TLC Identification of Agarwood and Sandalwood in Shiwuwei Longdanhua Pills

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Abstract [**Objectives**] To establish a thin layer chromatography (TLC) identification method for agarwood and sandalwood in the Tibetan medicine preparation Shiwuwei Longdanhua Pills. [**Methods**] Using reference medicinal materials of agarwood and sandalwood as the control, TLC identification was conducted on agarwood and sandalwood in Shiwuwei Longdanhua Pills, and methodological research was carried out. [**Results**] The established TLC method can distinguish agarwood and sandalwood, with clear spots and moderate Rf, negative without interference. The methodological investigation results show that the method has good adaptability. [**Conclusions**] This method is simple, feasible, reliable, has good reproducibility, and can be used as a quality control method for Shiwuwei Longdanhua Pills.

Key words Shiwuwei Longdanhua Pills, Thin layer chromatography (TLC), Agarwood, Sandalwood, Quality control

1 Introduction

Shiwuwei Longdanhua Pill is a classic Tibetan medicine preparation, included in the *Drug Standards of the Ministry of Health of the People's Republic of China · Tibetan Medicine* (WS3-BC-0195-95). It is composed of fifteen medicinal herbs such as Genfianae Szechenyii Flos, sandalwood, agarwood, Aucklandiae Radix, *etc.*, and has the effects of clearing heat, regulating lungs, stopping cough, and resolving phlegm. It is clinically used for cough, asthma, and hoarseness caused by bronchitis and emphysema[1]. In the prescription, Genfianae Szechenyii Flos is used as the monarch medicine to clear heat in the lungs, detoxify and clear the throat. It is supplemented with herbs such as Chebulae Fructus and agarwood to clear heat, promote lung function, relieve cough, and reduce phlegm, and combined with Aucklandiae Radix and sandalwood to strengthen the spleen and promote qi circulation. The entire prescription works together to clear heat, regulate the lungs, relieve cough, and reduce phlegm. However, the current quality standards only specify the characteristics and routine inspection items, and do not have exclusive qualitative methods for the precious fine medicines such as agarwood and sandalwood in the preparation, making it difficult to effectively control product quality. At present, research reports on the quality control of Shiwuwei Longdanhua Pills mainly focus on the determination of the contents of corilagin, gentiopicroside, eugenol, protocatechuic acid, glycyrrhizic acid, *etc*[2-7]. Although some scholars[8] have qualitatively identified agarwood, Aucklandiae Radix, and Glycyrrhizae Radix Et Rhizoma in the preparation using TLC and established a method for determining the content of agarotetrol, there has been no research on thin layer chromatography (TLC) identification of sandalwood.

Agarwood and sandalwood are both important medicinal flavors in Shiwuwei Longdanhua Pills. Agarwood has the functions of regulating qi, relieving pain, absorbing qi, and relieving asthma, while sandalwood is good at smoothing qi, warming spleen and stomach, stimulating appetite, and relieving pain[9]. The two work together in the prescription to promote qi circulation and aid in movement. Due to the fact that agarwood and sandalwood are both precious medicinal herbs, market adulteration often occurs. It is of great significance for ensuring the quality of preparations by establishing exclusive and reliable identification methods. Because of its simple operation, strong specificity and low cost, TLC has become a common means for qualitative identification of Chinese medicinal materials and traditional Chinese patent medicines and simple preparations[10-13]. On the basis of existing quality standards, for the two medicinal flavors of agarwood and sandalwood in Shiwuwei Longdanhua Pills, this study established a specialized TLC identification method, in order to improve the quality control system of the preparation and provide experimental basis for ensuring its clinical safety and effectiveness.

2 Instruments and reagents

2.1 Instruments CAMAG AUTOMATIC TLC SAMPLER 4 fully automatic thin-layer sampling instrument, CAMAG TLC Visualizer thin-layer imaging system (Swiss CAMAG Company); KQ5200DB type of ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.); ME55 type of electronic balance (Mettler Toledo Company); Puxi GWB-1T/2T ultra pure water device (Beijing Puxi General Instrument Co., Ltd.).

2.2 Reagents Shiwuwei Longdanhua Pills were provided by

Received: July 19, 2026 Accepted: September 28, 2026
Supported by the Science and Technology Program of Xizang Autonomous Region (XZ202501ZY0020).

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five enterprises, including Xizang Xiongbalaqu Shenshui Tibetan Medicine Co., Ltd., Xizang Shenhou Pharmaceutical Co., Ltd., and Xizang Jinhada Pharmaceutical Co., Ltd. The sample information was shown as Table 1.

Table 1 Sample information of Shiwuwei Longdanhua Pills

No.	Enterprise	Production lot No.
S1	A	20260101
S2	B	20230101
S3	B	20230102
S4	B	20230401
S5	B	20250101
S6	B	20250901
S7	C	260101
S8	D	20260105
S9	D	20251104
S10	D	20251201
S11	E	09260203
S12	E	09260201
S13	E	09260202
S14	E	09260204
S15	A	20260102

Sandalwood reference herb (lot No.: 121240-201703) and agarwood reference herb (lot No.: 121222-202104) were purchased from the China National Institute for Food and Drug Control. Silicone G thin laminates were purchased from Germany Merck Co., Ltd., Qingdao Marine Chemical Plant Branch, and Tianjin Silida Technology Co., Ltd. Trichloromethane, ether, petroleum ether (60 – 90 ℃), ethyl acetate, dimethylaminobenzaldehyde, glacial acetic acid, phosphoric acid, *etc.* were all analytical grade.

3 Methods and results

3.1 TCL identification of agarwood

3.1.1 Preparation of test solution. First, 3 g of sample was taken. After ground finely, 50 mL of ether was added, and it was treated for 60 min by ultrasound. After filtering, the filtrate was evaporated, and 1 mL of chloroform was added to dissolve the residue as the test solution.

3.1.2 Preparation of reference medicinal solution. First, 0.5 g of agarwood reference herb was taken, and 50 mL of ether was added. After treated for 60 min by ultrasound, it was filtered, and the filtrate was evaporated. The residue was dissolved by 1 mL of chloroform as a reference medicinal solution.

3.1.3 Preparation of negative solution. 1/10 of the prescription amount of Shiwuwei Longdanhua Pills lacking agarwood was taken and ground into fine powder. After sieved, it was mixed well, and a negative solution lacking agarwood was prepared according to Section **3.1.1**.

3.1.4 TLC conditions. According to the TLC method (General Rule 0502), 5 – 10 μL of each of the above two solutions were taken and dotted onto the same silica gel G thin-layer plate. Chloroform-ether (10∶1) was used as the developing agent, and they were developed. After taken out, it was dried naturally, and inspected under ultraviolet light (365 nm). In the chromatogram of the test samples, fluorescent spots of the same color appeared at the corresponding position as the chromatogram of the control medicinal material. The negative sample solution showed no fluorescent spots of the same color at the corresponding position on the chromatogram of the agarwood reference herb, indicating good method specificity (Fig. 1).

3.1.5 Sample size investigation. According to the above chromatographic conditions and methods, the sample sizes of the reference medicinal solution of agarwood and the test solution of Shiwuwei Longdanhua Pills were examined. The results showed that the spots of the reference medicinal solution of agarwood and the test solution of Shiwuwei Longdanhua Pills were clear at 5 – 10 μL, and the sample size was determined to be 5 – 10 μL, as shown in Fig. 2.

3.1.6 Durability assessment. (i) Comparison of different thin-layer plates. Silicone G plates from Germany Merck Co., Ltd., Qingdao Marine Chemical Plant, and Tianjin Silida Technology Co., Ltd. were selected for test according to the proposed testing methods. The results showed that all three types of thin-layer plates met the identification requirements, indicating good durability of the method (Fig. 3).

(ii) Investigation of different temperatures. The test solution and control herbal solution were prepared according to the selected method and dotted. The dotted silica gel G thin-layer plates (Qingdao Marine Chemical Plant) were taken and developed at 8 and 35 ℃, respectively. The results indicated that the method had good durability within the temperature range of 8 – 35 ℃ (Fig. 4).

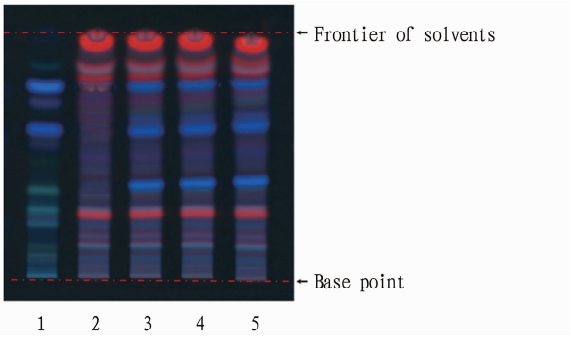
(iii) Investigation of different humidity levels. The test solution and reference herbal solution were prepared according to the selected method and dotted. The dotted silica gel G thin-layer plates (Qingdao Marine Chemical Plant) were taken and developed under 35% and 80% of humidity environments, respectively. The results indicated that the method had good durability within the humidity range of 35% to 80% (Fig. 5).

(iv) TLC identification of 15 lots of samples. According to the proposed method, 15 lots of Shiwuwei Longdanhua Pills were subjected to TLC identification, and the results were shown in Fig. 6. The results showed that, except for No. 2 – 6 samples, other lots of Shiwuwei Longdanhua Pills and agarwood reference medicinal materials displayed spots of the same color at the corresponding positions on their chromatograms.

3.2 TLC identification of sandalwood

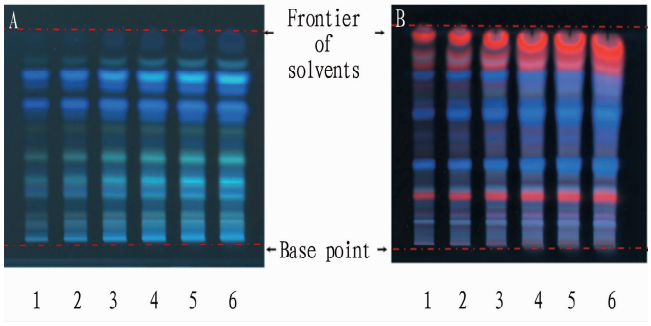
3.2.1 Solution preparation. The preparation of test solution was same as Section **3.1.1**. First, 0.5 g of sandalwood reference herb was taken, and 50 mL of ether was added. After treated for 60 min by the ultrasound, it was filtered, and the filtrate was evaporated. The residue was dissolved in 1 mL of chloroform as a reference herb solution. 1/10 of the prescription amount of Shiwuwei Longdanhua Pills lacking sandalwood was taken and ground into fine powder. After sieved, it was mixed well, and a negative solu-

tion lacking sandalwood was prepared according to Section **3.1.1**.



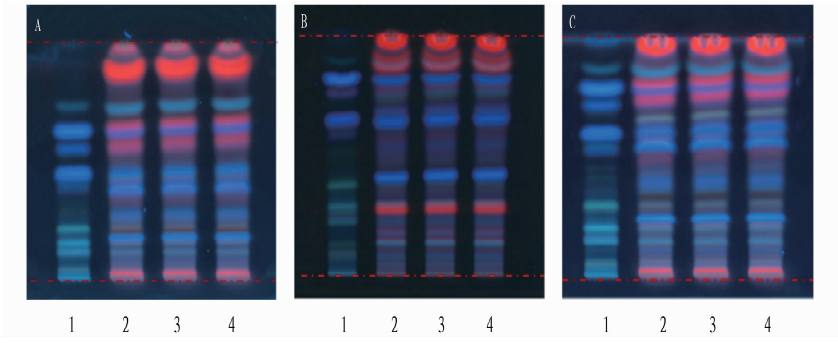
NOTE 1. Agarwood reference medicinal materials; 2. Negative sample; 3 – 5. Shiwuwei Longdanhua Pill samples.

Fig. 1 TLC of agarwood (T: 25 ℃, RH: 75%)



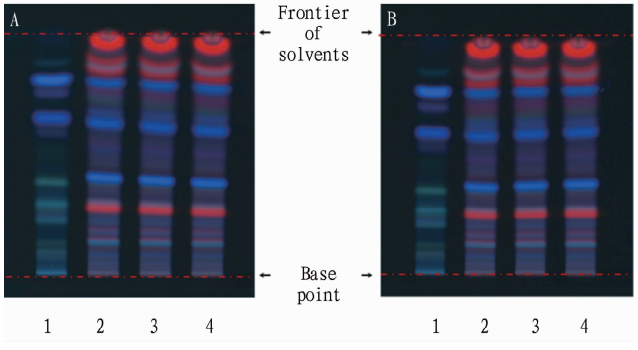
NOTE A. Reference medicinal herb of agarwood; B. Shiwuwei Longdanhua Pills sample. 1 – 6 were 1, 2, 4, 6, 8, 10 μL respectively.

Fig. 2 Chromatogram of sample size inspection for agarwood in Shiwuwei Longdanhua Pills (T: 25 ℃, RH: 75%)



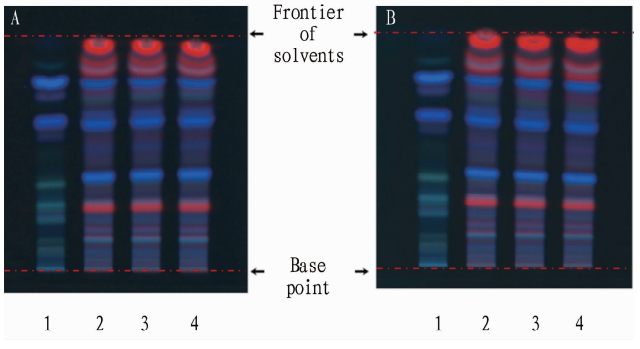
NOTE A. Silicone G plate from Germany Merck Co., Ltd.; B. Silicone G plate from Qingdao Marine Chemical Plant; C. Silicone G plate from Tianjin Silida Technology Co., Ltd. 1. Reference medicinal herb of agarwood; 2 – 4. Shiwuwei Longdanhua Pills sample.

Fig. 3 Comparative chromatograms of different thin-layer plates for agarwood in Shiwuwei Longdanhua Pills (T: 25 ℃, RH: 75%)



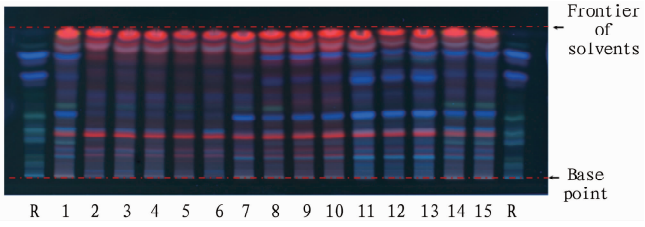
NOTE A. 8 ℃; B. 35 ℃. 1. Reference medicinal herb of agarwood; 2 – 4. Shiwuwei Longdanhua Pills sample.

Fig. 4 Results of investigation at different temperatures for agarwood in Shiwuwei Longdanhua Pills (RH: 75%)



NOTE A. RH 35%; B. RH 80%. 1. Reference medicinal herb of agarwood; 2 – 4. Shiwuwei Longdanhua Pills sample.

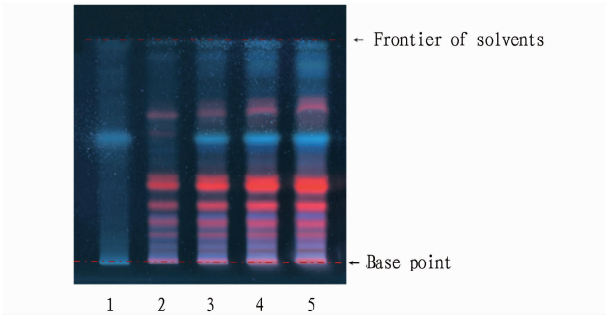
Fig. 5 Results of investigation at different humidity for agarwood in Shiwuwei Longdanhua Pills (T: 25 ℃)



NOTE R. Reference medicinal herb of agarwood; 1 – 15. Shiwuwei Longdanhua Pills sample (S1 – S15).

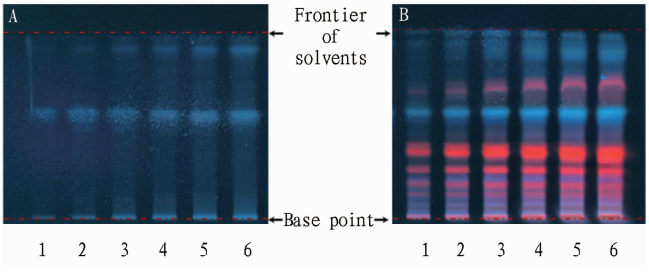
Fig. 6 TLC identification of 15 lots of Shiwuwei Longdanhua Pills (T: 25 ℃, RH: 75%)

3.2.2 TLC conditions. According to the TLC method (General Rule 0502), 5 – 10 μL of each of the above two solutions were taken and dotted on the same silica gel G thin-layer plate. Petroleum ether (60 – 90 ℃)-ethyl acetate (17 ∶ 3) was used as the developing agent. After developed, it was taken out to dry naturally. After sprayed with a solution of p-dimethylaminobenzaldehyde (0.25 g of p-dimethylaminobenzaldehyde was taken, 50 g of glacial acetic acid was added to dissolve, and 5 g of phosphoric acid and 20 mL of water were added to mix well), it was heated at



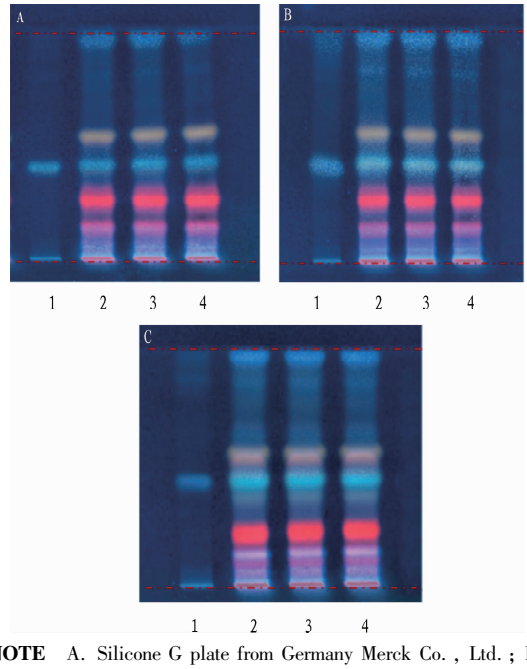
NOTE 1. Reference medicinal material of sandalwood; 2. Negative sample; 3 – 5. Shiwuwei Longdanhua Pills sample.

Fig. 7 Thin-layer chromatogram of sandalwood (T: 25 ℃, RH: 75%)



NOTE A. Reference medicinal material of sandalwood; B. Shiwuwei Longdanhua Pills sample. 1 – 6 were 1, 2, 4, 6, 8, 10 μL, respectively.

Fig. 8 Chromatogram of sample size inspection for sandalwood in Shiwuwei Longdanhua Pills (T: 25 ℃, RH: 75%)

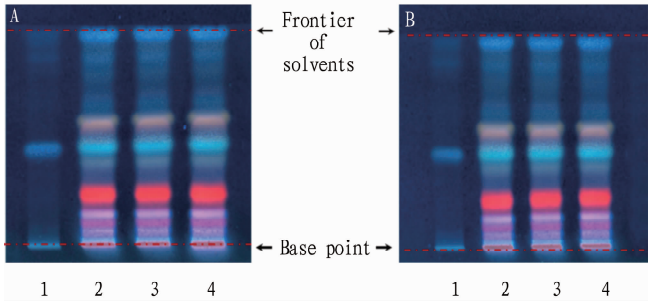


NOTE A. Silicone G plate from Germany Merck Co., Ltd.; B. Silicone G plate from Qingdao Marine Chemical Plant; C. Silicone G plate from Tianjin Silida Technology Co., Ltd. 1. Reference medicinal herb of sandalwood; 2 – 4. Shiwuwei Longdanhua Pills sample.

Fig. 9 Comparative chromatograms of different thin-layer plates for sandalwood in Shiwuwei Longdanhua Pills (T: 25 ℃, RH: 75%)

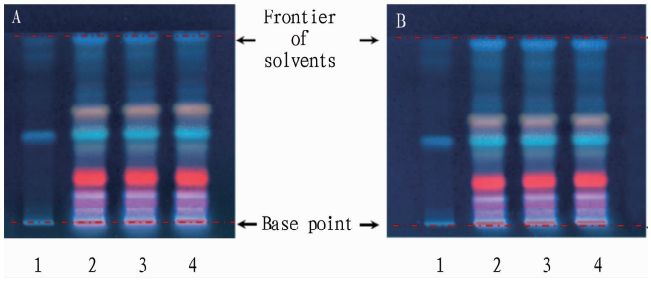
80 – 90 ℃ until the spots were clear, and inspected under ultraviolet light (365 nm). In the chromatogram of the test sample, fluorescent spots of the same color appeared at the corresponding position as the chromatogram of the reference medicinal material. The negative sample solution showed no fluorescent spots of the same color at the corresponding position on the chromatogram of the sandalwood reference medicinal material, indicating good specificity of the method (Fig. 7).

3.2.3 Sample size investigation. According to the above chromatographic conditions and methods, the sample sizes of the sandalwood reference medicinal solution and Shiwuwei Longdanhua Pills test solution were examined. The results showed that the spots of the sandalwood reference medicinal solution and Shiwuwei Longdanhua Pills test solution were clear at 5 – 10 μL, and the recommended sample size was 5 – 10 μL (Fig. 8).



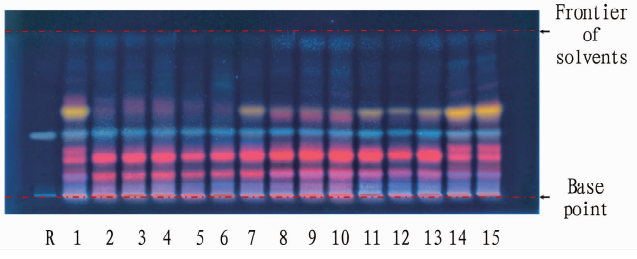
NOTE 1. Reference medicinal herb of sandalwood; 2 – 4. Shiwuwei Longdanhua Pills sample. A. 8 ℃; B. 35 ℃.

Fig. 10 Results of investigation at different temperatures for sandalwood in Shiwuwei Longdanhua Pills (RH: 75%)



NOTE A. RH 35%; B. RH 80%. 1. Reference medicinal herb of sandalwood; 2 – 4. Shiwuwei Longdanhua Pills sample.

Fig. 11 Results of investigation at different humidity for sandalwood in Shiwuwei Longdanhua Pills (T: 25 ℃)



NOTE R. Reference medicinal herb of sandalwood; 1 – 15. Shiwuwei Longdanhua Pills sample (S1 – S15).

Fig. 12 TLC identification of 15 lots of Shiwuwei Longdanhua Pills (T: 25 ℃, RH: 75%)

3.2.4 Durability assessment. (i) Comparison of different thin-layer plates. Silicone G plates from Germany Merck Co., Ltd., Qingdao Marine Chemical Plant, and Tianjin Silida Technology Co., Ltd. were selected for test according to the proposed testing methods. The results showed that all three types of thin-layer plates met the identification requirements (Fig. 9).

(ii) Investigation at different temperatures. The test solution and reference herbal solution were prepared according to the selected method and dotted. The dotted silica gel G thin-layer plates (Tianjin Silida Technology Co., Ltd.) were taken and developed at 8 and 35 ℃, respectively. The results indicated that the method had good durability within the temperature range of 8 – 35 ℃, as shown in Fig. 10.

(iii) Investigation of different humidity levels. The test solution and reference herbal solution were prepared according to the selected method and dotted. The dotted silica gel G thin-layer plates (Qingdao Marine Chemical Plant) were taken and developed under 35% and 80% of humidity environments, respectively. The results indicated that the method had good durability within the humidity range of 35% to 80% (Fig. 11).

3.2.5 TLC identification of 15 lots of samples. According to the proposed method, 15 lots of Shiwuwei Longdanhua Pills were subjected to TLC identification, and the results were shown in Fig. 12. The results showed that all lots of Shiwuwei Longdanhua Pills and sandalwood reference medicinal materials displayed spots of the same color at the corresponding positions on their chromatograms.

4 Conclusions and discussion

In this paper, a systematic investigation on TLC identification of agarwood and sandalwood in Shiwuwei Longdanhua Pills was conducted. The results showed that the established TLC method could display characteristic fluorescent spots at the corresponding positions of the control medicinal materials, with good separation, no interference from the negative control, strong specificity. It can provide reference for improving the quality standards of Shiwuwei Longdanhua Pills.

Through testing 15 lots of samples from multiple companies, it was found that except for 5 lots of samples from company B, which did not show the fluorescent spots of same color at the corresponding positions of the agarwood reference medicinal material, all other company samples showed positive results, indicating that the company may have problems such as missing or adulteration of agarwood medicinal materials. Further confirmation by combining fingerprint spectra, content determination and other methods is needed in the future.

The results of this study provide a reliable technical supplement for the quality control of Shiwuwei Longdanhua Pills. It is recommended to include this method in the quality standards of this variety and expand the sample size to examine the consistency between different sources of medicinal materials and preparations, thus providing a more comprehensive strategy for the authenticity identification of precious fine medicinal materials in compound preparations of ethnic medicines.

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