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Simultaneous Determination of 14 Compounds in Gegen Qinlian Tablet by HPLC

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A high performance liquid chromatographic method was established for the simultaneous determination of puerarin, daidzin, scutellarin, liquiritin, baicalin, jatrorrhizine, coptisine, daidzein, palmatine, berberine, wogonoside, glycyrrhizic acid, baicalein and wogonin in Gegen Qinlian Tablet. All the 14 compounds were separated on a Dikma C₁₈ column (150 × 4.6 mm i.d., 5 μm) by gradient elution. The mobile phase consisted of ammonium formate buffer solution (20 mmol L⁻¹, pH 3.2, adjusted by formic acid) (A) and acetonitrile (B) at a flow rate of 1 mL min⁻¹. The ultraviolet detection was accomplished at 254 and 275 nm. The RSD values for intraday precision (n = 6) and interday precision (n = 5) were less than 2.04 and 2.59 %, respectively. The recoveries were in the range of 91.6-101.3 %. Using ethanol-water (60:40, v/v) as extractant, this method was successfully applied to the quality evaluation of Gegen Qinlian tablets.

Keywords: HPLC, Simultaneous determination, Gegen Qinlian tablet.

INTRODUCTION

Gegen Qinlian decoction is a classical traditional Chinese medicine originated from eastern Han Dynasty Zhang Zhongjing's Treatise on Febrile Diseases¹, having the effect of clearing heat, relieving diarrhea, suppressing bacteria and enhancing immunologic function². Gegen Qinlian decoction is made by decocting Radix Puerariae, Radix Scutellariae, Coptis chinensis Franch and Radix Glycyrrhizae Preparata proportionally³. As the decocting process is time-consuming and improper operation will influence the efficacy of the decoction, so nowadays Gegen Qinlian tablet has become alternative to Gegen Qinlian decoction and has been listed in Chinese pharmacopoeia⁴.

For the quality control of Gegen Qinlian preparations (including tablets and pills, etc.), the existing studies mainly focus on two kinds of detection methods, one of which is to select several main components for determination⁵⁻⁷ and the other is to divide the main ingredients into groups and then analyze them, respectively under different chromatographic conditions⁸. Both of the detection methods have their deficiency. The former one can't give a comprehensive appraisal for the quality of the medicine and the latter is time consuming. Consequently, it is necessary to develop a highly efficient analytical method to achieve multicomponent simultaneous determination method. Existing literature reported some approaches for the multicomponent simultaneous determination^{9,10}.

However, simultaneous determination of 14 active compounds has not been performed.

In this paper, we used HPLC gradient elution with UV dual wavelength detection for simultaneous determination of 14 compounds in Gegen Qinlian tablet from different manufacturers. This simple and effective method is suitable for the quality evaluation of Gegen Qinlian preparations.

EXPERIMENTAL

Authentic standards of daidzin (dai), liquiritin (liq), coptisine (cop), jatrorrhizine hydrochloride (jat), wogonoside (wog), glycyrrhizic acid (gly) were purchased from Jingke corporation (Shanghai, China); Puerarin (pue), scutellarin (scu), baicalin (bai), daidzein (daid), palmatine hydrochloride (pal), hydroberbeaime sulfate (berb), baicalein (baic), wogonin (wogo) were purchased from National Institute for the Control of Food and Drug (Beijing, China). Gegen Qinlian tablets were purchased from Bai Autonomous Prefecture Chinese Medicine Pharmaceutical Co., Ltd., (specification: 0.5 g per piece, lot number 121202; Dali, China), Lijun Modern Chinese Medicine Co., Ltd., (specification: 0.5 g per piece, lot number 120910008; Shanxi, China) and Taiji Group Pharmaceutical Co., Ltd. (specification: 0.5 g per piece, lot number 55120003; Mianyang, China). Acetonitrile and methanol were of HPLC grade, other reagents were of analytical grade. Experimental water was doubly distilled.

All analyses were performed on an LC-20AD liquid chromatograph (Shimadzu, Japan) which consisted of two LC-20AD liquid chromatography pumps, a 7725i manual injector, an SPD-20A multi-wavelength detector and an LC solution Lite workstation. Diamonsil C18 column (150 × 4.6 mm i.d., 5 μm; Dikma, Beijing, China) was used for the separation of the compounds. A KQ2500E ultrasonic generator (10 L, 40 KHz; Kunshan ultrasonic instruments co., LTD, Jiangsu, China) was used for the ultrasonic-assisted extraction procedure.

Preparation of sample solutions: Ten tablets of Gegen Qinlian were accurately weighed and pulverized into fine powder in a mortar. 0.1 g powder were accurately weighed into a 15 mL centrifuge tube and extracted with 10 mL ethanol-water (60:40, v/v) in ultrasonic bath for 40 min. After centrifugation, the supernatant solution was collected and filtered through a 0.45 μm membrane filter before HPLC analysis.

Chromatographic conditions: The compounds were separated on a Diamonsil C18 column (150 × 4.6 mm i.d., 5 μm). The mobile phase consisted of ammonium formate buffer solution (20 mmol L⁻¹, pH 3.2, adjusted by formic acid) (A) and acetonitrile (B). The eluting conditions were as follows: 0-30 min: linear gradient from 15-25 % B; 30-35 min: linear gradient from 25-27 % B; 35-46 min: linear gradient from 27-80 % B. The flow rate was 1 mL min⁻¹. The detection wavelengths were set at 254 and 275 nm. The column temperature was kept at 30 °C. The injection volume was 5 μL.

RESULTS AND DISCUSSION

Selection of detection wavelength: The response signals of each compound were investigated at the detection wavelengths ranged from 245 to 370 nm. Eventually, 254 and 275 nm were selected because of better response and less interference. Puerarin, daidzin, scutellarin, daidzein and glycyrrhizic acid were detected at 254 nm, liquiritin, baicalin, jatrorrhizine, coptisine, palmatine, berberine, wogonoside, baicalein and wogonin were detected at 275 nm. Fig. 1 shows the chromatograms of standard solution and sample solution at 254 and 275 nm.

Optimization of extraction conditions

Effect of types of extractant: To find the best extractant, the extraction efficiencies of acetonitrile-water, ethanol-water and methanol-water were compared. The results showed that, the extraction efficiency of puerarin and daidzin by acetonitrile-water was much lower than either methanol-water or ethanol-water. In addition, there was a large interference peak close to the peak of puerarin, which might influence the determination of puerarin. Compared with methanol-water, the extraction efficiency of ethanol-water was better for the extraction of target compounds except for puerarin, daidzein, scutellarin, liquiritin and wogonoside. Besides, from the angle of green chemistry, ethanol-water is less toxic than methanol-water. Therefore, ethanol-water was selected as the extractant.

Effect of volume ratio of ethanol-water: The extraction efficiency of ethanol-water at different volume ratio of 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10 was investigated. The results are shown in Fig. 2. The extraction efficiency of puerarin and daidzin was in a downside trend with the increase

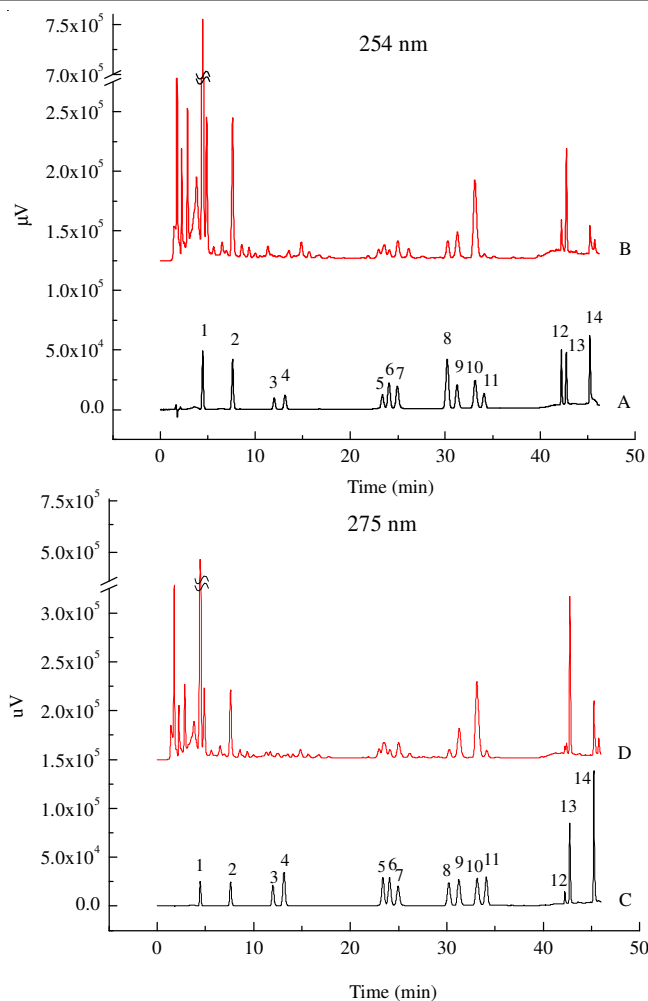


Fig. 1. Chromatograms of standard solution (A: 254 and C: 275 nm) and sample solution (B: 254 and D: 275 nm) (1) puerarin, (2) daidzin, (3) scutellarin, (4) liquiritin, (5) baicalin, (6) jatrorrhizine, (7) coptisine, (8) daidzein, (9) palmatine, (10) berberine, (11) wogonoside, (12) glycyrrhizic acid, (13) baicalein, (14) wogonin

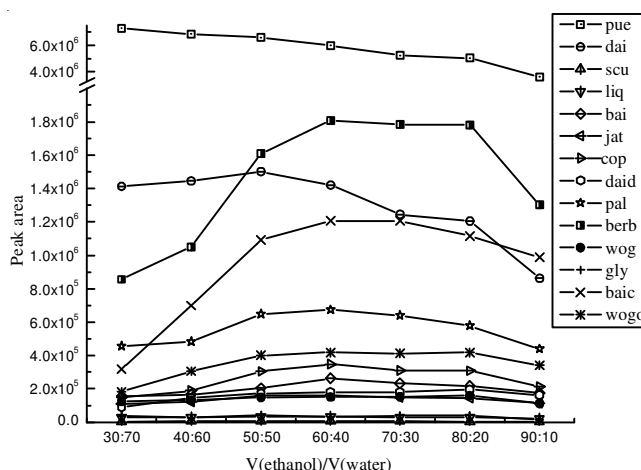


Fig. 2. Effect of volume ratio of ethanol-water on the extraction efficiency of the 14 compounds

of ethanol proportion. Other analytes reached their maximum value at volume ratio of 60:40. Therefore, volume ratio of 60:40 was employed in the following experiments.

Effect of ultrasonic extraction time: To optimize the ultrasonic extraction time, replicate samples were extracted

for 10, 20, 30, 40, 50, 60 and 70 min, respectively. Fig. 3 showed that the extraction efficiencies of the 14 compounds were improved as the extraction time increased from 10 to 40 min. After 40 min, extraction efficiencies of the compounds kept constant or even showed a trend of decline. Hence, 40 min was the proper ultrasonic extraction time.

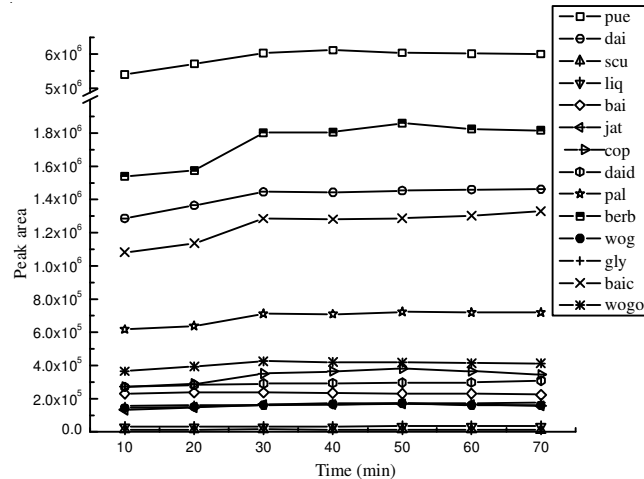


Fig. 3. Effect of ultrasonic extraction time on the extraction efficiency of the 14 compounds

Validation of method and quantification results: The regression equation, linear range, the limits of detection (LOD) and limit of quantification (LOQ) were evaluated. Working standard solutions containing each of the fourteen compounds were prepared by diluting the standard stock solutions with methanol to six different concentrations. The regression equation was obtained based on regression analysis of peak area (Y) versus concentration (X, $\mu\text{g mL}^{-1}$). The results are shown in Table-1.

The limits of detection and quantification were calculated based on signal-to-noise ratio of 3 and 10, respectively. The results are also listed in Table-1.

Precision and reproducibility test: The intraday precision of the method was determined by injecting the standard solution six times in one day. The interday precision of the method was investigated by injecting the standard solution

Compound	Sample1 (mg g ⁻¹)	Sample2 (mg g ⁻¹)	Sample3 (mg g ⁻¹)	Average recovery (%)	RSD (%)
Puerarin	38.39	33.10	36.52	97	2.02
Daidzin	2.70	7.01	6.44	95	0.99
Scutellarin	-	0.25	0.71	98	2.02
Liquiritin	0.073	0.40	0.44	100	1.45
Baicalin	8.70	2.19	2.80	95	0.68
Jatrorrhizine	0.61	0.71	0.47	97	2.04
Coptisine	2.55	14.64	0.82	93	2.36
Daidzein	2.51	0.87	2.12	101	2.03
Palmatine	2.15	2.84	1.81	92	4.19
Berberine	8.50	7.92	7.27	95	2.22
Wogonoside	2.05	0.70	0.56	99	2.17
Glycyrrhizic acid	0.28	3.97	1.10	94	2.40
Baicalein	0.51	3.76	1.18	97	2.04
Wogonin	0.25	1.30	0.21	94	1.99

once in a day for 5 consecutive days. The RSD values for intra-day precision (n = 6) and interday precision (n = 5) were less than 2.04 % and 2.59 %, respectively.

Reproducibility test was carried out by analyzing five replicate samples and RSD values for retention time and peak area were less than 0.55 and 2.70 %, respectively.

Sample analysis and recovery test: The proposed method was applied to the determination of 14 compounds in Gegen Qinlian tablets from 3 different manufacturers. Sample 1: Bai Autonomous Prefecture Chinese Medicine Pharmaceutical Co., Ltd; Sample 2: Taiji Group Pharmaceutical Co., Ltd; Sample 3: Lijun Modern Chinese Medicine Co., Ltd. The analytical results are shown in Table-2.

For the recovery test, 160 mg puerarin, 24 mg daidzin, 15 mg scutellarin, 9 mg liquiritin, 24 mg baicalin, 12 mg jatrorrhizine, 9 mg coptisine, 12 mg daidzein, 12 mg palmatine, 24 mg berberine, 9 mg wogonoside, 12 mg glycyrrhizic acid, 15 mg baicalein and 6 mg wogonin were spiked into 0.1 g sample and processed. The average recoveries are also shown in Table-2.

Conclusion

The experimental results indicate that the proposed method of HPLC-UV with gradient elution and dual wavelength

Compound	Linear regression equation	R	Linear range ($\mu\text{g mL}^{-1}$)	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)
Puerarin	$Y = 19434.45X - 57429.36$	0.9997	5-400	0.043	0.14
Daidzin	$Y = 21212.86X + 8759.72$	0.9999	0.8-80	0.071	0.24
Scutellarin	$Y = 4927.77X + 613.52$	0.9999	0.5-50	0.13	0.43
Liquiritin	$Y = 10004.00X + 2450.83$	0.9997	0.3-30	0.071	0.24
Baicalin	$Y = 19210.46X + 25994.37$	0.9994	0.8-120	0.10	0.35
Jatrorrhizine	$Y = 21820.07X + 434.29$	0.9999	0.4-40	0.10	0.35
Coptisine	$Y = 18679.27X + 1334.29$	0.9997	0.3-30	0.061	0.20
Daidzein	$Y = 32611.76X + 12249.40$	0.9996	0.4-40	0.030	0.10
Palmatine	$Y = 22538.38X + 4553.77$	0.9996	0.4-40	0.060	0.20
Berberine	$Y = 21298.30X + 1064.41$	0.9996	0.8-120	0.026	0.088
Wogonoside	$Y = 22473.93X + 5964.07$	0.9996	0.3-30	0.072	0.24
Glycyrrhizic acid	$Y = 5117.95X - 735.84$	0.9998	0.4-60	0.044	0.15
baicalein	$Y = 30588.15X + 8133.76$	0.9992	0.5-50	0.032	0.11
wogonin	$Y = 34820.74X + 9512.25$	0.9997	0.2-20	0.021	0.070

detection is efficient for the analysis of the fourteen compounds simultaneously in Gegen Qinlian tablet. Ethanol-water was chosen as extractant and the fourteen compounds were detected within 46 min. So this method is effective, simple, environmentally friendly and being well suitable for the quality evaluation of Gegen Qinlian tablet.

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