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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31658.12-2021

National food safety standard - Determination of cyromazine in animal derived food by high performance liquid chromatographic method

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Table of Contents

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:

National food safety standards

Determination of cypromethine residues in animal foods

HPLC

1 Scope

This document specifies the sample preparation and high-performance liquid chromatography determination methods for the detection of cipropromazine residues in animal foods:

This document is applicable to the detection of cipropromazine residues in sheep and poultry muscles, fat, liver and sheep kidney tissues:

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 cypromethazine in the sample was extracted with trichloroacetic acid/acetonitrile solution, purified with a mixed cation exchange solid phase extraction column, and subjected to high performance liquid chromatography:

Determination, external standard method quantification:

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):

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

5:1:3 Hydrochloric acid (HCl):

5:1:4 Ammonium acetate (CH3CONH3):

5:1:5 Acetic acid (CH3COOH):

5:1:6 n-Hexane (C6H14):

5:1:7 Ammonia (NH3H2O):

5:1:8 Trichloroacetic acid (CCl3COOH):

5:2 Solution preparation

5:2:1 25mmol/L ammonium acetate solution: Take 0:19g of ammonium acetate, add 950mL of water to dissolve, adjust the pH to 5:0 with acetic acid, and add water to dilute to 1000mL:

5:2:2 1% trichloroacetic acid solution: Take 1g of trichloroacetic acid, dissolve it in water and dilute it to 100mL:

5:2:3 Extraction solution: Take 15 mL of 1% trichloroacetic acid solution and dilute to 100 mL with acetonitrile:

5:2:4 0:1mol/L hydrochloric acid solution: Take 9mL of hydrochloric acid and dilute it to 1000mL:

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

5:2:6 Mobile phase: Take 40:0 mL of 25 mmol/L ammonium acetate solution and adjust the volume to 1000 mL with acetonitrile:

5:3 Standard products

Cyromazine (Cyromazine, C6H10N6, CAS number: 66215-27-8), content >= 97:0%:

5:4 Preparation of standard solutions

5:4:1 Standard stock solution (1 mg/mL): Accurately weigh the cipropromazine standard equivalent to 25 mg of cipropromazine, dissolve it with methanol and dilute it to a 25 mL volumetric flask, and prepare a concentration of 1 mg/mL: mL standard stock solution: Store below -18 degrees C, valid for 1 year:

5:4:2 Standard working solution (10 mg/L): Precisely measure 1 mL of the standard stock solution, put it in a 100 mL volumetric flask, dilute it to the mark with methanol, and prepare a standard working solution of ciprofloxacin with a concentration of 10 mg/L: - Stored below 18 degrees C, the validity period is 6 months:

5:5 Materials

5:5:1 Mixed cation exchange solid phase extraction column: 60mg/3mL, or equivalent:

5:5:2 Universal filter membrane for syringe filter: nylon material, pore size 0:45μm or equivalent performance:

6 Instruments and equipment

6:1 High performance liquid chromatograph: equipped with UV detector:

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

6:3 Nitrogen blowing instrument:

6:4 Solid phase extraction device:

6:5 Homogenizer:

6:6 Vortex mixer:

6:7 Centrifuge: the maximum speed is not less than 10000r/min:

6:8 pH meter:

6:9 Rotary evaporator:

6:10 Separating funnel: 100mL:

6:11 Chicken heart bottle: 100mL:

6:12 Graduated centrifuge tube: graduation value is 0:1mL:

7 Preparation and preservation of specimens

7:1 Preparation of specimens

Take an appropriate amount of fresh or thawed blank or test tissue, mince it, and homogenize it:

a) Take a homogeneous test sample as a test material;

b) Take a homogeneous blank sample as a blank sample;

c) Take a homogeneous blank sample, add 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 5g of the sample (accurate to +/-0:02g), put it in a 50mL centrifuge tube, add 15mL of the extraction solution, homogenize at high speed to evenly disperse, centrifuge at 5000r/min for 5min, take the supernatant into a 100mL separatory funnel, and add the residue to Extract 10 mL, repeat the extraction once, combine the supernatants, add 30 mL of n-hexane, shake for 2 minutes, and let stand for stratification: Collect the lower liquid in a 100 mL chicken heart bottle, and rotary evaporate it in a 50°C water bath to 1 mL (the vacuum needs to be controlled (to prevent bumping), transfer to a 10 mL graduated centrifuge tube, rinse the chicken heart bottle with the extract twice, 2 mL each time, combine the extracts, centrifuge at 10,000 r/min for 5 min, take the supernatant and set aside:

8:2 Purification

The solid-phase extraction column was activated with 3 mL of methanol and 3 mL of water in sequence: Pass the reserve solution through the column and control the flow rate to 1 mL/min: Elute with 3 mL of methanol, 3 mL of 0:1 mol/L hydrochloric acid solution, 3 mL of water, and 3 mL of methanol in sequence, and drain: Elute with 5 mL of ammonia and