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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31658.13-2021

National food safety standard - Determination of robenidine residues in animal derived food by liquid chromatography-tandem mass spectrometric method

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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 detection methods for the detection of chlorpheniramine residues in animal foods:

This document is applicable to the determination of chlorpheniramine residues in chicken muscle, liver, kidney and skin + fat tissue:

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 chlorphenanthrene in the sample was extracted with 0:1% formic acid and acetonitrile, purified with a solid phase extraction column, and measured by liquid chromatography-tandem mass spectrometry, using the internal standard method:

Quantitative:

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 Acetonitrile (CH3CN): chromatographically pure:

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

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

5:1:4 n-Hexane (C6H14):

5:1:5 Dimethyl sulfoxide (C2H6OS):

5:2 Solution preparation

5:2:1 0:1% formic acid in acetonitrile solution: Take 0:1mL of formic acid and dilute to 100mL with acetonitrile:

5:2:2 85% acetonitrile solution: Take 85mL of acetonitrile and dilute to 100mL with water:

5:2:3 0:1% formic acid aqueous solution: Take 0:1mL of formic acid and dilute to 100mL with water:

5:2:4 0:3% formic acid aqueous solution: Take 0:3 mL of formic acid and dilute to 100 mL with water:

5:2:5 50% acetonitrile formic acid aqueous solution: Take 50 mL of acetonitrile and dilute to 100 mL with 0:3% formic acid aqueous solution:

5:3 Standard products

5:3:2 Robenidine-D8hydrochloride (C15H6Cl3D8N5, CAS number: 1173097-77-2): content >=99:0%:

5:4 Preparation of standard solution

5:4:1 Standard stock solution of chlorpheniramine: Take an appropriate amount of chlorpheniramine hydrochloride standard (equivalent to 10 mg of chlorpheniramine), weigh it accurately, add an appropriate amount of methanol to dissolve and dilute it to a 10mL volumetric flask, and prepare it to a concentration It is a standard stock solution of 1 mg/mL: Stored below -18°C, it is valid for 6 months:

5:4:2 Chlorbenguanide standard working solution: Accurately measure 1 mL of chlorbenguanide standard stock solution, place it in a 100 mL volumetric flask, dilute to the mark with methanol, and prepare a chlorbenguanide standard working solution with a concentration of 10 μg/mL: Store at 2 degrees C~8 degrees C, valid for 3 months:

5:4:3 Chlorbenguanil-D8 internal standard stock solution: Take an appropriate amount of chlorbenguanide-D8 standard (equivalent to 10 mg of chlorbenguanil-D8), add an appropriate amount of dimethyl sulfoxide to dissolve, dilute with methanol to a constant volume: In a 10mL volumetric flask, prepare a chlorpheniramine-D8 internal standard stock solution with a concentration of 1 mg/mL: Store below -18°C and is valid for 6 months:

5:4:4 Chlorbenguanil-D8 internal standard intermediate solution: Accurately measure 1 mL of chlorbenguanil-D8 internal standard stock solution, place it in a 100 mL volumetric flask, dilute it to the mark with methanol, and prepare a chlorine solution with a concentration of 10 μg/mL: Benzene-D8 internal standard intermediate solution: Store at 2 degrees C~8 degrees C, valid for 1 month:

5:4:5 Chlorbenguanil-D8 internal standard working solution: Accurately measure 1 mL of chlorbenguanil-D8 internal standard intermediate solution, place it in a 10 mL volumetric flask, dilute it to the mark with methanol, and prepare a chlorine solution with a concentration of 1 μg/mL: Phenylguanidine-D8 internal standard working solution: Ready for use:

5:5 Materials

1) The HLB solid-phase extraction columns listed here are only for reference and are not for commercial purposes: Standard users are encouraged to try solid-phase extraction columns of different manufacturers or models:

5:5:1 HLB solid phase extraction column 1)::200mg/3mL, or equivalent:

5:5:2 Neutral alumina solid-phase extraction column: 500mg/3mL, or equivalent:

5:5:3 Nylon microporous filter membrane: 0:22μm:

5:5:4 Centrifuge tube: 50mL:

5:5:5 Graduated test tube: 10mL, graduation value 0:1mL:

6 Instruments and equipment

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

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

6:3 Low-temperature high-speed centrifuge: the speed can reach 8000r/min:

6:4 Tissue homogenizer:

6:5 Vortex mixer:

6:6 Solid phase extraction device:

6:7 Nitrogen blower:

7: Preparation and preservation of samples

7:1 Preparation of samples

Take fresh or thawed blank or test tissue, mince it, and homogenize it:

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

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 degrees C:

8 Measurement steps

8:1 Extraction

Take 2g of the sample (accurate to +/-0:02g), put it in a 50mL centrifuge tube, add accurately 100μL of chlorpheniramine-D8 internal standard working solution, add 5mL of 0:1% formic acid acetonitrile solution, vortex to mix, and oscillate at medium speed for 10 minutes: Centrifuge at 8000r/min for 10min, take the supernatant, add 1mL of water, mix well, and use it as backup solution 1:

8:2 Purification

Take a neutral alumina solid-phase extraction column, activate it with 2 mL of acetonitrile, pass the standby solution 1 through the column, collect all the effluent in a 10 mL test tube, and use

Elute with 2 mL of 85% acetonitrile solution, combine the effluents, blow with nitrogen at 40°C until the volume is less than 2 mL, add 8 mL of water, mix well, and use it as a backup solution: 2: Take the HLB solid phase extraction column and activate it with 3 mL of