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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31658.21-2022

National Food Safety Standard Determination of Levamisole Residues in Animal Foods by Liquid Chromatography-Tandem Mass Spectrometry

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foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1.Structure and Drafting Rules for Standardization Documents"

drafting.

This document is published for the first time.

National Food Safety Standards

Determination of levamisole residues in food of animal origin

Liquid chromatography-tandem mass spectrometry

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry method for the detection of levamisole residues in animal foods.

This document is applicable to the determination of levamisole residues in muscle, liver, kidney and adipose tissue of pigs, poultry, cattle and sheep.

2 Normative references

The content in the following documents constitutes the essential provisions of this document through normative references in the text. Among them, the dated reference documents,

Only the version corresponding to the date applies to this document; for undated references, the latest version (including all amendments) applies to this document

document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Terms and Definitions

This document does not have terms and definitions that need to be defined.

4 principles

The remaining levamisole in the sample was extracted with ethyl acetate under alkaline conditions, extracted with 0.1mol/L hydrochloric acid solution, mixed cation exchange

The solid-phase extraction column was replaced for purification, determined by liquid chromatography-tandem mass spectrometry, and quantified by external standard method.

5 Reagents or materials

Unless otherwise specified, all reagents are of analytical grade, and the water is first-class water in accordance with GB/T 6682.

5.2 Solution preparation

5.2.1 Saturated solution of sodium bicarbonate. take 100mL of water, add anhydrous sodium bicarbonate until it is insoluble, take the supernatant, and make it now.

5.2.2 Saturated solution of sodium carbonate. take 100mL of water, add sodium carbonate until it is insoluble, take the supernatant, and make it now.

5.2.3 Carbonate buffer solution. Take 90 mL of saturated sodium bicarbonate solution and 10 mL of saturated sodium carbonate solution, and mix them evenly.

5.2.4 0.1mol/L hydrochloric acid solution. take 9mL of hydrochloric acid, add water to dilute to 1000mL, and mix well.

5.2.5 4% ammonia water in methanol solution. Take 4 mL of ammonia water, dilute it with methanol to 100 mL, mix well, and prepare immediately after use.

5.2.6 0.1% formic acid solution. Take 500 μL of formic acid, dilute to 500 mL with water, and mix well.

5.2.7 10% acetonitrile formic acid solution. take 10mL of acetonitrile, dilute to 100mL with 0.1% formic acid aqueous solution, and mix well.

5.3 Standards

Levamisole hydrochloride (Levamisolehydrochloride, C11H12N2S.HCl, CAS number. 16595-80-5), content >=99%.

5.4 Preparation of standard solution

5.4.1 Standard stock solution. Take an appropriate amount of levamisole hydrochloride standard substance (equivalent to about 10 mg of levamisole), weigh it accurately, add an appropriate amount of methanol to make the solution

solution and dilute to a 10mL volumetric flask, and prepare a standard stock solution of levamisole with a concentration of 1mg/mL.

6 months.

5.4.2 Standard working solution. Accurately measure 1mL of standard stock solution, put it in a 100mL volumetric flask, dilute it to the mark with methanol, and prepare a concentration of

6 Instruments and equipment

6.1 Liquid chromatography-tandem mass spectrometer. distribution of electrospray ionization source.

7.1 Sample preparation

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 sample;

b) Take the homogenized blank sample as the blank sample;

c) Take the homogenized blank sample, add a standard solution of appropriate concentration, and add the sample as a blank.

7.2 Sample storage

Store below -18°C.

8.1 Extraction

Take 2 g of the sample (accurate to +/-0.05 g), put it in a 50 mL polypropylene centrifuge tube (the fat sample needs to be heated in a water bath at 60 °C for 20 min),

Add a ceramic homogenate, add carbonate buffer 0.5mL, anhydrous sodium sulfate 2g, ethyl acetate 10mL, vortex for 1min, shake for 5min,

Centrifuge at 6000r/min for 5min, take the upper layer of ethyl acetate to another centrifuge tube, repeat the extraction of the residue once with 10mL of ethyl acetate, and combine twice

Add 5 mL of 0.1 mol/L hydrochloric acid solution to the extract, shake for 5 min, centrifuge at 6000 r/min for 5 min, take the lower aqueous phase into another centrifuge tube,

Add 5 mL of 0.1 mol/L hydrochloric acid solution to the organic phase to repeat the extraction once, combine the two extracts, and set aside.