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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31658.2-2021

National Food Safety Standard Determination of Chloramphenicol Residues in Animal Foods Liquid Chromatography-Tandem Mass Spectrometry

Issued on: September 16, 2021 **Implemented on: February 1, 2022**

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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 chloramphenicol residues in animal foods

Liquid chromatography-tandem mass spectrometry

1 Scope

This document specifies sample preparation and liquid chromatography-tandem mass spectrometry for the detection of chloramphenicol residues in pig, chicken muscles, livers and edible tissues of fish and shrimp:

Determine the method:

This document is applicable to the determination of chloramphenicol residues in muscles, livers of pigs and chickens, and edible tissues of fish and shrimps:

2 Normative reference documents

The contents of the following documents constitute essential provisions of this document

through normative citations in the text: Among them, referenced 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:

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

For residual chloramphenicol in the sample, meta-chloramphenicol or deuterated chloramphenicol was used as the internal standard, followed by deproteinization with acetonitrile, 4% sodium chloride, and n-hexane:

Degreasing, ethyl acetate extraction, solid phase extraction column purification, nitrogen blow-drying, liquid chromatography-tandem mass spectrometry determination, and internal 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 Acetonitrile (CH3CN): chromatographically pure:

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

5:1:3 n-Hexane (C6H14):

5:1:4 Ethyl acetate (CH3COOC2H5):

5:1:5 Sodium chloride (NaCl):

5:2 Standard products

5: Chloramphenicol (CAP, Chloramphenicol, C11H12Cl2N2O5, CAS number: 56-75-7), content >= 98:0%:

5.

No:: 202480-68-0), content >= 98:0%:

5:3 Solution preparation

5:3:1 4% sodium chloride solution: Take 4g of sodium chloride, dissolve in water and dilute

to 100mL, prepare before use:

5:3:2 Water-saturated ethyl acetate solution: Take 400mL of ethyl acetate, put it in a 500mL brown reagent bottle, add 50mL of water, cover, shake and let stand:

Place and take the upper solution when using:

5:4 Preparation of standard solution

5:4:1 Chloramphenicol standard stock solution: Take about 10 mg of chloramphenicol, weigh it accurately, dissolve it with appropriate amount of methanol and sonicate it, and dilute it to 100 mL:

Volumetric flask, make a standard stock solution with a concentration of 100 μg/mL: Store at -18°C, valid for 1 year:

5:4:2 Internal standard stock solution: Take 10 mg of the internal standard, weigh it accurately, dissolve it with an appropriate amount of methanol by ultrasonic and dilute it to a 100 mL volumetric flask to make a concentrated solution:

Internal standard stock solution with a concentration of 100 μg/mL: Store at -18°C and is valid for 1 year:

5:4:3 Chloramphenicol standard working solution: Precisely measure an appropriate amount of chloramphenicol standard stock solution and dilute it with mobile phase to a concentration of 10ng/mL:

50ng/mL standard working solution: Store at 4°C and is valid for 3 months:

5.

Save, valid for 3 months:

5:5 Materials

C18 solid phase extraction column: 500mg/3mL, or equivalent:

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 Vortex mixer:

6: Tissue homogenizer:

6: Low temperature centrifuge:

6:6 Sample concentrator:

6:7 Solid phase extraction device:

7: Preparation and preservation of samples

7:1 Preparation of samples

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 Preservation of samples

Store below -18 degrees C:

8 Measurement steps

8:1 Extraction

Take 5g of the sample (accurate to +/-0:02g), add 250μL of internal standard working solution, 5mL of acetonitrile, and 5mL of 4% sodium chloride solution, and vortex

Centrifuge for 2 minutes at 4000 r/min for 10 minutes, take the supernatant, repeat the extraction of the residue once, and combine the supernatants: Add 5 mL of n-hexane and vortex:

Centrifuge for 1 min at:2000 r/min for 10 min: Discard the supernatant and repeat the treatment with n-hexane once: Add 5 mL of water-saturated ethyl acetate solution and vortex:

Rotate for 1 min, centrifuge at:2000 r/min for 10 min, transfer the supernatant liquid to a 15 mL centrifuge tube, repeat the extraction once, combine the extracts, and use nitrogen:

Blow dry, add 3 mL of water-acetonitrile (95:5) to dissolve, and set aside:

8:2 Purification

Take the solid-phase extraction column and pre-wash it with 10 mL of methanol and 10 mL of water in sequence: Pass the backup solution through the column, add water to wash the column 2 times, 3 mL each time, and add mobile phase:

4 mL, elute at 1 mL/min, collect the eluate: Add 4 mL of water-saturated ethyl acetate solution, vortex for 1 min,:2000 r/min

Centrifuge for 5 minutes, take the supernatant liquid, repeat the process once, combine the supernatant solutions, and blow dry with nitrogen: Add 1:0 mL of mobile phase to dissolve,