Determination of Paeonol in Traditional Chinese Medicine 'Jing-Shu' Capsule by HPLC*

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ABSTRACT Objective: To establish the HPLC method for the determination of paeonol in 'JingShu' Capsule. Methods: The separation was performed on Platisil ODS (250 mm× 4.6 mm,5 μm) column; The mobile phase was methanol-water (45:55) at a flow rate of 1.0ml·min-1; The detection wavelength was 274 nm, and the column temperature was 40℃. Results: The calibration curve showed good linearity in the range of 0.08487~0.50922 μg (r=0.9999). The average recovery was 98.75%, RSD was 1.74%. Conclusion: The method is convenient, rapid and accurate. It could be used for control the quality of 'JingShu' Capsule.

Key words: HPLC; Paeonol; 'JingShu' Capsule

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Introduction

'Jing-Shu' capsule is a compound preparation which is traditional Chinese medicine made by combining the theory of traditional Chinese medicine and modern processing technology. It contains fourteen Chinese traditional herbs, including Cortex Moutan, White Peony Roots, Angelica, Corydalis yanhusuo and Chinese trumpet creeper and so on. In clinical practice, 'Jing-Shu' capsule is commonly used for treating primary dysmenorrheal [1]. Epidemiological studies have shown that primary dysmenorrheal was a common gynecological disease [2]. From the results of investigation, it was found that the incidence of dysmenorrheal is 20% ~90% [3,4]. It has a torturing influence on women's health and their living quality [5,6]. The major function of 'Jing-Shu' capsule is blood-activating and stasis-dissolving, which can obviously relieve the symptoms of dysmenorrhea (painful menstruation), light Bleeding and amenorrhea (no Bleeding), the breasts swell, pain in chest and hypochondrium and so on[7].

Cortex Moutan is the chief component of 'Jing-Shu' capsule, which is first recorded 'Shen Nong's Herbal Classic', is the dry root-bark of peony (plants of the genus Paeonia)[8,9]. The major function of Cortex moutan is eliminating pathogenic heat from the blood and promoting blood circulation to remove blood stasis [10-12]. What is more, paeonol is one of the mostly effective composes of Cortex moutan. Many lines of pharmacological evidences suggest that paeonol show better treatment effect on preventing arrhythmia, curing atherosclerosis, protecting a decrease in the blood supply to a bodily tissue and promoting micro loop [13,14]. A variety of formulations of paeonol have been used to cure angina attack and coronary heart disease treatment in clinical practice. Quality control of paeonol is very important to Cortex Moutan. It have been reported that there was some methods of determination and quality controls of paeonol for Cortex Moutan[15-17].

'Jing-Shu' capsule is one of the Chinese patent drugs which are our country's unique formula. They have complex compositions and most of them are lack of detecting methods of quality control. As an analysis method, the HPLC method has good reproducibility, high accuracy, sensitivity [18-20]. It is suitable for content determination and the quality control of the Chinese patent drugs. This study determined paeonol in 'Jing-Shu' capsule.

1 Materials and Methods

1.1 Main reagents

Paeonol chemical reference substance (Batch No. 110708-200505) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and the purity was greater than 95%. The products of 'Jing-Shu' capsule were kindly provided by Pharmaceutical Corporation of Shanxi Guilong. HPLC grade methanol was purchased from Merck (DE); Water was purified by a Milli-Q water purification system (Millipore, USA); All other reagents of analytical reagent grade or higher.

1.2 Instrument and chromatographic conditions

All analysis were carried out on a HPLC system (Shimadzu, Kyoto, Japan) equipped with two LC-20AT pumps and a model SPD-20A UV detector. Data integration was performed using Shimadzu Class-vp software. Chromatographic separation was performed on a Platisil ODS (4.6 mm× 250 mm, 5 μm) and the column temperature was maintained at 40℃. The mobile phase was methanol-water (45:55) at a flow rate of 1.0 mL·min^{-1}. UV detector was set at 274 nm. The solvents were filtered trough a 0.5 μm Millipore filter and degassed prior to use. The injection volume

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was set at 10 μL.

1.3 Preparation of standard solution

The stock solution of paeonol was prepared as follows: paeonol (9.43mg) was accurately weighed and transferred into a 100 mL grass volumetric flask. Absolute methanol was added to dissolve the chemical and the final solution volume was 100 mL. Next, 3 mL paeonol was transferred into a 10 mL grass volumetric flask, mixed with methanol to obtain the concentration required in the experiments. The prepared stock solution was degassed in an ultrasonic bath and filtered through a 0.22 μm membrane.

1.4 Preparation of sample solutions

The sample solutions were prepared as follows: Ten 'Jing-Shu' capsules were crashed into fine powder. Then accurately weighed powder (about 0.4 g) was transferred into a 100 mL conical flask with stopper and extracted with 50 mL methanol in an ultrasonic processor for 30 minutes, covered and weighed. After the sample was cooled to room temperature, then weighed and used water to make up the loss weight. The obtained solution was filtered though a 0.22 μm filter membrane and the filtrate was injected to HPLC for analysis [21]. After suitable dilution, the drug contents in three batches of capsules were determined by HPLC.

1.5 Preparation of blank solution

Chinese traditional medicinal materials of 'Jing-Shu' capsule except for peony, tree peony bark were crashed into fine powder, and accurately weighed according to formula of 'Jing-Shu' capsule. Then they were prepared as described in Section 1.4.

1.6 Calibration curve

The prepared stock solutions (4,8,10,12,14,16 μL) were injected to generate a six-point calibration curve. The calibration curve was constructed by plotting the peak area versus the concentrations of analyte, and calibration curve of the analytes (paeonol) was performed with 6 different concentrations in triplicate.

2 Results

2.1 Specificity

The specificity study of the proposed method demonstrated that the other substances present in the preparation did not interfere with the separation of paeonol. The well resolved peaks also indicated the specificity of the method and the blank test showed no interference (Fig.1).

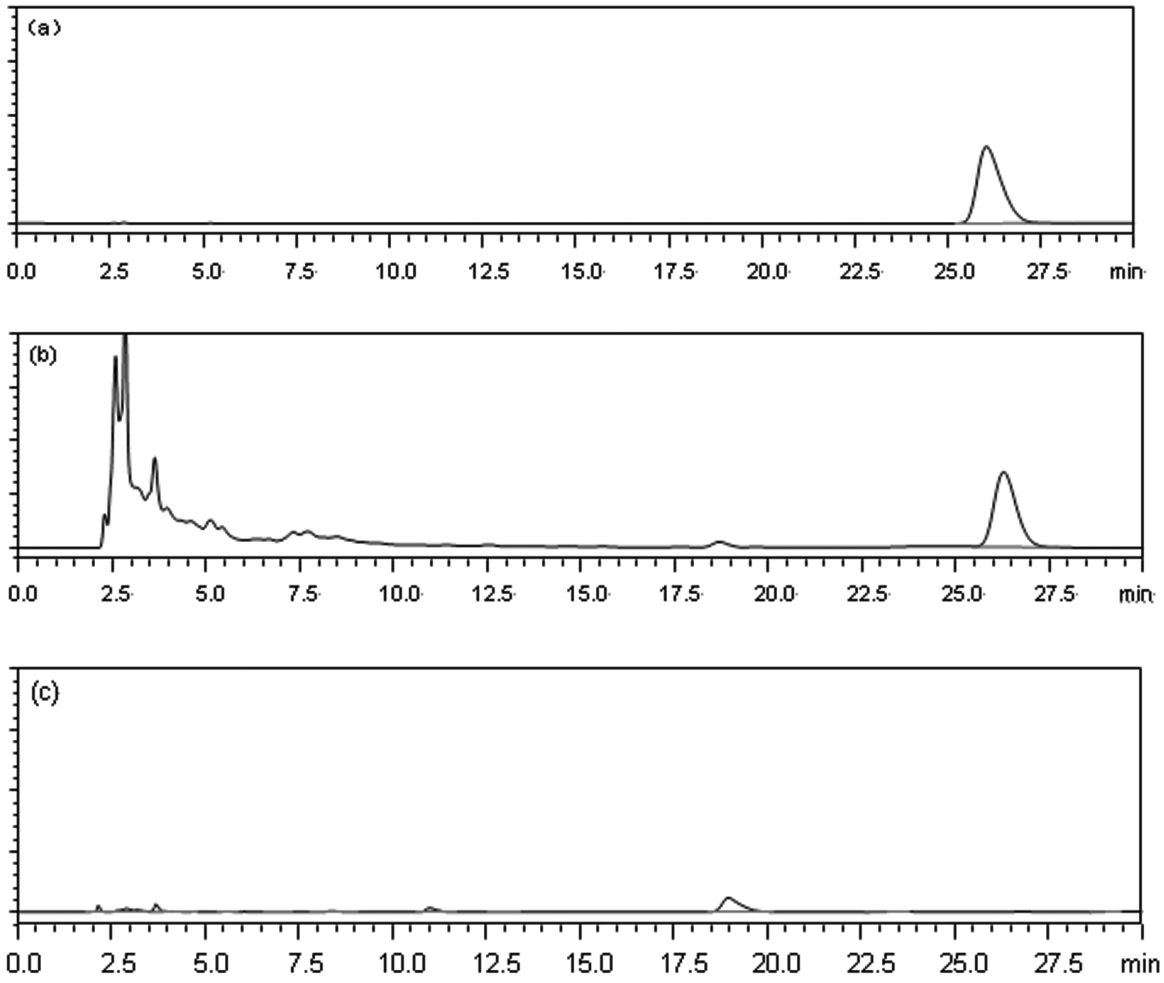

Fig.1 The different HPLC Chromatograms of specificity test

(a) HPLC Chromatogram of a reference solution of paeonol; (b) HPLC Chromatogram of a sample solution of 'Jing-Shu' capsule; (c) HPLC Chromatogram of a blank solution.

2.2 Linearity

The regression equation obtained for paeonol was y=57279.58x-5377.737, r=0.9999(n=5), respectively (x is the concentration, y is the peak area). The result indicated that there was a good correlation between the peak area and drug concentration within the linear range of (0.08487~0.50922) μg.

2.3 Precision

To investigate the reproducibility, the intra- and inter-day precision were estimate by five replicates containing the standard compound at four different concentrations in a single day and repeating this analysis for five days, respectively. Concentrations were determined using the calibration curve prepared on each day. The intra- and inter-day precisions were calculated to be 0.10% and 0.43% , respectively, which indicated that the developed HPLC method is precise enough for the quantitative evaluation of paeonol in 'Jing-Shu' capsule.

2.4 Stability

The stability of the sample solutions was also evaluated. The concentrations of the paeonol were determined at 0, 1, 4, 8, 12, 24 h, respectively, and fit was found that the solution were rather stable. The RSD value of paeonol kept at 0.14% (n=6) .

2.5 Recovery

The recovery property of paeonol was examined by adding certain of each standard solution to the samples prior to the extraction, and all the samples were processed with the same method as described in Section 1.4. The results were shown in Table 1. The above methodological studies showed that the current method was accurate and feasible in determining the content of paeonol in 'Jing-Shu' capsule.

Table 1 Result of recovery test

No.	Sample amount (mg)	Add amount (mg)	Found amount (mg)	Recovery (%)	Mean recovery (%)	RSD (%)
1	0.3193	1.020	1.3158	97.70	98.75	1.74
2	0.3231	1.020	1.3166	97.40		
3	0.6348	0. 680	1.2975	97.45		
4	0.6348	0. 680	1.3087	99.10		
5	0.9551	0.340	1.2915	98.94		
6	0.9557	0.340	1.3022	101.91		

2.6 Analysis of the samples

Three batches of 'Jing-Shu' capsule from Pharmaceutical Corporation of Shanxi Guilong, were detected as described Section 1.4, and the percentages of standard substances were calculated. The results were shown in Table 2. It show that the average concentration of Paeonol in the extract of 'Jing-Shu' capsule was 3.171~3.204 $mg \cdot g^{-1}$.

Table 2 Quantitative analytical result of the various batches of 'Jing-Shu' products

No. of batches	Average content ($mg \cdot g^{-1}$)	RSD (%)
110201	3.171	0.49
110202	3.187	0.15
110203	3.204	0.57

3 Discussions

There are many supplementary materials in the 'Jing-Shu' capsule. In order to easily extract paeonol form 'Jing-Shu' capsule, the drug must be fine triturated before preparing the test sample solution. We have also examined the extraction efficiency in 50 mL of three solvent: methanol, 95% ethanol, and absolute ethyl alcohol, respectively, and tested three different extraction time (20, 30 and 40 min). The results showed that 30 min of ultrasound extraction in methanol had the highest extraction efficiency.

Ch.p is provided by UV spectrophotometric method for the determination of paeonol content, but it can only be the determination of Paeonol in single medical herb. It can not apply to the determination of compound preparation. This test determination of standard solution and negative control solution by using HPLC. The separation of UPLC chromatographic was clear and the reproducible of method was good. There are also no interference of negative control. So the method of this test can control the content of paeonol in 'Jing-Shu' capsule.

4 Conclusions

A simple, convenient, sensitive and reliable HPLC analytical

method for the quantitative analysis of paeonol in Chinese medicine 'Jing-Shu' capsule was established. High linearity, specificity, accuracy and precision were presented in the method validation procedure. The developed method was then successfully applied in the quantification of paeonol in three batches of 'Jing-Shu' capsule. The proposed method could be used to improve the quality control of many products and ensure the stability. In addition, it enables us to distinguish them and use them correctly.

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高效液相色谱法测定经舒胶囊中丹皮酚的含量 *

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摘要 目的:建立以高效液相色谱法测定经舒胶囊中丹皮酚含量的方法。**方法:**采用 Platisil ODS 色谱柱(4.6 mm× 250 mm 5 μm);以甲醇 - 水(45∶55)为流动相;柱温:40℃;流速:1.0ml·min^{-1};检测波长:274nm。**结果:**在建立的色谱条件下,丹皮酚进样量在 0.08487~0.50922 μg 范围内与峰面积呈良好的线性关系(r=0.9999),平均回收率(n=6)为 98.75%(RSD=1.74%)。**结论:**方法简便可靠,分离度好,可用于经舒胶囊的质量控制。

关键词:高效液相色谱法;丹皮酚;经舒胶囊

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