GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

ICS 67.050

X 04

GB/T 20756-2006

Method for the Determination of Chloramphenicol, Thiamphenicol, and Florfenicol Residues in Edible Animal Muscles, Liver and Aquatic Products – LC-MS-MS Method

可食动物肌肉、肝脏和水产品中氯霉素、

甲砜霉素和氟苯尼考残留量的测定 液相色谱－串联质谱法

Issued on: December 31, 2006 **Implemented on: March 01, 2007**

Issued by: General Administration of Quality Supervision, Inspection and Quarantine;

Standardization Administration of the People's Republic of China.

Table of Contents

Method for the Determination of Chloramphenicol, Thiamphenicol, and Florfenicol Residues in Edible Animal Muscles, Liver and Aquatic Products – LC-MS-MS Method

1 Scope

This Standard specifies the LC-MS-MS method for the determination of chloramphenicol, thiamphenicol and florfenicol residues in edible animal muscle, liver, fish and shrimp.

This Standard applies to the determination of chloramphenicol, thiamphenicol and florfenicol residues in edible animal muscle, liver, fish and shrimp.

The detection limit of this Standard method: 0.1μg/kg for chloramphenicol, and 1.0μg/kg for thiamphenicol and florfenicol.

2 Normative References

The provisions in following documents become the provisions of this Standard through reference in this Standard. For dated references, the subsequent amendments (excluding corrigendum) or revisions do not apply to this Standard, however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 6379.1 Accuracy (trueness and precision) of measurement methods and results - Part 1: General principles and definitions (GB/T 6379.1-2004, ISO 5725-1:1994, IDT)

GB/T 6379.2 Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method (GB/T 6379.2-2004, ISO 5725-2:1994, IDT)

GB/T 6682 Water for laboratory use – Specifications (GB/T 6682-1992, neq ISO 3696:1987)

3 Principle

Chloramphenicol, thiamphenicol and florfenicol in the sample are extracted with ethyl acetate

under alkaline conditions. After the extract was spun to dryness; the residue is dissolved in water and degreased by n-hexane liquid-liquid partitioning. Take liquid chromatography-tandem mass spectrometer for detection.

4 Reagents

Unless otherwise specified, the used reagents are of analytically pure; and the water is the first-grade water specified in GB/T 6682.

4.1 Methanol: chromatographically pure.

4.2 Ethyl acetate

4.3 n-hexane.

4.4 Ammonium hydroxide: 25% ~ 28%.

4.5 Anhydrous sodium sulfate: burn at 650 °C for 4 h; and store in a desiccator for later-use.

4.6 Standard substances of chloramphenicol, thiamphenicol and florfenicol: with purity ≥99.5%.

4.7 Internal standard solution of Chloramphenicol D5: 100μg/mL.

4.8 Standard stock solution: 100μg/mL. Accurately weigh appropriate amounts of standard substances of chloramphenicol, thiamphenicol and florfenicol, respectively; and use methanol to make a standard stock solution of 100μg/mL. The solution can be stored at -18 °C and can be used for 1 year.

4.9 Mixed standard stock solution: 1μg/mL. Accurately pipette 1 mL of chloramphenicol, thiamphenicol and florfenicol standard stock solution (4.8) respectively into a 100 mL volumetric flask; and dilute to the mark with methanol. The solution can be stored at -18°C and can be used for 6 months.

4.10 Intermediate concentration mixed standard solution: 20ng/mL. Accurately pipette 1 mL of the mixed standard stock solution (4.9) in a 50 mL volumetric flask and dilute with water to the mark. The solution can be stored at 4°C and can be used for 3 months.

4.11 Internal standard stock solution: 1μg/mL. Accurately pipette 100μL of chloramphenicol-D5 (d_5-chloramphenicol) standard solution (4.7) into a 10 mL volumetric flask; and dilute to the mark with methanol. The solution is stored at -18°C and can be used for 6 months.

4.12 Intermediate concentration internal standard solution: 20ng/mL. Accurately pipette 1 mL of internal standard stock solution (4.11) into a 50 mL volumetric flask; and dilute to the mark with water. The solution is stored the at 4°C and can be used for 3 months.

4.13 Matrix mixed standard working solution: according to the sensitivity of each standard and

the linear range of the instrument, pipette a certain amount of intermediate concentration mixed standard solution (4.10) and intermediate concentration internal standard solution (4.12); and use the blank sample extract to prepare a series of matrix mixed standard working solutions; and the internal standard concentration is 0.3ng/mL. It shall be prepared in the same day.

4.14 Filter membrane: 0.2μm.

5 Instruments

5.1 Liquid chromatography-tandem mass spectrometer: equipped with an electrospray ion source.

5.2 Analytical balance: with sensitivity of 0.1 mg and 0.01 g.

5.3 Centrifuge: 4000 r/min.

5.4 High-speed desktop centrifuge: 13000 r/min.

5.5 Tissue masher.

5.6 Homogenizer.

5.7 Rotary evaporator.

5.8 Ultrasound.

5.9 Liquid mixer.

5.10 Polypropylene centrifuge tubes: 50mL, 1.5mL, with stoppers.

5.11 Heart-shaped bottle: 25mL.

5.12 Colorimetric tube: 50mL, with stopper.

6 Preparation and Storage of Specimen

6.1 Preparation of specimen

Take a sample of about 500g; grind it with a meat tissue grinder; put it into a clean container as a specimen; seal it; and mark it.

6.2 Storage of specimen

Store the specimen in a refrigerator at -18°C.

7 Test Procedures

7.1 Extraction

Weigh 5g of specimen, accurate to 0.01g. Place it in a 50 mL polypropylene centrifuge tube; add 75.0μL of intermediate concentration internal standard solution (4.12); add 15 mL of ethyl acetate; add 0.45 mL of ammonium hydroxide (4.4); and add 5 g of anhydrous sodium sulfate; and extract homogeneously for 30 s; centrifuge at 4000 r/min for 5 min; and transfer the supernatant to a 50 mL colorimetric tube. Take another 50 mL centrifuge tube; add 15 mL of ethyl acetate and 0.45 mL ammonium hydroxide; wash the homogeneous knife head for 10 s; transfer the washing liquid into the first centrifuge tube; stir the residue with a glass rod; and place it in a liquid mixer (5.9) to vortex for 1 min; ultrasonically extract for 5 min; centrifuge at 4000 r/min for 5 min; and put the supernatant into a 50 mL colorimetric tube. Add 15 mL of ethyl acetate to the residue; repeat the above operation; combine all supernatants into a 50 mL colorimetric tube; and make constant volume to 50 mL with ethyl acetate. After shaking well, pipette 10 mL of ethyl acetate extract into a 25 mL heart-shaped bottle; and concentrate to dryness at 45°C with rotation.

7.2 Purification

Dissolve the residue in the heart-shaped bottle with 3 mL of water; sonicate for 5 min; add 3 mL of n-hexane and vortex mix for 30 s; let stand to separate layers; discard the upper layer of n-hexane. Then add 3 mL of n-hexane and vortex mix for 30 s; after standing and separating layers, pipette 1 mL of the aqueous phase into a 1.5 mL polypropylene centrifuge tube; centrifuge at 1 3000 r/min for 5 min; pass through a 0.2μm filter membrane; and use it for liquid chromatography-tandem mass spectrometry.

7.3 Chromatographic determination

7.3.1 Liquid chromatography conditions

a) Chromatographic column: Discovery C_{18} chromatographic column; 5μm, 150 mm × 2.1 mm (inner diameter) or equivalent;

b) Column temperature: 40°C;

c) Mobile phase: methanol + water (40 + 60);

d) Flow rate: 0.30 mL/min;

e) Injection volume: 20μL.

7.3.2 Mass spectrometry conditions

a) Ion source: electrospray ion source;

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