Simultaneous Determination of Four Active Ingredients in Wuji Pellet by HPLC

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Abstract: **Objective** To apply the high performance liquid chromatography (HPLC) method to the determination of four ingredients including paeoniflorin, berberine, evodiamine, and rutaecarpin in Wuji Pellet. **Methods** Using Thermo-ODS2-Hypersil column (250 mm × 4.6 mm, 5 μm) and taking acetonitrile-0.05 mol/L monopotassium phosphate as mobile phase for elution, the conditions were as follows, flow rate of 1.0 mL/min, UV detector wavelength of 225 nm, and column temperature of 25 ℃. **Results** The standard curves of paeoniflorin, berberine, evodiamine, and rutaecarpine showed a good linear relationship in 0.0124—0.124, 0.0349—0.349 mg/mL, and 0.6—6, 0.5—5 μg/mL. The average recovery rates were 99.45%, 97.69%, 98.18%, and 98.46%, respectively. **Conclusion** The method is of high sensitivity and nice reproducibility, and it could be used for the simultaneous determination of the four ingredients in Wuji Pellet.

Key words: berberine; evodiamine; paeoniflorin; rutaecarpin; Wuji Pellet
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Introduction

Wuji Pellet first appeared in *Prescription of Peaceful Benevolent Dispensary* (Tai Ping Hui Ming He Ji Ju, Imperial Pharmaceutical Bureau) in Song Dynasty and recorded in *Pharmacopoeia of People's Republic of China 2010* (I). Mainly comprising of *Coptidis Rhizoma* (*Huanglian*), *Paeoniae Radix Alba* (*Baishao*), and *Evodiae Fructus* (*Wuzhuyu*) in a ratio of 6:6:1, the pellet is famous for soothing liver and regulating spleen, clearing away heat to harmonize stomach, and is mainly used for treating stomachache, acid regurgitation, bellyache, and diarrhea caused by disharmony of liver and spleen (Tan *et al*, 2010). Currently, the effective components in Wuji Pellet are already known. In addition to paeoniflorin and berberine, evodiamine and rutaecarpine are also important components in Wuji Pellet (Tan *et al*, 2002). Nevertheless, as mentioned in *Pharmacopoeia of People's Republic of China 2010* and relevant literatures, the quality control method of Wuji Pellet mainly focuses on HPLC for measuring the contents of berberine and paeoniflorin, yet there is not any controlling requirements on the content of *Evodiae Fructus* and its related components (Pharmacopoeia Committee of People's Republic of China, 2010). The current experiment tries to establish a method for measuring the effective components of Wuji Pellet so as to lay a firm foundation for its quality controlling research.

Materials and methods

Materials and reagents

Aeoniflorin (Batch No.: 110802-200606), berberine hydrochloride (Batch No.: 110736-20093; purity: 95.7%), evodiamine (Batch No.: 110802-200606), and rutaecarpine (Batch No.: 11081-201006) reference substances were supplied by National Institute for Food and Drug Control. Acetonitrile and methanol (Tianjin Kemiou Chemistry Reagent Ltd.) were of chromatographical grade, and other reagents and ultrapure water were of analytical grade. *Coptidis Rhizoma* (20111003), *Paeoniae Radix Alba* (20111023), and *Evodiae Fructus* (20111012) were bought from Guiyang Tongrentang Company. According to *Pharmacopoeia of People's*

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Republic of China 2010 (I), Wang Q, associate professor in Pharmacognosy Department, Guiyang College of Traditional Chinese Medicine (Guizhou, China), appraised that *Coptidis Rhizoma*, *Evodiae Fructus*, and *Paeoniae Radix Alba* were quality products. Wuji Pellet was self-prepared according to *Pharmacopoeia of People's Republic of China 2010*.

Apparatus and chromatographic conditions

Shimadzu LC — 20A Liquid Chromatograph, Shimadzu SPD—20A UV Detector, HT—20 Column Compartment, Auy 220 Electronic Balance, SK8210HP Desktop Ultrasonic Cleaning Machine (Shanghai Kudos Ultrasonic Instrument Co, Ltd.), applying Thermo-ODS2-hypersil column (250 mm × 4.6 mm, 5 μm), acetonitrile-0.05 mol/L monopotassium phosphate as mobile phase for elution, flow rate of 1.0 mL/min, UV detector wavelength of 225 nm, column temperature of 25 ℃. A gradient elution was introduced using 0.05 mol/L monopotassium phosphate (A) and MeCN (B): 0—20 min, 11% B; 20—22 min, 11%—28% B; 22—44 min, 28% B; 44—46 min, 28%—40% B; 46 — 67 min, 40% B. The presentative chromatograms obtained in the current study were shown in Fig. 1. Four active ingredients could be separated satisfactorily in 70 min.

Preparation of reference solution

Paeoniflorin (1.24 mg), berberine (3.49 mg), evodiamine (0.06 mg), and rutaecarpine (0.05 mg) were weighed accurately and transferred into a 10 mL volumetric flask, dissolved and diluted to graduation with methanol.

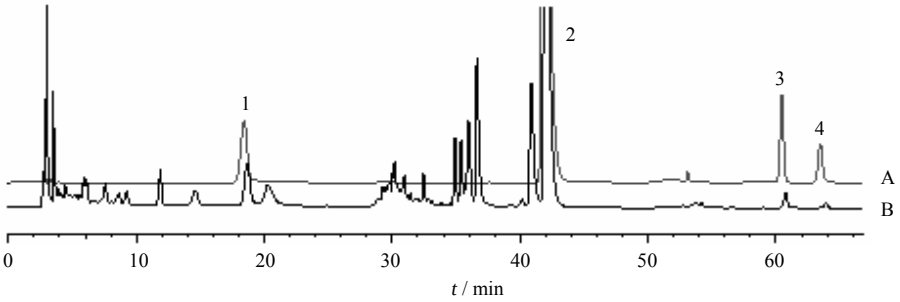

Fig. 1 HPLC chromatograms of reference substances (A) and samples (B)

1: paeoniflorin 2: berberine 3: evodiamine 4: rutaecarpine, same as below

Sample preparation

Wuji Pellets were powdered to a homogeneous size in a mill, and sieved through a No. 80 mesh. The powder (0.5 g) was placed into conical flask, added with 50 mL MeOH, then ultrasonically extracted for 30 min. After cooling to room temperature, the sample was weighed, methanol was used to make up for the loss weight, and then shake up. Finally the test material was filtrated through a 0.45 μm membrane before injecting 10 μL samples for analysis.

Results and discussion

Calibration

To prepare a reference solution containing paeoniflorin, berberine hydrochloride, evodiamine, and rutaecarpine, the amounts of each compound were accurately weighed and dissolved in methanol to give serial concentration with the ranges of 0.0124—0.124, 0.0349—0.349 mg/mL, and 0.6—6, 0.5—5 μg/mL, respectively. Reference solution (10 μL each) was injected in triplicate into the HPLC column, graphs of peak area versus concentration of standard solutions (six points) were plotted, and calibration curves were obtained by fitting the data using linear regression analysis. All four HPLC calibration curves of components exhibited good linearity with excellent correlated coefficients. In general, each component gave a wide calibration range for routine analysis (Table 1).

Negative control

Wuji Pellet with different contents of *Coptidis Rhizoma* (*Huanglian*), *Paeoniae Radix Alba* (*Baishao*), and *Evodiae Fructus* (*Wuzhuyu*) were prepared respectively. Following the method mentioned above, negative control solutions free from *Coptidis Rhizoma*, *Paeoniae Radix Alba*, and *Evodiae Fructus* were also prepared respectively in 10 μL sample injection and chromatographic condition was performed, while chromatographic graphs were recorded. The chroma-

tograms obtained in negative contrast test were shown in Fig. 2. The result indicated that one ingredient did not interfere with the determination of others.

Precision

Within-day variability of the assay was determined by repeated analysis of known concentration samples on the same day. The RSD values of peak areas for the four compounds were 1.35%, 1.07%, 1.59%, and 1.74% (n = 5), and these results indicated that this method exerted a good precision.

Table 1 Calibration curves, limits of detection, and linear ranges of four components

Components	Regression equations	Correlation coefficients	Linear ranges / (μg·mL^{-1})
paeoniflorin	y = 10 000 000 x − 60 962	0.9991	12.4—124.0
berberine	y = 40 000 000 x − 534 770	0.9993	34.9—349.0
evodiamine	y = 139 606 x + 29 869	0.9991	0.6—6
rutaecarpine	y = 9 000 000 x + 0.2749	0.9990	0.5—5

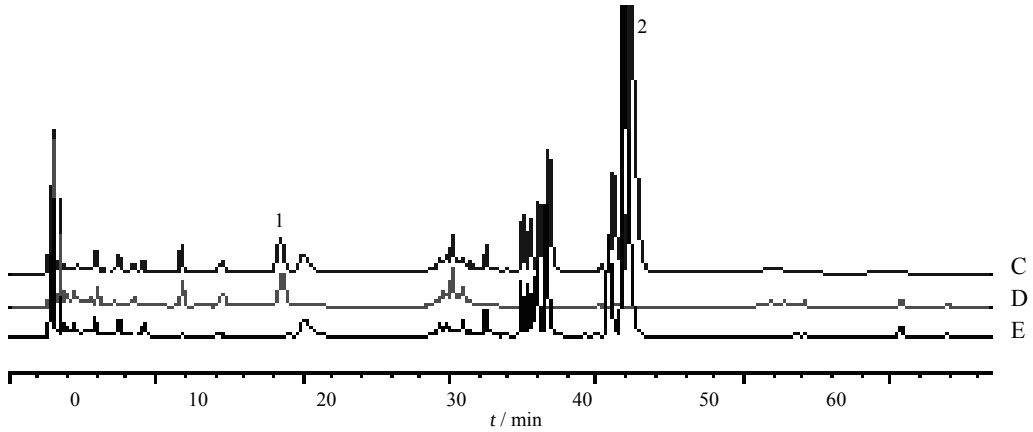

Fig. 2 HPLC chromatograms of negative control samples

C: negative control sample of *Evodiae Fructus* D: negative control sample of *Coptidis Rhizoma* E: negative control sample of *Paeoniae Radix Alba*

Reproducibility

Wuji Pellet powder (0.5 g) was prepared and the peak area integral values of the four ingredients were recorded according to the mentioned chromatograph condition. The contents of the four compounds in Wuji Pellet were calculated, and the RSD values were 1.17%, 1.14 %, 1.72%, and 1.12%, respectively. The results of the experiment indicated that the reproducibility was good.

Stability

The stability of the extracts from Wuji Pellet sample solution was studied by HPLC at room temperature. The test sample solutions were made at different time intervals, i.e. 0, 2, 4, 6, 8, and 12 h. The peak areas were recorded. The RSD values of peak areas were less than 3% (1.17%, 1.16%, 1.23%, and 1.07%). The results of the experiment indicated that the samples are stable in 12 h.

Recovery

An appropriate amount of Wuji Pellet powder was weighed and spiked with known amount of each standard component. The peak area integral values of the four ingredients were recorded according to the mentioned chromatograph conditions, and the average recoveries (n = 6) of the four components were 99.45%, 97.69%, 98.18%, and 98.46%. The results are shown in Table 2.

Content determination

Three samples of Wuji Pellet powder were taken in the same preparation, and the corresponding solutions were obtained. The analysis results of the chromatographic condition were illustrated in Table 3, The contents of paeoniflorin, berberine, evodiamine, and rutaecarpine in Wuji Pellet were 8.344, 20.800, 0.1382, and 0.0659 mg/g, respectively.

Discussion

Preparation of sample solutions

After a comparison of the influences on the components of paeoniflorin, berberine, evodiamine, and rutaecarpine in Wuji Pellet with the different extract methods, extract solutions, and extract time, the final

Table 2 Recovery rates of four components (n = 6)

Components	Weight / g	Content / mg	Added / mg	Amount / mg	Recovery / %	Average recovery/ %	RSD / %
paeoniflorin	0.2502	2.1021	2.1000	4.1961	99.72	99.45	1.15
	0.2503	2.1029	2.1000	4.1913	99.45		
	0.2505	2.1046	2.1000	4.1515	97.47		
	0.2507	2.1063	2.1000	4.2225	100.77		
	0.2502	2.1021	2.1000	4.1814	99.01		
	0.2512	2.1105	2.1000	4.2650	100.27		
berberine	0.2502	5.0864	5.0864	10.0078	96.76	97.69	1.20
	0.2503	5.0884	5.0864	10.0078	96.72		
	0.2505	5.0925	5.0864	10.0766	97.99		
	0.2507	5.0966	5.0864	10.1691	99.73		
	0.2502	5.0864	5.0864	10.0144	96.89		
	0.2512	5.1067	5.0864	10.0953	98.08		
evodiamine	0.2502	0.0348	0.0348	0.0694	99.60	98.18	1.83
	0.2503	0.0348	0.0348	0.0694	99.56		
	0.2505	0.0348	0.0348	0.0690	98.37		
	0.2507	0.0348	0.0348	0.0695	99.61		
	0.2502	0.0348	0.0348	0.0683	96.43		
	0.2512	0.0349	0.0348	0.0681	95.52		
rutaecarpine	0.2502	0.0167	0.0167	0.0333	99.30	98.46	1.44
	0.2503	0.0167	0.0167	0.0334	100.08		
	0.2505	0.0167	0.0167	0.0330	97.51		
	0.2507	0.0167	0.0167	0.0332	98.92		
	0.2502	0.0167	0.0167	0.0332	98.86		
	0.2512	0.0168	0.0167	0.0328	96.12		

Table 3 Determination of four components in Wuji Pellet

No.	Paeoniflorin		Berberine		Evodiamine		Rutaecarpine	
	Content / ($mg \cdot g^{-1}$)	RSD / %	Content / ($mg \cdot g^{-1}$)	RSD / %	Content / ($mg \cdot g^{-1}$)	RSD / %	Content / ($mg \cdot g^{-1}$)	RSD / %
1	8.355	1.51	20.694	1.21	0.139	0.34	0.066	0.84
2	8.348	0.82	20.752	1.02	0.138	0.87	0.066	1.07
3	8.344	0.86	20.800	0.73	0.138	1.17	0.066	1.60

method is decided as adding 50 mL methanol to 0.5 g Wuji Pellet powder to form a mixture which then underwent 30 min ultrasonic processing.

Selection of chromatographic condition

In this experiment, solvent systems of acetonitrile-0.1% phosphoric acid, acetonitrile-0.05 mol/L monopotassium phosphate, and acetonitrile-0.3% triethylamine phosphate were selected, and acetonitrile-0.05 mol/L monopotassium phosphate displayed the best performance in chromatographic peak separation of Wuji Pellet. Three wavelengths of 220, 225, and 230 nm were investigated, respectively. Giving equal consideration to the optimal absorption wavelengths of paeoniflorin, berberine, evodiamine, and rutaecarpine chromatographic peaks, 225 nm was selected for the experimental test.

Method selection

This method could be applied to measuring the contents of the four components in Wuji Pellet and lay a firm foundation for the quality control research of Wuji Pellet due to its simplicity, reliability, and good repeatability.

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