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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31656.1-2021

National food safety standard - Determination of mebendazole and its metabolites residue in fishery products by high performance liquid chromatography

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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:

1 Scope

This document specifies sample preparation and liquid chromatography for the detection of mebendazole and its metabolites aminomebendazole and hydroxymebendazole residues in aquatic products:

Spectral measurement method:

This document applies to the determination of residues of mebendazole and its metabolites aminomebendazole and hydroxymebendazole in the edible tissues of fish, shrimp and crabs:

Detection:

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 Rules and test methods for water use in analytical laboratories

GB/T 30891-2014 Sampling specifications for aquatic products

3 Terms and definitions

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

4 Principles

The remaining mebendazole and its metabolites in the sample were extracted with ethyl acetate, degreased with n-hexane, purified with a cationic solid-phase extraction column, and high-performance liquid phase

Determination by chromatography-UV detector and quantification by external standard method:

5 Reagents and materials

The reagents used below are of analytical grade unless otherwise noted, 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 Ethyl acetate (CH3COOCH2CH3): chromatographically pure:

5:1:5 N,N-dimethylformamide (C3H7NO): chromatographically pure:

5:1:6 Phosphoric acid (H3PO):

5:1:7 Ammonium dihydrogen phosphate (NH4H3PO,):

5:1:8 Triethylamine [(C2H5)3N]:

5:1:9 Formic acid (HCOOH):

5:1:10 Ammonia (NH3.H2O):

5:2 Standard products

Mebendazole (C16H13N3O3, CAS number: 31431-39-7), hydroxymebendazole (5-hydroxymebendazole,

5:3:2 1% formic acid solution: Take 1mL of formic acid, add water to 100mL, and mix well:

5:3:3 0:05mol/L ammonium dihydrogen phosphate-triethylamine solution: Take 5:8g of ammonium dihydrogen phosphate, add water to dissolve and dilute to 1000mL, add 1mL of triethylamine, and mix:

5:3:4 0:05mol/L ammonium dihydrogen phosphate buffer: Take 2:9g of ammonium dihydrogen phosphate, add 450 mL of water to dissolve, adjust the pH to 2:0 with phosphoric acid, and dilute to 500 mL with water:

5:3:5 5% ammoniated methanol solution: Take 5 mL of ammonia water and 95 mL of methanol, mix well; prepare before use:

5:3:6 Dissolution solution: Take 150mL of N,N-dimethylformamide and dilute to 500mL with 0:05mol/L ammonium dihydrogen phosphate buffer:

5:4 Preparation of standard solution

5:4:1 Standard stock solution (100 μg/mL): Take 10 mg each of mebendazole, aminomebendazole and hydroxymebendazole, and weigh them accurately:

Add 5 mL of formic acid to dissolve, dilute with methanol to a 100 mL volumetric flask, shake well, and prepare standard stock solutions with a concentration of 100 μg/mL:

Store away from light below -18 degrees C, valid for 6 months:

5:4:2 Mix standard intermediate solution: Accurately measure appropriate amounts of mebendazole, aminomebendazole and hydroxymebendazole standard stock solutions, and dilute with methanol:

Release and prepare a mixed standard intermediate solution containing aminomebendazole 5 μg/mL, hydroxymebendazole 2:5 μg/mL and mebendazole 2:5 μg/mL:

Store away from light below -18 degrees C, valid for 3 months:

5:5 Materials

5:5:1 Mixed cationic solid phase extraction column: 60mg/3mL, or equivalent:

5:5:2 Aqueous polysulfone ether needle filter: 0:45μm:

6 Instruments and equipment

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

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

6:3 Centrifuge: 6000r/min:

6:4 Rotary evaporator:

6:5 Solid phase extraction device:

6:6 Ultrasonic cleaning instrument:

6:7 Nitrogen blower:

6:8 Chicken heart bottle: 100mL:

7 Preparation and preservation of specimens

7:1 Preparation of specimens

Prepare samples according to the requirements of Appendix B in GB/T 30891-2014:

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

7:2 Storage of specimens

Stored below -18 degrees C, the validity period is 3 months:

8 Measurement steps

8:1 Extraction

Take 3g of the sample (accurate to +/-0:03g) in a 50mL centrifuge tube, add 2mL of water, vortex for 30s to disperse; add ethyl acetate

10 mL, shake for 2 min, ultrasonic for 5 min, centrifuge at 4500 r/min for 10 min, transfer ethyl acetate to a 100 mL chicken heart bottle: Add ethyl acetate to the residue:

Add 10 mL of ester and repeat the extraction twice as described above: Combine the ethyl acetate into a 100 mL chicken heart bottle and rotary evaporate it to dryness at 40°C: Add 80%

Add 3 mL of methanol solution, vortex and mix for 1 min, add 3 mL of n-hexane, mix for 1 min, transfer the solution to a 10 mL centrifuge tube, 6000 r/min

Centrifuge for 5 minutes, remove the n-hexane layer; add 3 mL of n-hexane to the lower layer, and repeat degreasing once: Add 1% formic acid aqueous solution to the lower layer:

3 mL, mix well, centrifuge at 6000r/min for 5min, and set aside the