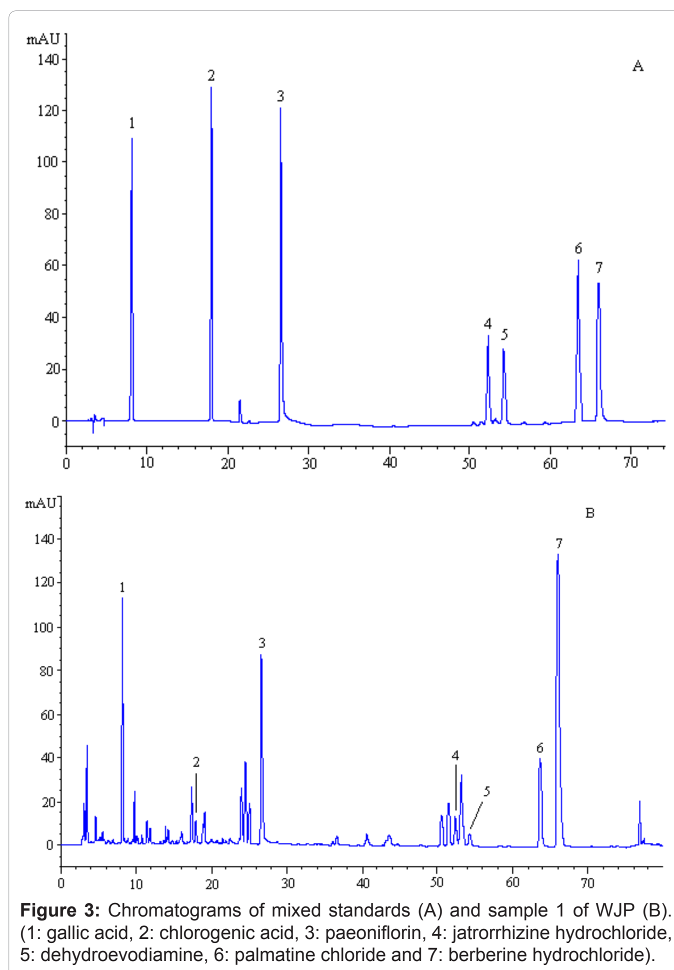

Figure 3: Chromatograms of mixed standards (A) and sample 1 of WJP (B). (1: gallic acid, 2: chlorogenic acid, 3: paeoniflorin, 4: jatrorrhizine hydrochloride, 5: dehydroevodiamine, 6: palmatine chloride and 7: berberine hydrochloride).

from DAD. The maximum absorption wavelengths of the seven compounds were different, but all of them could be dissolved with baseline separation and better resolution at 340 nm, which was therefore used as detection wavelength for all the seven compounds.

Validation of the HPLC method

Calibration curves, LOD and LOQ: The stock solution containing the seven analytes was prepared and diluted to appropriate concentration ranges for establishment of calibration curves. The calibration graphs were plotted after linear regression of the peak areas versus the corresponding concentrations. The linear regressions and ranges were described as following: gallic acid, $y = 3623.4\,x + 12.148$, $r = 0.9995$, 0.0203 ~ 0.4068 mg; chlorogenic acid, $y = 1822.9\,x + 5.008$, $r = 0.9995$, 0.0119 ~ 0.0952 mg; paeoniflorin, $y = 1388.2\,x - 2.5732$, r

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= 0.9995, 0.0421 ~ 0.8420 mg; jatrorrhizine hydrochloride, $y = 5684.8x + 0.5425$, $r = 0.9998$, 0.0079 ~ 0.0797 mg; dehydroevodiamine, $y = 3648.3x - 6.7303$, $r = 0.9993$, 0.0165 ~ 0.0496 mg; palmatine chloride, $y = 4170.6x + 10.747$, $r = 0.9998$, 0.0391 ~ 0.3910 mg; berberine hydrochloride, $y = 5191.6x + 49.152$, $r = 0.9998$, 0.1181 ~ 1.7707 mg, where y is the peak area, x is the concentration, and r is the correlation coefficient.

LOD was determined at signal-to-noise ratios (S/N) of about 3. The values were 0.2712, 0.1606, 0.9172, 0.4028, 0.3447, 0.5587 and 0.4722 ng for gallic acid, chlorogenic acid, paeoniflorin, jatrorrhizine hydrochloride, dehydroevodiamine, palmatine chloride and berberine hydrochloride, respectively. LOQ were determined at S/N of about 10. The values were 0.8136, 0.4817, 2.7516, 1.2085, 1.0341, 1.6761, 1.4166 ng for gallic acid, chlorogenic acid, paeoniflorin, jatrorrhizine hydrochloride, dehydroevodiamine, palmatine chloride and berberine hydrochloride, respectively.

Precision, repeatability and stability: Precision was evaluated with sample solution under the selected optimal conditions six times in one day for inter-day variation and twice a day on three consecutive days for intra-day variation. Repeatability was confirmed with six different working solutions prepared from sample No. 1 and each of them was injected into the apparatus every 2 h within 10 h to evaluate the stability of the solution. All the results were expressed as relative standard deviations (*RSD*) which were shown in Table 1.

Recovery: The recovery was performed by adding known amounts of the seven standards at low (80% of the known amounts), medium (same as the known amounts) and high (120% of the known amounts) levels. The spiked samples were then extracted, processed, and quantified in accordance with the methods mentioned above. The results were shown in Table 2.

Robustness: Method robustness was tested on Hypersil C18 column (250 mm × 4.6 mm I.D., 5 μm) and Agela promosil C18 column (250 mm × 4.6 mm I.D., 5 μm). The same sample solution was separately analyzed and the contents of the seven characteristic constituents were calculated. The contents of gallic acid, paeoniflorin and dehydroevodiamine were 2.14 ± 0.42, 7.68 ± 0.48, 6.72 ± 0.32 mg/g for Hypersil C18 column and 2.04 ± 0.22, 7.78 ± 0.28, 6.77 ± 0.61 mg/g for Agela promosil C18 column. No significant difference existed between the results from the two columns by t test (P>0.05),

Analytes	Precision				Repeatability		Stability	
	Intra-day (n=6)		Inter-day (n=6)					
	Average peak area	*R.S.D.* (%)	Average peak area	*R.S.D.* (%)	Mean (mg/g)	*R.S.D.* (%)	Average peak area	*R.S.D.* (%)
Chlorogenic Acid	50.80	0.35	52.30	2.33	0.27	2.97	51.40	1.58
Gallic Acid	561.95	0.20	546.22	2.36	1.96	1.37	561.00	0.24
Paeoniflorin	788.30	0.06	791.62	0.66	7.75	1.03	793.50	1.07
Jatrorrhizine Hydrochloride	202.50	0.59	205.60	0.66	0.49	2.49	202.90	1.68
Dehydroevodiamine	77.02	1.96	78.46	0.70	0.32	2.76	77.80	2.02
Palmatine Chloride	625.90	0.29	628.63	0.75	2.07	2.84	626.20	0.95
Berberine Hydrochloride	2295.75	0.12	2310.46	0.77	6.78	2.25	2304.40	0.73

Table 1: Precision, repeatability and stability of the HPLC method for determination of the seven analytes.

Analytes	Sample	Concentration				Mean Recovery (%) ± R.S.D
		Sample Content (mg)	Added (mg)	Found (mg)	Recovery (%)	
	S1[a]		0.3392	0.7733	103.39	
Gallic Acid	S2[b]	0.4226	0.4240	0.8651	104.35	102.99 ± 1.45
	S3[c]		0.5088	0.9376	101.22	
	S1		0.0689	0.1567	102.35	
Chlorogenic Acid	S2	0.0862	0.0862	0.1770	101.99	102.39 ± 1.85
	S3		0.1034	0.1903	102.82	
	S1		1.2040	2.7621	104.40	
Paeoniflorin	S2	1.5050	1.5050	3.0820	104.78	103.84 ± 1.35
	S3		1.8061	3.3536	102.35	
	S1		0.0857	0.1965	96.18	
Jatrorrhizine Hydrochloride	S2	0.1071	0.1071	0.2212	95.67	96.19 ± 1.13
	S3		0.1285	0.2337	96.72	
	S1		0.0566	0.1303	100.28	
Dehydroevodiamine	S2	0.0707	0.0707	0.1403	98.42	99.19 ± 2.99
	S3		0.0848	0.1546	98.87	
Palmatine Chloride	S2	0.4400	0.4400	0.8648	96.56	100.11 ± 2.79
	S3		0.5279	0.9752	101.39	
	S1		1.0890	2.4853	104.64	
Berberine Hydrochloride	S2	1.3691	1.3613	2.7122	98.67	102.63 ± 2.93
	S3		1.6335	3.0775	104.59	

Recovery (%) = (found−original) ×100 /added. The results indicated that the developed method was reliable and accurate for the measurement of the seven analytes.

[a] The samples added known amounts of standards at low level (80% of the known amounts).

[b] The samples added known amounts of standards at medium level (same as the known amounts).

[c] The samples added known amounts of standards at high level (120% of the known amounts).

Table 2: Recovery test of the seven analytes.

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Sample No.	Contents (Mean ± SD)						
	Gallic Acid	Chlorogenic Acid	Paeoniflorin	Jatrorrhizine Hydrochloride	Dehydroevodiamine	Palmatine Chloride	Berberine Hydrochloride
1.(A1[a]+B1[b]+C1[c])	1.904 ± 0.042	0.352 ± 0.009	7.707 ± 0.206	0.380 ± 0.025	0.292 ± 0.011	1.545 ± 0.020	6.630 ± 0.079
2.(A1+B1+C2)	1.637 ± 0.058	0.363 ± 0.011	7.637 ± 0.272	0.355 ± 0.016	0.267 ± 0.009	1.419 ± 0.051	6.369 ± 0.059
3.(A1+B1+C3)	1.770 ± 0.043	0.359 ± 0.015	7.442 ± 0.255	0.325 ± 0.013	0.263 ± 0.005	1.351 ± 0.044	6.073 ± 0.062
4.(A2+B1+C1)	1.589 ± 0.061	0.317 ± 0.019	7.184 ± 0.247	0.395 ± 0.021	0.245 ± 0.008	1.403 ± 0.037	6.211 ± 0.079
5.(A2+B1+C2)	1.667 ± 0.056	0.335 ± 0.015	7.036 ± 0.225	0.353 ± 0.020	0.264 ± 0.009	1.412 ± 0.052	6.054 ± 0.071

[a] Two batches of the *E. rutaecarpa* were collected from Guizhou (A-1) and Hunan (A-2).
[b] One batch of the *P. lactiflora* was collected from Anhui (B-1).
[c] Three batches of the *C. chinensis* were collected from Sichuan (C-1), Yunnan (C-2) and Guizhou (C-3).
All of them were purchased from Ji-Ren-Tang Pharmaceutical Corporation (Guiyang, China).

Table 3: Contents of the seven analytes in the tested samples(mg/ml).

indicating that the developed method was capable of producing results with acceptable performance.

Assessment of peak purity

Peak purity was assessed by Agilent ChemStation for LC 3D software. The purity factors of the seven characteristic constituents were 995.217, 991.265, 997.118, 996.4496, 997.228, 996.328, and 993.578, respectively.

Sample analysis

The established analytical method was then applied to determine the seven chemical compounds in five batches of preparations. The contents were listed in Table 3.

Conclusion

An HPLC method was developed for simultaneous determination of gallic acid, chlorogenic acid, paeoniflorin, jatrorrhizine hydrochloride, dehydroevodiamine, palmatine chloride and berberine hydrochloride to evaluate the quality of WJP. Gallic acid and paeoniflorin were derived from *P. lactiflora*, chlorogenic acid and dehydroevodiamine were derived from *E. rutaecarpa*, jatrorrhizine hydrochloride, palmatine chloride and berberine hydrochloride were derived from *C. chinensis*. The method has been validated and could be used with reasonable confidence, for quantification of the seven bioactive markers found in WJP. Five batches of self-manufactured WJP were analyzed, which showed the proposed HPLC method was durable and could be used to assess the WJP preparations and applied for quality control of this TCM.

Acknowledgements

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References

1. Cao Y, Wang L, Yu X, Ye J (2006) Development of the chromatographic fingerprint of herbal preparations Shuang-Huang-Lian oral liquid. J Pharm Biomed Anal 41: 845-856.
2. van Nederkassel AM, Vijverman V, Massart DL, Vander Heyden Y (2005) Development of a ginkgo biloba fingerprint chromatogram with UV and evaporative light scattering detection and optimization of the evaporative light scattering detector operating conditions. J Chromatogr A 1085: 230-239.
3. Xie Y, Jiang ZH, Zhou H, Cai X, Wong YF, et al. (2007) Combinative method using HPLC quantitative and qualitative analyses for quality consistency assessment of a herbal medicinal preparation. J Pharm Biomed Anal 43: 204-212.
4. Zhao Y, Chen P, Lin LZ, Harnly JM, Yu LL, et al. (2011) Tentative identification, quantitation, and principal component analysis of green pu-erh, green, and white teas using UPLC/DAD/MS. Food Chem 126: 1269-1277.
5. Zhou X, Zhao Y, Lei P, Cai Z, Liu H (2010) Chromatographic fingerprint study on Evodia rutaecarpa (Juss.) Benth by HPLC/DAD/ESI-MS(n) technique. J Sep Sci 33: 2258-2265.
6. Xie Z, Zhao Y, Chen P, Jing P, Yue J, et al. (2011) Chromatographic fingerprint analysis and rutin and quercetin compositions in the leaf and whole-plant samples of di- and tetraploid Gynostemma pentaphyllum. J Agric Food Chem 59: 3042-3049.
7. Li M, Zhou X, Zhao Y, Wang D, Hu X (2009) Quality assessment of *Curcuma longa* L. by gas chromatography-mass spectrometry fingerprint, principal components analysis and hierarchical clustering analysis. Bull Korean Chem Soc 30: 2287-2293
8. Chen P, Ozcan M, Harnly J (2007) Chromatographic fingerprint analysis for evaluation of Ginkgo biloba products. Anal Bioanal Chem 389: 251-261.
9. WHO (1991) Guidelines for the assessment of herbal medicines: World Health Organization, Geneva
10. Drug Administration Bureau of China (2000) Requirements for studying fingerprint of Traditional Chinese Medicine.
11. Pharmacopoeia of People's Republic of China (2010) The State Pharmacopoeia Commission of People's Republic of China. Chemical Industry Press, Beijing, China.
12. Wang YJ, Dong Y, Zhu XX (2007) Experimental studies of effects of Wujiwan extracts in different compatibilities on motility of isolated colon in guinea-pig. Zhongguo Zhong Yao Za Zhi 32: 2161-2165.
13. Weng X, Li Y, Yang Q, Wang Y, Liu X, et al. (2010) Effects of Wuji Pill with different compatibility on the activity of cytochrome P450 CYP31A2 in rat liver microsomes in vitro. Zhongguo Zhong Yao Za Zhi 35: 1164-1169.
14. Wu SR, Peng C, Guo CX, Cai R, Bai YG, et al.(2006) Effect of Wuji Pills decoction on the Content of NO in the mouse internal body infected by helicobacter pylori. Guiding J Tradit Chin Med 12: 65-66.
15. Tan SJ, Zhao BC, Wang HT, Li JL, Chu F (2002) Determination of evodiamine and rutaecarpine in Wuji Wan by HPLC. Pharm J Chin PLA 18 : 202-204.
16. Shu-tai MA (2009) Determination of Berberiue hydrochloride and Palmatine hydrochloride in Wuji Pills by HPLC. Pharmacy Today.
17. Zhan XF, Zhang CP, Lu Qi (2004) Determination of berberine hydrochloride in Wuji Pills by HPLC. Pharm Affairs 23: 27-28.