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

GB 31658.5-2021

**National Food Safety Standard Determination of Florfenicol and
Florfenicol Residues in Animal Foods Liquid
Chromatography-Tandem Mass Spectrometry**

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China, State Administration for Market Regulation**

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Foreword

This document complies with the provisions of GB/T 1:1-2020 "Standardization Work Guidelines Part 1: Structure and Drafting Rules of Standardization Documents"

Drafting:

This document is published for the first time:

National food safety standards

Determination of florfenicol and florfenicolamide residues in animal foods

Liquid chromatography-tandem mass spectrometry

1 Scope

This document specifies sample preparation and liquid chromatography-tandem mass spectrometry for the detection of florfenicol and florfenicolamine residues in animal foods:

method:

This document is applicable to the determination of florfenicol and florfenicol amide residues in muscle, skin + fat, liver and kidney tissue of chicken, pig, cattle and sheep:

Determination:

2 Normative reference documents

The contents of the following documents constitute essential provisions of this document through normative citations in the text: Among them, the cited documents with dates are:

Only the version corresponding to the date applies to this document; for undated referenced documents, the latest version (including all amendments) applies to this document:

document:

GB/T 6682 Specifications and test methods for water used in analytical laboratories

3 Terms and definitions

There are no terms or definitions that need to be defined in this document:

4 Principles

The remaining florfenicol and florfenicolamide in the sample were extracted with alkalized ethyl acetate, degreased with n-hexane, purified with a solid phase extraction column, and liquid chromatographed:

Determination by spectrum-G tandem mass spectrometry and quantification by internal standard method:

5 Reagents and materials

Unless otherwise specified, all reagents are of analytical grade and the water is first-grade water that complies with GB/T 6682:

5.1 Reagents

5.1 Acetonitrile (CH_3CN): chromatographically pure:

5.1:2 Methanol (CH_3OH): chromatographically pure:

5.1:3 Formic acid (HCOOH): chromatographically pure:

5.1:4 Ethyl acetate ($\text{CH}_3\text{COOC}_2\text{H}_5$):

5.1:5 Ammonia ($\text{NH}_3\cdot\text{H}_2\text{O}$):

5.1:6 Acetic acid (CH_3COOH):

5.2 Solution preparation

5.2:1 5% acetic acid aqueous solution: Take 5mL of acetic acid, dissolve it in water and dilute it to 100mL:

5.2: 10% ammonia-methanol solution: Take 5 mL of ammonia water, dissolve it in methanol and dilute it to 50 mL: Prepare it now:

5.2:3 30% acetonitrile aqueous solution: Take 30mL of acetonitrile, dissolve it in water and dilute it to 100mL:

5.2:4 Ethyl acetate-ammonia solution: Take 98mL of ethyl acetate, add 2mL of ammonia,

and mix:

5:3 Standard products

5:3:1 Florfenicol (Florfenicol, $C_{12}H_{14}Cl_2FNO_4S$, CAS number: 73231-34-2): content $\geq 99.0\%$:

5: Florfenicolamine ($C_{10}H_{14}FNO_3S$, CAS number: 76639-93-5): Content $\geq 97.6\%$:

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5: Deuterated chloramphenicol (Chloramaphenicol D5, d5-CAP, CAS number: 202480-68-0): content is 100ng/mL:

5:4 Preparation of standard solution

5:4:1 Florfenicol and florfenicol amine standard stock solutions: Take 25 mg of florfenicol and florfenicol amine standard solutions, weigh them accurately, and add acetonitrile appropriately:

Dissolve and dilute to a 25mL volumetric flask to prepare standard stock solutions of florfenicol and florfenicolamine respectively: Store below $-18^{\circ}C$:

Valid for 6 months:

5:4:2 10 $\mu g/mL$ mixed standard working solution: Precisely measure 1 mL each of florfenicol and florfenicol amine standard stock solutions, and place in 100 mL

Dilute the volumetric flask with acetonitrile to the mark to make a mixed standard working solution with a concentration of 10 $\mu g/mL$: Store it at $2^{\circ}C \sim 8^{\circ}C$ and is valid for one month:

5:4:3 1 $\mu g/mL$ mixed standard working solution: Precisely measure 1mL of 10 $\mu g/mL$ mixed standard working solution, put it in a 10mL volumetric flask, and add acetonitrile to it:

Dilute to the mark to make a mixed standard working solution with a concentration of 1 $\mu g/mL$: Store at $2^{\circ}C \sim 8^{\circ}C$ and is valid for 1 month:

5:5 Materials

MCX solid phase extraction column 60mg/3mL, or equivalent:

6 Instruments and equipment

6:1 Ultra-high performance liquid chromatography-tandem mass spectrometer: equipped with electrospray ion source:

6:2 Analytical balance: sensitivity 0:00001g and 0:01g:

6: High speed centrifuge:

6: Vortex mixer:

6:5 horizontal oscillator:

6: Homogenizer:

6:7 Solid phase extraction device:

6: 8 Nitrogen blowing instrument:

6:9 Filter membrane: organic phase, 0:22 μ m:

6:10 chicken heart bottle: 100mL:

7: Preparation and preservation of samples

7:1 Preparation of samples

Take an appropriate amount of fresh or thawed blank or test tissue, mince it, and homogenize it:

- a) Take a homogeneous test sample as a test material;
- b) Take a homogeneous blank sample as a blank sample;
- c) Take a homogeneous blank sample, add standard working solution of appropriate concentration, and add the sample as a blank:

7:2 Preservation of samples

Store below -18 degrees C:

8 Measurement steps

8:1 Extraction

Weigh 2g of the sample (accurate to ± 0.02 g), put it into a 50mL centrifuge tube, add 100 μ L of deuterated chloramphenicol internal standard solution, and vortex to mix:

Mix well and let stand for 15 minutes: Add 10 mL of ethyl acetate-ammonia solution, vortex for 1 minute, centrifuge at 3000 r/min for 5 minutes, and take the supernatant:

In a 100 mL chicken heart bottle, the residue was repeatedly extracted twice, and the three extracts were combined: Add 2 mL of 5% acetic acid to the chicken heart bottle, shake and mix, and

Concentrate to 1:5 mL in a 40°C water bath: Transfer to another 50 mL centrifuge tube, wash the chicken heart bottle with 2 mL of 5% acetic acid, and transfer the washing solution to

In the same centrifuge tube, add 5 mL of n-hexane for defatting, vortex for 1 min, and centrifuge at 3000 r/min for 5 min: Discard the upper layer and repeat the degreasing of the lower layer extract: