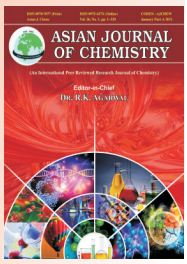
Asian Journal of Chemistry; Vol. 26, No. 15 (2014), 4868-4870

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16314



Simultaneous Determination of Six Ingredients in Wenweishu Capsule by RP-HPLC

NINGYUAN ZHONG[1,2], JIE GAO[1], WEIMING CHENG[3], JUN CHAI[2], CHAO YUE[1] and WENTING ZHANG[3,*]

[1]Zhejiang Medical University, No. 548 Binwen Road, Binjiang Dist. 310053, Hangzhou Zhejiang Province, P.R. China
[2]Shaoxing Institute for Food and Drug Control, No. 286 Shiji Road, Paojiang Dist. 312071, Shaoxing Zhejiang Province, P.R. China
[3]Zhejiang Institute for Food and Drug Control, No. 86 Lane 1, Jichang Road, Jianggan Dist. 310004, Hangzhou Zhejiang Province, P.R. China

*Corresponding author: Tel: +86 15858177067; E-mail: leozhwt@163.com

Received: 9 September 2013; *Accepted*: 6 December 2013; *Published online*: 16 July 2014; AJC-15588

This study established the HPLC method for the simultaneous determination of six ingredients in Wenweishu capsule. The sample was extracted with methanol by ultrasonication. The HPLC separation was performed on a zorbax SB-C18 (4.6 × 250 mm, 5 μm) column, using acetonitrile (A)-0.1 % formic acid (B) as the mobile phase with gradient elution in a flow of 1 mL min^{-1}. The column temperature was at 30 °C and the detector wavelength was 330 nm. The results showed that the six components were separated well and had good linear relationships: Calycosin glycoside in the range of 0.1012-2.024 μg (R^2 = 0.9997); Echinacoside in the range of 0.01974-0.3984 μg (R^2 = 0.9998); Verbascoside in the range of 0.01067-0.2134 μg (R^2 = 0.9996); Chlorogenic acid in the range of 0.02014-0.4028 μg (R^2 = 0.9994); Vitexin rhamnoside in the range of 0.02250-0.4500 μg (R^2 = 0.9993) and hesperidin in the range of 0.009440-0.1888 μg (R^2 = 1.0000) (n = 6). Their average recoveries were between 95-105 % (n = 6). This method was steady with high precision and good repeatability and non-interference in the negative control. It could be used for the determination of the six active ingredients and quality control of Wenweishu capsule.

Keywords: Wenweishu capsule, Calycosin glycoside, Echinacoside, Verbascoside, Chlorogenic acid, Vitexin rhamnoside, Hesperidin.

INTRODUCTION

Wenweishu capsule was made up of twelve Chinese medicinal herb such as *Codonopsis pilosula*, *Radix aconiti praeparata*, honey-fried *Radix astragali, Herba cistanche*, *Crataegi fructus*, *Citri reticulatae pericarpium*, *etc.*, which was recorded by Chinese Pharmacopeia[1], possessing the functions of warming the *Middle-jiao* and nourishing the stomach, promoting *Qi* circulation and relieving pain. Clinically, it always used for the treatment of stomach ache, abdominal distention, belch, anorexia, chilly and acratia induced by chronic atrophic gastritis and superficial gastritis. Honey-fried *Radix astragali*, as a traditional tonic medicine, has the function of tonifying *Qi* and lifting *Yang*, reinforcing the stomach and consolidating the surface. Calycosin glycoside (CG) is one effective constituent among flavonoids in *Radix astragali*[2]. *Herba cistanche* has the functions of strengthening the middle warmer, nourishing liver and kidney, tonifying *Yang* and relaxing bowel, playing a key role in the function of toni-fying *Middle-Jiao* and *Qi*. Its main active ingredients are phenylethanoid glycosides. Echinacoside (EC) and Verbascoside (VE) are regarded as the most active constituents among phenylethanoid glycosides[3]. Crataegi F has the functions of promoting digestion and invigorating stomach, promoting the circulation of *Qi* and dispersing stasis, in which Chlorogenic acid (CA) and vitexin rhamnosid (VR) are the main effective constituents[4-5]. Citri RP has the functions of dredging *Qi* flow and tonifying spleen, promoting digestion and its quality control is dependent on the determination of hesperidin (HE).

However, at present, there is no exact quantitative determination criterion for the quality control of Wenweishu capsule, except for *Psoraleae fructus*. In the experiment, an HPLC method was established for the simultaneous determination of six ingredients (Calycosin glycoside, Echinacoside, Verbascoside, Chlorogenic acid, Vitexin rhamnosid, Hesperidin) in Wenweishu capsule, which could provide relatively comprehensive for the quality control of Wenweishu capsule.

EXPERIMENTAL

The separation was carried out on an Agilent Zorbax SB C18 (250 mm × 4.6 mm, 5 μm) column. The solvents used for HPLC separation were acetonitrile (A) and 0.1 % formic acid (B) at a flow rate of 1 mL min^{-1} with gradient elution (0-20 min, 12-28 % A; 20-25 min, 28-12 % A) and the analysis was monitored at 330 nm with the column temperature at 30 °C and the injection volume was 5 μL.

The results showed that the peaks of the six effective components were separated well. The theoretical plates were all over

3000 and resolutions were all upper than 1.5. The comparison between standards and the sample was showed on Fig. 1.

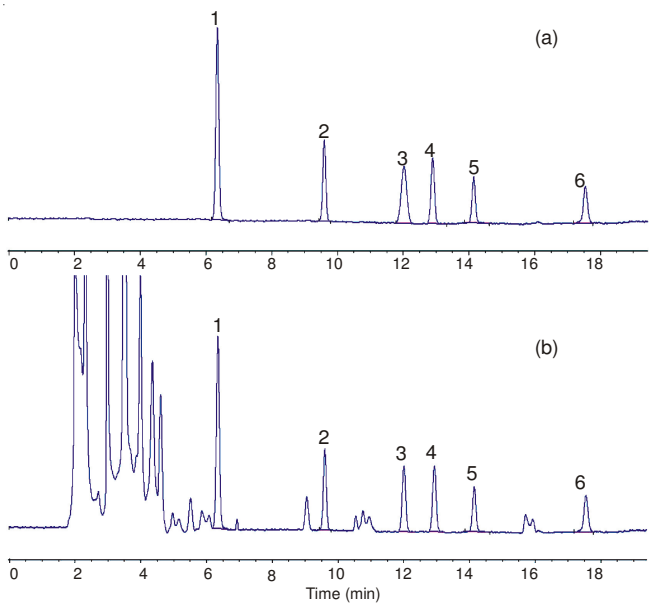


Fig. 1. HPLC chromatogram of the six ingredients mixture (a) and Wenweishu capsule (b) a: standard; b: sample; 1. Chlorogenic acid; 2. Echinacoside; 3. vitexin rhamnosid; 4. Calycosin glycoside; 5. Verbascoside; 6. Hesperidin

Standard solution: Certain amounts of the 6 standard compounds were dissolved with methanol into volumetric flasks, respectively. Then measured and diluted to the mixture solution with concentrations of 104.20, 19.56, 10.67, 20.14, 22.50 and 19.30 μg mL^{-1}, respectively.

Sample solution: Taken 10 grains of Wenweishu capsule, mixed the contents and accurately weighed 0.8 g into a cone flask with plug. Precisely measured 20 mL methanol, performed ultrasonic for 30 min after being weighed, after cool to the room temperature, weighed for supplying the lose weight with methanol. The solution was ready for chromatographic analysis after 10 min high speed centrifugation.

Precision: The standard mixture solution was injected into HPLC six times continuously and the area of each peak was used for the calculation of precision. The results showed that relative stand deviations (RSDs) of peak areas of calycosin glycoside, echinacoside, verbascoside, chlorogenic acid, vitexin rhamnosid and Hesperidin were 0.8, 0.5, 0.3, 0.2, 0.5 and 0.9 %, respectively.

Linear range: Linear regression analysis for each component was performed by the external standard method. Prepared certain amounts of the 6 standard compounds into a volumetric flask with concentrations of 404.80, 78.96, 42.68, 80.56, 90 and 37.76 μg mL^{-1}, respectively. Precise amount of the solution of 1, 2, 5, 10 mL were added into 20 mL volumetric flasks, respectively. Then the suspension was metered to the volume with methanol. The mixture solution of above six different concentrations was accurately injected 5 μL, respectively. The linearity of each compound was calculated by plotting the peak area (Y) *vs.* concentration (X). (Table-1). All the 6 components showed good linearities in wide concentration ranges.

Repeatability: Repeatability was carried out using six samples solution after the same treatment procedure. The results showed that RSD of each peak area was 0.8, 0.5, 1.0, 0.6, 1.2 and 0.2 %, respectively.

Stability: For stability test, the same sample solution was analyzed at designated time points in 0, 1, 2, 4, 8, 12, 24 h. The results showed that RSDs of peak area were 0.3, 0.7, 0.2%, 0.4, 1.4 and 0.3 % and found to be stable in 24 h for the experiment.

Recovery test: The sample with known targeted contents was spiked with certain amounts of the 6 standards. Then the spiked sample was processed in accordance with the established method for the HPLC detection. The average recoveries of the 6 standard compounds were showed in Table-2.

Determination of samples: The experiment determined three samples of different batches of the same manufacturer by the above method. The contents of the 6 components showed in Table-3.

RESULTS AND DISCUSSION

By scanning the ultraviolet absorption wavelength of 6 different reference substances, we found that calycosin glycoside, echinacoside, verbascoside, chlorogenic acid, vitexin rhamnosid and hesperidin had maximum absorption peak in 260, 330, 330, 327, 335 and 283 nm, respectively. By comparison, 330 nm was selected as the determination wavelength, under which each ingredient separated well and had few impurities interference.

On the basis of referencing to related literature[1,5-7], three different solvent systems: acetonitrile-0.1 % phosphate, methanol-0.1 mol L^{-1} sodium dihydrogen phosphate and acetonitrile-0.1 % formic acid were tested. By comparing isocratic elution with gradient elution, at last, gradient elution program was established in our study. Chromatographic peak of 6 different constituents had a baseline separation and analysis time could be shortened effectively by this program.

Four samples lacking of honey-fried *Radix astragali*, *Herba cistanche*, *Crataegi F* and *Citri RP* were prepared as negative controls for further verification, respectively. The result showed that the fourth chromatographic peak was the ingredient of flavonoids in *Astragali radix*, the second and the

TABLE-1
LINEAR REGRESSION EQUATION AND LINEAR RANGES

Component	Regression equation	Correlation coefficient (R^2)	Linear range/μg
Calycosin glycoside	Y = 160.69X + 15.510	0.9997	0.1012-2.024
Echinacoside	Y = 424.01X + 21.741	0.9998	0.01974-0.3948
Verbascoside	Y = 707.62X + 15.564	0.9996	0.01067-0.2134
Chlorogenic acid	Y = 1417.5X + 46.276	0.9994	0.02014-0.4028
Vitexin rhamnoside	Y = 875.79X + 25.043	0.9993	0.02250-0.4500
Hesperidin	Y = 131.22X + 5.049	1.0000	0.009440-0.1888

TABLE-2
RECOVERY RESULTS OF THE SIX ACTIVE INGREDIENTS

Component	Contents in samples (mg)	Added (mg)	Found (mg)	Recovery (%)	Mean (%)	RSD (%)
Calycosin glycoside	293.0	300.40	588.5	98.32	97.5	1.4
	299.3		589.1	96.46		
	292.2		590.3	99.21		
	296.9		588.7	97.11		
	295.8		591.7	98.48		
	298.6		586.1	95.69		
Echinacoside	96.34	96.32	192.8	100.15	100.4	1.1
	98.40		196.9	102.22		
	96.07		191.6	99.18		
	97.61		194.0	100.07		
	97.25		193.2	99.62		
	98.16		195.5	101.04		
Verbascoside	48.17	51.76	100.9	102.02	100.8	2.0
	49.20		102.1	102.32		
	48.04		100.3	101.05		
	48.80		99.3	97.41		
	48.62		101.6	102.50		
	48.08		100.5	99.31		
Chlorogenic acid	52.18	52.28	103.5	98.16	97.6	1.0
	53.30		103.9	96.85		
	52.04		102.7	96.89		
	52.87		104.3	98.39		
	52.68		103.1	96.48		
	53.17		104.9	98.97		
Vitexin rhamnoside	72.25	75.00	148.9	102.28	101.5	0.5
	73.80		150.2	101.90		
	72.05		147.7	100.90		
	73.21		149.2	101.36		
	72.94		148.6	100.91		
	73.63		149.9	101.74		
Hesperidin	1244.3	1216.44	2500.8	103.22	102.1	1.8
	1271.0		2473.1	98.87		
	1240.9		2488.9	102.54		
	1260.8		2530.6	104.24		
	1256.1		2492.0	101.55		
	1267.9		2512.1	102.19		

TABLE-3
CONTENTS DETERMINATION OF THE SIX ACTIVE INGREDIENTS IN 3 BATCHES

Batch number	Calycosin glycoside		Echinacoside		Verbascoside		Chlorogenic acid		Vitexin rhamnoside		Hesperidin	
	C (mg g^{-1})	RSD (%)	C (mg g^{-1})	RSD (%)	C (mg g^{-1})	RSD (%)	C (mg g^{-1})	RSD (%)	C (mg g^{-1})	RSD (%)	C (mg g^{-1})	RSD (%)
1204109	0.73	0.8	0.24	0.5	0.12	1.0	0.13	0.6	0.18	1.2	3.1	0.2
1208105	0.80	0.4	0.26	0.6	0.12	0.1	0.15	0.9	0.17	0.3	3.3	0.8
1211201	0.56	0.3	0.18	0.3	0.08	0.4	0.16	1.2	0.16	0.6	3.2	1.1

fifth peaks belonged to *Herba cistanche*; the first and third peaks belonged to *Crataegi F* and the sixth peak was the ingredient of *Citri RP*. Other ingredients in the prescription had no interference.

Selection of solvent and ultrasonic time: Samples were extracted with different concentrations of methanol (25, 50, 75, 100 %), we found that chlorogenic acid was more stable in pure methanol, so it was selected as extraction solvent. Also, we compared the analysis effects under different ultrasonic time (20, 30, 40 min), the results showed that all constituents had been separated completely under 0.5 h ultrasonic time.

Prospection: This method was steady with high precision and good repeatability. It could be used for the determination of the six active ingredients and quality control of Wenweishu capsule. Besides capsules, it could also provide basis for the quality control of other dosage forms of Wenweishu series, such as tablets and granules.

REFERENCES

1. State Pharmacopeia Committee of China, Chinese Pharmacopoeia, 2010 version Part I. pp. 29-30, 126, 176-177, 284, 1175-1176 (2010).
2. L.J. Liang, K.J. Zhao and P.F. Tu, *China Pharmacy* , **21**, 1385 (2010).
3. Z. Xuan, L. Xin and D.N. Sheng, *China J. Pharm. Anal.*, **23**, 254 (2003).
4. R.S. Liang, C. Zhang and H. Zao, *J. Pharm. Practice*, **30**, 457 (2012).
5. Y.J. He, J. Su and Q. Yian, *China J. Chin. Mater. Med.*, **37**, 829 (2012).
6. J. Wang, X.Z. Zhang and X.Y. Wu, *China J. Chin. Mater. Med.*, **35**, 1702 (2010).
7. H.T. Xu, J.F. Lu and H.Y. Lan, *Chin. J. Exp. Tradit. Med. Formulae*, **16**, 44 (2010).