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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

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National food safety standards-Determination of anticoccidial drug residues in chicken edible tissues-liquid chromatography-tandem mass spectrometry

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foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1.Structure and Drafting Rules for Standardization Documents"

drafting.

This document is published for the first time.

National Food Safety Standards

Determination of anticoccidial drug residues in chicken edible tissues by liquid chromatography-tandem mass spectrometry

1 Scope

This document regulates the levels of hemosanone, chlorhexenidine, salinomycin, monensin, methyl salinomycin, maduramicin ammonium and lasaloxil in the edible tissues of chickens

Sample preparation and liquid chromatography-tandem mass spectrometry method for the detection of seven kinds of coccidial drug residues.

This document is applicable to poultry muscle, liver and sebum (skin + fat) containing ketone, chlorhexenidine, salinomycin, monensin, methyl salinomycin, and madomi

Determination of residues of seven coccidiostats including startrimonium and lasaloxil.

2 Normative references

The content in the following documents constitutes the essential provisions of this document through normative references in the text. Among them, the dated reference documents,

Only the version corresponding to the date applies to this document; for undated references, the latest version (including all amendments) applies to this document

document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Terms and Definitions

This document does not have terms and definitions that need to be defined.

4 principles

The residual coccidiostats in the sample were hydrolyzed by trypsin, extracted with ethyl acetate, purified by solid phase extraction column, and detected by liquid chromatography-tandem mass spectrometry.

Quantification by matrix-matched external standard method.

5 Reagents and materials

Unless otherwise specified, all reagents are of analytical grade, and the water is first-class water in accordance with GB/T 6682.

5.2 Solution preparation

5.2.1 10% sodium carbonate solution. Take 20g of sodium carbonate, add appropriate amount of water to dissolve and dilute to.200mL, mix well.

5.2.2 1% acetic acid solution. Take 1 mL of acetic acid, dilute it with water to 100 mL, and mix well.

5.2.3 20% methanol solution. Take 20mL of methanol, dilute to 100mL with water, and mix well.

5.3 Standards

The content of fushenone, chlorhexidine, salinomycin, monensin, methyl salinomycin, maduromycin ammonium, and lasaloxil was >=95%. See Appendix A for details.

5.4 Preparation of standard solution

5.4.1 Standard stock solution (1 mg/mL). take hemosanone, chlorhexenidine, salinomycin, monensin, methyl salinomycin, madomicin ammonium, lasalol

An appropriate amount of western standard substance (equivalent to 10 mg of each active ingredient) was accurately weighed, and an appropriate amount of methanol was added to dissolve and dilute to a volume of 10 mL.

The standard stock solution with a concentration of 1 mg/mL was prepared and stored in the dark below -18 degrees C, and the validity period was 6 months.

5.4.2 Mixed standard working solution (10μg/mL). Accurately measure 0.1mL of each standard stock solution, put it in a 10mL volumetric flask, dilute with methanol

Diluted to the mark, and prepared into a mixed standard working solution with a concentration of 10 μg/mL. Stored in the dark below -18°C, the validity period is 6 months.

5.4.3 Mixed standard working solution (1μg/mL). Accurately measure 1mL of 10μg/mL mixed standard working solution in a 10mL volumetric flask,

Dilute to the mark with methanol, and prepare a mixed standard working solution with a concentration of 1 μg/mL. Store in the dark below -18°C, and the validity period is 3 months.

5.4.4 Mixed standard working solution (0.1μg/mL). Accurately measure 1mL of mixed standard working solution of 1μg/mL in a 10mL volumetric flask.

Dilute with methanol to the mark, and prepare a mixed standard working solution with a concentration of 0.1 μg/mL. Store in the dark below -18°C, and the validity period is 1 month.

7.1 Preparation of samples

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

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

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°C.

8.1 Extraction

Weigh 1 g of the sample (accurate to +/-0.01 g), add 25 mg of trypsin, 5 mL of water, vortex for 1 min, and adjust the pH with 10% sodium carbonate solution.

to 7.5, 40°C water bath overnight for enzymatic hydrolysis. Take it out and let it cool to room temperature, add 1mL of 10% sodium carbonate solution, vortex for 1min, add 9mL of ethyl acetate, vortex

Rotate for 5 min, centrifuge at 4°C and 10000r/min for 10 min, transfer the supernatant, and repeat the extraction once for the residue.

Dilute the ester to 20.0mL and mix well. Take 2.0mL of the extract and dry it with nitrogen in a water bath at 35°C, add 1mL of acetonitrile and vortex for 1min, add water

10mL, mix well, set aside.

8.2 Purification

The solid-phase extraction column was activated with 5 mL of methanol and 5 mL of 1% acetic acid solution in turn, and all the spare liquid was passed through the column, and the