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**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

WS/T 685-2020

**Detection methods and evaluation requirements for antifungal
drugs in disinfectants and antibacterial agents
(English Translation)**

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

Drafting organizations of this standard. Shandong Provincial Center for Disease Control and Prevention, China Center for Disease Control and Prevention, Environmental and Health Related Product Safety Institute, Qingdao Food and Drug Inspection and Research Institute of Shandong Province, Jiangsu Province Center for Disease Control and Prevention, Shandong Province Dezhou Center for Disease Control and Prevention, Shandong Province Jining City Center for Disease Control and Prevention, Beijing Municipal Center for Disease Control and Prevention.

1 Scope

This standard specifies the testing methods and evaluation requirements for antifungal drugs in disinfectants and antibacterial agents.

This standard applies to griseofulvin, econazole nitrate, fluconazole, ketoconazole, clotrimazole, bifonazole, Determination and evaluation of miconazole nitrate, flucytosine, naftifine hydrochloride and other antifungal drugs.

2 Normative references

The following documents are indispensable for the application of this document. For dated reference documents, only the dated version applies to this document.

For undated references, the latest version (including all amendments) applies to this document.

GB/T 6682 Analytical laboratory water specifications and test methods Pharmacopoeia of the People's Republic of China

3 Terms and definitions

The following terms and definitions apply to this document.

Antifungal drugs

Drugs that have antibacterial and/or bactericidal properties and are used to prevent and treat fungal infections, including antifungal drugs and prescription drugs the rational chemical synthesis of drugs.

4.1 Principle

After the sample is ultrasonically extracted with 1% formic acid acetonitrile, the cream formulation sample needs to be purified by N-propyl ethylenediamine, and other formulation samples do not need to be purified Measured by ultra performance liquid chromatography-tandem mass spectrometer, and quantified by external standard method.

4.2 Reagents and materials

Unless otherwise stated, the reagents used are all chromatographically pure, and the experimental water is the first grade water specified in GB/T 6682.

4.2.1 Reagents

4.2.1.1

Acetonitrile (CH_3CN).

4.2.1.2

Formic acid (HCOOH).

4.2.1.3

N-propyl ethylenediamine ($\text{C}_5\text{H}_{14}\text{N}_2$).

4.2.2 Reagent preparation

4.2.2.1 0.1% formic acid aqueous solution. take 1.0 mL of formic acid, dilute to 1000 mL with water, and mix.

4.2.2.2 1% formic acid aqueous solution. take 10.0 mL of formic acid, dilute to 1000 mL with water, and mix.

4.2.2.3 0.1% formic acid acetonitrile solution. take 1.0 mL of formic acid, dilute to 1000 mL with acetonitrile, and mix.

4.2.2.4 1% formic acid acetonitrile solution. take 10.0 mL of formic acid, dilute to 1000 mL with acetonitrile, and mix.

4.2.2.5 0.1% formic acid acetonitrile aqueous solution. Take 300 mL of acetonitrile, add 1.0 mL of formic acid, dilute to 1000 mL with water, and mix.

4.2.3 Standard products

Griseofulvin, ketoconazole, clotrimazole, econazole nitrate, miconazole nitrate, fluconazole, bifonazole, flucytosine, naphthalene hydrochloride Fen has a purity greater than 98.5% or a standard substance certified by the country and granted a standard substance certificate. For other relevant information, see Appendix A.

4.2.4 Standard solution preparation

4.2.4.1

Standard stock solution (1000 µg/mL). Weigh 50.0 mg (accurate to 0.1 mg) of the standard respectively, and dissolve it with a small amount of acetonitrile.

After the solution, transfer to a 50 mL volumetric flask, add acetonitrile to dissolve and dilute to the mark, and mix well, and the mass concentration will be 1000 µg/mL the standard stock solution, stored at -20°C±2°C, is valid for 3 months.

4.2.4.2

Mixed standard intermediate solution. Take an appropriate amount of the above standard stock solution and use acetonitrile to prepare the ketoconazole mass concentration of 10 µg/mL, the mass concentration of pyrimidine is 5 µg/mL, the mass concentration of fluconazole, griseofulvin, and bifonazole is 1 µg/L, econazole nitrate, miconazole nitrate, the mixed standard intermediate solution of naftifine hydrochloride and clotrimazole with a mass concentration of 0.5 µg/mL, stored in cold storage and protected from light, is valid for 1 month.

4.2.4.3

Mixed standard series solution. draw different volumes of mixed standard intermediate solution, and use the blank sample extract to prepare the appropriate quality concentration the standard working solution of ketoconazole is 10 µg/L, 25 µg/L, 50 µg/L, 100 µg/L, 150 µg/L, 200 µg/L.

The mass concentration of flucytosine is 5 µg/L, 12.5 µg/L, 25 µg/L, 50 µg/L, 75 µg/L, 100 µg/L, fluconazole, griseofulvin, the mass concentration of bifonazole is 1 µg/L, 2.5 µg/L, 5 µg/L, 10 µg/L, 15 µg/L, 20 µg/L, econazole nitrate, miconazole nitrate the mass concentrations of azole, naftifine hydrochloride and clotrimazole are 0.5 µg/L, 1.25 µg/L, 2.5

µg/L, 5 µg/L, 7.5 µg/L, and 10 µg/L, which are prepared for immediate use.

4.2.5 Materials

Organic filter membrane. pore size is 0.22 µm.

4.3 Apparatus and equipment

4.3.1

Ultra-high performance liquid chromatography-tandem mass spectrometer. charged electrospray ion source (ESI source).

4.3.2

Analytical balance. the smallest division value is 0.1 mg.

4.3.3

Centrifuge. speed ≥ 9000 r/min.

4.3.4

Vortex mixer.

4.3.5

Ultrasonic cleaner.

4.4.1.1 Purification treatment of cream dosage form samples

Weigh 0.5 g (accurate to 1 mg) of sample into a 15 mL centrifuge tube, add 8.0 mL of 1% formic acid acetonitrile, vortex for 1 min, add 50 mg N-propyl ethylene diamine, ultrasonic extraction for 30 min, centrifugation (9000 r/min) for 5 min, transfer the supernatant to a 10 mL volumetric flask, 1% ethyl formic acid Nitrile is made to volume. After mixing, take 1.0 mL and place it in a 10 mL volumetric flask, dilute to the mark with 0.1% formic acid acetonitrile aqueous solution, mix and transfer to Centrifuge the tube (9000r/min) for 5 minutes, take the supernatant and filter it through the membrane for use.

4.4.1.2 Sample processing of other dosage forms

Weigh 0.5 g (accurate to 1 mg) of the sample into a 15 mL centrifuge tube, add 8.0 mL of 1% formic acid acetonitrile, vortex for 1 min, and ultrasonically extract After 30 minutes, centrifuge (9000 r/min) for 5 minutes, transfer the supernatant to a 10 mL volumetric flask, and dilute to the mark with 1% formic acid acetonitrile. Take after mixing Place 1.0 mL in a 10 mL volumetric flask, dilute the volume to the mark with the initial mobile phase, transfer to a centrifuge tube after mixing, and centrifuge (9000 r/min) for 5 min.

Take the supernatant and filter it through the membrane for use.