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Simultaneous Determination of Multi Components in Weitongding Particle by HPLC

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An HPLC method for the simultaneous determination of multi components in Weitongding particle is established. HPLC conditions included Agilent Eclipse XDB-C_{18} column (4.6 mm × 250 mm, 5 μm) and the mobile phase was a mixture of buffer solution (A, containing 0.1 % formic acid) and methanol (B) at a flow rate of 1 mL min^{-1} for the gradient elution and the column temperature was maintained at 35 °C. Five active ingredients had good linear relationships: astragalin in the range of 0.0036-0.1800 μg (R^2 = 0.9991), quercetin in the range of 0.0045-0.2250 μg (R^2 = 0.9992), isorhamnetin in the range of 0.0113-0.5650 μg (R^2 = 0.9990), α-tractlone in the range of 0.0108-0.5400 μg (R^2 = 0.9993) and hyperoside in the range of 0.0165-0.8250 μg (R^2 = 0.9992). Their average recoveries were 95.1 % (RSD = 2.4 %), 93.5 % (RSD = 2.2 %), 96.2 % (RSD = 2.2 %), 94.9 % (RSD = 1.9 %) and 97.6 % (RSD = 3.0 %), respectively. This method was steady with high precision and good repeatability and could be used for the determination of the five active ingredients and quality control of Weitongding particle.

Keywords: Weitongding particle, HPLC, Astragalin, Quercetin, Isorhamnetin, α-Tractlone, Hyperoside.

INTRODUCTION

Weitongding particle was developed based on an old Chinese medicine prescription, composing of *Codonopsis pilosula*, *Radix Astragali mongolici*, *Dioscorea batatas*, *Rhizoma Atractylodis macrocephalae*, *Pericarpium Citri reticulatae*, *etc*., written by the famous medical scientist named Zhongjing Zhang of Han Dynasty, possessing the bioactivities of warming stomach and expelling pathogentic cold, improving digestive functions, enhancing the abilities of immunity[1,2], clinically mainly used against digestic ulcer, gastritis, middle region of the upper abdomen, dspepsodynia, gastrospasm, gaseous distention, *etc*[3,4]. Modern pharmacology has proved that the active ingredients in Weitongding particle are mainly flavones, such as quercetin, isorhamnetin and atractlone having the functions of improving digestive functions, enhancing the abilities of immunity and antibacterial antiinflammatory effect[5-9]. Others like astragalin and hyperoside, have also many bioactivities[10-14]. In recent years, determination of multi-components was increasingly used for the quality control of Chinese material medica preparation. In this experiment, an HPLC method was established for the simultaneous determination of the 5 active ingredients (astragalin, quercetin, isorhamnetin, α-tractlone and hyperoside) in Weitongding particle. This method was stable with high precision, which could be used for the determination of the five ingredients and quality control of Weitongding particle.

EXPERIMENTAL

Agilent 1100 series HPLC system (Agilent Technologies, USA) was equipped with degasser (G1322), pump (G1312A), automatic sampler (G1313A), UV variable-wavelength detector (1314A-UV) and column oven (G1316A); PA2251 electronic analytical balance (Sartorius Group, Germany); TW20 constant temperature bath box (Julabo Labortechnik GmbH company, Germany).

Standards of astragalin, quercetin, isorhamnetin, α-tractlone and hyperoside were isolated in our lab, or purchased from other companies. Weitongding particle was developed according to the preparation of manufacture procedure and 3 different batches were developed in different time and manufacture working place, with the batch number of 120501, 121004, 121119. Acetonitrile was of HPLC grade and other reagents used were of analytical grade. Deionized water was prepared using a Millipore water purification system.

HPLC conditions: The separation of the five ingredients was carried out on an Agilent Eclipse XDB-C_{18} (250 mm × 4.6 mm, 5 μm). The solvents used for HPLC separation were

0.1 % formic acid (A) and methanol (B) at a flow rate of 1 mL/min with gradient elution (0-10 min, 95-84 % A; 10-25 min, 84-55 % A, 25-35 min, 55-35 % A, 35-40 min, 35-25 % A) and the analysis was monitored at 254 nm with the column temperature of 35 °C and the injection volume was 10 μL.

Preparation of standard solution: Certain amounts of the 5 standard compounds were dissolved with methanol into volumetric flasks, respectively. Then measured 1 mL astragalin, 1 mL quercetin, 3 mL isorhamnetin, 2 mL α-tractlone and 2 mL hyperoside standard solution, respectively, into a 50 mL volumetric flask, then accurately measured 10 mL the mixed liquid to a 20 mL volumetric flask and diluted to the concentrations needed for the HPLC analysis with methanol

Preparation of sample solution: 5 g Weitongding particle was pulverized into fine powder, after passing through the 80 mesh sieve, about 2 g sample was accurately weighed, then added into a 100 mL conical flask. 40 mL 10 % formic acid was added into the flask and placed in constant temperature bath box for 2 h circulation reflux at 90 °C. After refrigerating, the suspension was moved to a separating funnel for 3 times extraction with chloroform, each time with 40 mL and merged the $CHCl_3$ layer into suspension instrument for recycling $CHCl_3$ and then dissolved the residue with methanol into a 50 mL volumetric flask and metered the volume. The solution was ready for chromatographic analysis after passing through a 0.45 μm membrane filter.

RESULTS AND DISCUSSION

Linear range: Linear regression analysis for each component was performed by the external standard method. The above five-compounds solution was accurately injected 1, 2, 5, 10, 20, 30 and 50 μL, respectively. The linearity of each compound was calculated by plotting the peak area (Y) *vs.* concentration (X). (Table-1). All the five components showed good linearities in wide concentration ranges.

TABLE-1
LINEAR REGRESSION EQUATION AND LINEAR RANGES

Components	Regression equation	Correlation coefficient (R^2)	Linear range (μg mL^{-1})
Astragalin	Y = 124X +12.21	0.9991	0.0036-0.1800
Quercetin	Y = 26.1X + 3.41	0.9992	0.0045-0.2250
Isorhamnetin	Y = 241X - 5.65	0.9990	0.0113-0.5650
α-Tractlone	Y = 175X + 2.14	0.9993	0.0108-0.5400
Hyperoside	Y = 56.4X - 2.87	0.9992	0.0165-0.8250

Precision: The standard mixture solution of five components 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 standard deviation (RSD) of peak area of each standard was 2.5, 2.2, 2.7, 1.7 and 1.9 %, respectively (Fig. 1).

Stability: For stability test, the same sample solution was analyzed at designated time points in 24 h. The results showed that RSDs of peak area were 1.3, 0.9, 2.1, 2 and 1.8 % and found to be stable for the experiment.

Repeatability: Repeatability was carried out using 5 samples solution after the same treatment procedure. The results showed that RSD of each peak area was suitable for the analysis.

TABLE-2
RECOVERY RESULTS OF THE FIVE ACTIVE INGREDIENTS

Component	Contents in samples (mg)	Added (mg)	Found (mg)	Recovery (%)	Mean (%)	RSD (%)
Astragalin	1.38	1.25	2.55	93.4	95.1	2.4
	1.51	1.25	2.72	96.7		
	1.16	1.25	2.37	96.9		
	0.98	1.25	2.14	93.1		
	1.06	1.25	2.22	92.6		
	1.11	1.25	2.33	97.9		
Quercetin	2.32	2.11	4.25	91.4	93.5	2.2
	2.16	2.11	4.14	93.9		
	2.05	2.11	4.04	94.5		
	1.96	2.11	4.00	96.7		
	1.93	2.11	3.90	93.3		
	1.84	2.11	3.76	91.2		
Isorhamnetin	9.91	9.58	19.23	97.3	96.2	2.2
	10.31	9.58	19.24	93.2		
	9.56	9.58	18.98	98.3		
	9.93	9.58	19.14	96.1		
	11.45	9.58	20.85	98.1		
	10.23	9.58	19.25	94.2		
α-Tractlone	8.61	8.51	16.81	96.4	94.9	1.9
	8.05	8.51	16.33	97.3		
	8.99	8.51	17.07	95		
	9.05	8.51	17.00	93.4		
	7.69	8.51	15.57	92.6		
	8.03	8.51	16.09	94.7		
Hyperoside	8.82	8.12	16.50	94.6	97.6	3.0
	7.56	8.12	15.46	97.3		
	7.33	8.12	15.17	96.6		
	8.10	8.12	15.89	95.9		
	9.04	8.12	17.02	98.3		
	8.56	8.12	16.92	102.9		

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

Batch	Astragalin (mg/g)	Quercetin (mg/g)	Isorhamnetin (mg/g)	α-Tractlone (mg/g)	Hyperoside (mg/g)
120501	5.61	4.97	20.89	15.41	12.78
121001	5.55	4.32	18.73	13.22	9.54
121119	5.23	4.66	19.65	13.46	11.45

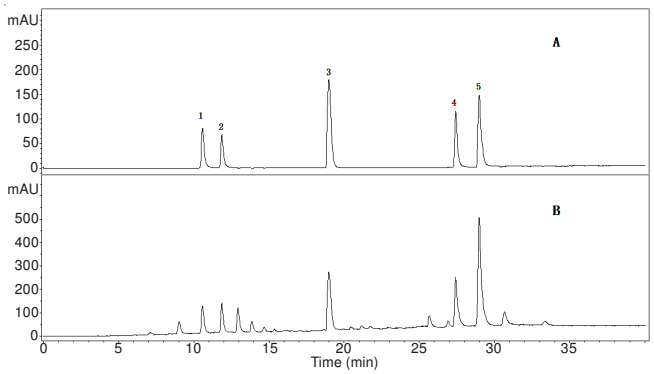

Fig. 1. HPLC chromatograms of the five ingredients mixture (A) and *Weitongding* particle (B) 1. astragalin, 2. quercetin, 3. isorhamnetin, 4. α-tractlone, 5. hyperoside

Recovery test: The sample with known targeted contents was spiked with certain amounts of the five standards. Then the spiked sample was processed in accordance with the established method for the HPLC detection. The recoveries of the five standards were 93.5-97.6 % (Table-2).

Application of HPLC method for quantitation studies: The experiment determined three samples of different batches by the above method. The contents of the five components are showed in Table-3.

Optimization of HPLC separation conditions: In order to get a separation with better resolution of targeted components with shorter analytical time, we compared three different column temperature: 25, 35 and 40 °C, then we found that the higher temperature, the shorter retention time with almost the same resolution, so we chose 35 °C finally for protecting the lifespan of the column.

Besides, reflux extraction and ultrasonic extraction were investigated in the experiments. Considering the advantages of high efficiency, we chose reflux extraction as the way to prepare sample solution. In the meantime, we compared the efficiency of different elution solvents, namely methanol-formic acid and acetonitrile-formic acid. The results indicated that the latter was slightly better. We also tried different reflux time: 1, 2 and 3 h. Finally, taken the determination of targeted components into consideration, 2 h. reflux extraction was better.

Five chromatographic columns of same specification were compared in the experiment: Phenomen Luna-C_{18}, Agilent Zorbax SB-C_{18}, Agilent Eclipse XDB-C_{18}, Waters Sunfire-C_{18} and Agilent Extend-C_{18}, according to the effect of separation, Agilent Eclipse XDB-C_{18} (250 mm × 4.6 mm, 5 μm) was used for the further research of the methodology.

Conclusion

In this study, five active components were quantitatively determined in Weitongding particle. On the whole, this developed method was simple, accurate for the determination of astragalin, quercetin, isorhamnetin, α-tractlone and hyperoside simultaneously and reliable for the quality control and further efficacy study of Weitongding particle in manufacture.

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