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

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**National food safety standards-Determination of residues of
amidoalcohol drugs and their metabolites in animal
foods-liquid chromatography-tandem mass spectrometry**

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Table of Contents

foreword

1 Scope

2 Normative references

3 Terms and Definitions

4 principles

5 Reagents and materials

5.1 Reagents

5.2 Solution preparation

5.3 Standards

5.4 Preparation of standard solution

100 µg/mL internal standard stock solution. Stored below -18 degrees C, valid for 12 months.

5.5 Materials

6 Instruments and equipment

6.1 Liquid chromatography-tandem mass spectrometer. with electrospray ionization source (ESI).

6.2 Balance. Sensitivity 0.00001g and 0.01g.

6.3 Homogenizer.

6.4 Vortex mixer.

6.5 Multi-tube vortex shaker.

6.6 High-speed refrigerated centrifuge. the speed can reach 8000r/min.

6.7 Nitrogen blowing instrument.

7 Preparation and storage of samples

7.1 Preparation of samples

7.2 Storage of samples

8 Measurement steps

8.1 Extraction

8.2 Purification

8.3 Preparation of standard curve

8.4 Determination

8.5 Blank test

9 Calculation and presentation of results

10 Method sensitivity, accuracy and precision

10.1 Sensitivity

10.2 Accuracy

10.3 Precision

foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1. Structure and Drafting Rules for Standardization Documents" drafting.

This document is published for the first time.

National Food Safety Standards

Determination of residues of amidoalcohol drugs and their metabolites in food of animal origin

Liquid chromatography-tandem mass spectrometry

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry for the detection of amide alcohols and their metabolite residues in animal foods.

method.

This document is applicable to muscle, liver, kidney, and adipose tissue of pigs, chickens, cattle, and sheep, as well as chloramphenicol, thiamphenicol, and chloramphenicol in eggs, milk, and goat milk.

Determination of florfenicol and florfenicol amine residues.

2 Normative references

The content in the following documents constitutes the essential provisions of this document through normative references in the text. Among them, the dated reference documents,

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

document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Terms and Definitions

This document does not have terms and definitions that need to be defined.

4 principles

The residual chloramphenicol, thiamphenicol, florfenicol and florfenicol amine in the sample were extracted with 2% ammoniated ethyl acetate solution, degreased with n-hexane,

Hydrogenated ethyl acetate was back extracted, determined by liquid chromatography-tandem mass spectrometry, and quantified by internal standard method.

5 Reagents and materials

Unless otherwise specified, all reagents are of analytical grade, and the water is first-class water in accordance with GB/T 6682.

5.2 Solution preparation

5.2.1 2% ammoniated ethyl acetate solution. Take 20mL ammonia water and dilute it to 1000mL with ethyl acetate.

5.2.2 4% sodium chloride solution. Take 4g of sodium chloride, dissolve it in water and dilute to 100mL.

5.2.3 4% sodium chloride saturated n-hexane. Take an appropriate amount of 4% sodium chloride solution, add excess n-hexane, mix, let stand to separate layers, take the upper layer of n-hexane

Hexane.

5.2.4 20% methanol solution. Take 20mL of methanol and dilute with water to 100mL.

5.2.5 10mmol/L ammonium formate solution. Take 0.63g of ammonium formate, dissolve it in water and dilute to 1000mL.

5.3 Standards

Chloramphenicol, thiamphenicol, florfenicol, florfenicol amine, the content of which was $\geq 99\%$, chloramphenicol-D5, thiamphenicol-D3, florfenicol-D3, fluorfenicol

Benicolamide-D3 internal standard, the contents were all $\geq 98\%$, see Appendix A for details.

5.4 Preparation of standard solution

5.4.1 Standard stock solution. Take an appropriate amount of each standard substance of

chloramphenicol, thiamphenicol, florfenicol, and florfenicol amine (equivalent to about 10mg), accurately weighed, respectively add appropriate amount of methanol to dissolve and dilute to a volume of 100mL volumetric flask, and prepare a concentration of 100µg/mL Standard stock solution. Store below -18°C, valid for 12 months.

5.4.2 Internal standard stock solution. take appropriate amount of internal standard of chloramphenicol-D5, thiamphenicol-D3, florfenicol-D3, florfenicol amine-D3 (equivalent to each active

(about 1 mg of active ingredients), accurately weighed, add appropriate amount of methanol to dissolve and dilute to volume into a 10mL volumetric flask, and prepare the concentration of

100 µg/mL internal standard stock solution. Stored below -18 degrees C, valid for 12 months.

5.4.3 Mixed standard intermediate solution. Accurately measure 0.1 mL of chloramphenicol standard stock solution, thiamphenicol, florfenicol, and florfenicol amine standard solution respectively.

0.5mL of each stock solution was diluted to the mark with methanol in a 10mL volumetric flask, and the concentration of chloramphenicol was 1 µg/mL.

Benicol and florfenicol amine concentration was 5 µg/mL mixed standard intermediate solution. Stored below -18 degrees C, valid for 3 months.

5.4.4 Mixed internal standard intermediate solution. Accurately measure 0.1 mL of internal standard stock solution of chloramphenicol-D5, thiamphenicol-D3, florfenicol-D3,

Benicolamine-D3 internal standard stock solution, 0.5 mL each, was diluted with methanol to the mark in a 10 mL volumetric flask, and the concentration of chloramphenicol-D5 was prepared as

1 µg/mL, thiamphenicol-D3, florfenicol-D3, and florfenicol amine-D3 at a concentration of 5 µg/mL mixed internal standard intermediate solution.

deposit, valid for 3 months.

5.4.5 Mixed internal standard working solution. Take the mixed internal standard intermediate solution and dilute it with 20% methanol solution to make the concentration of chloramphenicol-D5 10ng/mL, methylsulfone

The concentrations of mymycin-D3, florfenicol-D3 and florfenicol amine-D3 were 50 ng/mL mixed internal standard working solution, which was ready for immediate use.