CCS X 04

NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31658.14-2021

National food safety standard - Determination of o-trenbolone, B-trenbolone residues in animal derived food by liquid chromatography-tandem mass spectrometric method

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

Issued by: National Health Commission of the People's Republic of China, State Administration for Market Regulation

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:

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry detection methods for the determination of alpha-trenbolone and beta-trenbolone residues in animal foods:

This document is applicable to the determination of alpha-trenbolone and beta-trenbolone residues in muscle, fat, liver and kidney tissues of pigs, cattle, sheep and chickens, as well as rabbit meat, eggs, milk and goat milk:

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 alpha-trenbolone and beta-trenbolone in the sample were enzymatically hydrolyzed under weakly acidic conditions, extracted with tert-butyl methyl ether, degreased with n-hexane, HLB and ammonia:

Based on solid phase extraction column purification, liquid chromatography-tandem mass spectrometry determination, and external standard method for 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 Acetonitrile (CH3CN): chromatographically pure:

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

5:1:3 Tert-butyl methyl ether (C5H12O):

5:1:4 n-Hexane (C6H14):

5:1:5 Glacial acetic acid (CH3COOH):

5:1:6 Ammonia (NH3:H2O): content (NH3) is about 25%:

5:1:7 Sodium acetate (C2H3NaO2):

5:1:8 Acetone (C3H6O):

5:1:9 beta-glucuronidase/arylsulfatase: >100000U/mL:

5:2 Solution preparation

5:2:1 0:04mol/L sodium acetate solution: Take 3:28g of sodium acetate, dissolve it in water and dilute it to 1000mL:

5:2:2 80% methanol aqueous solution: Take 80mL of methanol and dilute to 100mL with water:

5:2:3 10% methanol aqueous solution: Take 10 mL of methanol and dilute to 100 mL with water:

5:2:4 2% ammonia solution: Take 8 mL of ammonia water and dilute it with water to 100 mL:

5:2:5 Methanol-2% ammonia solution (5:95): Take 5 mL of methanol, add 95 mL of 2% ammonia solution (5:2:4), and mix well:

5:2:6 Methanol-2% ammonia solution (40:60): Take 40 mL of methanol, add 60 mL of 2% ammonia solution (5:2:4), and mix:

5:2:7 Acetone-methanol solution (80:20): Take 80 mL of acetone, add 20 mL of methanol, and mix:

5:3 Standard products

5:3:1 alpha-Trenbolone (17alpha-Trenbolone, C18H22O2, CAS number: 80657-17-6): content >= 98:5%:

5:3:2 beta-Trenbolone (17beta-Trenbolone, C18H22O2, CAS number: 10161-33-8): content >= 98:0%:

5:4 Preparation of standard solution

5:4:1 Standard stock solution: Take about 10 mg each of alpha-trenbolone and beta-trenbolone standard products, weigh them accurately, dissolve them with an appropriate amount of methanol and dilute them to a 10 mL volumetric flask, and prepare them to a concentration of 1mg/mL alpha-trenbolone and beta-trenbolone standard stock solution: Store below -18 degrees C and is valid for 6 months:

5:4:2 Mix standard intermediate solution: Accurately measure 1 mL each of alpha-trenbolone and beta-trenbolone standard stock solutions in a 10 mL volumetric flask, and dilute with methanol

Release to the mark and prepare a mixed standard intermediate solution with a concentration of 100 μg/mL: Store below -18°C and has a validity period of 3 months:

5:4:3 Mixed standard working solution: Precisely measure appropriate amounts of mixed standard intermediate solutions, dilute with methanol, and prepare concentrations of 20ng/mL, 50ng/mL, 100ng/mL,:200ng/mL, 500ng/mL and 1000ng respectively: /mL mixed standard working solution: Ready for use:

5:5 Materials

5:5:1 HLB solid phase extraction column 1)::200mg/3mL, or equivalent:

1) The HLB solid-phase extraction columns listed here are only for reference and are not for commercial purposes: Standard users are encouraged to try solid-phase extraction columns of different manufacturers or models:

5:5:2 Amino solid-phase extraction column: 500mg/6mL, 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 sensitivity 0:01g:

6:3 pH meter:

6:4 Centrifuge: the speed can reach 5000r/min:

6:5 Vortex mixer:

6:6 Constant temperature shaker:

6:7 Horizontal oscillator:

6:8 Solid phase extraction device:

6:9 Nitrogen blower:

6:10 Nylon microporous filter membrane: 0:22μm:

7: Preparation and preservation of samples

7:1 Preparation of samples

7:1:1 Muscle, fat, liver and kidney tissue

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

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:

7:1:2 Milk

Take an appropriate amount of fresh or thawed blank or test milk and mix evenly:

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:

7:1:3 Eggs

Take an appropriate amount of fresh or refrigerated blank or test eggs, peel them, and homogenize them: