

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

SN/T 5121-2019

**Determination of Ivermectin residue in live animal and feed for
import and export - LC-MS/MS method**

进出口食用动物、饲料中伊维菌素残留测定 液相色谱-质谱/质谱法

(English Translation)

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Foreword

This standard was drafted according to GB/T 1.1-2009.

Please note that some content of this document may involve patents, and the issuing authority of this document is not responsible for identifying these patents.

This standard is proposed and managed by the General Administration of Customs of the People's Republic of China.

This standard was drafted. Chongqing Customs, People's Republic of China.

1 Scope

This standard specifies liquid chromatography-mass spectrometry/mass spectrometry for determination of ivermectin residues in imported and exported food animals and feed.

This standard applies to the determination of ivermectin in the blood and animal feed of imported and exported food animals (such as pigs, cattle, sheep, rabbits, chickens, ducks)

and animal feed.

Confirmation.

2 Normative references

The following documents are essential for the application of this document. For dated references, only the dated version applies to this document.

file. For undated references, the latest version (including all amendments) applies to this document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Principle

After the sample was extracted by acetonitrile vortex, it was detected and confirmed by liquid chromatography-mass spectrometry/mass spectrometry, and quantified by internal standard peak area method.

4 Reagent materials

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

4.1

Acetonitrile. HPLC grade.

4.2

Formic acid.

4.3

Ammonium acetate.

4.4

Sodium chloride.

4.5 5mol/L ammonium acetate + 0.1% formic acid aqueous solution. Weigh 0.3385g of ammonium acetate in a beaker, dissolve in water, and transfer to 1000mL in a volumetric flask, wash the beaker with water at least 3 times until it is washed, and transfer all the washing liquid to a 1000 volumetric flask, then add 1 mL of formic acid in a 1000mL volumetric flask, dilute with water.

4.6

Reference material. Ivermectin (CAS number. 7028-86-7), purity is greater than 96%; Ivermectin isotope internal standard (Ivermetin-d2), purity is greater than 98%.

4.7

Standard stock solution. Accurately weigh 0.1g of ivermectin standard (accurate to 0.0001g), fully dissolve with acetonitrile (4.1), and dilute to volume.

In a 100mL volumetric flask, a standard stock solution with a concentration of 1mg/mL was prepared; in addition, the ivermectin internal standard was 10mg.

Dissolve fully in acetonitrile (4.1), dilute to volume in a 10mL volumetric flask, and prepare an ivermectin internal standard with a concentration of 1mg/mL.

For the standard stock solution, pour the volume of the standard stock solution into the brown reagent bottle, and store it in the dark below -18 ° C.

4.8

Standard intermediate stock solution. Accurately transfer 100 µL of ivermectin standard stock solution (4.7) and 100 µL of ivermectin internal standard stock solution (4.7) in two 100 mL volumetric flasks with acetonitrile Dilute to a constant volume and prepare 1 µ/mL ivermectin standard intermediate stock solution and 1 µg/mL ivermectin internal standard intermediate stock solution. The solution is placed in a brown reagent bottle, and stored in a dark place below -18 ° C... 4.9 Standard working solution with internal standard. Dilute ivermectin standard intermediate stock solution (4.8) with acetonitrile (4.1) to appropriate concentration as needed Mixed standard working curve solution, at the same time each standard solution is added with an equal amount of ivermectin internal standard intermediate stock solution standard intermediate SN/T 5121-2019 ?????, ?????, ?????, ??

Stock solution (4.8). The concentration of the internal standard of ivermectin in each standard solution was 20 µg/L. The reference linear concentration range of ivermectin was 5 µg/L to 50 µg/L. 4.10 Matrix standard working solution. Select 5 samples of ivermectin-free sample and process according to this standard 7.1 to "take the supernatant 5mL nitrogen "Air blow dry" (without the addition of ivermectin internal standard), add 1 mL of standard working solution (4.9) with internal standard at different concentrations, dissolve the slag, after passing through a 0.22 µm filter membrane (4.11), is configured into a matrix standard working solution, which is now ready for use. 4.11 Microporous membrane. organic, 0.22 µm.

5 Instruments and equipment**5.1**

Liquid chromatography-mass spectrometry/mass spectrometer. Power distribution spray ion source (ESI).

5. Balance. Sensitivity is 0.0001g and 0.01mg.

5.3

Refrigerated centrifuge. The rotation speed is not less than 8000r/min.

5.4

Vortex mixer.

5.5

Ultrasonic cleaner.

5.6

Nitrogen blowing instrument.

6.1 Sample preparation

6.1.1

Feed. take an appropriate amount of a representative sample from the sample taken, grind and mix it, and put it into a clean plastic bag. The number of samples 15g, sealed and marked.

6.1.2

Blood. Animal blood was collected with heparin sodium anticoagulant blood collection tubes, the sample size was 15mL, sealed and labeled.

6.2 Sample preservation

Feed was stored at room temperature. Blood samples to be tested were stored at 4 ° C. Blood samples were stored frozen at -18 ° C.

7.1.1 Blood

Weigh 2.0g (accurate to 0.01g) sample and 0.5g sodium chloride (4.4) into a 50mL centrifuge tube, add 40μL of ivermectin internal standard intermediate stock solution (4.8, Equivalent to the internal standard mass of ivermectin is 4ng), 3mL of water and 10mL of acetonitrile (4.1), vortex Mix for 2 min, shake for 30 min, freeze and centrifuge for 5 min at 8000 r/min below 10 ° C, and take 5 mL of nitrogen from the supernatant.

Dry, add 1.0 mL of acetonitrile (4.1), dissolve the residue with ultrasound, pass through a 0.22 μm filter membrane (4.11), and use for liquid chromatography-mass spectrometry/mass spectrometry for determination and confirmation.

7.1.2 Feed

Weigh 2.0g (accurate to 0.01g) sample and 3.0g sodium chloride (4.4) into a 50mL centrifuge tube, add 40μL of ivermectin isotope internal standard intermediate stock solution (4.8, It is equivalent to the internal standard mass of ivermectin is 4ng), 7.5mL of