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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

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National food safety standard - Determination of avermectins residues in animal derived food by high performance liquid chromatography and liquid chromatography-tandem mass spectrometric method

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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 HPLC determination methods for avermectin, ivermectin, doramectin and acetamidoavermectin in animal foods:

This document is applicable to the determination of avermectin, ivermectin, doramectin and

acetamidoavermectin residues in muscles, livers, kidneys and adipose tissues of pigs, cattle and sheep:

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 residual avermectin drugs in the sample were extracted with acetonitrile, purified with a C18 solid-phase extraction column, fluorescently derivatized, and determined by high-performance liquid chromatography:

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

5:1:1 Acetonitrile (CH3CN): chromatographically pure:

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

5:1:3 n-hexane (C6H14): chromatographically pure:

5:1:4 Acetic acid (CH3COOH): chromatographically pure:

5:1:5 Trifluoroacetic anhydride (C4F6O3): chromatographically pure:

5:1:6 Triethylamine (C6H15N):

5:1:7 1-N-methylimidazole (C4H6N2):

5:2 Solution preparation

5:2:1 Derivatization reagent solution A: Take 5 mL of 1-N-methylimidazole and 5 mL of acetonitrile, and mix well:

5:2:2 Derivatization reagent B solution: Take 5 mL of trifluoroacetic anhydride and 10 mL of acetonitrile, and mix well:

5:2:3 Washing solution: Take 80 mL of acetonitrile, 120 mL of water, and 0:2 mL of triethylamine, and mix well:

5:3 Standard products

The contents of avermectin, ivermectin, doramectin and acetamido-avermectin are all >=95%, see Appendix A for details:

5:4 Preparation of standard solution

5:4:1 Standard stock solution (1 mg/mL): Take appropriate amounts of each of the avermectin, ivermectin, doramectin, and acetamido-avermectin standard products (equivalent to about 10 mg of each active ingredient), Weigh accurately, add acetonitrile to dissolve in a 10 mL volumetric flask and dilute to the mark to prepare a standard stock solution with a concentration of 1 mg/mL: Store in the dark below -18°C and is valid for 6 months:

5:4:2 Mixed standard working solution (10 μg/mL): Precisely measure 0:1 mL of each standard stock solution in a 10 mL volumetric flask, dilute to the mark with acetonitrile, and prepare a mixed standard working solution with a concentration of 10 μg/mL: : Store below -18 degrees C away from light, valid for 3 months:

5:4:3 Mixed standard working solution (1 μg/mL): Precisely measure 1 mL of the mixed standard working solution of 10 μg/mL, put it in a 10 mL volumetric flask, dilute it to the mark with acetonitrile, and prepare a mixed standard with a concentration of 1 μg/mL: Working fluid: Store in a dark place below -18°C and is valid for 3 months:

5:5 Materials

5:5:1 Solid phase extraction column: triple bonded C18, 500mg/6mL, or equivalent:

5:5:2 Microporous nylon filter membrane: 0:2μm:

6 Instruments and equipment

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

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

6:3 Centrifuge:

6:4 Tissue homogenizer:

6:5 Vortex mixer:

6:6 Solid phase extraction device:

6:7 Nitrogen blower:

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 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

Take 2g of the sample (accurate to +/-0:02g), put it into a 50mL centrifuge tube, add 8mL of acetonitrile, vortex for 2min, and centrifuge at 4000r/min:

5min: Collect the supernatant, add 8mL of acetonitrile to the residue, and repeat the extraction once: Combine the two extracts and mix well: Take 4mL of the extract and add 6 ml of water:

mL and 10 μL of triethylamine, mix well, and set aside:

8:2 Purification

The solid-phase extraction column was activated with 4 mL of acetonitrile and 4 mL of washing solution in sequence, and the backup solution was passed through the column, and then rinsed with 5 mL of washing solution and 3 mL of water:

Drain, rinse with 4 mL of n-hexane, drain, and elute with 8 mL of acetonitrile: Collect the eluate and blow dry with nitrogen in a 40°C water bath:

8:3 Derivatization

Add 100 μL of derivatization reagent A, 150 μL of derivatization reagent B, 50 μL of acetic acid, and 50 μL of triethylamine, vortex for 30 s, and let stand in the dark at 20°C for 15 min: Add 650 μL of methanol, vortex for 10 s, and let stand in the dark at 20°C for 15 min: , filtered and then measured by high performance liquid chromatography: