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

GB 31656.12-2021

**National food safety standard - Determination of Penicillin
multi-residues in aquatic products by liquid
chromatography-tandem mass spectrometry method**

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China, State Administration for Market Regulation**

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1 Scope

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

This document applies to amoxicillin, ampicillin, penicillin G, penicillin V, and oxacillin in the edible tissues of aquatic products such as fish, shrimp, turtle, and sea cucumber:

Determination of single or multiple drug residues of lin, cloxacillin, dicloxacillin, nafcillin, piperacillin, azlocillin and methicillin:

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 penicillins in the sample were extracted with acetonitrile aqueous solution,

purified with a solid phase extraction column, and measured by liquid chromatography-tandem mass spectrometry, using the internal standard method:

Quantitative:

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 Acetonitrile (CH₃CN): chromatographically pure:

5:1:2 Formic acid (HCOOH): chromatographically pure:

5:2 Solution preparation 5:2:1 30% acetonitrile aqueous solution: Take 30mL of acetonitrile and 70mL of water, mix:

5:2:2 80% acetonitrile aqueous solution: Take 400mL of acetonitrile and 100mL of water, mix:

5:2:3 0:05% formic acid aqueous solution: Take 250 uL of formic acid and dilute to 500 mL with water:

5:2:4 0:05% formic acid acetonitrile solution: Take 250 uL of formic acid, add acetonitrile and dilute to 500 mL:

5:3 Standard products 5:3:1 Penicillins: amoxicillin, ampicillin, penicillin G, penicillin V, oxacillin, cloxacillin, dicloxacillin, nafcillin, piperacillin, azlocillin and methicillin, the content is $\geq 95\%$, please see Appendix A for details:

5:3:2 Internal standards: amoxicillin Gd4, ampicillin Gd5, penicillin Gd7, nafcillin Gd6, the contents are all $\geq 95\%$, see Appendix A for details:

5:4 Preparation of standard solution 5:4:1 Penicillin standard stock solution (0:10 mg/mL): Take about 10 mg of penicillin drug standard, weigh it accurately, and use 30% acetonitrile water. Dissolve and dilute the solution to a volume of 100 mL, and prepare a standard stock solution with a concentration of 0:10 mg/mL. Store in the dark at -18°C to be effective:

Issue 5d:

5:4:2 Internal standard stock solution (0:10 mg/mL): Take about 10 mg of the internal standard standard, weigh it accurately, dissolve it in 30% acetonitrile aqueous solution and dilute it to the final concentration:

GB 31656:12-2021 The volume is increased to 100 mL, and the internal standard stock solution is prepared with a concentration of 0:10 mg/mL. Store in the dark at -18°C and has a validity period of 5 days:

5:4:3 Mix standard intermediate solution (1:0ug/mL): Precisely measure an appropriate

amount of penicillin standard stock solution and dilute it with water to prepare amoxicillin, ammonia and ammonia:

Benzillin, Penicillin G, Penicillin V, Oxacillin, Cloxacillin, Dicloxacillin, Nafcillin, Piperacillin, Azlocillin and Methicillin Concentrate A mixed standard solution with a concentration of 1:0 ug/mL: Store in the dark at -4°C and have a validity period of 5 days:

5. d5, penicillin G-d7, and nafcillin-d6 are all internal standard working solutions with a concentration of 1:0 ug/mL: Store in the dark at -4°C and have a validity period of 5 days:

5:5 Materials 5:5:1 Pass-through reversed-phase mixed hydrophilic-lipophilic solid-phase extraction column::200mg/6mL, or equivalent:

5:5:5: Ultrafiltration tube: 10kD, 0:05mL:

6 Instruments and equipment

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

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

6:3 High-speed refrigerated centrifuge:

6:4 Solid phase extraction device:

6: Ultrasonic cleaning instrument:

6: Tissue homogenizer:

6: Vortex mixer:

6: 8 Nitrogen blowing instrument:

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 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 Preservation of samples Store below -18 degrees C and conduct analysis and testing within 3 months:

8 Measurement steps 8:1 Extraction Take 2:5g of the sample (accurate to +/-0:02g), add 100uL of internal standard working solution, let it stand for 10min, add 5mL of 80% acetonitrile aqueous solution, and vortex Mix for 1 minute, ultrasonic for 10 minutes, centrifuge at 10,000 r/min for 5 minutes at 4°C, take the supernatant, add 4 mL of 80%

acetonitrile aqueous solution to the residue, and repeat the extraction:

Take once, combine the supernatants, dilute to 10:0 mL with 80% acetonitrile aqueous solution, and set aside:

8:2 Purification Take about 1 mL of 80% acetonitrile aqueous solution to rinse the solid phase extraction column, discard the effluent, and pass 2:0 mL of the reserve solution through the column, keeping the flow rate at 1 drop/s:

Collect the effluent, blow it with nitrogen at 35°C to less than 0:5 mL, add water to adjust the volume to 0:5 mL, centrifuge with an ultrafiltration tube at 12000 r/min for 10 min, and take the filter liquid for determination by liquid chromatography-tandem mass spectrometry:

8:3 Preparation of standard curve Precisely measure appropriate amounts of the mixed standard intermediate solution and internal standard working solution, and prepare penicillin drugs with water concentrations of 2 ug/L, 5 ug/L, Mixed standard working solutions of 10 ug/L, 25 ug/L, 50 ug/L, 150 ug/L and 300 ug/L, in which the concentrations of penicillin internal standard solutions are all 40 ug/L: Take the measured characteristic ion mass chromatography peak external standard and internal standard peak area ratio as the ordinate, and the corresponding standard solution concentration as the abscissa, Draw a standard curve and find the regression equation and correlation coefficient:

8: Measurement 8:4:1 Liquid Chromatography Reference Conditions a) Chromatographic column: C18 chromatographic column (2:1mmx100mm, 2:6um), or one with equivalent performance;

b) Column temperature: 35 degrees C;

c) Flow rate: 0:4mL/min;

d) Injection volume: 10uL;

e) Mobile phase: A is 0:05% formic acid aqueous solution, B is 0:05% formic acid acetonitrile solution: Gradient elution conditions are shown in Table 1:

8:4:2 Mass Spectrometry Reference Conditions a) Ionization mode: electrospray ion source (ESI), multiple reaction monitoring (MRM), positive ion mode;

b) Spray voltage: 5:5kV;

c) Curtain air pressure: 0:24MPa;

d) Collision gas pressure: 0:02MPa;

e) Ion source temperature: 500 degrees C;

f) Collision chamber inlet voltage: 10V;

g) Collision chamber outlet voltage: 12V;