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

GB 31656.16-2022

**National food safety standards - Determination of
chloramphenicol, thiamphenicol, florfenicol and florfenicol
amine residues in aquatic products - gas chromatography**

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foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1. Structure and Drafting Rules for Standardization Documents" drafting.

This document is published for the first time.

National Food Safety Standards

Determination of chloramphenicol, thiamphenicol, florfenicol and florfenicol amine residues in aquatic products

gas chromatography

1 Scope

This document specifies the sample preparation and gas chromatographic determination for the determination of chloramphenicol, thiamphenicol, florfenicol and florfenicol amine residues in aquatic products

method.

This document is applicable to the residues of chloramphenicol, thiamphenicol, florfenicol and florfenicol amine in edible tissues of fish, shrimp, turtle, shellfish, sea cucumber and other aquatic products.

Quantitative determination.

2 Normative references

The content in the following documents constitutes the essential provisions of this document through normative references in the text. Among them, the dated reference documents,

Only the version corresponding to the date applies to this document; for undated references, the latest version (including all amendments) applies to this document

document.

GB/T 6682 Analytical laboratory water specifications and test methods

GB/T 30891-2014 Sampling Specifications for Aquatic Products

3 Terms and Definitions

This document does not have terms and definitions that need to be defined.

4 principles

The residual chloramphenicol, thiamphenicol, florfenicol and florfenicol amine in the sample were extracted with ethyl acetate under alkaline conditions and removed with n-hexane.

Lipid, solid phase extraction column purification, silanization reagent derivatization, gas chromatography detection, external standard method quantification.

5 Reagents and materials

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

5.2 Solution preparation

5.2.1 Ethyl acetate-ammonia water. Take 2mL of concentrated ammonia water and dilute it to 100mL with ethyl acetate.

5.2.2 Methanol-ammonia water. take 2mL of concentrated ammonia water and dilute to 100mL with methanol.

5.2.3 5% acetic acid solution. Take 5mL of acetic acid and dilute it with water to 100mL.

5.2.4 Derivatization reagent. take N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS),

Ratio of 99:1 mixed. Now ready to use.

5.3 Standards

Chloramphenicol, thiamphenicol, florfenicol, and florfenicol amine, the content of which was $\geq 98\%$. See Appendix A for details.

5.4 Preparation of standard solution

5.4.1 Standard stock solution. take 15 mg each of chloramphenicol, thiamphenicol, florfenicol, and florfenicol amine standard products, weigh them accurately, add acetonitrile

Appropriate amount to dissolve and dilute to a 100mL brown volumetric flask, and prepare a standard stock solution with a concentration of 150 μ g/mL. Below -18°C

Stored and valid for 3 months.

5.4.2 Mixed standard working solution. Accurately measure 1 mL of chloramphenicol standard stock solution, thiamphenicol, florfenicol and florfenicol amine respectively.

10mL in a 50mL volumetric flask, dilute to the mark with acetonitrile to make chloramphenicol concentration 3 μ g/mL, thiamphenicol, florfenicol and florfenicol

The mixed standard working solution with chloramine concentration of 30 μ g/mL was stored below -18 degrees C, and the validity period was 1 month.

7.1 Preparation of samples

Prepare samples according to the requirements of Appendix B of 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 standard working solution of appropriate concentration, and add the sample as a blank.

7.2 Storage of samples

Store below -18°C.

8.1 Extraction

Take 5g of the sample (accurate to +/-0.05g), put it in a 50mL stoppered glass centrifuge tube, add 20mL of ethyl acetate-ammonia water, vortex immediately

1 min, ultrasonication for 15 min, centrifugation at 4000r/min for 5 min, the supernatant was taken into a 100 mL chicken heart bottle, the extraction was repeated once, and the supernatant was combined.

Add 3 mL of 5% acetic acid solution, oscillate to mix, and evaporate at 40 °C to about 1.5 mL, set aside.

8.2 Purification

Take the spare solution in a 10mL centrifuge tube, wash the chicken heart bottle with 1.5mL of 5% acetic acid, and put the washing solution in the centrifuge tube. Add 5mL of n-hexane, vortex

Mix for 1 min, centrifuge at 4000r/min for 5 min, take the water layer, repeat degreasing once with 5 mL of n-hexane, take the water layer, and set aside.

Take the MCX solid-phase extraction column, activate it with 3mL of methanol and 3mL of water successively, take the spare solution degreased by n-hexane, pass it through the column, and wash it with 5% ethyl alcohol.