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# NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA



# GB 31659.1-2021

## National food safety standard--Determination of xylazine residue in milk by liquid chromatography -tandem mass spectrometric method

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

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## Foreword

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

This document is published for the first time:

## 1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry determination methods for the detection of xylazine residues in milk:

This document is suitable for the qualitative and quantitative detection of xylazine residues in milk:

## 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 residual xylazine in milk was extracted with acetonitrile and protein precipitated, purified with a solid phase extraction column, measured by liquid chromatography-tandem mass spectrometry, and matrix matched:

Quantification by external standard method with standard solution:

## 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

5:1:1 Methanol (CH3OH): chromatographically pure:

5:1:2 Acetonitrile (CH3CN): chromatographically pure:

5:1:3 Concentrated ammonia (NH3:H2O):

5:1:4 Formic acid (HCOOH):

5:2 Preparation of solution

5:2:1 2% formic acid in methanol solution: Take 2mL of formic acid, add methanol to dilute to 100mL, mix well, and get it:

5:2:2 0:5% ammonia solution: Take 0:5 mL of concentrated ammonia solution, add water to dilute to 100 mL, mix well, and it is ready:

5:2:3 20% acetonitrile solution: Take:200mL of acetonitrile, add water to dilute to 1000mL, mix well, and it is ready:

5:2:4 Mobile phase A: 0:1% formic acid in acetonitrile solution: Take 0:1 mL of formic acid, add acetonitrile to dilute to 100 mL, and mix well:

5:2:5 Mobile phase B: 0:1% formic acid solution: Take 0:1mL of formic acid, add water to dilute to 100mL, mix well, and get it:

5:3 Standard products

Xylazine (C12H16N2S, CAS number: 7361-61G7), content >= 98:0%:

5:4 Preparation of standard solutions

5:4:1 Standard stock solution (1mg/mL): Take about 10mg of xylazine standard, weigh it

accurately, dissolve it in acetonitrile and dilute it to a 10mL volumetric flask, shake it well, and store it below -18 degrees C: The validity period 3 months:

5:4:2 Standard intermediate solution (10 μg/mL): Precisely measure 100 μL of the above standard stock solution, put it into a 10 mL volumetric flask, add acetonitrile to dilute to the mark, shake well, and get it: Store at 2 degrees C~8 degrees C, valid period 1 months:

5:4:3 Standard working solution (100ng/mL): Precisely measure 100μL of the above standard intermediate solution, put it into a 10mL volumetric flask, add 20% acetonitrile solution to dilute to the mark, shake well, and store at 2 degrees C~8 degrees C , valid for 1 month:

5:5 Materials

5:5:1 Solid phase extraction column: HLB (60mg/3mL), or equivalent:

5:5:2 Microporous filter membrane: 0:22μm:

## 6 Instruments and equipment

6:1 Liquid chromatography-tandem mass spectrometer: equipped with electrospray ion source:

6:2 Balance: sensitivity 0:01g:

6:3 Analytical balance: sensitivity 0:00001g:

6:4 Vortex mixer:

6:5 Oscillator:

6:6 Centrifuge:

6:7 Nitrogen blower:

6:8 Ultrasonic instrument:

7: Preparation and preservation of samples

7:1 Preparation of samples

Sample preparation includes:

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

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 degrees C:

## 8 Measurement steps

8:1 Extraction

Weigh 2 (+/-0:02) g of the sample, add 8 mL of acetonitrile, vortex and mix for 20 s, oscillate and extract for 5 min, centrifuge at 8000 r/min for 5 min at 5°C, put the supernatant into a 10mL centrifuge tube, and concentrate in a 40°C water bath under nitrogen flow: to less than 2 mL, add 4 mL of 0:5% ammonia solution, vortex to mix, and set aside:

8:2 Purification

Activate the solid phase extraction column with 3 mL of methanol, 3 mL of water, and 3 mL of 0:5% ammonia solution in sequence: Pass all the reserve solution through the column and add 3 mL of water:

Rinse, vacuum dry, and elute with 3 mL of 2% formic acid in methanol solution: Dry the eluate with nitrogen in a 40°C water bath, add 0:5 mL of 20% acetonitrile solution to the residue, vortex, and dissolve with ultrasonic for 5 min: After filtration through a 0:2 μm filter membrane, it was analyzed by liquid chromatography-tandem mass spectrometer:

8:3 Preparation of matrix matching standard curve

Precisely measure an appropriate amount of standard working solution and dilute it with 20% acetonitrile solution to 0:5ng/mL, 1:0ng/mL, 2:0ng/mL,

A series of working solutions of 5:0ng/mL, 10ng/mL, 20ng/mL, and 50ng/mL: After the blank milk sample was extracted, purified, and dried with nitrogen, it was dissolved in 0:5mL of the above series of standard working solutions to make a series of Concentration matrix matching standard solution: Use liquid chromatography-tandem mass spectrometry

For measurement, take the characteristic ion mass chromatographic peak area of the analyte as the ordinate and the concentration of the matrix-matched standard solution as the abscissa, draw a standard working curve, and calculate the linear equation and regression coefficient:

8:4 Determination

8:4:1 Liquid chromatography reference conditions

a) Chromatographic column: C18 chromatographic column (50mmx2:1mm, 1:7μm) or equivalent;

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b) Mobile phase: A is 0:1% formic acid acetonitrile solution, B is 0:1% formic acid solution;

c) Flow rate: 0:3mL/min;