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Simultaneous Determination of Five Active Ingredients in Huangshixiangsheng Pills by HPLC

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To establish an HPLC method for the simultaneous determination of five active ingredients in a famous Chinese clinical complex prescription. HPLC conditions included Hypersil-C_{18} column (4.6 mm × 250 mm, 5 μm) and the mobile phase was a mixture of acetonitrile and 0.1 % phosphoric acid for gradient elution. The flow rate was 1 mL/min and the column temperature was at 30 ºC. Five active ingredients had good linear relationships: Aloe-emodin in the range of 0.0081-0.4045 μg (R^2 = 0.9997), rhein in the range of 0.0065-0.3255 μg (R^2 = 0.9997), emodin in the range of 0.0124-0.6220 μg (R^2 = 0.9997), chrysophanol in the range of 0.0138-0.6910 μg (R^2 = 0.9999) and physcion in the range of 0.0032-0.1615 μg (R^2 = 0.9994). Their average recoveries were 99.1 % (RSD = 1.2 %), 98.6 % (RSD = 1.7 %), 97.4 % (RSD = 1.4 %), 97.4 % (RSD = 2.6 %) and 97.0 % (RSD = 0.6 %), 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 Huangshixiangsheng pills.

Key Words: HPLC, Huangshixiangsheng Pill, Aloe-emodin, Rhein, Emodin, Chrysophanol, Physcion.

INTRODUCTION

Huangshixiangsheng pills was developed based on an 800-year-ago secret complex prescription provided by the famous laryngologist Shennong Huang, composing of 12 traditional Chinese medicine, including *Herba menthae*, *Forsythia suspensa* (thunb.) vahl, *Radix et rhizoma* Rhei, *Bulbus fritillariae* Thunbergii, *Semen sterculiae* Lychnophorae, *Radix platycodi, Periostracum cicadae, Rhizoma chuanxiong, Catechu, Fructus chebulae, Radix glycytthizae* and *Mentholum*. It has the functions of dispersing wind heat, reducing phlegm and removing stasis, relieving sore-throat and opening voice[1], usually clinically used in China, Japan and Korea against exterior wind heat, acute and chronic pharyngitis caused by excessive interior phlegm heat, such syndromes as hoarseness, swelling and pain of throat, pharyngeal dry heat, phlegm dampness accumulated in pharynx, chills and fever on head, astriction and dark urine, *etc.*[2-5]. Modern pharmacology has proved that the active ingredients in Huangshixiangsheng pills are mainly anthraquinones, such as emodin, aloe-emodin and chrysophanol, which could dredge and regulate stasis, anti bacteria and inflammation[6-9]. In addition, the metabolites of rhein, namely rhein anthrone, has the effect of purging excessive heat[10]. Referring to the determination method of rheum in Chinese Pharmacopoeia 2010 edition[11], the research established the method of simultaneous determination of 5 active ingredients (aloe-emodin, rhein, emodin, chrysophanol and physcion) in Huangshixiangsheng pills. This method was stable with high precision, which could be used for the determination of the five ingredients and further quality control.

EXPERIMENTAL

Agilent 1100 series HPLC system (Agilent Technologies, USA) was equipped with quaternary pump (G1311A), automatic sampler (G1313A), UV variable-wavelength detector (1314A-UV) and column oven (CTO-10ASVP); Pa2251 electronic analytical balance (Sartorius Group of Germany); 4020P ultrasonic cleaner (JAC company of Korea).

Standards of aloe-emodin, rhein, emodin, chrysophanol and physcion were supplied by National Institute for Food and Drug Control (NIFDC, Beijing, China) with the batch number of 110795-200203, 110757-200206, 110756-200210, 110796-200309, 110758-200307, respectively. Huangshixiangsheng pills was purchased from Jimin Pharmaceutical Co., LTD (Wuxi, China). 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 aloe-emodin, rhein, emodin, chrysophanol and physcion was performed on

Hypersil SB-C18 (250 mm × 4.6 mm, 5 μm). The solvents used for HPLC separation were acetonitrile (A) and 0.1 % phosphoric acid (B) at a flow rate of 1.0 mL/min with gradient elution (0-10 min, 44 % A; 10-25 min, 44-82 % A; 25-30 min, 82-90 % A; 30-35 min, 90 % A) and the analysis was monitored at 254 nm with the column temperature of 30 ºC 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 aloe-emodin, 2 mL rhein, emodin and physcion and 3 mL chrysophanol standard solution, respectively, into a 25 mL volumetric flask and diluted and formed the mixture standard solution at the concentrations of 206.4, 81.4, 155.5, 115.6 and 40.48 μg/mL, respectively.

Preparation of sample solution: 30 Huangshixiangsheng pills were pulverized into fine powder, about 1 g sample was accurately weighted, then added into a 150 mL conical flask. Accurate 50 mL methanol was added into the flask and placed in ultrasound bath for 15 min sonicating at room temperature. This extraction process was repeated twice under the same conditions, then filtered and merged the extraction solution, then evaporated the filtrate at 75 ºC on water bath kettle. Next, about 20 mL 10 % hydrochloric acid solution was added into the above residue, then moved the suspension to a separating funnel for extraction with $CHCl_3$ 3 times, each time with 60 mL and merged the $CHCl_3$ layer for solvent recycling and then dissolved the residue with methanol into a 25 mL volumetric flask. The solution was ready for chromatographic analysis after passing through a 0.45 μm membrane filter.

RESULTS AND DISCUSSION

Validation of 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 into HPLC, respectively. The linearity of each compound was calculated by plotting the peak area (Y) *vs.* concentration (X) (Table-1). All the 5 components showed good linearities in wide concentration ranges (Fig. 1).

TABLE-1
LINEAR REGRESSION EQUATION AND LINEAR RANGES

Components	Regression equation	Correlation coefficient (R^2)	Linear range (μg/mL)
Aloe-emodin	Y=0.3433X-8.0943	0.9997	0.81-40.45
Rhein	Y=0.2301X-5.2009	0.9997	0.65-32.55
Emodin	Y=0.3842X-10.1846	0.9997	1.24-62.20
Chrysophanol	Y=0.6075X-10.0514	0.9999	1.38-69.10
Physcion	Y=0.1004X-2.8249	0.9994	0.32-16.15

Precision: The standard mixture solution was injected into HPLC six times continuously and the peak areas of compound were used for the calculation of precision. The results showed that relative stand deviation (RSD) of each standard was 0.1, 0.1, 0.4, 0.1, 0.3 %, respectively, indicating good precision of the method.

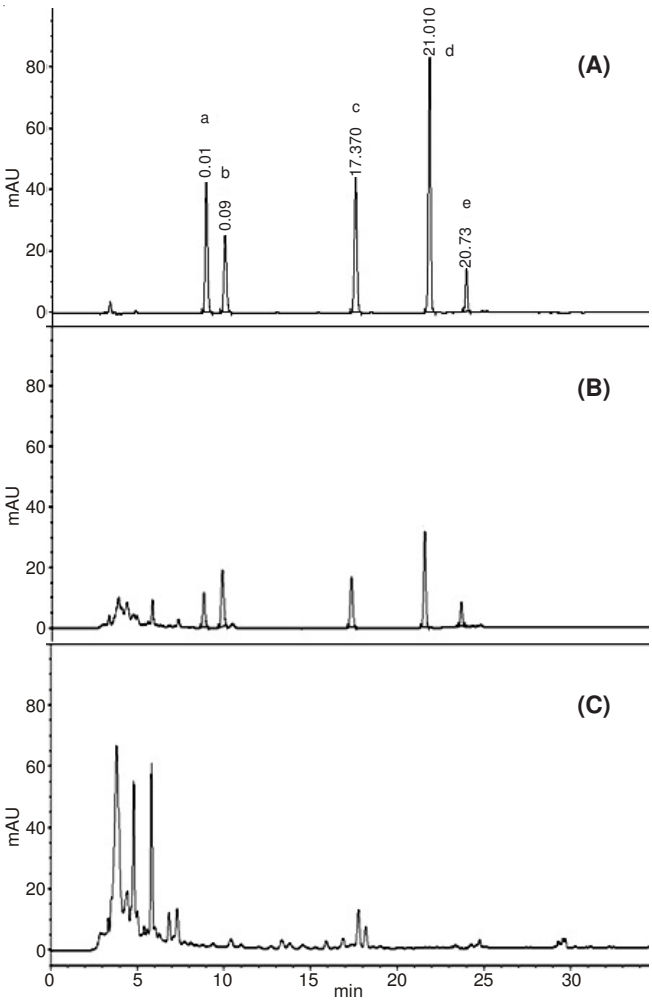

Fig. 1. HPLC chromatograms of the mixture standard solution (A), Huangshixiangsheng pills (B) and negative sample (C); a: Aloe-emodin; b: Rhein; c: Emodin; d: Chrysophanol; e: Physcion

Stability: For stability test, the same sample solution was analyzed at designated time points in 48 h. The results showed that RSDs of peak area were 1.2, 0.7, 1.4, 1.1, 2.2 % and found to be stable for the whole research duration.

Repeatability: Repeatability was carried out using six samples solution after the same treatment procedure. The results showed that RSD of each peak area was 1.9, 1.3, 1.7, 1.7 and 2.0 %, respectively, suggesting the method repeatability was suitable for the analysis.

Recovery test: The samples with known contents of the targeted components were spiked with certain amounts of the 5 standards. Then the spiked samples were processed in accordance with the established method for the HPLC detection. The average recoveries were 97.0-99.1 % (Table-2).

Application of the method for quantitation studies: The experiment determined 3 samples of different batches of the same manufacturer by the above method. The contents of the 5 components was shown 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 with three different column temperature: 20, 30 and 40 ºC, then we found that the higher temperature, the shorter retention time with

TABLE-2
RECOVERY RESULTS OF THE FIVE ACTIVE INGREDIENTS

Component	Contents in samples (mg)	Added (mg)	Found (mg)	Recovery (%)	Mean (%)	RSD (%)
Rhein	0.0636	0.0847	0.1496	101.53	98.6	1.7
	0.0882	0.0847	0.1721	99.06		
	0.0603	0.0847	0.1432	97.87		
	0.0752	0.0847	0.1583	98.11		
	0.0641	0.0847	0.1477	98.70		
	0.0699	0.0847	0.1516	96.46		
Emodin	0.0720	0.0964	0.1678	99.38	97.4	1.4
	0.0803	0.0964	0.1724	95.54		
	0.0913	0.0964	0.1853	97.51		
	0.0945	0.0964	0.1873	96.27		
	0.0896	0.0964	0.1845	98.44		
	0.0928	0.0964	0.1866	97.30		
Chrysophanol	0.1071	0.0670	0.1705	94.63	97.4	2.6
	0.1044	0.0670	0.1723	101.34		
	0.0821	0.0670	0.1479	98.21		
	0.0764	0.0670	0.1421	98.06		
	0.0741	0.0670	0.1395	97.61		
	0.0801	0.0670	0.1436	94.78		
Physcion	0.0414	0.0397	0.0801	97.48	97.0	0.6
	0.0575	0.0397	0.0959	96.73		
	0.0628	0.0397	0.1014	97.23		
	0.0549	0.0397	0.0931	96.22		
	0.0491	0.0397	0.0875	96.73		
	0.0773	0.0397	0.1161	97.73		

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

Batch number	Aloe-emodin (mg/g)	Rhein (mg/g)	Emodin (mg/g)	Chrysophanol (mg/g)	Physcion (mg/g)
100914	0.085	0.181	0.206	0.306	0.594
110632	0.077	0.185	0.178	0.143	0.089
110634	0.080	0.193	0.185	0.148	0.092

almost the same resolution, so we chose 30 ºC finally for protecting the lifespan of the column.

Besides, reflux extraction and ultrasonic extraction were investigated in the experiment. Considering the advantages of high efficiency and easy operation, we chose ultrasonic extraction as the way to prepare sample solution. In the meantime, we compared the efficiency of different elution solvents, namely methanol-phosphoric acid and acetonitrile-phosphoric acid. The results indicated that the latter was slightly better. We also tried different ultrasonic time: 15, 25 and 30 min, and 15 min ultrasonic extraction was chosen for there was no significant difference among 3 periods experimented.

Six chromatographic columns of the same specification were compared in the experiment: Zorbax SB-C_{18}, Kromasil C_{18}, Eclipse-C_{18}, Symmetry Shield RP_{18}, Hypersil C_{18} and Extend-C_{18}, according to the effect of separation, Hypersil 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 Huangshixiangsheng pills. To our best of knowledge, this work remarkably shortened the analysis time when compared with other methods. On the whole, this developed method was simple, accurate for the simultaneous determination of aloe-emodin, rhein, emodin, chrysophanol and physcion and reliable for the quality control and further efficacy study of Huangshixiangsheng pills in clinic.

REFERENCES

1. State Pharmacopeia Committee of China. Chinese Pharmacopoeia, Version part I. 2010: 1064 (2010).
2. D.D. Zhang, *Chin. Med. Modern Distance Educ. China*, **9**, 68 (2011).
3. Y.H. Zeng and R.Q. Huang, *Modern Chin. Med. Appl.*, **3**, 72 (2009).
4. X.M. Chen, *Modern J. Integr. Tradit. Chin. West. Med.*, **17**, 2346 (2008).
5. Y. Shi, *Lishizhen Med. Mater. Med. Res.*, **10**, 461 (1999).
6. Y.Y. Li, Z.Y. Xue, L.L. Mo and Y.Q. Qu, *J. Guangzhou College Tradit. Chin. Med.*, **3**, 11 (1997).
7. K. Liu and H.S. Zheng, *Chin. Arch. Tradit. Chin. Med.*, **22**, 1732 (2004).
8. J. Li, L.Y. Zhang and Z.Z. Jiang, *Process Pharm. Sci.*, **29**, 542 (2005).
9. J.D. Yan, *J. Mudanjiang Medical College*, **27**, 62 (2006).
10. D.N. Liang, *Informat. Tradit. Chin. Med.*, **5**, 43 (1986).
11. State Pharmacopeia Committee of China, Chinese Pharmacopoeia, Version part I.2010: 22 (2010).