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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31658.7-2021

National food safety standard - Determination of 17B-estradiol, estriol, ethinylestradiol and estrone residues in animal products by gas chromatography-mass spectrometry 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

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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

17betaG estradiol, estriol, ethinyl estradiol and

Determination of estrone residue gas chromatography-mass spectrometry

1 Scope

This document stipulates the steroid hormone drugs (17beta-estradiol, estriol, ethinyl estradiol, ethinyl estradiol, etc:)

Sample preparation and gas chromatography-mass spectrometry method for detection of alcohol, estrone) residues:

This document is applicable to the determination of 17beta-estradiol, estriol, ethinyl estradiol, and estrone residues in beef, sheep, pig, and chicken muscles and pig liver, kidney, and fat:

Detection:

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 17beta-estradiol, estriol, ethinyl estradiol and estrone in the sample were determined by gas chromatography-mass spectrometry after enzymatic hydrolysis, extraction, purification and derivatization:

Quantification by 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 Acetonitrile (CH3CN): chromatographically pure:

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

5:1:3 Ethyl acetate (CH3COOC2H5):

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

5:1:5 Toluene (C6H5CH3):

5:1:6 Sodium hydroxide (NaOH):

5:1:7 Sodium acetate (NaC2H3O2:3H2O):

5:1:8 Ammonium acetate (NH4C2H3O2):

5:1:9 Glacial acetic acid (C2H4O2):

5:1:10 Dithioerythritol (C4H10O2S2, DTE):

5:1 Trimethyliodosilane (C3H9ISi, TMIS):

5:1:12 Glucuronidase/aryl sulfatase:

5:1:13 N-methyltrimethylsilyltrifluoroacetamide (MSTFA):

5:2 Standard products

5:17beta-Estradiol (17beta-Estradiol, C18H24O2, CAS number: 50-28-2): content >=98:0%:

5: 2: Estriol (Estriol, C18H24O3, CAS number: 50-27-1): content >= 98:0%:

5: Ethinylestradiol (C20H24O2, CAS No:: 57-63-6): Content >= 98:0%:

5: Estrone (C18H22O2, CAS number::200-164-5): content >= 98:0%:

5:3 Solution preparation

5:3:1 Sodium hydroxide solution (1:0mol/L): Take 40g of sodium hydroxide, dissolve it in water and dilute it to 1000mL:

5:3:2 Sodium acetate buffer (pH 5:2): Take 19:0g of sodium acetate, dissolve it in water and dilute it to 500mL, and adjust the pH to 5:2 with acetic acid:

5:3 Ammonium acetate solution (2:0mol/L): Take 46:25g of ammonium acetate, dissolve it in water and dilute it to 300mL:

5:3:4 Methanol aqueous solution: Take 400mL of methanol and add water to 1000mL:

5:3:5 Ethyl acetate n-hexane solution: Take 10mL of ethyl acetate, add n-hexane to dissolve and dilute to 100mL:

5:3:6 Derivatization reagent: Weigh 0:01g of dithioerythritol, dissolve it with 5mL of N-methyltrimethylsilyl trifluoroacetamide, and add it under the liquid surface:

Add 10 μL of trimethylsilane iodide, mix well, and place it overnight at 2°C to 8°C: Store it sealed and protected from light and moisture: The derivatization reagent should be colorless: If browning occurs,

Color changes such as red indicate that the reagent has failed:

5:4 Preparation of standard solution

5:4:1 Standard stock solution (100 μg/mL): Take about 10 mg each of 17beta-estradiol, estriol, ethinyl estradiol and estrone standards, weigh them accurately, and divide

Add an appropriate amount of methanol to dissolve and dilute to a 100mL volumetric flask to prepare a standard stock solution with a concentration of 100μg/mL: -18 degrees C or below

Save, valid for 6 months:

5:4:2 Mix standard working solution (1μg/mL): Precisely measure each standard stock

solution of 17beta-estradiol, estriol, ethinyl estradiol and estrone:

Put 1 mL in a 100 mL volumetric flask, dilute to the mark with methanol, and prepare a mixed standard working solution with a concentration of 1 μg/mL: Keep at 2°C to 8°C:

Deposit, valid for 1 month:

5:5 Materials

5:5:1 C18 solid phase extraction column: 500mg/3mL, or equivalent:

5:5:2 Silica gel solid-phase extraction column: 500mg/3mL, or equivalent:

6 Instruments and equipment

6:1 Gas chromatograph-mass spectrometer: equipped with EI source:

6: Analytical balance: sensitivity 0:00001g and 0:001g:

6:3 Nitrogen blowing instrument:

6:4 Solid phase extraction device:

6: Pipette: range:200μL~1000μL:

6: Pipette: range 20μL~100μL:

6: Homogenizer:

6: Vortex mixer:

6: Centrifuge: 10000r/min:

6:10 oven:

6:11 pH meter:

6:12 Rotary evaporator:

6:13 Chicken heart bottle: 100mL:

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 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: