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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31656.13-2021

National Food Safety Standard Determination of Multi-residue Nitrofuran Metabolites in Aquatic Products Liquid Chromatography-Tandem Mass Spectrometry

Issued on: September 16, 2021 **Implemented on: February 1, 2022**

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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 multiple residues of nitrofuran metabolites in aquatic products

Liquid chromatography-tandem mass spectrometry

1 Scope

This document specifies the nitrofuran metabolites 3-amino-2-oxazolidinone (AOZ) and 5G in aquatic products:

Morpholinomethyl-3-amino-2-oxazolidinone (5-morpholinomethyl-3-amino-2-oxazolidinone, AMOZ), 1-amino-2G hydantoin

Methods for sample preparation and liquid chromatography-tandem mass spectrometry determination of (1-aminohydantoin, AHD) and semicarbazide (SEM) residues:

This document applies to AOZ, AMOZ, AHD and SEM residues of nitrofuran metabolites in edible tissues of fish, sea cucumbers, turtles and other aquatic products:

Determination of retention amount, as well as determination of AOZ, AMOZ and AHD in edible tissues of crustaceans such as shrimps and crabs:

2 Normative reference documents

The contents of the following documents constitute essential provisions of this document through normative citations in the text: Among them, referenced 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:

GB/T 6682 Specifications and test methods for water used 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 nitrofuran protein-bound metabolites in the sample were hydrolyzed under acidic conditions, derivatized with 2-nitrobenzaldehyde, and treated with ethyl acetate:

Ester liquid-liquid extraction, high-speed centrifugal purification, liquid chromatography-tandem mass spectrometry, and internal standard method for quantitation:

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 Methanol (CH3OH): chromatographically pure:

5:1:2 Ammonium acetate (CH3COONH4) is chromatographically pure:

5:1:3 Ethyl acetate (C4H8O2): chromatographically pure:

5:1:4 Dimethyl sulfoxide [(CH3)2SO]: chromatographically pure:

5:1:5 2-nitrobenzaldehyde (C7H5NO3): chromatographically pure:

5:1:6 Anhydrous dipotassium hydrogen phosphate (K2HPO):

5:1:7 Hydrochloric acid (HCD:

5:2 Solution preparation

5:2:1 0:5 mol/L hydrochloric acid solution: Take 42mL of hydrochloric acid, dilute it with water to 1000mL, and mix well:

5:2:20:002 mol/L ammonium acetate solution: Take 0:15g of ammonium acetate, dissolve it in water and dilute it to 1000mL, and mix well:

5:2:30:05 mol/L 2-nitrobenzaldehyde solution: Take 0:076 g of 2-nitrobenzaldehyde, dissolve it in dimethyl sulfoxide and dilute it to 10 mL, mix well, and prepare it now:

5:2:41:0 mol/L dipotassium hydrogen phosphate solution: Take 87:1 g of anhydrous dipotassium hydrogen phosphate, dissolve and dilute to 500mL with water, and mix well:

5:2:55% methanol solution: Take 5mL of methanol, dilute to 100mL with water, and mix well:

5:3 Standard products

5:3:1 Nitrofuran metabolites: AOZ, AMOZ, AHD.HCl and SEM.HCl, the content is >=99:0%, see Appendix A for details:

5:3:2 Internal standards: AOZ-D4, AMOZ-D, AHD-3C3 and SEM.HCl-3C, 15N2, the contents are all >=99:0%, see the attachment for details

Record A:

5:4 Preparation of standard solution

5:4:1 Standard stock solution: Take an appropriate amount of each of the AOZ, AMOZ, AHD.HCl, and SEM.HC1 standards (equivalent to 10 mg of active ingredient), weigh it accurately, add an appropriate amount of methanol to dissolve it, and dilute it to a 10mL brown volumetric flask: Prepare a standard stock solution with a concentration of 1:0 mg/mL: Store at -18 degrees C away from light, valid for 6 months:

5:4:2 Mixed standard intermediate solution: Accurately measure an appropriate amount of the standard stock solution, dilute it step by step with methanol, and prepare a mixed standard intermediate solution with a concentration of 1:0 μg/mL: Store at 2 degrees C~8 degrees C away from light, valid for 1 month:

5:4:3 Mixed standard working solution: Accurately measure 1 mL of the mixed standard intermediate solution into 10 mL and 100 mL brown volumetric flasks, dilute to the mark with methanol, and prepare mixed standard working solutions with concentrations of 100 μg/L and 10 μg/L: Ready for use:

5:4:4 Isotope internal standard standard stock solution: Take appropriate amounts of each of AOZ-D4, AMOZ-D5, AHD-3C3 and SEM.HCI-3C, 5N2 standards (equivalent to 10 mg of active ingredient), weigh them accurately, and add an appropriate amount of methanol: Dissolve and dilute to a 10mL brown volumetric flask, and prepare to a concentration of

1:0 mg/mL internal standard standard stock solution: Store at -18 degrees C away from light, valid for 6 months:

5:4:5 Mixed internal standard standard working solution: Accurately measure an appropriate amount of the isotope internal standard standard stock solution, dilute it step by

step with methanol, and prepare a mixed internal standard standard working solution with a concentration of 100 μg/L: Store at -18 degrees C away from light, valid for 3 months:

6Instruments and equipment

6:1 Liquid chromatography-tandem mass spectrometer: equipped with electrospray ionization source:

6:2 Analytical balance: sensitive to 0:01g and 0:00001g:

6:3 Centrifuge: 6000 r/min:

6:4 High-speed centrifuge: 14000 r/min:

6:5 Vortex mixer:

6:6 Constant temperature oscillator:

6:7 Nitrogen blower:

7 Preparation and preservation of samples

7:1 Preparation of samples

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

a) Take the homogeneous test material as the 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 Hydrolysis and derivatization

Take 2g of the sample (accurate to +/-0:01g), put it into a 50mL polypropylene centrifuge tube, add exactly 50μL of the mixed internal standard standard working solution, vortex and mix for 1 minute, add 5mL of hydrochloric acid solution and 0:15mL of 2-nitrobenzaldehyde solution: , vortex and mix for 1 min, place in a constant temperature oscillator, and oscillate at 37°C in the dark for 16 h:

8:2 Extraction and purification

Take out the centrifuge tube and cool it to room temperature, adjust the pH to 7:0~7:5 with dipotassium hydrogen phosphate solution, add 8 mL of ethyl acetate, vortex for 30 s,