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# NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA



# GB 31659.5-2022

## National food safety standards-Determination of rifaximin residues in milk by liquid chromatography-tandem mass spectrometry

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## 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 rifaximin residues in milk 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 rifaximin residues in milk.

This document applies to the determination of rifaximin residues in milk.

## 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 are only

The version corresponding to the date applies to this document; for undated references, the latest version (including all amendments) applies to this 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 rifaximin in the sample was extracted with acetonitrile, purified with a solid phase extraction column, and determined by liquid chromatography-tandem mass spectrometry.

Quantification by solution external standard method.

### 5.1 Reagents

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

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

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

### 5.3 Standards

Rifaximin (Rifaximin, C43H51N3O11, CAS number. 80621-81-4), content >= 94.0%.

### 5.4 Preparation of standard solution

5.4.1 Standard stock solution. take about 10mg of rifaximin standard substance, weigh it accurately, dissolve it in a 10mL volumetric flask with acetonitrile and dilute to the mark.

The standard stock solution of rifaximin with a concentration of 1.0 mg/mL was prepared and stored below -18°C, and the validity period was 6 months.

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

Prepare a standard intermediate solution with a concentration of 10 μg/mL and store it at 2°C to 8°C, with a validity period of 3 months.

5.4.3 Standard working solution. Accurately measure 0.1mL of rifaximin standard intermediate solution, put it in a 10mL volumetric flask, dilute it with 20% acetonitrile solution to

The standard working solution with a concentration of 100ng/mL was prepared and stored at 2°C to 8°C, with a validity period of 3 months.

## 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, and mix well.

a) Take the mixed test sample as the test sample;

b) Take the mixed blank sample as the blank sample;

c) Take the mixed 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), put it in a 50 mL centrifuge tube, add 8 mL of acetonitrile, add 2 g of anhydrous sodium sulfate, vortex

Afterwards, shake horizontally at.200 rpm for 10 min and centrifuge at 6000 r/min for 8 min, take the supernatant and set aside.

### 8.2 Purification and concentration

After the hydrophilic-lipophilic solid-phase extraction column was activated with 5 mL of acetonitrile, the spare liquid was passed through the column and connected to a 15-mL glass tube, and then 3 mL of acetonitrile was used to

They were eluted together in the same glass tube, squeezed dry, and blown dry at 50°C with nitrogen.

After microporous membrane filtration, it was tested on the machine.



### 8.3 Preparation of matrix-matched standard curve

To the residue of the blank sample after extraction, purification, and nitrogen blow-drying, sequentially add the concentration prepared with 20% acetonitrile solution

1.0 mL each of 10 ng/mL, 20 ng/mL, 50 ng/mL, 100 ng/mL, and.200 ng/mL series of standard solutions were fully dissolved to obtain matrix

Match the standard solution, and measure it on the machine after filtering the membrane. Take the characteristic ion mass chromatographic peak area as the ordinate, and the concentration of the matrix matching standard solution as the abscissa

to draw a standard curve.

#### 8.4.1 Reference conditions for liquid chromatography

a) Chromatographic column. C18 (50mmx2.1mm, 1.7μm), or equivalent.

b) Mobile phase. A is 0.1% formic acid acetonitrile solution, B is 0.1% formic acid solution. Gradient elution program. 0min~3min, 30%A

Change linearly to 80% A; 3min~4.5min maintain 30%A.

c) Flow rate. 0.3mL/min.

d) Injection volume. 10 μL.