

CCS X 22



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

GB 31613.4-2022

**National Food Safety Standard Determination of Perilimycin
Residues in Bovine Edible Tissues by Liquid
Chromatography-Tandem Mass Spectrometry**

Issued on: September 20, 2022

Implemented on: February 1, 2023

**Issued by: National Health Commission of the People's Republic of
China, State Administration for Market Regulation**

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

5.5 Materials

6 Instruments and equipment

6.2 Analytical balance. Sensitivity 0.01g and 0.00001g.

6.3 Vortex mixer.

6.4 High speed centrifuge.

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 matrix-matched 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

800 µg/kg, and fat 10 µg/kg~200 µg/kg, the recoveries were 60%~110%.

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 Pirinomycin Residues in Bovine Edible Tissues by Liquid Chromatography-Tandem Mass Spectrometry

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry method for the detection of pilimycin residues in bovine edible tissues.

This document is applicable to the determination of pilithromycin residues in beef muscle, liver, kidney and fat.

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 pyrilimycin in the sample was extracted with 5% formic acid acetonitrile solution, purified by solid phase extraction column, and determined by liquid chromatography-tandem mass spectrometry.

Quantification by matching standard solution external 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 5% formic acid in acetonitrile solution. Take 5 mL of formic acid and dilute to 100 mL with acetonitrile.

5.2.2 0.1% formic acid in acetonitrile solution. Take 1 mL of formic acid and dilute it to 1000 mL with acetonitrile.

5.2.3 0.1% formic acid solution. Take 1 mL of formic acid and dilute it with water to 1000 mL.

5.2.4 80% methanol solution. Take 80 mL of methanol and dilute with water to 100 mL.

5.3 Standards

Pirimycin (Pirimycin, C₁₇H₃₁ClN₂O₅S, CAS number. 79548-73-5), content ≥99.0%.

5.4 Preparation of standard solution

5.4.1 Standard stock solution. Accurately weigh about 10 mg of standard pirinomycin, put it in a 10 mL volumetric flask, dissolve it with methanol and dilute to the mark.

Prepare the standard stock solution of pirinomycin with a concentration of 1 mg/mL, store it below -18 degrees C, and have a validity period of 12 months.

5.4.2 Standard intermediate solution. Accurately measure 0.1 mL of standard stock solution of pirinomycin, put it in a 10 mL volumetric flask, dilute with methanol to the mark, and prepare

A standard intermediate solution of pirimycin with a concentration of 10 µg/mL was prepared, stored at 2°C to 8°C, and valid for 6 months.

GB31613.4-2022

5.4.3 Standard working solution. Accurately measure 0.1 mL of the standard intermediate solution of pirinomycin, put it in a 10 mL volumetric flask, and dilute it with 80% methanol to the mark.

The standard working solution of pirimycin with a concentration of 100 ng/mL was prepared and stored at 2 degrees C to 8 degrees C, and the validity period was 6 months.

5.5 Materials

5.5.1 Solid-phase extraction column. matrix-enhanced fat removal column (300mg/3mL), or

equivalent.

6 Instruments and equipment

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

7.1 Preparation of samples

Take an appropriate amount of fresh or thawed blank or test sample, mince and homogenize.

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

7.2 Storage of samples

Store below -18°C.

8.1 Extraction

Weigh 2 g of the sample (accurate to ± 0.05 g) into a 50 mL centrifuge tube, add ceramic homogenizers, add 10 mL of 5% formic acid acetonitrile solution,

Vortex for 30 s, add 4 g of anhydrous sodium sulfate and 1 g of sodium chloride, vortex for 30 s, centrifuge at 8000 r/min for 8 min at 4 °C, and quickly pipette the upper layer B.

Add 2.4 mL of nitrile to another centrifuge tube, add 0.6 mL of water, mix well, and set aside.

8.2 Purification

Pass the spare liquid directly through the solid phase extraction column, flow out naturally, collect the filtrate, and squeeze it dry.

Spectrometer-tandem mass spectrometry.

8.3 Preparation of matrix-matched standard curve

Precisely measure an appropriate amount of standard working solution of pyriithycin, and dilute it with the extracted and purified blank sample solution to contain drug concentrations of

1 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/mL, 100 ng/mL and 400 ng/mL matrix-matched series of