Establishment of a Microbial Limit Test Method for Wuteng Qufeng Zhitong Powder

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Abstract [**Objectives**] To establish a microbial limit test method for Wuteng Qufeng Zhitong Powder, ensuring its compliance with the relevant requirements of the *Chinese Pharmacopoeia* 2020 Edition. [**Methods**] In accordance with the *Chinese Pharmacopoeia* 2020 Edition, General Chapters Part IV, 1105 and 1106, method suitability tests were conducted for the total aerobic microbial count (TAMC), total yeasts and molds count (TYMC), and for the specified microorganisms (*Staphylococcus aureus*, *Pseudomonas aeruginosa*). Representative test strains, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, and *Aspergillus niger*, were employed in spiked recovery tests using the pour plate method to calculate the recovery ratio. Validation was performed on three batches of the product. [**Results**] The recovery ratios for all test strains fell within the range of 0.5 to 2.0. In the specified microorganism tests, the corresponding strains were successfully detected in the test groups, indicating that the established test sample preparation method effectively neutralized the sample's potential antimicrobial activity. The microbial limit test results for all three batches complied with the specifications. [**Conclusions**] The established microbial limit test method is accurate, reliable, and suitable for the microbiological quality control of Wuteng Qufeng Zhitong Powder.

Key words Wuteng Qufeng Zhitong Powder, Microbial limit test, Methodology validation, Antimicrobial activity

1 Introduction

Traditional Chinese medicine (TCM) compound formulations possess complex compositions, and some active ingredients may exhibit inherent antimicrobial activity, which can interfere with the accuracy of microbial limit test results. As mandated by the *Chinese Pharmacopoeia*, it is essential to establish and validate test methods capable of eliminating or neutralizing this antimicrobial activity for such products[1-3]. Therefore, guided by the *Chinese Pharmacopoeia* 2020 Edition, General Chapters Part IV, this study systematically conducted method suitability research for the microbial limit testing of Wuteng Qufeng Zhitong Powder. The aim was to establish a scientific and standardized testing protocol, thereby providing a foundation for enhancing its quality standards.

2 Materials

2.1 Samples Wuteng Qufeng Zhitong Powder (specification: 10 g per bag) was provided by the Zhuang-Yao Medicine Preparation Center of Guangxi International Zhuang Medicine Hospital, batch numbers: 230701, 230702, 230703.

2.2 Test strains *Staphylococcus aureus* [CMCC(B) 26003], *Pseudomonas aeruginosa* [CMCC(B)10104], *Bacillus subtilis* [CMCC(B)63501], *Candida albicans* [CMCC(F)98001], *Aspergillus niger* [CMCC(F)98003]. All strains were purchased from the National Institutes for Food and Drug Control (NIFDC), China. Following revival in the laboratory, the third subculture generation was used for testing.

2.3 Major equipment Biological Safety Cabinet (Model BSC-1604 II A2, Sujing Suzhou Antai Air Technology Co., Ltd.); Vertical High-Pressure Steam Sterilizer (Model SQ510C, Chongqing Yamato Scientific Co., Ltd.); Mold Incubator (Model MJ-250-II, Shanghai Yiheng Scientific Instrument Co., Ltd.).

2.4 Media and diluents Tryptic Soy Agar (TSA) (Production Batch No.: 2304A17A10), Tryptic Soy Broth (TSB) (Production Batch No.: 230411A20), Sabouraud Dextrose Agar (SDA) (Production Batch No.: 230414A10), Sabouraud Dextrose Broth (SDB) (Production Batch No.: 1102045), Mannitol Salt Agar (MSA) (Production Batch No.: 1096991), Cetrimide Agar, Sterile pH 7.0 Sodium Chloride - Peptone Buffer. All media were produced by Guangdong Huankai Microbial Sci. & Tech. Co., Ltd. Media and diluents were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121 ℃ for 30 min prior to use.

3 Methods and results

3.1 Preparation of microbial suspensions Following the method described in the *Chinese Pharmacopoeia*, bacterial or spore suspensions of each test strain were individually prepared. Their concentrations were adjusted to approximately 1.0×10^4 CFU/mL using sterile pH 7.0 sodium chloride-peptone buffer, and the suspensions were set aside for use.

3.2 Preparation of the test solution A 10 g sample was weighed and added to sterile pH 7.0 sodium chloride-peptone

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buffer to make a total volume of 100 mL. The mixture was shaken vigorously to yield a homogeneous 1∶10 test solution.

3.3 Method suitability test for total aerobic microbial count (TAMC) and total yeasts and molds count (TYMC)

3.3.1 Preliminary test. A preliminary test was performed using the pour plate method, with *S. aureus* as the indicator strain for TAMC and *C. albicans* as the indicator strain for TYMC. Following the design of "Test Solution + Microbial Suspension" (Test Group), "Test Solution + Diluent" (Product Control Group), and "Diluent + Microbial Suspension" (Microbial Suspension Control Group), the corresponding media were poured into plates. After incubation, colonies were counted, and the recovery ratio was calculated as: [(Colony Count of Test Group − Colony Count of Product Control Group)/Colony Count of Microbial Suspension Control Group]. The results (Table 1) showed that when using the 1∶10 test solution (1 mL/plate), the recovery ratios for both indicator strains met the specified range of 0.5 to 2.0, preliminarily indicating the feasibility of the method.

Table 1 Results of recovery ratios in the preliminary test (n = 2)

Batch No.	Test strain	Dilution ratio 1 mL/plate	A	B	C	Recovery ratio
230701	*Staphylococcus aureus*	1∶10	85.0	0	55.5	0.65
	Candida albicans	1∶10	63.5	0	93.5	1.47

NOTE A. Mean value of bacterial suspension control group (CFU); B. Mean value of product control group (CFU); C. Mean value of test group (CFU). The same in Tables 2 − 3.

3.3.2 Formal validation test. Based on the conditions established in the preliminary test, three batches of samples were used to concurrently validate the TAMC method using the five specified test strains (*S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, *A. niger*) and the TYMC method using *C. albicans* and *A. niger*. The results (Tables 2 and 3) demonstrated that the recovery ratios for all test strains across the three batches fell within the range of 0.5 to 2.0, meeting the requirements of the *Chinese Pharmacopoeia*.

Table 2 Recovery ratios for TAMC in three batches of samples (n = 2)

Batch No.	Test strain	A	B	C	Recovery ratio
230701	*Pseudomonas aeruginosa*	87.0	0	84.5	0.98
	Staphylococcus aureus	41.0	0	42.0	1.02
	Bacillus subtilis	42.0	0	33.5	0.80
	Candida albicans	86.5	0	88.0	1.02
	Aspergillus niger	77.5	0	63.5	0.82
230702	*P. aeruginosa*	87.0	0	79.5	0.91
	S. aureus	41.0	0	44.5	1.09
	B. subtilis	42.0	0	37.5	0.89
	C. albicans	86.5	0	82.5	0.95
	A. niger	77.5	0	65.0	0.83
230703	*P. aeruginosa*	87.0	0	81.0	0.93
	S. aureus	41.0	0	45.0	1.10
	B. subtilis	42.0	0	40.5	0.96
	C. albicans	86.5	0	80.5	0.93
	A. niger	77.5	0	69.0	0.89

Table 3 Recovery ratios for TYMC in three batches of samples (n = 2)

Batch No.	Test strain	A	B	C	Recovery ratio
230701	*Candida albicans*	74.0	0	85.0	1.15
	Aspergillus niger	85.5	0	63.0	0.73
230702	*C. albicans*	74.0	0	90.5	1.22
	A. niger	85.5	0	57.5	0.67
230703	*C. albicans*	74.0	0	90.5	1.22
	A. niger	85.5	0	57.5	0.67

3.4 Method suitability test for specified microorganisms

3.4.1 Method suitability. Method suitability tests were conducted separately for *S. aureus* and *P. aeruginosa*. A 10 mL aliquot of the 1∶10 test solution was added to 100 mL of Tryptic Soy Broth (TSB). The test strain (≤100 CFU) was inoculated into this mixture (Test Group). A Product Control Group (without inoculum) and a Negative Control Group (without product or inoculum) were also set up. After incubation, portions of the enrichment broth were streaked onto Mannitol Salt Agar (MSA) plates (for *S. aureus*) or Cetrimide Agar plates (for *P. aeruginosa*). The results (Table 4) showed that the corresponding specified microorganisms were detected in the Test Groups, while no growth was observed in the Product Control Group or the Negative Control Group. This indicates that the conventional enrichment method using 100 mL TSB effectively neutralized the antimicrobial activity of the sample against these two specified microorganisms.

Table 4 Results of the method suitability test for specified microorganisms

Test item	Culture media	Test group	Product control group	Negative control
Staphylococcus aureus	Tryptic Soy Broth (TSB) Enrichment Liquid	−	−	−
	Mannitol Salt Agar Plate	+	−	−
Pseudomonas aeruginosa	Tryptic Soy Broth (TSB) Enrichment Liquid	+	−	−
	Cetrimide Agar Plate	+	−	−

NOTE '+' indicates positive detection or turbidity; '−' indicates negative detection or clarity. The same applies below.

3.4.2 Validation with three batches. The method established above was validated for specified microorganism testing using the three batches of samples. The results (Table 5) confirmed that for each batch, the test strains were detected in the inoculated test groups, while *S. aureus* and *P. aeruginosa* were not detected in the uninoculated product groups themselves, demonstrating the feasibility of the method.

Table 5 Validation results of specified microorganism testing for three batches of samples

Strain	Sample Batch No.	Test culture media	Test results		
			Test group	Product control group	Negative control
Staphylococcus aureus	230701	Tryptic Soy Broth (TSB)	–	–	–
		Mannitol Salt Agar Plate	+	–	–
	230702	Tryptic Soy Broth (TSB)	–	–	–
		Mannitol Salt Agar Plate	+	–	–
	230703	Tryptic Soy Broth (TSB)	–	–	–
		Mannitol Salt Agar Plate	+	–	–
Pseudomonas aeruginosa	230701	Tryptic Soy Broth (TSB)	+	–	–
		Cetrimide Agar Plate	+	–	–
	230702	Tryptic Soy Broth (TSB)	+	–	–
		Cetrimide Agar Plate	+	–	–
	230703	Tryptic Soy Broth (TSB)	+	–	–
		Cetrimide Agar Plate	+	–	–

3.5 Microbial limit testing of samples The established method was applied to perform comprehensive microbial limit testing on three batches of finished Wuteng Qufeng Zhitong Powder. Results (Table 6) showed that the total aerobic microbial counts (TAMC) and total yeasts and molds counts (TYMC) for all three batches were below the specified limits. Furthermore, *S. aureus* and *P. aeruginosa* were not detected. These results comply with the requirements of the *Chinese Pharmacopoeia* and relevant preparation standards.

Table 6 Microbial limit test results for three batches of Wuteng Qufeng Zhitong Powder

Batch No.	Total aerobic microbial count CFU/g	Total molds and yeasts count CFU/g	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	Conclusion
230701	$< 10^1$	$< 10^1$	Not detected	Not detected	Meet the specification
230702	$< 10^1$	$< 10^1$	Not detected	Not detected	Meet the specification
230703	$< 10^1$	$< 10^1$	Not detected	Not detected	Meet the specification

4 Discussion and conclusion

This study strictly adhered to the guiding principles of the *Chinese Pharmacopoeia* 2020 Edition to systematically establish and validate a microbial limit test methodology for Wuteng Qufeng Zhitong Powder. Employing a 1∶10 test solution in the pour plate method effectively neutralized the antimicrobial activity of the sample, as evidenced by the recovery test results for all five specified test strains meeting the acceptance criteria; the conventional enrichment method (adding 10 mL of test solution to 100 mL TSB) proved reliable for detecting *S. aureus* and *P. aeruginosa*, as confirmed by both method suitability tests and validation across three batches; the compliant results obtained from testing three batches of the finished product further substantiate the practicality and reliability of the established method.

In summary, this study successfully established a microbial limit test method for Wuteng Qufeng Zhitong Powder. This method is characterized by standardized operation and accurate results, enabling effective control of the microbiological quality of this preparation. It provides a scientific foundation for monitoring the production process, releasing finished products, and enhancing quality standards.

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