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Determination of Total Lignanoids in Tangjiangshenkang Granules by Two-Wavelength Ultraviolet Spectrophotometry

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Abstract [**Objectives**] To establish a determination method for the content of total lignanoids in Tangjiangshenkang granules. [**Methods**] Two-wavelength ultraviolet spectrophotometry (TWBS) was used to scan arctiin control solution, chlorogenic acid control solution and Tangjiangshenkang granule test solution in the range of 200 - 400 nm. In the ultraviolet scanning diagram of arctiin reference solution, the maximum absorption wavelength of 280 nm was determined as the determination wavelength λ_1, the detection wavelength in the ultraviolet scanning diagram of chlorogenic acid reference solution (λ_1 = 280 nm) was determined, and 350 nm was the reference wavelength λ_2; the content of total lignosides in Tangjiangshenkang granules was determined with arctiin as the reference substance. [**Results**] The precision, accuracy, and durability of this method were fine. The concentration of arctiin was linearly correlated with the absorbance difference in the range of 0.007 95 - 0.071 55 mg/mL (r = 0.999 9). The average recovery of arctiin was 100.8%, and the *RSD* value was 1.04% (n = 6). Calculated as arctiin, three batches of Tangjiangshenkang granules contain no less than 20% of total lignosides. [**Conclusions**] The method has the advantages of simple operation, good accuracy, precision and reliable stability. It can be used as the content determination and quality control method of total lignosides in Tangjiangshenkang granules.

Key words Tangjiangshenkang granule, *Fructus arctii*, Total lignanoids, Two-wavelength ultraviolet spectrophotometry

1 Introduction

Tangjiangshenkang granule is a traditional Chinese medicine preparation made from *Fructus arctii*, which is extracted and processed by modern technology. At present, it is the only drug with clear mechanism and composition in clinic to treat diabetes and nephropathy[1]. Its main active components are lignanoids in the extract of *F. arctii*[2]. Therefore, the content of total lignanoids in Tangjiangshenkang granules is an important quality index of this variety.

In the standard of Tangjiangshenkang granule and related literature[3-4], the content of total lignanoids was determined by ultraviolet spectrophotometry with arctiin as the control substance. However, according to the extraction process of *F. arctii*, Tangjiangshenkang granules not only contain lignanoids, but also contain a small quantity of organic acids including chlorogenic acid, cryptochlorogenic acid, caffeic acid, caffeoyl quinic acid and so on[5-7]. Under the detection wavelength 280 nm, the ultraviolet absorption intensity of organic acid is high, which has a great influence on the detection results. Therefore, the two-wavelength ultraviolet spectrophotometry was used to detect the content of total lignanoids in Tangjiangshenkang to eliminate the interference of organic acids at the determination wavelength and improve the accuracy of the determination of total lignanoids.

2 Instruments and materials

UV-2600 ultraviolet spectrophotometer (Shimadzu, Japan); KQ-500DV ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd., China); XS105DU electronic balance (1/100 000, Mettler Toledo); MS204S electronic balance (1/10 000, Mettler Toledo).

Tangjiangshenkang granule [Chongqing Kerui Pharmaceutical (Group) Co., Ltd., batch number: 2004L04979]; arctiin (National Institutes for Food and Drug Control, batch number: 110819-201611); chlorogenic acid (National Institutes for Food and Drug Control, 110753-201415); methanol, analytical purity.

3 Methods and results

3.1 Solution preparation

3.1.1 Reference solution. Preparation of chlorogenic acid reference solution: the chlorogenic acid reference substance was precisely weighed and dissolved with methanol to prepare a solution containing 8 μg per 1 mL. Preparation of arctiin reference solution: the reference substance of arctiin was precisely weighed and dissolved with methanol to prepare a solution containing 0.08 mg per 1 mL.

3.1.2 Sample solution. Appropriate amount of Tangjiangshenkang granule was taken and ground. 0.10 g of fine powder was weighed accurately, placed in a 25 mL volumetric flask, and treated by ultrasonic waves with proper amount of methanol for 30 min. After being taken out, it was placed at room temperature, methanol was added, shaken well, and filtered. 1 mL of filtrate was taken with precision, and put into the 25 mL volumetric flask, mixed with methanol, shaken well, and used as the test solution.

3.2 Determination of reference wavelength In the range of 200 - 400 nm, the reference solution of arctiin, the reference solution of chlorogenic acid and the sample solution of Tangjiangsh-

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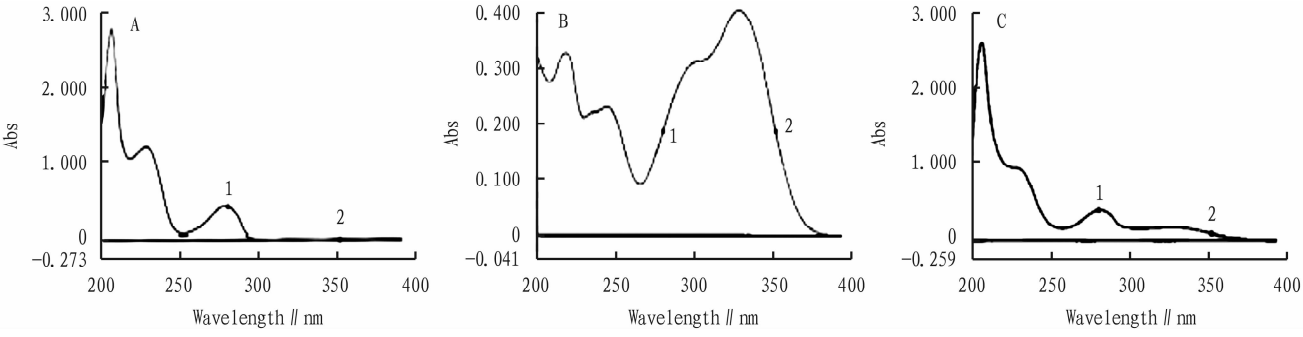
enkang granule were scanned. The results showed that arctiin had the maximum absorption around 280 nm, chlorogenic acid had the maximum absorption around 330 nm, and chlorogenic acid also had great absorption around 280 nm (Fig. 1). The two-wavelength ultraviolet spectrophotometry was used in the experiment.

In the UV scanning picture of chlorogenic acid reference solution, the equivalent absorption point wavelength of detection wavelength (λ_1 = 280 nm) near 350 nm was determined as the reference wavelength λ_2; then the absorbance of the sample solution at the detection wavelength λ_1 (280 nm) and the reference wavelength (λ_2) was determined, and the content of total lignanoids was calculated by the difference of absorbance, so as to eliminate the effect of organic acids on the determination of total lignanoids.



Note: A. arctiin; B. chlorogenic acid; C. Tangjiangshenkang granule.

Fig. 1 UV scanning image

3.3 Drawing of standard curve 1.0, 3.0, 5.0, 7.0 and 9.0 mL of arctiin reference solutions were measured in 10 mL flask, methanol was added, shaken evenly, and the absorbance was measured at the determination wavelength λ_1 (280 nm) and the reference wavelength λ_2 (351.4 nm). The standard curve was drawn and the regressive calculation was carried out with the absorbance difference ΔA ($\Delta A = A\lambda_1 - A\lambda_2$) as the ordinate and the concentration C as the abscissa. The linear regression equation was $\triangle A = 10.019C + 0.0086$. The concentration of arctiin was linearly correlated with $\triangle A$ in the range of 0.007 95 − 0.071 55 mg/mL, and the correlation coefficient r was 0.999 9.

3.4 Determination method A proper amount of the test solution was taken, and the absorbance at the determination wavelength λ_1 (280 nm) and reference wavelength λ_2 (351.4 nm) was determined by UV-vis spectrophotometry. The absorbance difference $\triangle A$ ($\triangle A = A\lambda_1 - A\lambda_2$) was substituted into the linear regression equation to calculate the content of arctiin, and the content of arctiin was taken as the content of total lignanoids.

3.5 Precision test 5.0 mL of arctiin reference solution was taken and placed in a 10 mL flask, methanol was added and shaken well. The absorbance at the determination wavelength λ_1 (280 nm) and the reference wavelength λ_2 (351.6 nm) was determined continuously for 6 times, and the absorbance difference ΔA ($\Delta A = A\lambda_1 - A\lambda_2$) and the *RSD* value of ΔA were calculated. The average value of absorbance difference ΔA for 6 times was 0.405 and the *RSD* (n = 6) was 0.19%.

3.6 Reproducibility test Tangjiangshenkang granule (batch No.: 2004L04979) was used to prepare the sample solution according to Section **3.1.2**, and 6 samples were prepared in parallel. The solution of each test sample was detected, and the content of total lignanoids was calculated, and the *RSD* value of the content was calculated according to the determination method in Section **3.4**. The average content of total lignanoids in Tangjiangshenkang granules was 17.97%, and the *RSD* value was 0.67%.

3.7 Sample recovery test About 50 mg of fine powder of Tangjiangshenkang granule (batch No.: 2004L04979) with known content of total lignanoids was taken, precisely weighed, placed in a 25 mL volumetric flask, and a certain amount of arctiin reference substance was precisely added (the ratio of the addition to the total content of lignanoids in the sample was about 1∶1). According to the preparation method in Section **3.1.2**, the sample solution was prepared, and 6 samples were prepared in parallel. The solution of each sample was detected according to the determination method in Section **3.4**, and the recovery rate was calculated. The results showed that the average recovery rate of total lignanoids in Tangjiangshenkang granules was 100.8% and the *RSD* (n = 6) was 1.04%.

The results are shown in Table 1.

Table 1 Recovery test results of Tangjiangshenkang granules

Sampling amount // mg	Addition amount // mg	Measured amount // mg	Content of total lignanoids in samples // mg	Recovery rate // %	Average // %	*RSD* // %
50.39	10.040 7	19.183 07	9.216 33	99.26	100.8	1.04
50.76	10.069 5	19.433 55	9.284 00	100.79		
50.80	10.424 3	19.934 49	9.291 32	102.10		
50.65	10.740 8	20.184 96	9.263 89	101.68		
50.85	10.127 0	19.433 55	9.300 47	100.06		
50.88	10.079 1	19.496 16	9.305 95	101.10		

3.8 Stability test Appropriate amount of Tangjiangshenkang granule (batch No.:2004L04979) was taken, and the sample solution was prepared according to Section **3.1.2**. The sample solution was determined according to the determination method in Section **3.4** at 0, 0.5, 1, 2, 3 and 4 h, respectively, and the absorbance difference ΔA and *RSD* value at each determination time point were calculated. The results showed that within 4 h, the average absorbance difference ΔA of total lignanoids in Tangjiangshenkang granules was 0.320, *RSD* ($n=6$) was 0.67%, indicating that the sample solution was stable within 4 h.

3.9 Determination of sample content In addition, three batches of Tangjiangshenkang granules were taken to prepare the test solution according to Section **3.1.2**, and the solution of each sample was determined according to the determination method in Section **3.4**. The absorbance difference ΔA was substituted into the linear regression equation, and the content of total lignanoids was calculated. At the same time, the single wavelength method was used to calculate the content of total lignanoids in the sample at the detection wavelength of 280 nm. The detection results of three batches of samples by the two methods are shown in Table 2.

Table 2 Determination results of sample content

Batch No.	Content of total lignanoids//%	
	Single wavelength	Dual wavelength
150821	22.48	16.55
287021	22.64	18.25
925021	21.32	16.38

4 Discussion

According to the related literature[3-4], the structure of lignanoids was similar, and both total lignanoids and arctiin were absorbed at the same wavelength (280 nm). At the same time, arctiin was the main component of lignanoids in *F. arctii*, and the basic ratio of arctiin to total lignanoids in granules was 1∶2[4], that is, the content of arctiin in total lignanoids was 50%. Therefore, arctiin was selected as the control substance, and the content of total lignanoids in Tangjiangshenkang granules was determined by ultraviolet spectrophotometry.

The extract from *F. arctii* was studied, and the results showed that there were about 10% of organic acids in the extract, mainly chlorogenic acid, caffeic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 1,5-dicaffeoylquinic acid[5]. As shown in the UV scanning map of Tangjiangshenkang granule solution (as shown in Fig. 1), the UV spectra of lignanoids and organic acids were superimposed to form the UV spectrum of test solution. Although the content of organic acids in *F. arctii* extract accounted for only about 10%[5], at the same concentration, the UV absorption intensity of organic acids was higher than that of lignanoids, so that at the detection wavelength of 280 nm, organic acids still had a great influence on the determination of total lignanoids. Therefore, the equal absorption dual-wavelength method was selected to eliminate the effect of organic acids on the determination of content of total lignanoids. At the same time, the UV absorption spectra of the above organic acids were similar, they all had the maximum absorption near 330 nm[8]. Therefore, referring to the determination method of "Flos Lonicerae Extract" in the *Standard of Traditional Chinese Medicine in Shandong Province*[9] and related literature[10], chlorogenic acid was selected as the control substance for the determination of organic acids.

The total lignanoids in Tangjiangshenkang granules were determined by two-wavelength ultraviolet spectrophotometry, which eliminated the effect of organic acids. As can be seen from Table 2, the content of total lignanoids measured by equal absorption dual-wavelength method was about 5% lower than that by single-wavelength method. Through the methodology validation, the precision, stability and reproducibility of the method are good, and it is simple and easy, which provides a new idea for the quality control of Tangjiangshenkang granules.

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