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Preparation Process and Thin Layer Chromatography Identification of Wufang Babu Poultice

Junjun LIU[1], Luyao XIE[1], Chuhui ZHOU[1], Zhengteng YANG[2*], Xiongbin GUI[2*]

1. Guangxi University of Chinese Medicine, Nanning 530200, China; 2. The First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning 530023, China

Abstract [**Objectives**] To establish the process flow of preparation of Wufang Babu Poultice and the identification method of thin layer chromatography (TLC). [**Methods**] For the forming process of Wufang Babu Poultice, the preparation method of mixed pharmaceutical powder with suitable pharmaceutical excipients was adopted. Qualitative identification of medicinal materials in Wufang Babu Poultice (Strychni Semen, Rhei Radix Et Rhizoma, Dipsaci Radix, and Angelicae Sinensis Radix) was carried out by TLC. [**Results**] Mixed pharmaceutical powder mixed with glycerol, gelatin and other pharmaceutical excipients can be prepared for forming. The test solution chromatography of each medicinal material (Strychni Semen, Rhei Radix et Rhizoma, Dipsaci Radix, Angelicae Sinensis Radix) showed pigment spots of the same color at the same position as its corresponding control medicinal materials and reference chromatography, and the display was clear. [**Conclusions**] The preparation process is simple and feasible, and can be used as the forming process of Wufang Babu Poultice. The TLC determination method is simple to operate, has good specificity, and has no effect on negative results, and can be used for identification of Wufang Babu Poultice.

Key words Wufang Babu Poultice, Preparation process, Thin layer chromatography (TLC)

1 Introduction

Soft tissue injury refers to the pathological damage of soft tissues caused by the local release of vasoactive substances and inflammatory mediators such as histamine. The soft tissue injury causes a series of microcirculation changes at the injury site and seriously affects people's health[1]. Because its disease is located in a superficial area, it is more suitable for external treatment. External treatment can directly reach the lesion from the body surface, especially external ointment, plaster, paste, poultice, *etc.* are the most extensive, reliable in efficacy and convenient to use. Besides, external drugs have few toxic side effects on the human body, high safety, and high clinical acceptance by the majority of patients. The soft tissue injury site absorbs some bioactive substances contained in external Chinese medicine through transdermality, and maintains a relatively stable blood concentration in the injured area, so as to achieve the effects of activating blood circulation and removing blood stasis, easing pain and reducing swelling[2-4].

Wufang Babu Poultice is the changed dosage form of the secret medicine of Professor Liang Xi'en, who was a famous orthopaedic master of the First Affiliated Hospital of Guangxi University of Chinese Medicine. It has the effects of promoting blood circulation, removing blood stasis, clearing away heat, relieving inflammation, relaxing muscle and tendons, activating collaterals, and strengthening tendons and bones. In the prescription, Lycopi Herba is sovereign medicinal, Rhei Radix et Rhizoma is minister medicinal, Carthami Flos is assistant medicinal, and Eupolyphaga Steleophaga is courier medicinal. The combined efficacy of each drug in the prescription promotes the repair of acute soft tissue injury[5-7]. The original prescription and Wufang Powder have obtained a large number of clinical effect data, and have good therapeutic effects on promoting blood circulation, dissipating blood stasis, relieving swelling and pain, and setting bones[8-10]. In order to improve the preparation process and quality standards of Wufang Babu Poultice and ensure the safety and effectiveness of clinical medication, we established of a pharmaceutical process system. In this study, we identified and analyzed some medicinal materials (Strychni Semen, Rhei Radix Et Rhizoma, Dipsaci Radix, Angelicae Sinensis Radix) in the preparation by thin layer chromatography (TLC), so as to provide a basis for the improvement of its preparation technology and quality standards.

2 Materials

2.1 Instruments DHG-9240A electric constant temperature water bath (Shanghai Qixin Scientific Instrument Co., Ltd.); SQP analytical balances (Sartorius Scientific Instruments (Beijing) Co., Ltd.); TGL-16G high speed centrifuge (Shanghai Anting Scientific Instrument Factory); XM-P06H stepless power ultrasonic cleaning machine (Xiaomei Ultrasonic Instrument (Kunshan) Co., Ltd.); Eppendorf pipette pen (Eppendorf Research plus, Germany); TH-II type CNC thin layer chromatography heater (Shanghai Kezhe Biochemical Technology Co., Ltd.).

2.2 Reagents Methanol (Tianjin Fuyu Fine Chemical Co., Ltd., batch No.:20200327), water was ultrapure water, and other reagents were analytically pure; strychninee (China National Institutes for Food and Drug Control, batch No.:110705-201307); Brucine (China National Institutes for Food and Drug Control, batch

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* Corresponding author. E-mail: 674632459@qq.com; guiXB2008@163.com

No.: 112030-201601); Rhein (Chengdu Refmedic Technology Co., Ltd, batch No.: RP210516); Chrysophanol (Chengdu Refmedic Technology Co., Ltd, batch No.: RP210718); Emodin (China National Institutes for Food and Drug Control, batch No.: 110756-200110); Asperosaponin VI (China National Institutes for Food and Drug Control, batch No.: 111685-201707). Silica gel G thin layer plate (Qingdao Ocean Chemical Co., Ltd., batch No.: 20220902). Wufang Babu Poultice was provided by Guangzhou Heyi Medical Technology Co., Ltd.

3 Methods

3.1 Preparation process and parameters

3.1.1 Preparation process. Based on the compatibility of the original prescription, the project developed Wufang Powder into Wufang Poultice according to the relevant requirements of the application for registration of preparations in medical institutions. By keeping a large amount of active components in the original prescription, the effectiveness of the original prescription was retained, the stability of the preparation was improved, and the preparation was convenient to store, carry and use. After being prepared into the poultice dosage form, not only can the original medication effect be maintained in the application, but also the advantages of large drug loading of the Wufang poultice, good drug release characteristic, basically no sensitization and irritation, high compliance and the like can be shown.

3.1.2 Process parameters. Medicinal materials: 12.0 kg mixed powder. The process parameters of excipients are shown in Table 1.

Table 1 Process parameters of excipients

Name	Dosage//kg	Name	Dosage//kg	Name	Dosage//kg
Purified water	11.0	Glycerol	3.30	Kaolinite (Kaolin)	1.20
Sodium Polyacrylate	1.5	Gelatin	2.10	Sodium carboxyme thyl cellulose (CMC)	0.88
Carbomer	1.0	Benzoic acid	0.02	Ethanol	0.15
Menthol	0.1	Camphore	0.10	White vaselin	0.50

3.1.2 Process flow. Chinese medicine decoction pieces → crushed→ mixed well→ mixed with excipients, mixed and stirred → poultice → coated and formed → cutting → packaging → obtained.

3.1.3 Preparation process. Dissolved sodium polyacrylate, gelatin, sodium carboxymethylcellulose and carbomer in water to obtain phase A for later use. Added kaolin, glycerol, water, and ethanol solution of benzoic acid into that medicinal powder, and stirred to obtain phase B for later use. Heated and melted menthol, camphora, and vaselin to obtain phase C for later use. A, B and C were mixed and stirred evenly, that poultice was coated, formed and cut, and then was pasted on a non-woven fabric and packaged to obtain.

3.2 TLC identification

3.2.1 Strychni Semen. (i) Solution preparation. Took 5 g of this product, removed the lining film, added 1 mL of hydrochloric acid and 80 mL of ethanol after cutting, heated and refluxed for about one hour, poured the ethanol solution, recovered the ethanol to near dry, added 30 mL of water, heated in a water bath for about 10 min and filtered. The filtrate was shaken with an appropriate amount of ether, the aqueous solution was separated, and then ammonia was added to alkaline, extracted three times with chloroform solution, the extract was combined, heated and evaporated in a water bath, and the residue was dissolved by adding 1 mL of chloroform, that is, the test solution. Took 2 g of Strychni Semen control medicinal material and prepared the control solution in the same way as the test solution. Took 5 g of Wufang Babu Poultice sample not containing Strychni Semen and prepared a negative control solution of Strychni Semen using the same method. In addition, separately took the strychnine reference substance and Strychni Semen reference substance to make a mixed solution containing 2 mg/mL of chloroform as a reference solution.

(ii) Identification. The method of TLC of General Principles 0502 in Volume IV of the *Chinese Pharmacopoeia* (2015 Edition)[11] was used to carry out experimental research. Pipetted 7 μL each of the test solution, the control medicinal material solution, and the negative control solution, and 3 μL of the reference solution, and dropped on the same silica gel G thin layer plate in turn. Toluene-acetone-ethanol-concentrated ammonia test solution (4∶5∶0.6∶0.4) was used as the developing agent, developed, took out, dried and sprayed with dilute bismuth iodide potassium test solution (took 2 g of commercially available bismuth potassium iodide reagent, added 20 mL of glacial acetic acid to dissolve and dilute with 50 mL of water).

3.2.2 Rhei Radix Et Rhizoma. (i) Solution preparation. Took 2 g of this product, added 10 mL of 10% hydrochloric acid solution, sonicate for 30 min, extracted twice with chloroform shaking, 20 mL each time, and then combined chloroform aqueous solution, evaporated to dry, and added 2 mL of methanol to the residue to dissolve, that is, the test solution. Took 5 g of Rhei Radix et Rhizoma control medicinal material, and prepared the control medicinal material solution in the same way as the test solution. Took 2 g of Wufang Babu Poultice sample not containing Rhei Radix et Rhizoma and prepared a negative control solution of Rhei Radix et Rhizoma by the same method. Took the Rhein reference substance and added methanol to make a solution containing 1 mg per 1 mL as the reference solution I. Took an appropriate amount of Emodin, Rhein, Chrysophanol reference substance, added methanol to make a mixed solution containing 1 mg per 1 mL, as the reference solution II.

(ii) Identification. The method of TLC of General Principles 0502 in Volume IV of the *Chinese Pharmacopoeia* (2015 Edition)[11] was used to carry out experimental research. Separately pipetted 7 μL of the test solution, the control medicinal material

solution and the negative control solution, and 3 μL of the reference solution, and successively dropped on the same silica gel G thin layer plate, with petroleum ether (30 – 60 ℃) ethyl carboxylate monocarboxylic acid (15 ∶ 7 ∶ 1) upper solution as the developing agent, developed, took out, dry, and placed under an ultraviolet light (365 nm) for inspection.

3.2.3 Angelicae Sinensis Radix. (i) Solution preparation. Took 2 g of Wufang Babu Poultice, added 20 mL of ether, filtered it after sonication for 10 min, evaporated the filtrate, and added 1 mL of ethanol to the residue to dissolve, that is, the test solution. Took 5 g of Angelicae Sinensis Radix control medicinal material and prepared the control medicinal material solution in the same way as the test solution. Took 2 g of Wufang Babu Poultice sample not containing Angelicae Sinensis Radix, and prepare Angelicae Sinensis Radix negative control solution using the same method.

(ii) Identification. The method of TLC of General Principles 0502 in Volume IV of the *Chinese Pharmacopoeia* (2015 Edition)[11] was used to carry out experimental research. Pipetted 8 μL each of the test solution, the control medicinal material solution and the negative control solution, dropped on the same silica gel G thin layer plate in turn, took n-hexane-ethyl acetate (4 ∶ 1) as the developing agent, developed, took out, dried, and placed under ultraviolet light (365 nm) for inspection.

3.2.4 Dipsaci Radix. (i) Solution preparation. Took 3 g of this product, added 40 mL of methanol, treated it with ultrasonic reaction for 30 min, filtered, evaporated the filtrate, added 20 mL of water to dissolve, extracted 20 mL of water-saturated n-butanol, extracted three times, and combined the (upper) water-saturated n-butanol; washed three times with ammonia, 50 mL each time, discarded the ammonia, evaporated the n-butanol solution saturated with water, and add ed1 mL of methanol to the residue to dissolve to prepare the test solution. Took 5 g of Dipsaci Radix control medicinal material, and prepared the control medicinal material solution in the same way as the test solution. Took 2 g of Wufang Babu Poultice sample not containing Dipsaci Radix, and prepared Dipsaci Radix negative control solution using the same method. Took Asperosaponin VI reference substance, added methanol to make a solution containing 1 mg per 1 mL as a reference solution.

(ii) Identification. The method of TLC of General Principles 0502 in Volume IV of the *Chinese Pharmacopoeia* (2015 Edition)[11] was used to carry out experimental research. Pipetted 12 μL each of the test solution, the control medicinal material solution and the negative control solution, and successively dropped on the same silica gel G thin layer plate, and used the lower solution of chloroform-methanol-water-formic acid (13 ∶ 7 ∶ 2 ∶ 0.1) below 10 ℃ for more than 12 h as the developing agent, took out to dry, and then sprayed 10% ethanol sulfate solution, and heated up at 105 ℃ until the spots were clear.

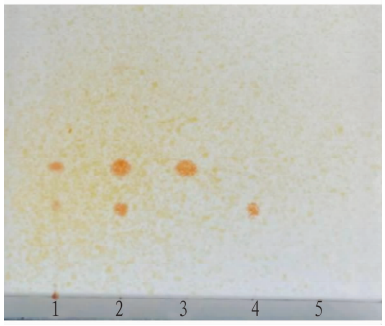
4 Results and analysis

4.1 Selection of TLC identification conditions for Strychni Semen Firstly, with reference to the method of Strychni Semen in the Volume I of the 2020 edition of the *Chinese Pharmacopoeia*, used 5 mL of chloroform-ethanol (10 ∶ 1) mixed solution and 0.5 mL of concentrated ammonia test solution, shook for 5 min and then stood for 2 h, then filtered, and used the filtrate as a test solution. This method did not appear spots, so we considered that it is not suitable for this compound preparation, after improving the method, fully removing impurities, adding hydrochloric acid and ethanol to heat and reflux to recover ethanol to near dry, we added water to heat the filtrate in a water bath and shook with ether, separated the aqueous solution, and then added ammonia to adjust to alkaline, extracted with chloroform solution, combined the extraction, heated and evaporated in a water bath, and added chloroform to dissolve the residue, that is, the test solution. In the test chromatography, plaques of the same color were shown at the corresponding positions of the control chromatography, but there was no plague compared with the negative solution of Strychni Semen. The results are shown in Fig. 1.

4.2 Selection of TLC identification conditions for Rhei Radix Et Rhizoma For the thin-layer identification of Rhei Radix et Rhizoma, we first added methanol for sonication, then added water and hydrochloric acid for refluxing, and finally extracted with ether, and after evaporation, added chloroform to the residue to obtain the test solution, the separation effect was not ideal and the tailing phenomenon was outstanding. After several pre-experiments, the preparation and extraction method of the test solution was improved: the sample was sonicated with 10% hydrochloric acid, and the test solution was concentrated in a water bath after filtration, but the effect was still not satisfactory, and there were too many impurities to interfere with the problem. Finally, the preparation and extraction method of the test solution was determined: the sample was extracted by sonication with 10% hydrochloric acid and chloroform after shaking, and the residue after evaporation was dissolved by methanol. The developing agent adopted upper solution of petroleum ether (30 – 60 ℃)-ethyl formate-formic acid (15 ∶ 7 ∶ 1). When viewed under UV light (365 nm), the spots were well separated, the color was clear, and there was no interference in the negative (Fig. 2).

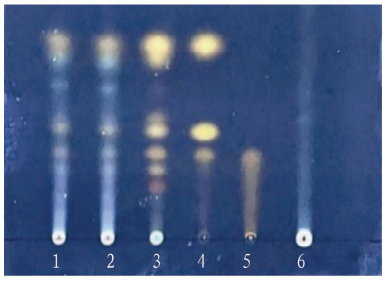
4.3 Selection of TLC identification conditions for Angelicae Sinensis Radix For the TLC of Angelicae Sinensis Radix, ether sonication and heating reflux were performed at the same time of extracting the test solution. Experimental results showed that both could be clearly displayed, so ether sonication was adopted to simplify the extraction process, save time and achieve the same results. In the chromatogram of test solution, fluorescent spots of the same color were displayed at the same position corresponding to the control medicinal material chromatography, and the Angelicae Sinensis Radix negative control did not have such spots. The results are shown in Fig. 3.

4.4 Selection of TLC identification conditions for Dipsaci Radix In the TLC study of Dipsaci Radix, we first referred to the preparation and extraction method of Dipsaci Radix in Volume I of the 2020 edition of the Chinese Pharmacopoeia, added methanol and sonicated, and then filtered, and dissolved the residue after evaporation of the filtrate with methanol. However, when used as a test solution, the separation effect is not ideal, and there are more



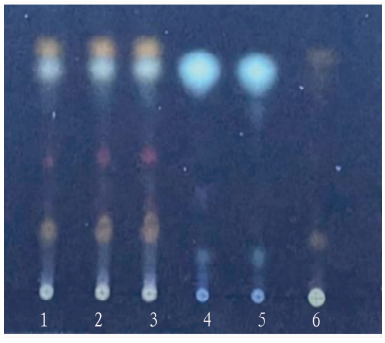
Note: 1. Test solution; 2. Strychni Semen reference substance; 3. Strychnine; 4. Brucine; 5. Negative.

Fig. 1 Thin layer chromatogram of Strychni Semen



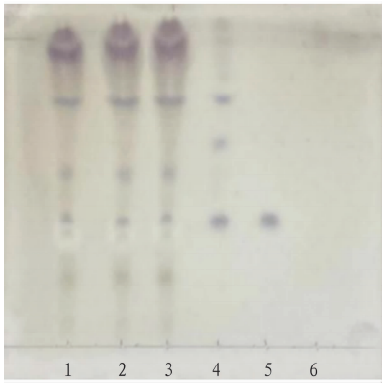
Note: 1 – 2. Test solution; 3. Rhei Radix Et Rhizoma control medicinal material; 4. Emodin, Rhein, Chrysophanol reference substances; 5. Rhein reference substance; 6. Negative.

Fig. 2 Thin layer chromatogram of Rhei Radix Et Rhizoma



Note: 1 – 3. Test solution; 4 – 5. Angelicae Sinensis Radix control medicinal materials; 6. Negative.

Fig. 3 Thin layer chromatogram of Angelicae Sinensis Radix



Note: 1 – 3. Test solution; 4. Dipsaci Radix control medicinal material; 5. Asperosaponin VI reference substance; 6. Negative.

Fig. 4 Thin layer chromatogram of Dipsaci Radix

impurities affecting the interference. After improving the extraction technology, we added methanol for sonication and then extracted n-butanol saturated with water for impurity removal, and finally washed with ammonia, the operation method indicates clear spots and less impurity interference (Fig. 4).

5 Conclusions

After further optimization design and verification, the TLC identification results of Strychni Semen, Rhei Radix et Rhizoma, Angelicae Sinensis Radix, and Dipsaci Radix constructed by us were clearly distinguished, and had no effect when compared with the negative control. The method is simple to operate and can be used as a qualitative identification technique for Wufang Babu Poultice. The preparation process is also compliant with pharmacopoeia regulations. In future, we will further carry out in-depth research on content detection to provide more comprehensive and reliable technical support for the quality of Wufang Babu Poultice products.

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