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Simultaneous Determination of Five Active Ingredients in Yiqing Tablet by HPLC

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To establish an HPLC method for the simultaneous determination of 5 active ingredients in Yiqing tablet. HPLC conditions included Agilent ZorbaxSB-C18 column (4.6 mm × 250 mm, 5 μm) and the mobile phase was a mixture of acetonitrile and 0.1 % formic acid for gradient elution.The flow rate was 1 mL/min and the column temperature was 35 ºC. Five active ingredients had good linear relationships: berberinein the range of 0.0024-0.1192 μg (R^2 = 0.9995), aloe-emodin in the range of 0.00103-0.0515 μg (R^2 = 0.9998), emodin in the range of 0.0052-0.0260 μg (R^2 = 0.9999), chrysophanol in the range of 0.0011-0.0560 μg (R^2 = 0.9999) and physcion in the range of 0.0011-0.0530 μg (R^2 = 0.9996). Their average recoveries were 101.4 % (RSD = 2.3 %), 100.6 % (RSD = 2.0 %), 100.3 % (RSD = 2.8 %), 102.0 % (RSD = 1.6 %)and 101.8 % (RSD = 1.8 %), 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 Yiqing tablet.

Key Words: HPLC, Yiqing tablet, Jatrorrhizine, Palmatine, Berberine, Aloe-emodin, Rhein, Wogonin, Emodin, Chrysophanol, Physcion.

INTRODUCTION

Yiqing tablet was developed based on the Xiexin Decoction in Medical Treasures of the Golden Chamber, written by the famous medical scientist named Zhongjing Zhang of Han Dynasty. It contains three traditional Chinese herbs with bitter cold properties, possessing the functions of clearing away the heat and expelling toxicity, discharging fire and relaxing bowels[1], clinically mainly used against upper respiratory tract infection, mouth ulcer, haemorrhoids, acne, constipation, allergic rhinitis, seborrheic dermatitis, upper gastrointestinal hemorrhage, hypertention, *etc.*[2]. Modern pharmacology has proved that the active ingredients in Yiqing tablet are mainly anthraquinones, such as emodin, aloe-emodin and chrysophanol, which have the function of dredging and regulating stasis and antibacterial antiinflammatory effect[3-7]. Others like berberine and wogonin, have antiviral effect[8-11]. 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 five active ingredients (berberine, aloe-emodin, emodin, chrysophanol and physcion) in Yiqing tablet. This method was stable with high precision, which could be used for the determination of the five ingredients and quality control of Yiqing tablet.

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 (JulaboLabortechnik GmbH company, Germany).

Standards of berberine, aloe-emodin, emodin, chrysophanol and physcion were supplied by National Institute for Food and Drug Control (NIFDC, Beijing, China) with the batch number of110713-200911, 110795-200203, 110756-200210, 110796-200309, 110758-200307, respectively. Yiqing tablet was purchased from Nanfeng Pharmaceutical Co., LTD (Taizhou, China), with the batch number of 110702, 110813, 110925. 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 5 ingredients was carried out on an Agilent ZorbaxSB-C_{18} (250 mm × 4.6 mm, 5 μm). The solvents used for HPLC separation were acetonitrile (A) and 0. 1 % formic acid (B) at a flow rate of 1.0 mL/min with gradient elution (0-15 min, 23-26 % A; 15-17 min, 26-40 % A, 17-35 min, 40-45 % A, 35-45 min, 45-85 % A, 45-50 min, 85 % A) and the analysis was monitored at 270

nm with the column temperature of 35 ºC and the injection volume was 10 µL.

Preparation of standard solution: Certain amounts of the five standard compounds were dissolved with methanol into volumetric flasks, respectively. Then measured 2 mL berberine, 1 mL aloe-emodin, 1 mL emodin, 2 mL physcion and 5 mL chrysophanol standard solution, respectively, into a 100 mL volumetric flask, then accurately measured 10 mL the mixed liquid to a 20 mL volumetric flask and diluted to the concentrations of 2.39, 1.03, 0.52, 1.12 and 1.06 µg/mL with methanol.

Preparation of sample solution: Ten pouches Yiqing tablet were pulverized into fine powder, after passing through the 80 mesh sieve, about 1 g sample was accurately weighted, then added into a 150 mL conical flask. Accurate 20 mL 10 % hydrochloric acid and 40 mL trichloromethane were added into the flask and placed in constant temperature bath box for 2 h circulation reflux at 80 ºC. After refrigerating, the suspension was moved to a separating funnel for 3 times extraction with trichloromethane solvent, 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. Accurate 1 mL above solution was measured to a 10 mL volumetric flask and metered the volume with methanol. The solution was ready for chromatographic analysis after passing through a 0.45 µm membrane filter.

RESULTS AND DISCUSSION

Validation of the chromatographic method

Linear range: Linear regression analysis for each component was performed by the external standard method. The above five compound 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})
Berberine	Y=3.79X+5.06	0.9995	0.00240-0.1192
Aloe-emodin	Y=2.89X+0.46	0.9998	0.00103-0.0515
Emodin	Y=4.02X-0.06	0.9999	0.00520-0.0260
Chrysophanol	Y=58.51X-10.42	0.9999	0.00110-0.0560
Physcion	Y=1.89X-0.96	0.9996	0.00110-0.0530

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 stand deviation (RSD) of peak area of each standard was 0.8, 1.7, 1.5, 1.5 and 1.5 %, respectively.

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 1.1, 1.5, 1.6, 1.5 and 1.5 % and found to be stable for the experiment.

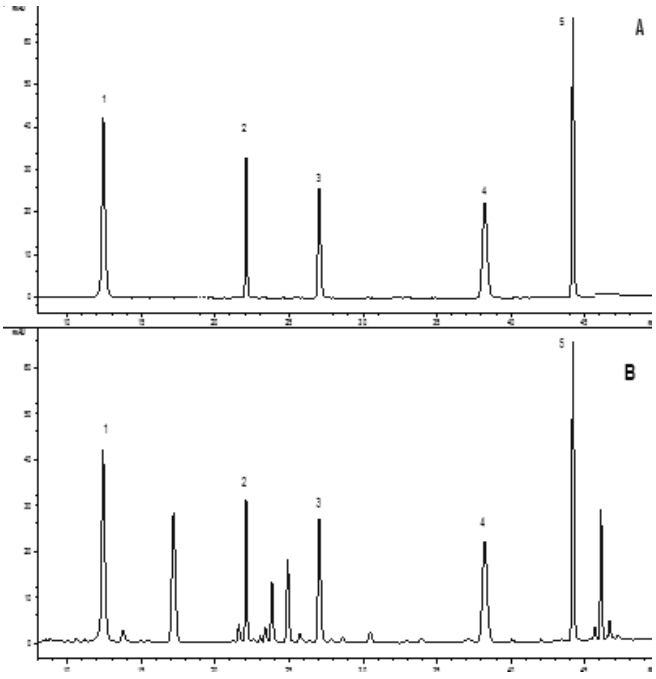

Fig. 1. HPLC chromatograms of the five ingredients mixture (A) and Yiqing tablet (B); 1: Berberine; 2: Aloe-emodin; 3: Emodin; 4: Chrysophanol; 5: Physcion

Repeatability: Repeatability was carried out using five samples solution after the same treatment procedure. The results showed that RSD of each peak area was 2.6, 2.0, 1.7, 2.2 and 1.3 %, respectively.

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 5 standards were 100.6-102.0 % (Table-2).

Application of the HPLC method for quantitation studies: The experiment determined three samples of different batches of the same manufacturer by the above method. The contents of the five components were 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 ºC, then we found that the higher temperature, the shorter retention time with almost the same resolution, so we chose 35 ºC 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 the same specification were compared in the experiment: Phenomen Luna-C_{18}, Agilent Zorbax SB-C_{18}, Agilent Eclipse XDB-C18, Waters Sunfire-C_{18} and Agilent Extend-C_{18}, according to the effect of

TABLE-2
RECOVERY RESULTS OF THE FIVE ACTIVE INGREDIENTS

Component	Contents in samples (mg)	Added (mg)	Found (mg)	Recovery (%)	Mean (%)	RSD (%)
Berberine	0.6951	0.7003	1.3871	98.81	101.4	2.3
	0.6691	0.7003	1.3726	100.45		
	0.6898	0.7003	1.4219	104.55		
	0.7008	0.7003	1.3935	98.92		
	0.6799	0.7003	1.3993	102.72		
	0.6873	0.7003	1.4083	102.95		
Aloe-emodin	0.1604	0.2146	0.3811	102.84	100.6	2.0
	0.1544	0.2146	0.3714	101.12		
	0.1591	0.2146	0.3764	101.27		
	0.2085	0.2146	0.3706	97.35		
	0.189	0.2146	0.3758	102.01		
	0.1851	0.2146	0.3709	98.92		
Emodin	0.1219	0.1813	0.2978	97.00	100.3	2.8
	0.1173	0.1813	0.2969	99.08		
	0.1210	0.1813	0.3072	102.72		
	0.1229	0.1813	0.3076	101.88		
	0.1192	0.1813	0.3090	104.70		
	0.1205	0.1813	0.3013	96.20		
Chrysophanol	0.2985	0.2914	0.5977	102.68	102.0	1.6
	0.2873	0.2914	0.5886	103.40		
	0.2962	0.2914	0.5880	100.12		
	0.3010	0.2914	0.5917	99.75		
	0.2920	0.2914	0.5919	102.91		
	0.2952	0.2914	0.5964	103.36		
Physcion	0.1474	0.1651	0.3105	98.77	101.8	1.8
	0.1418	0.1651	0.3121	103.15		
	0.1462	0.1651	0.3121	100.49		
	0.1486	0.1651	0.3177	102.44		
	0.1441	0.1651	0.3128	102.21		
	0.1457	0.1651	0.3165	103.48		

TABLE-3
CONTENTS DETERMINATION OF THE SEVEN ACTIVE INGREDIENTS IN THREE BATCHES

Batch	Berberine (mg/g)	Aloe-emodin (mg/g)	Emodin (mg/g)	Chrysophanol (mg/g)	Physcion (mg/g)
110702	4.70	1.14	2.59	3.75	1.70
110813	3.23	1.47	2.10	1.98	1.07
110925	6.54	0.74	2.67	2.42	1.32

separation, Agilent Zorbax SB-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 Yiqing tablet. On the whole, this developed method was simple, accurate for the determination of berberine, aloe-emodin, emodin, chrysophanol and physcion simultaneously and reliable for the quality control and further efficacy study of Yiqing tablet in manufacture.

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