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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31658.9-2021

National food safety standard - Determination of estrogen residues in animal derived foods and animal urine by liquid chromatography-tandem mass spectrometry method

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Table of Contents

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:

1 Scope

This document specifies the estrogenic drugs (estriol, estrone, ethinyl estradiol, ethinyl estradiol, Sample preparation and liquid chromatography-tandem mass spectrometry method for detection of residual amounts of 17a-estradiol, 17beta-estradiol, diethylstilbestrol, hexylbestrol and diethylstilbestrol):

This document applies to estrogenic drugs (estriol, estrone, ethinylestradiol, Determination of residual amounts of 17a-estradiol, 17beta-estradiol, diethylstilbestrol, hexestrol and diethylstilbestrol):

2Normative 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

3Terms and Definitions

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

4 Principles

The remaining drugs in the sample were extracted with acetonitrile after enzymatic

hydrolysis, purified with a solid-phase extraction column, measured by liquid chromatography-tandem mass spectrometry, and quantified by the internal standard method:

5Reagents 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):

5:1:3 Sodium hydroxide (NaOH):

5:1:4 n-Hexane (C6H1):

5:1:5 Dichloromethane (CH2Cl2):

5:1:6 Sodium chloride (NaCD):

5:1:7 Ammonium acetate (CH3COONH4):

5:1:8 Acetic acid (CH3COOH):

5:1:9 beta-glucuronidase/arylsulfatase (beta-glucuronidase/arylsulfatase): beta-glucuronidase 4:5 U/mL, arylsulfatase 14 U/mL

5:2 Preparation of solution

5:2:1 Dichloromethane-methanol solution: Take 70 mL of dichloromethane and 30 mL of methanol, and mix:

5:2:2 Methanol-water solution: Take 40 mL of methanol and 60 mL of water and mix:

5:2:3 Ammonium acetate solution (0:02 mol/L): Take 1:54g of ammonium acetate, add water to dissolve and dilute to about 800mL, adjust the pH to 5:2, dilute to 1000mL, and mix:

5:2:4 40% acetonitrile solution: Take:200 mL of acetonitrile and 300 mL of water, and mix well:

5:3 Standard products

Estriol, estrone, ethinyl estradiol, 17a-estradiol, 17beta-estradiol, diethylstilbestrol, hexestradiol, diethylstilbestrol Estriol-D3, estrone-D2, ethinylestradiol- D:, 17a-estradiol-D2, 17beta-estradiol-D2, diethylstilbestrol-Da, diethylstilbestrol-D and diethylstilbestrol-D6, the contents are all >=95%, see Appendix A:

5:4 Preparation of standard solution

5:4:1 Standard stock solution: Take 10 mg of each estrogen drug standard and isotope-labeled standard, weigh them accurately, add an appropriate amount of acetonitrile to dissolve and dilute to a 100 mL brown volumetric flask, and make a concentration of 100 μg/mL: Standard stock solution: Store at -18 degrees C, valid for 12 months:

5:4:2 Mixed standard stock solutions: Accurately pipette the standard stock solutions of estriol, estrone, ethinyl estradiol, 17a-estradiol, 17beta-estradiol, diethylstilbestrol, hexanebestrol and diethylstilbestrol respectively: 5mL, put in a 100mL brown volumetric flask, dilute to the mark with acetonitrile: Store at -18 degrees C, valid for 12 months:

5:4:3 Mix internal standard stock solution: accurately pipette estriol-D2, estrone-D2, ethinylestradiol-D4, 17a-estradiol-D2, 17beta-estradiol-D2, diethylstilbestrol-Da, Diethylstilbestrol-D, and Diethylstilbestrol-D: Put 5 mL of each standard stock solution into a 100 mL brown volumetric flask and dilute to the mark with acetonitrile: Store at -18 degrees C, valid for 12 months:

5:4:4 Mixed internal standard working solution: Accurately transfer 0:2 mL of mixed internal standard stock solution into a 10 mL brown volumetric flask, and dilute to the mark with acetonitrile: The concentration of each internal standard in this solution is 100 μg/L: Ready for use:

5:4:5 Mixed standard working solution: Accurately pipet an appropriate amount of mixed standard stock solution and mixed internal standard working solution, and dilute it with 40% acetonitrile solution to the concentration:

It is a series of standard working solutions of 1:0μg/L, 2:0μg/L, 5:0μg/L, 20μg/L, 50μg/L and:200μg/L: Each standard working solution contains an internal standard concentration of 5:0μg/L: Ready for use:

5:5 Materials

5:5:1 HLB solid phase extraction column::200 mg/6 mL, or equivalent:

5:5:2 Amino solid-phase extraction column: 500 mg/6mL, or equivalent, activated with 6mL of dichloromethane-methanol solution before use:

5:5:3 Syringe filter (universal filter membrane): made of nylon, with a pore size of 0:22 μm or equivalent performance:

6Instruments and Equipment

6:1 Liquid chromatography tandem mass spectrometer: equipped with automatic injector and electrospray ion source:

6:2 Analytical balance: sensitive to 0:00001g and 0:01g:

6:3 Nitrogen blower:

6:4 Solid phase extraction device:

6:5 Centrifuge tube: polypropylene plastic centrifuge tube, 50 mL:

6:6 Centrifuge: 10000 r/min or above:

6:7 pH meter:

6:8 Vortex mixer:

7 Preparation and preservation of samples

7:1 Preparation of samples

Take an appropriate amount of fresh or thawed air 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:

7:2 Storage of samples

Store below -18 degrees C and conduct analysis and testing within 3 months:

8 Measurement steps

8:1 Extraction

8:1:1 Animal tissue, milk, goat milk and eggs

Take 5g of sample (accurate to +/-0:05g), put it into a 50mL centrifuge tube, add exactly 50μL of mixed internal standard working solution, mix for 15 seconds, add 10mL of ammonium acetate solution, and 50μL of beta-glucuronidase/arylsulfatase , vortex for 1 min, cover, and shake for enzymatic hydrolysis at (37+/-1)°C for 12 h: Take it out, cool to room temperature, add 4g of sodium chloride, shake to dissolve, add 20mL of acetonitrile, vortex for 1 minute, centrifuge at 10000 r/min for 5 minutes, take the acetonitrile layer, add 15mL of acetonitrile, and repeat the extraction: Combine the two extracts, add 10 mL of n-hexane, vortex for 1 min, centrifuge at 10000 r/min for 2 min, take the acetonitrile layer, blow to dryness with nitrogen at 40°C, dissolve with 1 mL of methanol, add 9 mL of water, and set aside:

8:1:2 Animal urine

Take 5:00mL of the sample into a 50mL centrifuge tube, adjust the pH to 5:2 with acetic acid, accurately add 50μL of mixed internal standard working solution, mix for 15s, add 10mL of ammonium acetate solution, 50pL of beta-glucuronidase/arylsulfatase, and follow-up The extraction steps are the same as 8:1:1: