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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31656.7-2021

National food safety standard - Determination of niclosamide residues in aquatic products by liquid chromatography-tandem mass spectrometry

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

Issued by: National Health Commission of the People's Republic of China, State Administration for Market Regulation

Table of Contents

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry determination methods for the detection of niclosamide residues in aquatic products:

This document is suitable for the detection of niclosamide residues in edible tissues of fish, shrimp, turtle and other aquatic products:

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

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 niclosamide in the sample was extracted with ammoniated acetonitrile, purified with C18 adsorbent, detected by liquid chromatography-tandem mass spectrometer, and quantified by the 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:

5Reagents 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 (C2H3N): chromatographically pure:

5:1:2 Ammonia (NHa.H2O):

5:1:3 Anhydrous magnesium sulfate (MgSO4):

5:2 Solution preparation 5:2:1 Ammonia solution: Take 5mL of ammonia, add 5mL of water, and mix well:

5:2:2 Ammoniated acetonitrile solution: Take 100 uL of ammonia solution, add acetonitrile to 100mL, and mix well:

5:2:3 70% acetonitrile aqueous solution: Take 70mL of acetonitrile, add 30mL of water, and mix well:

5:3 Standard products Niclosamide (Niclosamide, C13 H8 Cl2 N2 O4, CAS number: 50G 65G 7), content >=96:0%:

5:4 Preparation of standard solution 5:4:1 Standard stock solution (0:1 mg/mL): Take 10 mg of niclosamide standard, weigh it accurately, add 10 mL of acetonitrile, dissolve it with ultrasound, and dissolve it with acetonitrile:

Dilute nitrile to a 100mL brown volumetric flask, shake well, and get it: - Store below 18 degrees C, valid for 2 months:

5:4:2 Standard working solution (1 ug/mL): Accurately measure 1 mL of the standard stock solution, put it into a 100 mL brown volumetric flask, and dilute it with acetonitrile to Temperature, shake well, and it is ready: - Store below 18 degrees C, valid for 1 month:

5:5 Materials C18 adsorbent: particle size 40 um~ 60 um, or equivalent:

6 Instruments and equipment

6:1 Liquid chromatography-tandem mass spectrometer: equipped with electrospray ion source (ESI):

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

6:3 Centrifuge: 8000r/min or above:

6:4 Vortex mixer:

6:5 Ultrasonic cleaning instrument:

6:6 Nitrogen blower:

6:7 Stoppered polypropylene centrifuge tube: 50mL:

6:8 Stoppered centrifuge tubes: 10mL, 25mL:

7: Preparation and preservation of samples 7:1 Preparation of samples Prepare samples according to the requirements of Appendix B in GB/T 30891-2014:

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 standard working 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 5g of the sample (accurate to +/-0:02g), put it into a 50mL centrifuge tube with a stopper, add 10mL of ammoniated acetonitrile solution, vortex and mix for 1 minute, and supernatate:

Sonicate for 10 minutes, add 3g of anhydrous magnesium sulfate, vortex and mix for 1 minute, centrifuge at 5000r/min for 5 minutes, take the supernatant and transfer it to a 25mL volumetric flask, repeat the extraction of the residue with 10mL of ammoniated acetonitrile, combine the supernatants, and dilute with acetonitrile Dilute to volume, mix, and set aside:

8:2 Purification Accurately measure 5 mL of the above reserve solution into a 10 mL centrifuge tube, blow dry with nitrogen at 45°C, and dissolve with 1:0 mL of 70% acetonitrile aqueous solution:

The residue was then added with 50 mg of C18 adsorbent, vortexed for 30 s, centrifuged at 8000 r/min for 6 min, and the supernatant was passed through a 0:22 um organic filter membrane for LC-MS/MS measurement:

8:3 Preparation of matrix matching standard curve Take 6 blank samples, extract according to step 7:1, and accurately measure 5 uL, 10 uL, 25 uL, 50 uL, niclosamide standard working solution:

Add 100 uL and:200 uL to 6 blank sample extracts respectively, purify according to step 8:2, take the supernatant, and prepare niclosamide concentrations of 0:5ng/mL, 1ng/mL, 2:5ng/mL, A series of matrix-matched standard solutions of 5ng/mL, 10ng/mL, and 20ng/mL were filtered and used for LC-MS/MS determination: Using the measured niclosamide quantitative ion peak area as the ordinate and the corresponding concentration as the abscissa, draw Matrix standard curve: Find regression equation and correlation coefficient:

8:4 Determination 8:4:1 Liquid chromatography reference conditions a) Chromatographic column: C18 (150mm x 2:1mm, 5um), or equivalent;

b) Column temperature: 35 degrees C;

c) Flow rate: 0:2mL/min;

d) Injection volume: 10uL;

e) Mobile phase: A is acetonitrile, B is water, and the gradient elution procedure is shown in Table 1:

8:4:2 Mass spectrometry reference conditions a) Ionization mode: electrospray ion source (ESI), negative ion mode;

b) Spray voltage: 2500V;

c) Sheath gas pressure: 40L/min;

d) Auxiliary air pressure: 5L/min;

e) Ion transfer tube temperature: 330 degrees C;

f) Scan mode: Selected Reaction Monitoring (SRM): Selected reaction monitoring precursor ions, product ions and collision energies are shown in Table 2;

8:4:3 Determination method Take the sample solution and the matrix-matched standard solution, conduct single-point or multi-point calibration, quantify the chromatographic peak area, and calculate by the external standard method: The sample solution and The characteristic ion mass chromatographic peak areas of niclosamide in the matrix-matched standard solution should be within the linear range of instrument detection: Chlorine in the sample solution The ratio of the retention time of nitlosamide to the retention time of niclosamide in the matrix-matched standard working solution is within +/-2:5%: In the sample solution Compared with the relative ion abundance in the matrix-matched standard solution, the relative ion abundance meets the requirements of Table 3: The characteristic ion mass of the standard solution See Appendix A for the chromatogram:

8:4:4 Blank test Take a blank sample and perform parallel operations using the same measurement steps except that no standard solution is added:

9 Calculation and presentation of results The residual amount of niclosamide in the sample is calculated according to the standard curve or formula (1):

10 Sensitivity, accuracy and precision of detection methods 10:1 Sensitivity The detection limit of this method is 0:2ug/kg, and the quantitation limit is 0:5ug/kg:

10:2 Accuracy The recovery rate of this method at the added concentration level of 0:5ug/kg~20ug/kg is 70%~110%:

10:3 Precision The intra-batch relative standard deviation of this method is <=15%, and the