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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31658.3-2021

National food safety standard - Determination of baclofen residue in swine urine by liquid chromatography-tandem mass spectrometry method

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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 baclofen residues in pig urine by liquid chromatography-tandem mass spectrometry

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry methods for the determination of baclofen residues in pig urine:

This document is applicable to the detection of baclofen residues in pig urine:

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 baclofen in the sample was acidified, extracted, purified with a solid phase extraction column, measured by liquid chromatography-tandem mass spectrometry, and quantified by the 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 (CH3CN): chromatographically pure:

5:1:2 Methanol (CH3OH): chromatographically pure:

5:1:3 Formic acid (HCOOH): chromatographically pure:

5:1:4 Hydrochloric acid (HCl):

5:1:5 Ammonia (NH3:H2O):

5:1:6 Sodium hydroxide (NaOH):

5:2 Solution preparation

5:2:1 10% hydrochloric acid solution: Take 10mL of hydrochloric acid and dilute it with water to 100mL:

5: 2: 0:1% formic acid solution: Take 0:5 mL of formic acid and dilute to 500 mL with water:

5:2% formic acid solution: Take 0:2mL of formic acid and dilute it with water to 100mL:

5:2% formic acid-acetonitrile solution: Take 80mL of 0:2% formic acid solution and add acetonitrile to dilute it to 100mL:

5:2:5 5% ammonia-methanol solution: Take 5 mL of ammonia water and dilute it with methanol to 100 mL:

5:2:6 1mol/L sodium hydroxide solution: Take 4g of sodium hydroxide, dissolve it in water and dilute it to 100mL:

5:3 Standard products

5:3:1 Baclofen (Baclofen, C10H12ClNO2, CAS number: 1134-47-0) content >= 99:0%:

5:3:2 Baclofen-D4: content >=99:0%:

5:4 Preparation of standard solution

5:4:1 Baclofen standard stock solution: Take about 10 mg of baclofen standard, weigh it accurately, and add 1 mL of 1 mol/L sodium hydroxide solution to dissolve it:

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Solution, dilute with methanol to a 10mL volumetric flask to prepare a baclofen standard stock solution with a concentration of 1mg/mL: Store below 4°C to be effective:

Period of 4 months:

5:4:2 Baclofen standard intermediate solution (10μg/mL): Precisely measure 100μL of baclofen standard stock solution, put it in a 10mL volumetric flask, and dilute with methanol

Release to the mark and prepare a baclofen standard intermediate solution with a concentration of 10 μg/mL: Store below 4°C and has a validity period of 1 month:

5:4:3 Baclofen standard working solution (1μg/mL): Precisely measure 1mL of the baclofen standard intermediate solution, dilute it with methanol to a volume of 10mL

bottle, prepare a standard working solution of baclofen with a concentration of 1 μg/mL: Prepare it for immediate use:

5: Baclofen-D4 stock solution (1 mg/mL): Take about 10 mg of baclofen-D4 reference substance, weigh it accurately, and add 1 mol/L sodium hydroxide solution

Dissolve in 1 mL, dilute with methanol to the volume of 10 mL volumetric flask, and prepare a baclofen-GD4 stock solution with a concentration of 1 mg/mL:

Store it under the following conditions and it is valid for 4 months:

5.

Release it into a baclofen-D4 intermediate solution with a concentration of 10 μg/mL: Store below 4°C and has a validity period of 1 month:

5:4:6 Baclofen-D4 working solution (1μg/mL): Precisely measure 1mL of baclofen-D4 intermediate solution, place it in a 10mL volumetric flask, and dilute with methanol

Release into baclofen-D4 working solution with a concentration of 1 μg/mL: Prepare now for immediate use:

5:4:7 Mix standard working solution: Precisely measure appropriate amounts of Baclofen standard working solution and Baclofen-D4 working solution, and dissolve them with 0:2%

formic acid-acetonitrile:

The liquid was prepared to contain baclofen-D410ng/mL, and the baclofen-containing concentrations were 1:0ng/mL, 2:0ng/mL, 5:0ng/mL, 10:0ng/mL, and

25:0ng/mL and 50:0ng/mL series mixed standard working solutions: Prepared and ready for use:

5:5 Materials

Mixed strong cation solid phase extraction column, 60mg/3mL, or equivalent:

6 Instruments and equipment

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

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

6: Centrifuge: 8000r/min:

6: Vortex oscillator:

6:5 Solid phase extraction device:

6:6 Nitrogen blower:

6:7 pH meter:

6: Centrifuge tube: 50mL:

6:9 Filter membrane: 0:22μm, water phase:

7: Preparation and preservation of samples

7:1 Preparation of samples

Take an appropriate amount of fresh or thawed blank or test sample, return to room temperature before use, mix well, if there is turbidity, centrifuge and take the supernatant for later use:

a) Take spare test samples as test materials;

b) Take a spare blank sample as blank material;

c) Take the spare blank sample, add the standard working solution of appropriate concentration, and add the sample as a blank:

7:2 Preservation of samples

Test samples or blank samples should be frozen and stored below -18°C as soon as possible: