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Original Paper

Determination of the Content of Hairy Vetch Isoflavone Glucoside in Qianliekang Tablets

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Abstract

Objective: To determine the content of maohui isoflavone glycoside based on its composition in Qianliekang tablets.

Methods: High-Performance Liquid Chromatography (HPLC) was employed for the determination, with a detection wavelength set at 260 nm; column temperature maintained at 25℃; and mobile phase consisting of acetonitrile-0.1% formic acid solution (14:86). The flow rate was standardized to 1.0 ml/minute.

Results: A good linear relationship between sample content and peak area was observed within the range of 0.051 μg to 0.816 μg for maohui isoflavone glycoside, with a relative standard deviation (RSD) of repeatability reaching 1.47%. The average recovery rate was found to be 98.21%, with an RSD of 2.31%.

Conclusion: This method demonstrates excellent stability, high accuracy, precision, and reproducibility, making it suitable for quality control of Qianliekang tablets.

Keywords

High-Performance Liquid Chromatography, Maohui Isoflavone Glycoside, Content Determination

1. Introduction

The Qianliekang tablets are primarily aimed at treating the syndrome of damp-heat and blood stasis (Zhou, 2 020). This formulation is a pure traditional Chinese medicine compound composed mainly of Huangbai (Phellodendron), Huangqi (Astragalus), and Kushen (Sophora flavescens). It possesses the functions of clearing heat, transforming dampness, promoting blood circulation, and unblocking meridians. Clinical applications have reached over two thousand cases with an efficacy rate exceeding 90%. In this formula, Huangbai serves as the principal ingredient, complemented by Kushen, Huangqi,

Niuxi (Achyranthes bidentata), among others. The combination aims to clear pathogenic factors while reinforcing the body's vital energy. Huangqi is a commonly used traditional Chinese medicine in clinical practice with extensive effects and applications. This article provides an overview of its advancements in both traditional Chinese medicine theory and modern medical science. Morus alba flavonoid glycosides are natural plant compounds found in various plants such as safflower and honeysuckle. In medicine, Morus alba flavonoid glycosides have been widely applied for treating multiple diseases and have garnered significant attention from researchers. Firstly, regarding pharmacological effects, Morus alba flavonoid glycosides exhibit antioxidant, antibacterial, and anti-inflammatory properties. Their primary components—flavonoids—can protect cells from damage by scavenging free radicals while also modulating immune system functions to enhance the body’s adaptability to external environments. Additionally, these compounds contribute to lowering blood pressure and improving cardiovascular health. Secondly, Morus alba flavonoid glycosides are utilized medically for addressing specific human health issues. For instance, in gynecology they can alleviate symptoms associated with menopause syndrome or irregular menstruation; whereas in urology they find application in treating benign prostatic hyperplasia or chronic prostatitis problems among men. Furthermore, within dermatology they may be employed to relieve allergic dermatitis or eczema.

This study focuses on Qianliekang tablets as its research subject aiming to establish accurate reliable quality content determination methods for their constituents. Preliminary experiments were conducted to formulate a method for measuring the content of Morus alba flavonoid glycosides within Huangqi according to detection standards outlined in the 2020 edition of "Pharmacopoeia of China." This research specifically investigates aspects such as stability accuracy precision repeatability concerning their measurement providing theoretical support for further development efforts. The research on the quality standards of Qianliekang tablets has been extensively reported in both domestic and international literature. Studies (Li, Li, Guo, et al., 2011; Zhou, Peng, Liang et al., 2018; Forrest, Nickel, & Moldwin, 2007) regarding the quality standards of Qianliekang tablets primarily involve conventional testing methods, supplemented by qualitative identification through thin-layer chromatography, as well as quantitative analysis using liquid chromatography and gas chromatography. These analyses typically focus on the main active ingredients or other components with higher concentrations present in the formulation. For varieties included in pharmacopoeias, adherence to pharmacopoeial regulations is more common. Moreover, high-performance liquid chromatography (HPLC) is frequently employed in both domestic and international studies; certain components are analyzed using gas chromatography based on their specific characteristics (Gou, Geng, Zhong, Wei, Liu, Deng, Li, Yuan, Wang, & Guo, 2021). Currently, various treatment options for prostatitis are available clinically, including commonly used medications, physical therapies (such as extracorporeal shock wave therapy and infrared treatment), biofeedback techniques, psychological counseling, and traditional Chinese medicine. Non-steroidal anti-inflammatory drugs (NSAIDs) and herbal preparations are among the most commonly utilized medications. In cases where patients experience anxiety or depression-related issues, oral anxiolytics or

antidepressants may be considered (Huo, Zhang, Liu et al., 2010; Li, 2011; Shi, Jia, Li et al., 2022). It is crucial to note that these medications can have serious adverse effects; therefore, careful attention must be paid to dosage and contraindications. At present, the efficacy of Western medicines remains limited—particularly when used alone—for recurrent prostatitis cases. Consequently, Qianliekang tablets have emerged as an effective therapeutic option for chronic prostatitis.

2. Chromatographic Conditions Investigation

2.1 Investigation of HPLC Conditions for the Determination of Genistin Glucoside Content

① Preparation of Standard Solution: Accurately weigh 1.3 mg of Maorui isoflavone glycoside standard using an analytical balance, and dissolve it in 100 ml of 70% methanol to prepare a solution with a concentration of 0.013 mg/ml. ② Preparation of Sample Solution: Grind the pilot test sample finely using a mortar and pestle, then accurately weigh 0.12 g and dissolve it in 100 ml of 70% methanol solution, followed by ultrasonic treatment for 30 minutes (ultrasonic conditions set at a frequency of 40 kHz and power output at 250 W) to obtain the sample solution. ③ Measurement: According to experimental requirements, transfer the prepared standard and sample solutions into quartz cuvettes for absorbance detection, with the reference solution being 70% methanol; measurements are conducted within a wavelength range from 190 nm to 400 nm, plotting absorption curves based on results obtained from both standard and sample solutions (Figure 1). The results indicate that, as shown in the figure above, the standard solution of Maorui Isoflavone Glucoside exhibits maximum absorption at 206 nm. The sample solution demonstrates significant absorption near 206.8 nm. In accordance with the relevant content under Astragalus in the current edition of the Pharmacopoeia, we have established a detection wavelength of 206 nm (Liu, Yu, Zhang, Chen, Cheng, Song, & Liu, 2018) (Table 1).

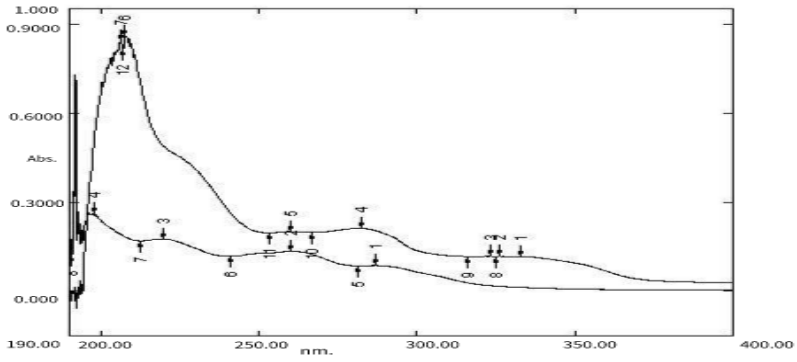

Figure 1. Maximum Absorption Wavelength Scan of Genistin Glucoside

Table 1. Wavelength Data

Standard substance No.	Wavelength nm.	Abs.	Sample No.	Wavelength nm.	Abs.
1	286.6	0.177	1	332.8	0.151
2	260	0.113	2	326	0.153
3	219.4	0.186	3	323	0.154
4	206	0.289	4	282.2	0.207
5	281	0.184	5	260.2	0.263
6	240.8	0.191	6	207.6	0.817
7	206.8	0.265	7	206	0.872
			8	325.2	0.151
			9	315.8	0.151
			10	266.8	0.259
			11	253.2	0.256
			12	206.8	0.723

2.2 Selection of Chromatographic Conditions for the Determination of Genistin Glucoside Content

① Preparation of Standard Solution: Accurately weigh 5.2 mg of maorui isoflavone glycoside reference substance, dissolve it in methanol to a final volume of 100 ml in a volumetric flask, resulting in a concentration of 0.052 mg/ml, which will serve as the standard solution. ② Preparation of Sample Solution: The study utilizes pilot samples from Prostaglandin tablets for analysis. First, grind the sample using a mortar and pestle until fine; then accurately weigh 1.52 g of the sample and place it into a 100 ml conical flask. Gradually add 50 ml methanol as solvent to the flask and subject the mixture to ultrasonic treatment for 40 minutes (ultrasonic conditions: power at 250 W, frequency at 40 kHz). Afterward, allow the sample to equilibrate with room temperature before weighing again; use methanol solution to compensate for any weight loss due to evaporation or absorption during this process, mix thoroughly, filter through appropriate means, and accurately measure out 25 ml of filtrate. Evaporate this filtrate slowly on a water bath until dry; then re-dissolve in methanol and dilute to a final volume of 5 ml before filtering again to obtain the test solution. ③ Selection of Liquid Chromatography Conditions: a) Optimization of Mobile Phase: A systematic evaluation was conducted comparing four mobile phases—acetonitrile:0.2% formic acid (15:85), acetonitrile:0.2% formic acid (17:83), acetonitrile: 0.2% formic acid (14:86), and acetonitrile:0.1% formic acid (14:86)—leading us to select acetonitrile-0.1% formic acid solution (14:86) as our optimal mobile phase. b) Optimization of Chromatographic Column: Four different chromatographic columns were systematically evaluated; ultimately, we identified Agilent ZORBAX XB-C18 column with octadecylsilyl bonded silica gel packing material (5 μm; dimensions =4.6*250 mm) as our preferred choice. c) Optimization of Column Temperature and Flow

Rate: Through single-factor investigations involving varying temperatures and flow rates while assessing peak shapes along with system performance comprehensively led us to determine that an optimal column temperature should be set at 25 ℃ with a flow rate maintained at 1.0 ml/min.

2.3 Selection of Sample Processing Methods

① Optimization of Sample Extraction Methods: The pilot samples were finely ground using a mortar and pestle, followed by extraction using methanol via Soxhlet extraction, 60% methanol ultrasonic extraction (30 min), and methanol heating reflux extraction (60 min). Samples and standard solutions were prepared according to the aforementioned methods for content determination. The results indicated that the methanol reflux method resulted in complex extracts, leading to interference from other components with the target component (maohui isoflavone glucoside). Both Soxhlet extraction and 60% methanol ultrasonic extraction yielded lower recovery rates for the target component compared to the methanol ultrasonic method, which provided the highest concentration of maohui isoflavone glucoside. As maohui isoflavone glucoside is identified as the primary active ingredient in pilot samples, higher extract concentrations are preferable; thus, the optimal detection method for maohui isoflavone glucoside was determined to be methanol ultrasonic extraction. ② Selection of Extraction Time: Following optimization of the above-mentioned extraction methods, it was necessary to optimize the extraction time. Using these optimized methods, extractions were performed at intervals of 20 min, 30 min, and 40 min respectively; sample solutions were prepared as per section 2.2.1.1.2 guidelines before being injected into a liquid chromatograph for measurement and calculation. The results showed that samples extracted for both 30 minutes and 40 minutes exhibited similar contents of maohui isoflavone glucoside (mg/g). From an efficiency perspective regarding extract yield, a shorter duration was preferred; therefore, an optimal time of 30 minutes was ultimately established. ③ Selection of Material-to-Solvent Ratio: The pilot samples were finely ground using a mortar and pestle with three different material-to-solvent ratios set at: 1.5 g/75 ml, 1.5 g/50 ml, and 1.5 g/25 ml while employing optimized methanol ultrasonic extraction over a minimum duration of 30 minutes following previously described procedures for preparing sample solutions used in measuring maohui isoflavone glucoside content. Results indicated that samples prepared with material-to-solvent ratios of both 1.5 g/50 ml and 1.5 g/75 ml yielded comparable measurements for maohui isoflavone glucoside content; hence considering raw material conservation aspects led us to finalize on a ratio setting of 1.5 g/50 ml (National Pharmacopoeia Commission, 2020).

3. Method

3.1 Blank Experiment

Following the relevant procedures, prepare standard solution, sample solution, negative sample solution, and reagent solution. The formulation samples that do not contain Astragalus are prepared as negative sample solutions according to the aforementioned method, which serves as the blank solution. Methanol is used as the reagent solution (Li, Li, Guo, et al., 2011; Liu, Yu, Zhang, Chen, Cheng, Song, & Liu,

2018). A precise volume of 5μl from each of the four types of solutions is taken for analysis testing. The results indicate that the corresponding standard peaks are present in the sample solution at specific retention times, while no standard peaks are observed in the blank solution. Furthermore, the solvent does not interfere with the standard peaks. Therefore, this method is feasible and possesses specificity, making it suitable for inclusion as a content determination standard (Figure 2).

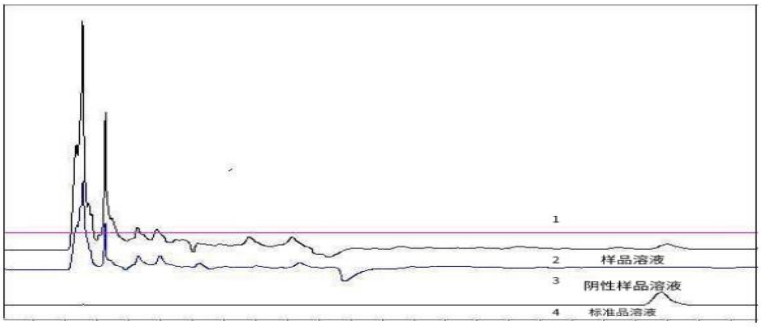

Figure 2. Doverlay of Daidzein Glucoside

3.2 Linear Investigation Experiment

Preparation of Standard Solution: A reference standard of 1.05 mg of Maohui Yihuangton Glucoside was dissolved in methanol to create a solution with a concentration of 0.105 mg/ml. Subsequently, sample solutions at the same concentration (0.105 mg/ml) were prepared. Volumes of 2 μl, 4 μl, 6 μl, and 7 μl were injected into the high-performance liquid chromatography (HPLC) system using the aforementioned detection method for analysis, and experimental results were recorded as shown in Figure 2.3 and Table 2.2. The results indicate that the linear equation obtained is Y = 2250.8X - 56.956, with R^2 = 0.9998 and R = 0.999. The injection amounts of the standard within the range of 0.052 μg to 0.817 μg exhibited a positive correlation with peak area, demonstrating a strong linear relationship in the results (Figure 3).

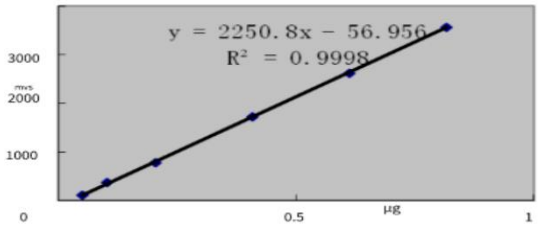

Figure 3. Standard Curve of Genistin Glucoside

Table 2. Linear Test Results of Genistin Glucoside

Sample volume (μg)	0.052	0.103	0.203	0.407	0.614	0.817
Peak area (mVs)	57.25	184.85	389.35	863.3	1313.46	1785.54

3.3 Stability Test

The test sample solution was analyzed at time intervals of 0 h, 2 h, 4 h, 6 h, 8 h, and 10 h to determine its content (Shi, Bao, Jiang et al., 2007). The results indicated that the relative standard deviation (RSD) was measured at 1.64%, which is below the threshold of 2%. This finding suggests that there are significant changes in the stability of the sample solution within the time frame of 0 to 10 hours, highlighting its importance (Table 3).

Table 3. Results of Stability Test for Genistin Glucoside

Place time (h)	0	2	4	6	8	10	RSD (%)
Content of Genistin (mg/g)	0.1408	0.1457	0.1448	0.1405	0.1453	0.1420	1.64

3.4 Recovery Rate Experiment

In samples with known content, a fixed amount of the reference compound, puerarin glycoside, was added to calculate its recovery rate (Huo, Zhang, Liu et al., 2010). The experimental findings indicate that the average recovery rate is 98.21%, with an RSD value of 2.31%, which is below the acceptable threshold of 3%. This demonstrates that the method employed for content determination is valid and reliable. Further quantification will be conducted based on the calculated recovery rates.

3.5 Reproducibility Test

The test samples were subjected to the aforementioned method, with six repetitions of sample application conducted. The variations in the content of hyperoside glucosides for each trial were compared to determine the relative standard deviation (RSD) as a measure of result reproducibility (Shi, Bao, Jiang et al., 2007). The results indicated that the RSD was 1.47%, which is less than 3%, demonstrating good reproducibility of the sample solution (Table 4).

Table 4. Results of the Reproducibility Test for Genistin Glucoside

Number of trials	1	2	3	4	5	6	RSD (%)
Content of Genistin (Genistein-7-O-glucoside)(mg/g)	0.1472	0.1519	0.1499	0.1455	0.1486	0.1493	1.47

3.6 Intermediate Precision Test

Samples from the same batch were analyzed using different testing instruments and varying durations of operation, with different operators performing the sample injections and result measurements to assess the precision of the instruments. Utilizing Shimadzu and Agilent 1100 liquid chromatography systems, we followed the aforementioned experimental protocol to continuously inject the test sample solution six times, measuring peak area RSD (Relative Standard Deviation) to reflect the repeatability of the results

(Ni & Wang, 2022). The results indicated RSD values of 1.55% and 1.76%, demonstrating that the intermediate precision is satisfactory (Table 5).

Table 5. Precision Test Results for Genistein Glucoside

Instrument	Sample content(mg)						RSD (%)
Agilent 1100	0.1479	0.1435	0.1448	0.1411	0.1455	0.1447	1.55
	0.1431	0.1401	0.1465	0.1408	0.1403	0.1407	1.76

4. Result and Discussion

4.1 Exploration of Liquid Phase Conditions for Content Determination

Based on the requirements outlined in the 2020 edition of the "Chinese Pharmacopoeia" and experimental conditions reported in published literature, a detailed analysis was conducted on the research subject, Prostatitis Tablets (Li, Yi, & Liu, 2022; National Pharmacopoeia Commission, 2020). Through careful selection of mobile phase components, flow rate, and column type, optimal chromatographic conditions were established. Additionally, considerations regarding extraction methods, solvent choice and volume, extraction time, and stability assessment of the test solution led to the determination of an ideal sample processing method.

4.2 Methodological Investigation

This experiment involved conducting blank tests with formulations containing Astragalus membranaceus compared to those without it to identify differences in standard peak responses; this ensured reliability in content measurement. A linearity study demonstrated a positive correlation between injection volume of standard solutions and peak area. Stability experiments identified significant variations over specific time intervals; further accuracy assessments calculated recovery rates to establish acceptable standards for content determination methods (Cheng, Xiahou, Deng et al., 2010; Liang, Zhao, Tu et al., 2010). Furthermore, specificity testing was performed on Astragalus membranaceus within traditional Chinese medicine contexts. The investigation into linear relationships confirmed that all methodological criteria for measuring the content of calycosin-7-O-glucoside were met satisfactorily. It was concluded that within a range from 0.052 μg to 0.817 μg, there exists a strong linear relationship between sample concentration and peak area; repeatability yielded an RSD (Relative Standard Deviation) of 1.47%, while average recovery rates reached 98.21% with an RSD of 2.31%. Employing high-performance liquid chromatography (HPLC), this method proved rapid yet straightforward while yielding reliable results for quantifying calycosin-7-O-glucoside in Prostatitis Tablets (Li, 2011; Shi, Jia, Li et al., 2022). The findings from this study are expected to advance understanding related to chronic prostatitis issues further; thus laying a theoretical foundation for future clinical applications concerning its treatment mechanisms.

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Institutional Review Board Statement: All data are publicly available GWAS datasets, therefore no additional ethical approval was required.

Conflicts of Interest: The authors declare no conflicts of interest.

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