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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 31613.2-2021

**National food safety standard--Determination of tylvalosin and
3-acetyltylosin residues in swine and chicken tissues by liquid
chromatography-tandem mass spectrometry method**

食品安全国家标准 猪、鸡可食性组织中泰万菌素和3-乙酰泰乐菌素残留量的测定
液相色谱-串联质谱法

(English Translation)

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Table of Contents

- 1 Scope
- 2 Normative reference documents
- 3 Terms and definitions
- 4 Principles
- 5 Reagents and Materials
- 6 Instruments and equipment
- 7 Preparation and preservation of samples
- 8 Measurement steps
- 9 Result calculation and presentation
- 10 Sensitivity, accuracy and precision of detection methods

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry methods for the detection of tivalisin and 3-acetyltylosin residues in edible tissues of pigs and chickens:

This document is applicable to the determination of tivalisin and 3-acetyltylosin residues in pig, chicken sebum, muscle, liver, kidney, and eggs:

2 Normative reference documents

The contents of the following documents constitute essential provisions of this document through normative references in the text: Among them, for dated reference documents, only the version corresponding to the date applies to this document; for undated reference 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 to be defined in this document:

4 Principles

The remaining tyversin and 3-acetyl tylosin in the sample were extracted with 50% acetonitrile, purified with n-hexane and acidic alumina, measured by liquid chromatography-tandem mass spectrometry, and quantified using the matrix-matched

standard solution external 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

5.1.1

Acetonitrile (CH_3CN)

Chromatographically pure:

5.1.2

Formic acid (HCOOH)

Chromatographically pure:

5:1:3 n-Hexane (C_6H_{14}):

5.1.4

Anhydrous magnesium sulfate (MgSO_4):

5.1.5

Sodium chloride (NaCl):

5.2

Solution preparation 5:2:1 0.02 mol/L tartaric acid aqueous solution: Take 0.3g of tartaric acid and add 100mL of water to dissolve:

5:2:20.1% formic acid aqueous solution: Take 0.5 mL of formic acid and dissolve it in 500 mL of water:

5:2:3 50% acetonitrile tartaric acid solution: Take 50mL of acetonitrile, dilute it to 100mL with 0.02 mol/L tartaric acid aqueous solution, and mix well:

5.3

Standard products

5.3.1

Tylvalosin ($\text{C}_{22}\text{H}_{35}\text{NO}$, CAS number 63409-12-1), content $\geq 93.0\%$:

5:3:23-acetyltylosin (3-acetyltylosin, $\text{C}_{23}\text{H}_{37}\text{NO}_6$, CAS number: 63409-10-9), content $\geq 95.0\%$:

5.4

Preparation of standard solution

5.4.1

Tylvalisin standard stock solution (1mg/mL)

Take 10 mg of Tylvalisin reference substance, weigh it accurately, dissolve it in acetonitrile and dilute it to a 10mL brown volumetric flask, shake well, and it is ready: Stored below -18°C, valid for 6 months:

5:4:23-Acetyl Tylosin Standard Stock Solution (1 mg/mL): Take 10 mg of 3-Acetyl Tylosin reference substance, weigh it accurately, and place it in 10 mL of brown solution:

Color volumetric flask, dissolve with acetonitrile and dilute to volume, shake well: Stored below -18 °C, valid for 6 months:

5.4.3

Mixed standard working solution (10 µg/mL)

Accurately draw 1 mL of each of the standard stock solutions of tylvalisin and 3-acetyl tylosin into 100 mL:

Brown volumetric flask, dilute to volume with acetonitrile, shake well, and it is ready: Stored below -18°C, valid for 3 months:

5.5

Materials

5.5.1

Acid alumina

100 mesh to:200 mesh:

5.5.2

Microporous filter membrane

Nylon, 0:22µm:

6 Instruments and equipment

6.1

Liquid chromatography-tandem mass spectrometer

Equipped with electrospray ion source:

6.2

Analytical balance

Sensitivity 0:01g and sensitivity 0:00001 g:

6.3

Centrifuge tubes

50 mL, 10 mL:

6.4

Vortex mixer:

6:5 horizontal oscillator,

6.6

High-speed centrifuge

The maximum speed is not less than 8000 r/min:

7 Preparation and preservation of samples

7.1

Preparation of samples Take an appropriate amount of fresh or refrigerated blank or test sample, homogenize and set aside:

- 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 and add standard working solution of appropriate concentration as a blank self-added sample:

7.2

Storage of samples Store below -20°C:

8 Measurement steps

8.1

Extraction

Take 2g of the sample (accurate to ± 0.02 g) in a 50mL centrifuge tube, add 5.0 mL of water and 5.0 mL of acetonitrile, vortex and mix, add 10mL of n-hexane, shake for 5 minutes, then add 2g each of anhydrous magnesium sulfate and sodium chloride: Shake for 5 minutes, centrifuge at 8000 r/min for 5 minutes, and transfer the middle acetonitrile layer to another centrifuge tube for later use:

8.2

Purification

Accurately pipette 0.5mL of the reserve solution and 0.5mL of the 0.02 mol/L tartaric acid solution, totaling 1.0mL: Add 0.2g of acidic alumina, vortex and mix for 30 s, centrifuge at 10000 r/min for 5 min, take the upper layer, pass through a 0.22 μ m microporous filter, and