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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31656.6-2021

National food safety standard - Determination of eugenol residues in aquatic products by gas chromatography mass spectrometry method

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

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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 eugenol residues in aquatic products by gas chromatography-mass spectrometry

1 Scope

This document specifies the sample preparation and gas chromatography mass spectrometry methods for detecting eugenol residues in aquatic products:

This document is suitable for the detection of eugenol residues in edible tissues of fish and shrimp:

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 residual eugenol in the sample was extracted with acetonitrile, purified with a solid phase extraction column, degreased with n-hexane, measured by gas chromatography mass spectrometry, and quantified by the internal standard method:

55:1 Reagents

5:1:1 Acetonitrile (CH3CN): chromatographically pure:

5:1:2 n-hexane (C5H): chromatographically pure:

5:1:3 Ethyl acetate (CH3COOCH2CH3): chromatographically pure:

5:1:4 Methanol (CH3OH): chromatographically pure:

5:2 Solution preparation

5:2:1 Methanol solution: Take 10mL of methanol and add water to 100mL:

5:2:2 Acetonitrile-saturated n-hexane: Put:200 mL of n-hexane into a 250 mL separatory funnel, add an appropriate amount of acetonitrile, shake vigorously, and after the distribution is balanced, discard the lower acetonitrile layer to obtain:

5:3 Standard products

5:3:1 Eugenol (Eugenol, C10H12O2, CAS number: 97-53-0), content >= 99%:

5:3:2 Deuterated eugenol (Eugenol-d3, C10H9D3O2, CAS number: 1335401-17-6), content >= 99%:

5:4 Preparation of standard solution

5:4:1 Eugenol standard stock solution: Take 10 mg of eugenol standard, weigh it accurately, dissolve it in ethyl acetate and dilute it to 100 mL brown volume:

Use a measuring flask to prepare a standard stock solution with a concentration of 100 μg/mL: Store in a dark place below -18°C and has a validity period of 2 months:

5:4:2 Internal standard stock solution: Take 10 mg of deuterated eugenol standard, weigh it accurately, dissolve it in ethyl acetate and set it to a 100 mL brown volumetric flask to

prepare an internal standard stock solution with a concentration of 100 μg/mL: Store away from light below -18 degrees C, valid for 2 months:

5:4:3 Eugenol standard working solution: Accurately pipet an appropriate amount of eugenol standard stock solution and dilute it with ethyl acetate to a standard working solution with a concentration of 1 μg/mL:

For liquid use, store at 4°C away from light, valid for 1 month:

5:4:4 Internal standard working solution: Take an appropriate amount of the internal standard stock solution and dilute the standard working solution with a concentration of approximately 1 μg/mL with ethyl acetate: Keep away from light at 4°C:

Save, valid for 1 month:

5:5 Materials

5:5:1 Solid phase extraction column (C18): 300mg/3mL, or equivalent:

5:5:2 Stoppered plastic centrifuge tube: 50mL:

5:5:3 Nylon microporous filter membrane: 0:22μm:

6 Instruments and equipment

6:1 Gas chromatograph-tandem mass spectrometer: equipped with electron bombardment ionization ion source:

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

6:3 Nitrogen blowing instrument:

6:4 Vortex mixer:

6:5 Centrifuge: 4000r/min:

6:6 Ultrasonic oscillator:

6:7 Homogenizer:

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 about 2g of the sample (accurate to +/-0:02g), put it into a 50mL plastic centrifuge tube with a stopper, add 0:1mL of internal standard working solution and 10mL of acetonitrile, and vortex

Spin for 1 minute, sonicate for 10 minutes, and centrifuge at 4000 r/min for 5 minutes: Put the supernatant into another 50 mL plastic centrifuge tube with a stopper: Repeat the extraction of the residue once with 10 mL of acetonitrile, and combine the supernatants: Add 10 mL of acetonitrile-saturated n-hexane, skim, and vortex: ,1min, centrifuge at 4000r/min

After 5 minutes, take the acetonitrile layer and repeat degreasing once as described above: Blow nitrogen in a 45°C water bath until it is almost dry, add 10 mL of methanol aqueous solution to dissolve:

spare:

8:2 Purification

The solid-phase extraction column was activated with 3 mL of methanol and 3 mL of water each in sequence: Pass the backup solution through the column, add 3 mL of water to rinse, drain for 5 min, and add 3 mL of methanol:

Elute, take the eluate, blow dry with nitrogen, add 1:0 mL of ethyl acetate to dissolve, filter with a 0:22 μm filter, and measure by gas chromatography and mass spectrometry:

8:3 Preparation of standard curve

Precisely measure an appropriate amount of eugenol standard working solution and internal standard working solution, and dilute it with ethyl acetate into a series of standard working solutions with concentrations of 4 μg/L, 10 μg/L, 40 μg/L, 100 μg/L, 400 μg/L, and 800 μg/L: :The content of deuterated eugenol in the standard working solution is 100 μg/L: It is ready for use: Inject a series of standard working solutions, take the peak area ratio of eugenol and deuterated eugenol as the ordinate, and the corresponding eugenol concentration is On the abscissa, draw the standard working curve: Find the regression equation and correlation coefficient:

8:4 Determination

8:4:1 Gas chromatography reference conditions

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