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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31656.2-2021

National food safety standard - Determination of Tylosin residues in aquatic products by high performance liquid chromatography

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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 Tylosin Residues in Aquatic Products by High Performance Liquid Chromatography

1 Scope

This document specifies the sample preparation and high-performance liquid chromatography determination methods for the detection of tylosin residues in aquatic products:

This document is applicable to the determination of tylosin residues in edible tissues of fish, shrimp, crab, soft-shell turtle and other aquatic products:

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

GB/T 30891-2014 Sampling specifications for aquatic products

3 Terms and definitions

There are no terms or definitions that need to be defined in this document:

4 Principles

The remaining tylosin in the sample was extracted with ethyl acetate, defatted with n-hexane, and purified with an SCX solid-phase extraction column under weakly alkaline conditions:

Determination by liquid chromatography and quantification by external standard method:

5 Reagents and materials

The reagents used below are all analytically pure reagents unless otherwise noted; 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 n-hexane (C6Hu): chromatographically pure:

5:1:4 Ethyl acetate (CH3COOC2H5): chromatographically pure:

5:1:5 Dipotassium hydrogen phosphate (K2HPO4):

5:1:6 Potassium dihydrogen phosphate (KH2PO):

5:1:7 Phosphoric acid (H3PO4)

5:2 Standard products

Tylosin tartrate (CAS number: 74610-55-2): content >=98%:

5:3 Solution preparation

5:3:1 Phosphate buffer solution I (pH 8:0): Take 0:52 g of potassium dihydrogen phosphate and 16:73 g of dipotassium hydrogen phosphate, dissolve in water and dilute to 500 mL:

5:3:2 Phosphate buffer solution II (pH 4:0): Use phosphoric acid to adjust the pH of

phosphate buffer solution I to 4:0:

5:3:3 Dipotassium hydrogen phosphate solution (0:1 mol/L, pH 9:0): Take 1:74 g of dipotassium hydrogen phosphate, dissolve it in water and dilute it to 100 mL:

5:3:4 Potassium dihydrogen phosphate solution (0:01 mol/L, pH 2:5): Take 1:36 g of potassium dihydrogen phosphate, dissolve it in water and dilute it to 1000mL, phosphoric acid

Adjust pH to 2:5:

5:4 Preparation of standard solution

Tylosin standard stock solution (100 μg/mL): Take about 10 mg of tylosin tartrate standard, weigh it accurately, and add an appropriate amount of acetonitrile to dissolve it:

Dissolve and dilute to volume into a 100mL brown volumetric flask, shake well, and get ready: Store in the dark at 2 degrees C~8 degrees C, valid for 1 month:

5:5 Materials

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

6 Instruments and equipment

6:1 Liquid chromatograph: equipped with UV detector:

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

6:3 High-speed tissue masher:

6:4 Centrifuge: 5000r/min:

6:5 Vortex mixer:

6:6 Oscillator:

6:7 Rotary evaporator:

6:8 Solid phase extraction device:

6:9 Chicken heart bottle: 100mL:

6:10 Centrifuge tube with stopper: 50mL:

7: Preparation and preservation of samples

7:1 Preparation of samples

Prepare samples according to the requirements of Appendix B in GB/T 30891-2014:

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

Stored below -18 degrees C, the validity period is 3 months:

8 Measurement steps

8:1 Extraction

Take 5g of the sample (accurate to +/-0:05g), put it into a 50mL centrifuge tube with a stopper, add 5mL of phosphate buffer solution I, and vortex to mix for 1 min:

Add 25 mL of ethyl acetate, shake and extract for 10 min, centrifuge at 4500 r/min for 5 min, transfer the supernatant to a 100 mL chicken heart bottle, add 25 mL of ethyl acetate, repeat the extraction once, combine the extracts, and evaporate under reduced pressure in a 45°C water bath until nearly dry:

8:2 Purification

Dissolve the residue with 1 mL of methanol, transfer to another 50 mL centrifuge tube, and wash with 10 mL of phosphate buffer solution II and 10 mL of n-hexane:

Wash the chicken heart bottle, vortex and mix for 2 minutes, combine the washing liquid in the centrifuge tube, vortex and mix, centrifuge at 4500 r/min for 5 minutes, discard the upper n-hexane layer, and take

The lower aqueous solution is reserved for later use: Take the SCX solid phase extraction column, activate it with 5 mL of methanol and 5 mL of phosphate buffer solution II, and pass the backup solution through the column (control the flow rate to about 1:5 mL/min): Add 3 mL of water, 5 mL of methanol, 3 mL of water, and hydrogen phosphate in sequence: Wash the column with 3 mL of dipotassium solution and 0:4 mL of methanol, discard the eluate, and drain it: Add 3:0 mL of methanol for elution, collect the eluate, vortex to mix, filter with a 0:45 μm microporous membrane, and wait until machine analysis:

8:3 Preparation of standard curve

Precisely measure an appropriate amount of tylosin standard stock solution and dilute it with methanol to make the concentrations 0:050 μg/mL, 0:10 μg/mL, 0:50 μg/mL, 1:00 μg/mL, 2:00 μg/mL, and 5 :00μg/mL series of standard working solutions for liquid chromatography analysis:

8:4 Determination

8:4:1 Liquid chromatography reference conditions

a) Chromatographic column: C18 chromatographic column (250mmx4:6mm, 5μm) or one with equivalent performance;