QUANTITATIVE ANALYSIS OF ACTEOSIDE AND JIONOSIDE B1 IN THE TUBEROUS ROOTS OF *Rehmannia glutinosa* BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

This study reports the quantitative determination of two phenylpropanoid glycosides, Acteoside and Jionoside B1, in the tuberous roots of *Rehmannia glutinosa* using high-performance liquid chromatography (HPLC). The analysis was carried out on an Agilent 1260 Infinity II system equipped with a Hydrosphere C18 column and a diode-array detector (DAD) at 198 nm. Chromatographic conditions were optimized to ensure effective separation and accurate quantification of the target compounds. Calibration curves exhibited good linearity over the concentration range of 100–5000 µg/mL with correlation coefficients (R^2) above 0.996. The validated method showed satisfactory precision, accuracy, and sensitivity in accordance with AOAC guidelines. The contents of Acteoside and Jionoside B1 in the total methanolic extract were 2.12% and 2.39%, respectively, corresponding to 0.59% and 0.66% in the dried tuberous roots of *Rehmannia glutinosa*. These results suggest that both compounds may serve as suitable chemical markers for the quality control and standardization of *Rehmannia glutinosa* and related medicinal products.

PHÂN TÍCH ĐỊNH LƯỢNG HỢP CHẤT ACTEOSIDE VÀ JIONOSIDE B1 TRONG RỄ CỦ LOÀI ĐỊA HOÀNG *Rehmannia glutinosa* BẰNG PHƯƠNG PHÁP SẮC KÝ LỎNG HIỆU NĂNG CAO

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TỪ KHÓA

Acteoside
Jionoside B1
Rehmannia glutinosa
Phân tích định lượng
HPLC

TÓM TẮT

Nghiên cứu này trình bày kết quả định lượng hai hợp chất phenylpropanoid glycoside là Acteoside và Jionoside B1 trong rễ củ loài Địa hoàng (*Rehmannia glutinosa*) bằng phương pháp sắc ký lỏng hiệu năng cao (HPLC). Phân tích được thực hiện trên hệ thống Agilent 1260 Infinity II sử dụng cột Hydrosphere C18 và detector mảng diode (DAD) tại bước sóng 198 nm. Các điều kiện sắc ký được tối ưu nhằm đảm bảo khả năng tách và định lượng chính xác các hợp chất mục tiêu. Đường chuẩn cho thấy tính tuyến tính tốt trong khoảng nồng độ 100–5000 µg/mL với hệ số tương quan (R^2) lớn hơn 0,996. Phương pháp đạt độ chính xác, độ đúng và độ nhạy phù hợp theo hướng dẫn AOAC. Hàm lượng Acteoside và Jionoside B1 trong cao chiết methanol tổng lần lượt là 2,12% và 2,39%, tương ứng với 0,59% và 0,66% trong mẫu rễ củ khô của *Rehmannia glutinosa*. Kết quả cho thấy hai hợp chất này có thể được sử dụng làm chất đánh dấu hóa học trong kiểm soát và chuẩn hóa chất lượng dược liệu Địa hoàng và các sản phẩm liên quan.

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1. Introduction

The tuberous roots of *Rehmannia glutinosa* have been widely used in traditional medicine for the treatment of diabetes, anemia, hemoptysis, cancer, and gynecological disorders [1]-[3]. Our previous scientific publications reported that the principal chemical constituents of *Rehmannia glutinosa* are iridoid glycosides and phenylpropanoid glycosides, among which the phenylpropanoid glycosides exhibit significantly stronger α-glucosidase inhibitory activity than the iridoid glycosides [4]-[7].

Our further phytochemical investigations revealed that two phenylpropanoid glycosides, Acteoside and Jionoside B1, are present in higher abundance compared to other constituents [6]. Therefore, this article aims to present the quantitative analysis of these two compounds in the tuberous roots of *Rehmannia glutinosa* using high-performance liquid chromatography (HPLC).

2. Materials and Methods

The tuberous roots of *Rehmannia glutinosa* were collected in Viet Tri, Phu Tho Province, Vietnam, in March 2020 and authenticated by Dr. Nguyen The Cuong (Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology). The plant material was cleaned, air-dried, and ground into powder (RG). The fresh and dried tuberous-root samples of *Rehmannia glutinosa* are presented in Figure 1.

Figure 1. *Fresh and dried tuberous root samples of Rehmannia glutinosa*

The powdered sample of 50.0 g was ultrasonically extracted three times (300 mL each extraction, sonicated for 30 minutes at room temperature). The methanolic extract was concentrated under reduced pressure to obtain 13.815 g of methanolic residue (RGM).

The RGM was subsequently dissolved in methanol to prepare the sample solution at a concentration of 30 mg/mL (C_B).

The pure compounds, Acteoside and Jionoside B1, previously isolated from the tuberous roots of *Rehmannia glutinosa*, were used as reference standard compounds [6]. Stock solutions of each compound were prepared at a concentration of 5 mg/mL. From these stock solutions, accurately measured volumes were diluted with methanol to obtain a series of solutions with decreasing concentrations. The chemical structures of Acteoside and Jionoside B1 are presented in Figure 2.

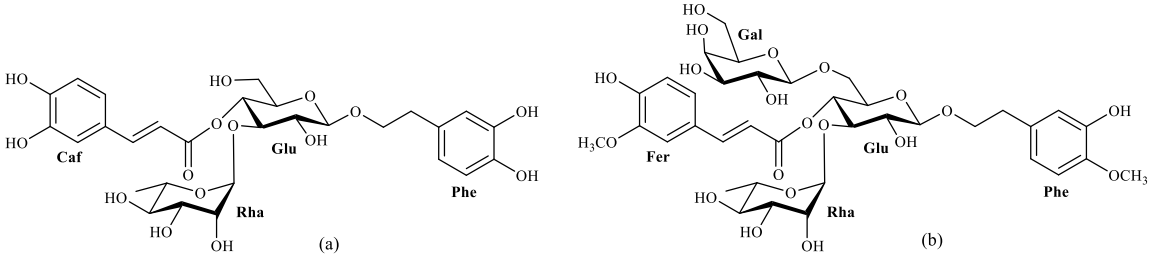


Figure 2. *Chemical structures of Acteoside (a) and Jionoside B1 (b)*

HPLC-grade solvents (Merck), including methanol (CH_3OH), acetonitrile (CH_3CN, ACN), and acetic acid, were used. Deionized water was obtained directly from a Milli-Q water purification system (Merck).

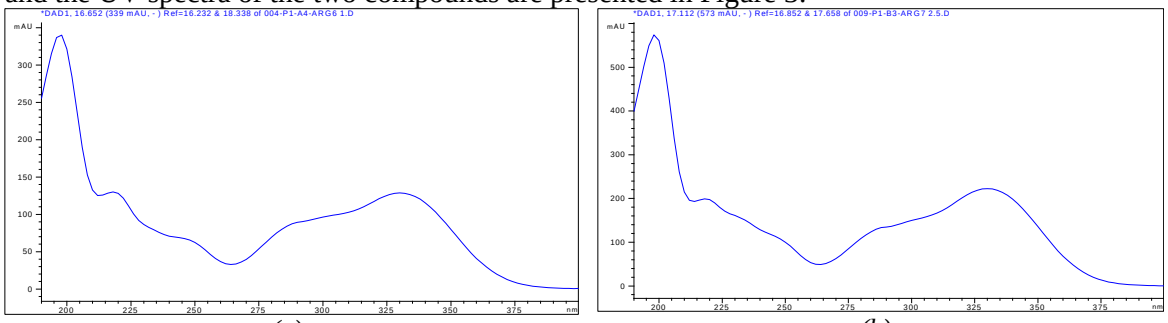
The amount of the target compounds in the samples was quantified using high-performance liquid chromatography (HPLC). The analysis was performed on an Agilent 1260 Infinity II system equipped with a binary solvent pump, an autosampler, a column oven, and a diode-array detector (DAD), using a Hydrosphere C18 analytical column (150 × 4.6 mm, 5 µm) at the Institute of Chemistry, Vietnam Academy of Science and Technology.

3. Results and Discussion

3.1. Optimization of HPLC analytical conditions

3.1.1. Selection of the optimal wavelength

The selected wavelength corresponded to the maximum absorption wavelength (λ_{max}) of the two compounds, which was determined by analyzing each reference standard individually under chromatographic conditions. The DAD detection wavelength was investigated experimentally, and the UV spectra of the two compounds are presented in Figure 3.



(a) *(b)*

Figure 3. *UV spectra of Acteoside (a) and Jionoside B1 (b)*

The experimental results obtained from the two reference standards indicated that both compounds exhibited maximum absorption at λ_{max} = 198 nm. Therefore, a wavelength of 198 nm was selected for the quantitative determination of Acteoside and Jionoside B1 in the tuberous root samples of *Rehmannia glutinosa.*

3.1.2. Selection of injection volume, flow rate, and mobile phase composition

Experimental evaluation of the injection volume indicated that an injection volume of 5 µL provided symmetrical peaks. Therefore, 5 µL was selected as the optimal injection volume for the chromatographic analysis.

After investigating several solvent systems, ACN–water was selected as the mobile phase. The suitable chromatographic conditions were established as follows:

- Chromatographic column: Hydrosphere C18 (150 × 4.6 mm, 5 µm)
- Flow rate: 1 mL/min
- Injection volume: 5 µL
- Mobile phase: ACN–water with gradient elution as follows in Table 1.

Table 1. *ACN–water mobile phase with gradient elution*

Time (min)	% ACN (0.1% acetic acid)	% H_2O (0.1% acetic acid)
0	15	85
10	15	85
35	100	0

3.1.3. Determination of the retention times of the two standard compounds

Each reference standard solution (at a concentration of 5 mg/mL) was individually injected and analyzed under the established chromatographic conditions. Under the selected conditions, the obtained chromatograms showed well-resolved peaks with good symmetry and stable baselines. The retention times of the two standard compounds are shown in Figure 4.

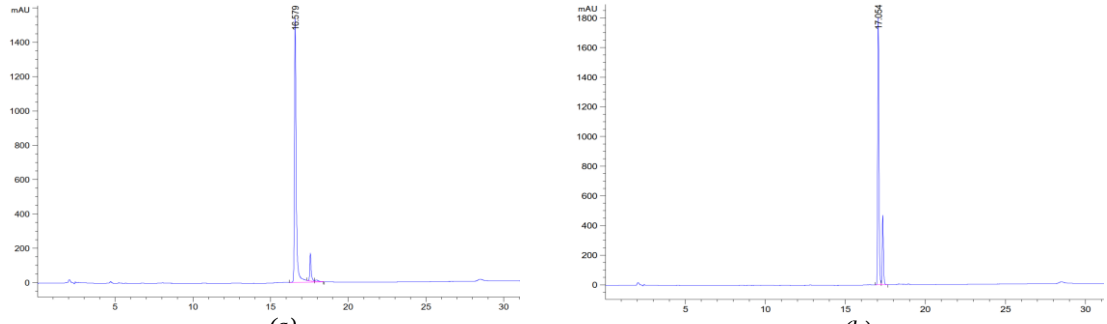


Figure 4. *Retention times of Acteoside (a) and Jionoside B1 (b)*

Each reference compound produced a single, symmetrical chromatographic peak. Both compounds exhibited distinct and stable retention times under the same chromatographic conditions. Thus, the selected analytical conditions allowed effective simultaneous separation of both compounds in the analytical sample. The retention times of the two reference compounds are presented in Table 2.

Table 2. *Retention times of the two reference compounds*

Compound	Retention time (min)
Acteoside	16.5 ÷ 16.6
Jionoside B1	17.0 ÷ 17.1

3.2. Construction of calibration curves and validation

From the 5 mg/mL stock solutions, accurately measured volumes were diluted with methanol to prepare calibration solutions, including 5000 µg/mL, 2500 µg/mL, 1000 µg/mL, 500 µg/mL, and 100 µg/mL for both Acteoside and Jionoside B1.

The HPLC analysis was performed under the optimized chromatographic conditions. The analytes signals were recorded at the previously determined maximum absorption wavelength (198 nm). Calibration curves were constructed by plotting peak area (Y) versus the corresponding concentration (X). The calibration curves for the two reference standards are presented in Figure 5.

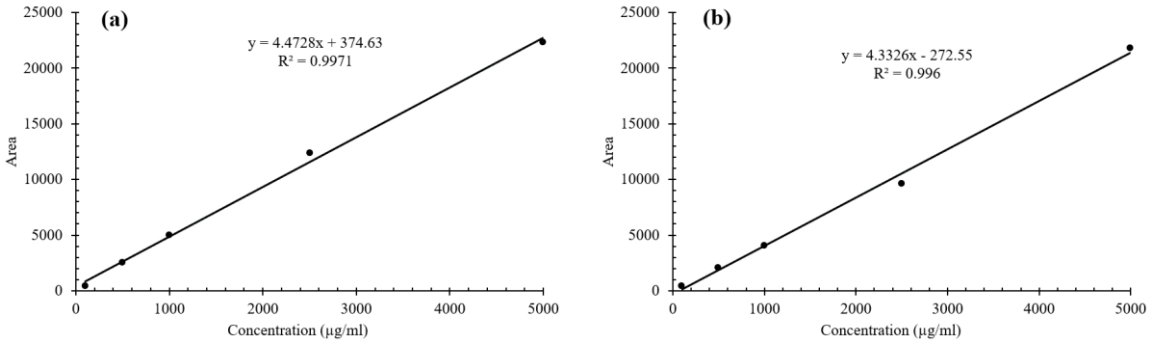


Figure 5. *Calibration curves of Acteoside (a) and Jionoside B1 (b) at 198 nm*

The calibration equations of the individual reference standards are summarized in Table 3.

Table 3. *Linear regression data, LOD and LOQ of Acteoside and Jionoside B1*

No.	Analytes	Regression equation (n = 3)	Test range (µg/mL)	R^2	LOD (µg/mL)	LOQ (µg/mL)
1	Acteoside	Y = 4.4728X + 374.6	100-5000	0.9971	5.44	16.48
2	Jionoside B1	Y = 4.3326X – 272.55	100-5000	0.9960	4.52	13.71

According to the guidelines of the Association of Official Analytical Chemists (AOAC) [8], calibration curves with correlation coefficients within $0.995 < R^2 < 1$ meet acceptable standards.

Therefore, both calibration curves demonstrated high linearity. The limits of detection (LOD) and quantification (LOQ) values were determined at a signal-to-noise ratio of 3.3 (LOD) and 10 (LOQ). Under the present conditions, the LOD and LOQ of Acteoside were 5.44 µg/mL and 16.48 µg/mL; and those of Jionoside B1 were 4.52 µg/mL and 13.71 µg/mL, respectively.

Furthermore, the calibration ranges were relatively wide (100–5000 µg/mL), indicating that the method provides reliable quantification and is suitable for the determination of the target compounds in *Rehmannia glutinosa* tuberous root samples.

The repeatability of the method was evaluated by assaying six replicate injections, three times for intra-day (0 h, 3 h, 6 h) and three for inter-day (1st, 2nd, and 3rd day) of each analyte at the same concentration (1000 µg/mL), under the same experimental conditions. Variations of peak areas were monitored as relative standard deviations (RSD). The results showed that reproducibility (RSD values) for Acteoside were 0.64% (intra-day) and 1.47% (inter-day); and reproducibility for Jionoside B1 were 0.37% (intra-day) and 0.91% (inter-day). A recovery test was used to evaluate the accuracy of the analysis. The methanolic extract solution of *R. glutinosa* (10,000 µg/mL) was spiked with known amounts of Acteoside and Jionoside B1. The mixtures were analyzed under the above-established method. For comparison, a vehicle sample (spiked with methanol) was prepared and analyzed. The percentage recoveries were evaluated by calculating the ratio of the detected amount to the added amount. As shown in Table 4, the recovery rates were in the range 97.72%–101.48% for Acteoside and 98.01–101.87% for Jionoside B1, and their RSD values were both less than 2% (1.93% and 1.97% respectively). The RSD values in both repeatability and recovery test were in the acceptable range of the AOAC guidelines.

Table 4. *Recoveries of Acteoside and Jionoside B1*

No.	Analytes	Spiked (µg)	Found (µg)	Recovery (%)	Mean (%)	RSD (%)
1	Acteoside	500	488.61	97.72	99.38	1.93
		1000	989.35	98.94		
		2500	2537.11	101.48		
2	Jionoside B1	500	490.04	98.01	100.18	1.97
		1000	1018.7	101.87		
		2500	2516.2	100.65		

3.3. Quantification of the two reference compounds in rehmannia glutinosa tuberous root samples

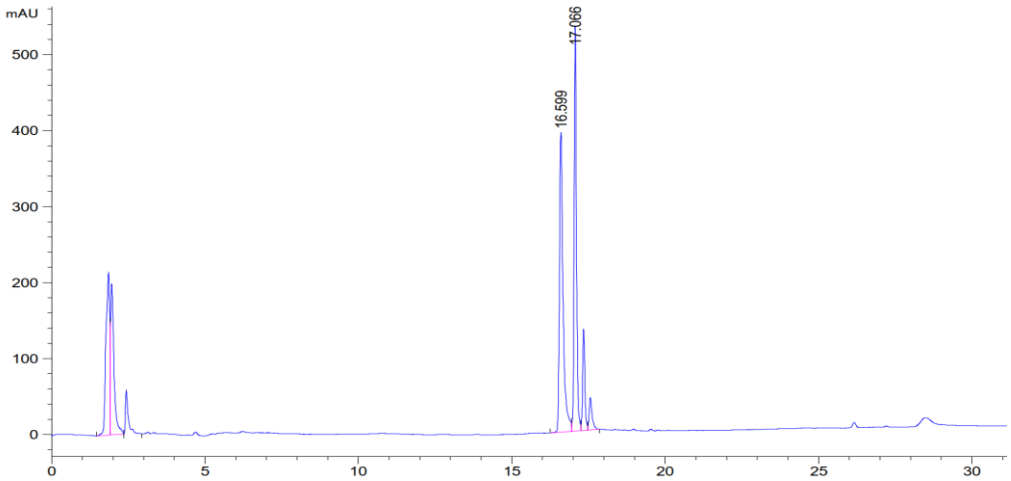


Figure 6. *HPLC chromatogram of methanolic extract (RGM) at 198 nm showing Acteoside (t_R = 16.6) and Jionoside B1 (t_R = 17.1)*

The RG powder (50 g) was ultrasonically extracted three times with methanol. The methanolic extract was concentrated under reduced pressure to obtain RGM (13.815 g),

corresponding to an extraction yield of 27.63%. The RGM residue was dissolved in methanol at 30,000 µg/mL and analyzed by HPLC. The HPLC chromatogram of the methanolic extract residue of the sample is presented in Figure 6.

The contents of the reference compounds were calculated according to Equation (1).

$$\%A = \frac{C_A}{C_B} \text{ x } C_{\%} \quad (1)$$

Where C_A is the concentration of the reference compound determined from the calibration curve (µg/mL), C_B is the concentration of the sample solution (30,000 µg/mL), $C\%$ is the percentage of methanolic extract in the dried *Rehmannia glutinosa* tuberous root sample.

Based on the calibration equations, the concentrations of the reference compounds were determined, and the data were processed according to equation (1). The resulting contents of the compounds are presented in Table 5.

Table 5. *Quantitative content of the two compounds in Rehmannia glutinosa samples*

Compound	Quantitative content (%)	
	Content in RGM (%)	Content in dried root (%)
Acteoside	2.12	0.59
Jionoside B1	2.39	0.66

HPLC quantitative analysis determined that the content of Acteoside was 2.12% and Jionoside B1 was 2.39% in the total methanolic extract (RGM), corresponding to 0.59% and 0.66%, respectively, in the dried *Rehmannia glutinosa* tuberous root powder (RG).

Several studies have reported HPLC-based quantification of Acteoside in *Rehmannia glutinosa*, particularly for quality control purposes. However, most of these studies focused on Acteoside as a single marker compound or in combination with other common glycosides [9]-[11].

The Acteoside content in the dried tuberous roots of *Rehmannia glutinosa* was 0.59% (w/w), which is markedly higher than previously reported levels in crude materials (0.07–0.11%) [1]. It is also comparable to the upper range reported under optimized conditions or specific tissues (~0.5–0.7%) [9]. This variation is likely due to differences in plant origin, harvesting conditions, and extraction procedures.

To the best of our knowledge, the quantitative analysis of Acteoside and Jionoside B1 in *Rehmannia glutinosa* has not been reported to date.

In a previous publication on phenylpropanoid glycosides, Acteoside and Jionoside B1 exhibited strong α-glucosidase inhibitory activity with IC_{50} values of 295.22 ± 13.72 µM and 408.74 ± 19.18 µM, respectively (compared to the positive control acarbose: 204.17 ± 19.90 µM) [6].

Several scientific studies have also reported α-glucosidase inhibitory activity of phenylpropanoid glycosides, indicating their potential for the development of antidiabetic therapeutic products [12]-[15].

4. Conclusion

This article presents a study on establishing quantitative analytical data for two compounds, Acteoside and Jionoside B1, in *Rehmannia glutinosa* samples using high-performance liquid chromatography (HPLC).

The obtained results showed that the contents of Acteoside and Jionoside B1 in the total methanolic extract were 2.12% and 2.39% at 198 nm, respectively, corresponding to 0.59% and 0.66% in the tuberous roots of *Rehmannia glutinosa*.

The study indicates that the two phenylpropanoid glycosides, Acteoside and Jionoside B1, are present in relatively high amounts and exhibit good α-glucosidase inhibitory activity, thereby demonstrating the potential application of *Rehmannia glutinosa* in the development of pharmaceutical products for the potential treatment of diabetes.

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