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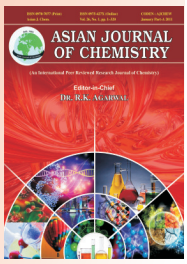

Simultaneous Determination of Five Active Ingredients in Yixintong Tablet by HPLC

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To establish an HPLC method for the simultaneous determination of five active ingredients (glucosyl-vitexin, rhamnosyl-vitexin, vitexin, rutin and hyperin). HPLC conditions included Welch material XB-C18 column (4.6 mm × 250 mm, 5 μm) and the mobile phase was a mixture of tetrahydrofuran (A)-acetonitrile (B)-methanol (C)-0.05 % phosphoric acid (D) at a flow rate of 1 mL/min for gradient elution. The column temperature was 30 °C. Five active ingredients had good linear relationships: glucosyl-vitexin in the range of 0.0369-1.85 μg (R^2 = 0.9998), rhamnosyl-vitexin in the range of 0.109-5.47 μg (R^2 = 0.9995), vitexin in the range of 0.00600-0.300 μg (R^2 = 0.9999), rutin in the range of 0.00510-0.255 μg (R^2 = 0.9997) and hyperin in the range of 0.00510-0.255 μg (R^2 = 0.9997). Their average recoveries were 97.55 % (RSD = 1.9 %), 98.27 % (RSD = 2.1 %), 98.22 % (RSD = 2.4 %), 97.55 % (RSD = 2.1 %) and 98.13 % (RSD = 2.6 %), respectively. This method was steady with high precision and good repeatability. It could be used for the determination of the five active ingredients and quality control of Yixintong tablet.

Keywords: HPLC, Yixintong tablet, Glucosyl-vitexin, Rhamnosylvitexin, Vitexin, Rutin, Hyperin.

INTRODUCTION

Yixintong tablet is a preparation of Chinese patent medicine with Hawthorne leaf P.E as its raw material, now being carried on the 2010 version of Pharmacopoeia of the P.R. China, possessing the function of promoting blood circulation to remove stasis, regulating the flow of vital energy and dredging collaterals, diffusing and smoothening heart vessels. Clinically, it has curative effects on Qi-stagnation and blood stasis, oppression in chest and breathlessness, cardiopalmus and amnesia, dizziness and tinnitus and many kinds of cardiovascular and cerebrovascular diseases such as coronary artery disease, angina pectoris, hyperlipidemia, blood insufficiency of cerebral arteries, *et al.*[1]. It was reported in literature, flavonoids as the main active ingredients including glucosyl-vitexin, rhamnosyl-vitexin, vitexin, rutin and quercetin[2-3], possessing the pharmacological effects of anti-inflammatory, analgesia, antioxygen, anti-aging, anti-myocardial ischemia, liver protection effect, *etc.*[4-15]. At present, there have been few reports on simultaneous determination in Yixintong tablet in literature. Moreover, the quality control for Yixintong tablet in Chinese Pharmacopoeia is restricted to determining hyperin. Its quality control is lack of specificity and could not monitor the determination of other active ingredients, existing large space for adulteration. This experiment established the method for the simultaneous determination of the five active ingredients (glucosyl-vitexin, rhamnosyl-vitexin, vitexin, rutin and hyperin) in Yixintong tablet. This method was stable with high precision, which could be used for the determination of the five ingredients and quality control of Yixintong tablet.

EXPERIMENTAL

Agilent 1260 series HPLC system (Agilent Technologies, USA) was equipped with Quat Pump (G1311), 1260 ALS (G1329B), 1260 TCC (G1316A) and 1260 DAD (G4212B); PA2251 electronic analytical balance (Sartorius Group, Germany); Centrifuge 5430R high speed centrifuge (USA, Eppendorf); 4020P ultrasonic cleaner (Korea, JAC Company) Standards of rhamnosyl-vitexin, vitexin, rutin and hyperin were supplied by National Institute for Food and Drug Control (NIFDC) (Beijing, China) with the batch number of 111668-200401, 111687-200602, 100080-200306 and 111521-200303, respectively. Standards of glucosyl-vitexin was extracted and purified by ourselves (purity > 98 %). Eleven batches of Yixintong tablets were provided by Taiji Group Zhejiang East Pharmaceutical Co., LTD (Shaoxing, China), Shanxi Taiyuan Pharmaceutical Co., LTD (Taiyuan, China), Shanxi Angsheng Pharmaceutical Co., LTD (Changzhi, China) and Shanxi Zhendong Pharmaceutical Co., LTD (Changzhi, China). Tetrahydrofuran, acetonitrile and methanol were 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 glucosyl-vitexin, rhamnosyl-vitexin, vitexin, rutin and hyperin was carried out on an Welch Material XB-C18 (250 mm × 4.6 mm, 5 µm) column. The solvents used for HPLC separation were tetrahydrofuran (A)-acetonitrile (B)-methanol (C)-0.05 % phosphoric acid (D) at a flow rate of 1 mL/min with gradient elution (Table-1) and the analysis was monitored at 363 nm with the column temperature of 30 °C and the injection volume was 10 µL.

TABLE-1
GRADIENT ELUTION OF HPLC CONDITION

Solvents	Time (min)			
	0-15	15-20	20-40	40-45
A (%)	8	19	30	8
B (%)	0.5	0.5	5	0.5
C (%)	0.5	0.5	5	0.5
D (%)	91	80	60	91

Preparation of standard solution: Certain amounts of the 5 standard compounds (glucosyl-vitexin, rhamnosyl-vitexin, vitexin, rutin and hyperin) were dissolved with 50 % methanol into volumetric flasks, respectively. Then measured 1 mL glucosyl-vitexin, 5 mL rhamnosyl-vitexin, 0 mL vitexin, 1 mL rutin and 1 mL hyperin standard solution, respectively, into a 10 mL volumetric flask and diluted to the concentrations of 36.9, 109.4, 6.0, 5.1 and 5.1 µg/mL.

Preparation of sample solution: Twenty slices Yiqing tablet were pulverized into fine powder, after passing through the 80 mesh sieve, about 0.2 g sample was accurately weighted, then added into a 50 mL volumetric flask. 40 mL 50 % methanol were added into the flask and placed in ultrasonic instrument for 0.5 h ultrasound. After refrigerating, the suspension was metered to the volume with 50 % methanol. The solution was ready for chromatographic analysis after 10 min high speed centrifugation.

RESULTS AND DISCUSSION

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-2). All the 5 components showed good linearities in wide concentration ranges (Fig. 1).

Precision: The standard mixture solution of glucosyl-vitexin, rhamnosyl-vitexin, vitexin, rutin and hyperin 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 0.9, 0.9, 0.3, 1.6 and 0.6 %, 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 1.9, 2.2, 2.1, 1.4 and 1.1 % and found to be stable for the experiment.

Repeatability: Repeatability was carried out using six samples solution after the same treatment procedure. The results showed that RSD of each peak area was 1.7, 2.7, 2.4, 2.2 and 2.6 %, respectively.

TABLE-2
LINEAR REGRESSION EQUATION AND LINEAR RANGES

Components	Regression equation	Correlation coefficient (R^2)	Linear range (µg)
Glucosyl-vitexin	Y = 0.1949X + 2.0641	0.9998	0.0369-1.85
Rhamnosyl-vitexin	Y = 0.2301X - 5.2009	0.9995	0.109-5.47
Vitexin	Y = 0.3842X -10.1846	0.9999	0.00600-0.300
Rutin	Y = 0.3137X + 0.3431	0.9997	0.00510-0.255
Hyperin	Y = 1.5591X + 2.4411	0.9997	0.00510-0.255

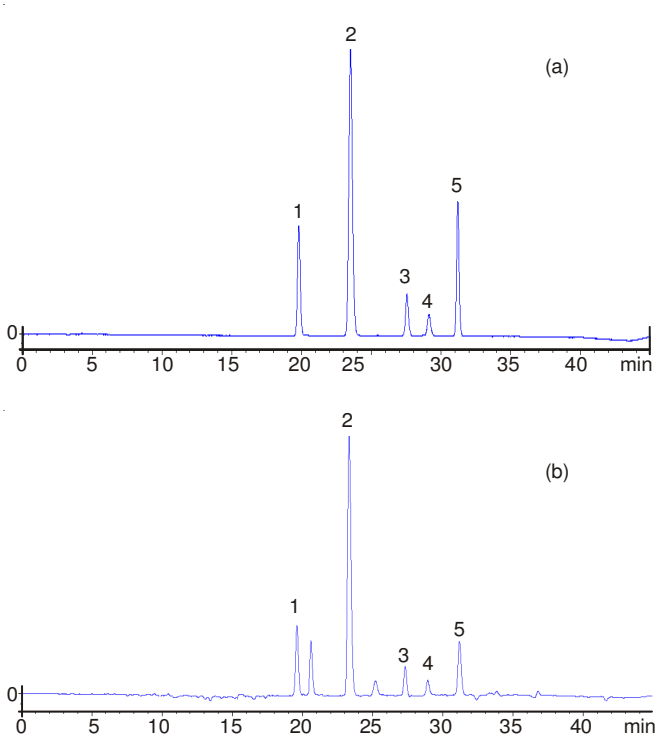

Fig.1. HPLC chromatograms of the five ingredients mixture (a) and Yixintong tablet (b) 1: glucosyl-vitexin; 2: rhamnosyl-vitexin; 3: vitexin; 4: rutin; 5: hyperin

Recovery test: The sample with known targeted contents was spiked with certain amounts of the 5 standards. Then the spiked sample was processed in accordance with the established method for the HPLC detection. The average recoveries for glucosyl-vitexin, rhamnosyl-vitexin, vitexin, rutin and hyperin determined were 97.55, 98.27, 98.22, 97.55 and 98.13 % (Table 3).

Application of 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 5 components were showed in Table-4.

Selection of sample preparation method: Different extraction solvents (methanol, 50 % methanol and pure water), different extraction means (ultrasound and reflux) and different extraction time (20, 30 and 40 min) were investigated on the selection of sample preparation method, respectively. The results indicated that the optimization was extracted by ultrasound for 0.5 h with 50 % methanol.

TABLE-3
RECOVERY RESULTS OF THE FIVE ACTIVE INGREDIENTS

Component	Contents in samples (mg)	Added (mg)	Found (mg)	Recovery (%)	Mean (%)	RSD (%)
Glucosyl-vitexin	1.0591	0.7388	1.7695	96.16	97.55	1.9
	1.0456	0.7388	1.7509	95.47		
	0.9914	0.7388	1.7093	97.17		
	1.0257	0.7388	1.7570	98.98		
	0.9977	0.7388	1.7394	100.39		
	0.9760	0.7388	1.6936	97.13		
Rhamnosyl-vitexin	3.9202	3.5008	7.3522	98.03	98.27	2.1
	3.8700	3.5008	7.2982	97.93		
	3.6695	3.5008	7.2285	101.66		
	3.7965	3.5008	7.2643	99.06		
	3.6929	3.5008	7.1013	97.36		
	3.6127	3.5008	6.9584	95.57		
Vitexin	0.1588	0.1199	0.2743	96.33	98.22	2.4
	0.1568	0.1199	0.2732	97.08		
	0.1487	0.1199	0.2692	100.50		
	0.1538	0.1199	0.2687	95.83		
	0.1496	0.1199	0.2715	101.67		
	0.1464	0.1199	0.2638	97.91		
Rutin	0.0725	0.1014	0.1723	98.42	97.55	2.1
	0.0716	0.1014	0.1684	95.46		
	0.0679	0.1014	0.1689	99.61		
	0.0702	0.1014	0.1716	100.00		
	0.0683	0.1014	0.1655	95.86		
	0.0668	0.1014	0.1641	95.96		
Hyperin	0.1263	0.1016	0.2262	98.33	98.13	2.6
	0.1247	0.1016	0.2228	96.56		
	0.1183	0.1016	0.2155	95.67		
	0.1223	0.1016	0.2245	100.59		
	0.119	0.1016	0.2223	101.67		
	0.1164	0.1016	0.2139	95.96		

TABLE-4
CONTENTS DETERMINATION OF THE FIVE ACTIVE INGREDIENTS IN 3 BATCHES

Batch number	Glucosyl-vitexin (mg/mL)	Rhamnosyl-vitexin (mg/mL)	Vitexin (mg/mL)	Rutin (mg/mL)	Hyperin (mg/mL)
121409	0.0284	0.0100	0.0303	0.0253	0.0098
121410	0.0293	0.0106	0.0316	0.0262	0.0104
121411	0.0286	0.0102	0.0305	0.0254	0.0100

Selection of chromatographic column and column temperature: Six chromatographic columns of the same specification were compared in the experiment: Agilent Zorbax SB-C18, Agilent Kromasil 100-5C18, Agilent Extend-C18, Phenomenex Luna-C18, Welch Material XB-C18 and Agilent Eclipse XDB-C18, according to the effect of separation, Welch Material XB-C18 (250 mm × 4.6 mm, 5 μm) was used for the further research of the methodology. In order to get a separation with better resolution of targeted components with shorter analytical time, we compared with three different column temperatures: 25, 30 and 35 °C, then we found that the higher temperature, the shorter retention time with almost the same resolution, so we chosed 30 °C finally for protecting the lifespan of the column.

Conclusion

In this study, five active components were quantitatively determined in Yixintong tablet. On the whole, this developed method was simple, accurate for the determination of glucosyl-vitexin, rhamnosyl-vitexin, vitexin, rutin and hyperin simultaneously and reliable for the quality control and further efficacy study of Yixintong tablet in clinic.

REFERENCES

1. State Pharmacopeia Committee of China, Chinese Pharmacopoeia, 2010 version, Part I, p. 1024 (2010).
2. D.F. Hao, R.P. Yang, Y.Z. Zhou, H. Chen, Z.-F. Li and Y.-H. Pei, *J. Shenyang Pharm. Univ.*, **26**, 282 (2009).
3. R.H. Liu, F. Shao, Y.Q. Deng, *et al.*, *J. Chin. Med. Mater.*, **31**, 1102 (2007).
4. Y.C. Quan and L.P. Guan, *Lishizhen Med. Mater. Med. Res.*, **22**, 463 (2006).
5. Q.H. Yu, P.L. Huang, S.H. Zhang and H. Wang, *Chin. J. Publ. Health*, **22**, 463 (2006).
6. B. Yu, H.Y. Li, L. Zhang, L. Xu and T.H. Fang, *Tradit. Chinese Drug Res. Clin. Pharmacol.*, **19**, 461 (2008).
7. L. Li, H. Lv and H. Pang, *Lishizhen Med. Mater. Med. Res.*, **18**, 2143 (2007).
8. Q. Min, Y.T. Bai, Z. Zhang and L.-Y. Shi, *Chinese J. Clin. Pharmacol. Therap.*, **12**, 800 (2007).
9. Y.-S. Ji, X.-D. Zhang, H. Li and S.-J. Yang, *J. Jilin Univ. (Medicine Ed.)*, **31**, 879 (2005).
10. X.T. Lv, J.R. Ma and E.L. Yi, *World Health Digest*, **4**, 526 (2007).
11. X.Y. Ye, L. Zhang, J. Shen and Y.F. Wang, *Chin. Tradit. Herbal Drugs*, **36**, 1683 (2005).
12. P. Li, J.N. Wang, S.J. Lu, J.H. Fu and J.X. Liu, *China J. Chinese Mater. Med.*, **34**, 96 (2009).
13. X.Y. Ju, T.H. Fang and W.T. Zhang, *J. Nanjing Univ. TCM*, **21**, 381 (2005).
14. Z.Y. Chen, M.X. Yan and P.H. He, *J. Med. Res.*, **36**, 33 (2007).
15. K.Y. Jang, Z.L. Gu and C.G. Ruan, *Chinese Tradit. Patent Med.*, **22**, 782 (2000).