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

WS/T 684-2020

**Detection methods and evaluation requirements of antibacterial
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. Jiangsu Provincial Center for Disease Control and Prevention, China Center for Disease Control and Prevention, Environmental and Health Related Product Safety Institute, Beijing Municipal Center for Disease Control and Prevention, Jiangsu Institute of Food and Drug Inspection, China Pharmaceutical University, National Center for Food Safety Risk Assessment, Shandong Provincial Center for Disease Control and Prevention, Xuzhou Center for Disease Control and Prevention, Zhongda Hospital affiliated to Southeast University.

1 Scope

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

This standard applies to tetracyclines (tetracycline, oxytetracycline, chlortetracycline, minocycline, doxycycline) in disinfectants and antibacterial agents, Macrolides (erythromycin), beta-lactams (cefradine), sulfa (sulfadiazine), quinolones (ofloxacin, norfloxacin) Determination and evaluation of lincosamide (clindamycin), chloramphenicol (chloramphenicol) and other antibacterial 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 article. 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.

Antibacterial drugs

Drugs that have antibacterial and/or bactericidal activity and are used to prevent and treat bacterial infections, including antibiotics and prescription drugs. Learn synthetic drugs.

4.1 Principle

The antibacterial drugs in the sample are ultrasonically extracted with methanol or acetonitrile. After the extract is filtered, it is mixed with water and used by ultra-high performance liquid chromatography-tandem mass spectrometry.

Measured by instrument and quantified by internal standard method.

4.2 Reagents and materials

Unless otherwise specified, 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 (CH₃CN).

4.2.1.2

Formic acid (HCOOH).

4.2.1.3

Methanol (CH₃OH).

4.2.1.4

Sodium chloride (NaCl). analytically pure.

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

Methanol aqueous solution. Take 800 mL of methanol, add.200 mL of water, and mix well.

4.2.4 Standard solution preparation

4.2.4.1

Standard stock solution (about 1.0 mg/mL). accurately weigh tetracycline, oxytetracycline, chlortetracycline, minocycline, doxycycline, About 10.0 mg of erythromycin, cefradine, sulfadiazine, ofloxacin, norfloxacin, clindamycin, and chloramphenicol in a 10 mL volumetric flask, Dissolve it with methanol and make it to the mark, prepare a standard stock solution with a concentration of approximately 1.0 mg/mL, and store it in a frozen state at $-20^{\circ}\text{C}\pm 2^{\circ}\text{C}$. The validity period is 3 months.

4.2.4.2

Mixed standard intermediate solution (10 $\mu\text{g/mL}$). accurately draw appropriate amounts of each single standard stock solution into a 25 mL volumetric flask, and use A Dilute with alcohol and make the volume up to the mark, prepare a mixed standard intermediate solution with a concentration of 10 $\mu\text{g/mL}$, store it in cold storage and avoid light, and the validity period is 1 month.

4.2.4.3

Internal standard compound standard stock solution (about 1.0 mg/mL). Weigh tetracycline-D6 and oxytetracycline-C22- respectively N2, minocycline-D6, doxycycline-D3, sulfadiazine-D6, cephalexin-D5, levofloxacin-D8, erythromycin-C-D3, Thiabendazole-D4, chloramphenicol-D5 about 10.0 mg in a 10 mL volumetric flask, dissolved in methanol and dilute to the mark, Prepare an internal standard stock solution with a concentration of about 1.0 mg/mL, at -20°C Frozen storage at $\pm 2^{\circ}\text{C}$, valid for 6 months.

4.2.4.4

Standard intermediate solution of mixed internal standard compound. draw appropriate amount of single internal standard stock solution into 10 mL volumetric flask and dilute with methanol and dilute to the mark, prepare a 1 $\mu\text{g/mL}$ mixed internal standard intermediate solution, store in refrigeration and avoid light, the validity period is 3 months.

4.2.4.5

Mixed standard solution. use methanol aqueous solution to prepare the standard intermediate solution of antibacterial drugs into a mixed standard series solution, such as 1 $\mu\text{g/L}$, 2 $\mu\text{g/L}$, 5 $\mu\text{g/L}$, 10 $\mu\text{g/L}$, 20 $\mu\text{g/L}$, 50 $\mu\text{g/L}$, 100 $\mu\text{g/L}$, the mixed internal standard

compound concentration in each series of solutions is 10 µg/L, Ultra performance liquid chromatography-tandem mass spectrometer determination. Now equipped for temporary 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. Sensitivity 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 Liquid dosage form

Weigh 0.2 g (accurate to 1 mg) of a well-mixed sample, place it in a 15 mL centrifuge tube, and add 10.0 µL of mixed internal standard compound standard Quasi-intermediate solution, add 3.0 mL methanol, vortex for 1 min to disperse the sample, ultrasonically extract for 10 min, add 0.15 g sodium chloride, vortex to mix, Centrifuge at 9000 r/min for 5 minutes, aspirate the supernatant, filter with a membrane, take 0.8 mL of filtrate into the sample bottle, add 0.2 mL of water, and mix well.

For ultra performance liquid chromatography-tandem mass spectrometry determination.

4.4.1.2.1 Gel dosage form

Weigh 0.2 g (accurate to 1 mg) of a well-mixed sample, place it in a 15 mL centrifuge tube, and add 10.0 µL of mixed internal standard compound standard Quasi-intermediate solution, add 3.0 mL acetonitrile, vortex for 1 min to disperse the sample, ultrasonically extract for 10 min, centrifuge at 9000 r/min for 5 min, and aspirate the supernatant Liquid, filter membrane, take 0.8 mL of filtrate into the sample bottle, then add 0.2 mL of water, mix well, for ultra performance liquid chromatography-tandem mass spectrometry