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

GB 31658.11-2021

**National food safety standard - Determination of albendazole
and its metabolites residues in animal derived food by high
performance liquid chromatography method**

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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 albendazole and its metabolites residues in animal foods

HPLC

1 Scope

This document specifies the sample preparation and HPLC determination methods for the detection of albendazole and its metabolites residues in animal foods:

This document applies to albendazole, albendazole sulfone, albendazole sulfoxide, and albendazole-2-aminosulfone in muscles, livers, kidneys, adipose tissues and milk of pigs, cattle, sheep, and chickens Detection of residual amounts:

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 albendazole and its metabolites remaining in the sample were alkalized and extracted with ethyl acetate (fat sample was extracted with hydrochloric acid water-acetonitrile solution, milk

The sample was extracted with acetonitrile and ethyl acetate), purified with acidic alumina solid phase extraction column and mixed cation exchange solid phase extraction column, and high performance liquid phase

Detection by chromatography-fluorescence detector and 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

5:2 Solution preparation

5:2:1 2% sodium carbonate solution: Take 2:0g of sodium carbonate, dissolve it in water and dilute it to 100mL:

5:2:2 0:2mol/L hydrochloric acid solution: Take 1:8mL of hydrochloric acid and dilute to 100mL with water:

5:2:3 0:2mol/L hydrochloric acid-acetonitrile solution: Take 10mL of 0:2mol/L hydrochloric acid solution, add 90mL of acetonitrile, and mix:

5:2:4 10mol/L sodium hydroxide solution: Take 4:0g of sodium hydroxide, dissolve and dilute to 10mL with water, and cool to room temperature:

5:2:5 0:004g/mL sodium metabisulfite solution: Take 0:4g of sodium metabisulfite, dissolve it in water and dilute it to 100mL:

5:2:6 0:1mol/L disodium ethylenediaminetetraacetate solution: Take 3:36g of disodium

ethylenediaminetetraacetate, dissolve it in water and dilute it to 100mL:

5:2:7 10% ammoniated acetonitrile solution: Take 10 mL of ammonia water, add 90 mL of acetonitrile, and mix well:

5:2:8 0:5mol/L ammonium acetate solution: Take 1:93g ammonium acetate, dissolve it in water and dilute it to 500mL, adjust the pH to 5:0 with glacial acetic acid, and mix well:

5:2:9 Reconstitute the solution: Take 10 mL of acetonitrile and 10 mL of methanol, add 80 mL of 0:5 mol/L ammonium acetate solution, and mix well:

5:2:10 Acetonitrile-dimethyl sulfoxide (9:1): Take 90 mL of acetonitrile and 10 mL of dimethyl sulfoxide, and mix well:

5:3 Standard products

5:3:1 Albendazole (Albendazole, $C_{12}H_{15}N_3O_2S$, CAS number: 54965-21-8): content $\geq 99:0\%$:

5:3:2 Albendazole Sulfone ($C_{12}H_{15}N_3O_4S$, CAS number: 75184-71-3): content $\geq 98:3\%$:

5:3:4 Albendazole-2-aminosulfone (Albendazole-2-aminosulfone, $C_{10}H_{13}N_3O_2S$, CAS number: 80983-34-2): content $\geq 90:0\%$:

5:4 Preparation of standard solutions

5:4:2 Mix standard intermediate solution: accurately pipette 2 mL of albendazole standard stock solution, 0:2 mL of albendazole sulfone standard stock solution, 2 mL of albendazole sulfoxide standard stock solution and albendazole respectively -2-Aminosulfone standard stock solution 1mL, in a 10mL volumetric flask, dilute to the mark with acetonitrile, prepare albendazole concentration of $20\mu\text{g/mL}$, albendazole sulfone concentration of $2\mu\text{g/mL}$, albendazole sulfone concentration Mixed standard intermediate solution with a sulfone concentration of $20\mu\text{g/mL}$ and albendazole-2-aminosulfone concentration of $10\mu\text{g/mL}$: Ready to use:

5:5 Materials

5:5:1 Acidic alumina solid phase extraction column: 1g/3mL, the filler is acidic alumina, or equivalent:

5:5:2 Mixed cation exchange solid phase extraction column: 150mg/6mL, or equivalent:

5:5:3 Nylon microporous filter membrane: 0:22 μm :

6 Instruments and equipment

6:1 High performance liquid chromatograph: equipped with fluorescence detector:

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

6:3 Tissue homogenizer:

6:4 Vortex mixer:

6:5 Vortex oscillator:

6:6 Centrifuge: the speed can reach 10000r/min:

6:7 Solid phase extraction device:

6:8 Nitrogen blower:

7: Preparation and preservation of samples

7:1 Preparation of samples

7:1:1 Organization

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

- 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, add a standard 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 sample and mix evenly:

- 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, add a standard solution of appropriate concentration, and add the sample as a blank:

7:2 Storage of samples

Store below -18 degrees C:

8 Measurement steps

8:1 Extraction

8:1:1 Muscle, liver, kidney

Take 2g of the sample (accurate to ± 0.02 g), place it in a 50mL centrifuge tube, add 0.5mL of 2% sodium carbonate solution, 1mL of dimethyl sulfoxide,

Add 1 mL of 0.004 g/mL sodium metabisulfite solution, vortex for 1 min: Add 10 mL of ethyl acetate, vortex for 1 min, shake for 5 min, and centrifuge at 10000 r/min: