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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31656.11-2021

National Food Safety Standard Determination of Oxytetracycline, Tetracycline, Chlortetracycline and Doxycycline Residues in Aquatic Products

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

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1 Scope

This document specifies a high-performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline residues in aquatic products:

This document is applicable to the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline residues in the edible tissues of aquatic products such as fish, shrimp, crab, turtle and sea cucumber:

Determination:

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

After the oxytetracycline, tetracycline, chlortetracycline and doxycycline in the sample were extracted with citric acid buffer solution, the solid phase extraction column was purified, and the liquid chromatography fluorescence Detection by detector and quantification by external

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standard method:

5 Reagents and materials

The reagents used below are of analytical grade unless otherwise noted; 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 Magnesium acetate (C4H6O4Mg.4H2O):

5:1:3 Imidazole (C3H4N2):

5:1:4 Potassium dihydrogen phosphate (KH2PO):

5:1:5 Citric acid (C6H8O3.H2O): excellent grade pure:

5:1:6 Disodium hydrogen phosphate (Na2HPO4): superior grade pure:

5:1:7 Disodium ethylenediaminetetraacetate (Na2EDTA.2H2O): superior grade pure:

5:1:8 Glacial acetic acid (CH3COOH):

5:2 Solution preparation 5:2:1 Potassium dihydrogen phosphate solution: Take 13:6 g of potassium dihydrogen phosphate, add an appropriate amount of water to dissolve and dilute to 1000 mL:

5:2:2 Methanol solution: Take 5 mL of methanol and 95 mL of water, and mix well:

5:2:3 Citric acid buffer: Take 6:30 g of citric acid, 13:8 g of disodium hydrogen phosphate, and 1:86 g of disodium ethylenediaminetetraacetate, add 400 mL of water to dissolve, adjust the pH to 4:0 with glacial acetic acid, and then dilute to 500 mL with water: :

5:2:4 Imidazole buffer solution: Take 68:0g of imidazole, 0:40g of disodium ethylenediaminetetraacetate, and 10:0g of magnesium acetate, add 800mL of water to dissolve, and dissolve with Adjust the pH to 7:2 with glacial acetic acid, dilute it with water to 1000mL, filter it through a 0:45um aqueous phase microporous membrane and set aside:

5:2:5 Methanol-imidazole buffer solution: Take 20 mL of methanol and 80 mL of imidazole buffer solution, and mix well:

5:3 Standard products The contents of tetracycline hydrochloride, chlortetracycline hydrochloride, oxytetracycline hydrochloride, and doxycycline hydrochloride are all >=95%: See Appendix A for details:

5:4 Preparation of standard solution 5:4:1 Standard stock solution (100pg/mL): Accurately weigh appropriate amounts of standards, dissolve and dilute to constant volume with methanol to prepare standards with concentrations of 100 ug/mL for oxytetracycline, tetracycline, chlortetracycline and doxycycline: Stock solution: Store away from light below

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-18 degrees C, valid for 1 month:

5:4:2 Mix standard intermediate solution: accurately pipette 1:0mL, 1:0mL, oxytetracycline, tetracycline, chlortetracycline and doxycycline standard stock solutions respectively:

2:0 mL and 2:0 mL in a 100 mL volumetric flask, dilute with methanol and adjust to volume, and prepare the concentrations of oxytetracycline, tetracycline, chlortetracycline and doxycycline Mixed standard intermediate solutions of 1:0ug/mL, 1:0ug/mL, 2:0ug/mL and 2:0ug/mL respectively: Store away from light below -18 degrees C, validity period is 7 days:

5:5 Materials 5:5:1 Solid phase extraction column: 500 mg/6 mL, Oasis HLB or equivalent:

5:5:2 Water phase microporous filter membrane: 0:22um:

6Instruments and equipment 6:1 High performance liquid chromatograph: equipped with fluorescence detector:

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

6:3 Analytical balance: sensitivity 0:00001 g:

6:4 Vortex Oscillator, 6:5 Centrifuge: speed 6000 r/min or above:

6:6 Solid phase extraction device (with negative pressure suction filter):

6:7 Nitrogen blower:

6:8 Tissue homogenizer:

7 Preparation and preservation of specimens 7:1 Preparation of specimens 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 standard working solution of appropriate concentration, and add the sample as a blank:

7:2 Storage of specimens Store below -18 degrees C:

8 Measurement steps 8:1 Extraction Take 5g of sample (accurate to +/-0:05g), add 20mL of citric acid buffer, vortex for 2 minutes, extract with ultrasonic for 10 minutes, centrifuge at 6000 r/min for 10 minutes, and take the supernatant in another 50mL centrifuge tube: : Add 10 mL of citric acid buffer to the residue, repeat the extraction twice, and combine the supernatants from three times for purification:

8:2 Purification Activate the Oasis HLB solid-phase extraction column with 10 mL of methanol, 10 mL of water, and 5 mL of citric acid buffer in sequence, transfer the solution to be purified, rinse with 10 mL of methanol solution, 10 mL of water, drain, add 10 mL of methanol for elution, and collect the eluate , blow dry with nitrogen at 40 degrees C, add

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potassium dihydrogen phosphate to dissolve Dissolve the residue in 1:0 mL of solution, sonicate for 1 min, and pass through a 0:22 um aqueous phase microporous membrane for high-performance liquid chromatography measurement:

8:3 Preparation of standard curve Accurately transfer an appropriate amount of the mixed standard intermediate solution of oxytetracycline, tetracycline, chlortetracycline and doxycycline, and dilute it with methanol-imidazole buffer solution to make the concentrations of oxytetracycline and tetracycline respectively 50 ng/mL, 100 ng/mL and:200 ng/mL, 500ng/mL, 1000ng/mL, mixed standard series working solutions with chlortetracycline and doxycycline concentrations of 100ng/mL,:200ng/mL, 400ng/mL, 1000ng/mL, and:2000ng/mL respectively: High performance liquid chromatography determination: Draw a standard curve with the peak area of the analyte as the ordinate and the corresponding standard solution concentration as the abscissa: Calculate the regression equation and correlation coefficient:

8:4 Determination 8:4:1 Liquid Chromatography Reference Conditions a) Chromatographic column: Cu chromatographic column (250mmx4:6mm, 5um), or equivalent;

b) Column temperature: 40 degrees C;

c) Injection volume: 30gL;

d) Flow rate: 0:8mL/min;

e) Excitation wavelength: 380 nm;

f) Emission wavelength: 520 nm;

g) Mobile phase: methanol-imidazole buffer solution:

8:4:2 Determination method Take the sample solution and the corresponding standard solution, perform single-point or multi-point calibration, quantify the chromatographic peak area, and calculate using the external standard method: The response values of oxytetracycline, tetracycline, chlortetracycline and doxycycline in the standard working solution and sample solution should be within the linear range of instrument detection: The HPLC chromatogram of the standard solution is shown in Figure B:1 in Appendix B:

8:5 Blank test Take the empty sample and perform parallel operations using the same measurement steps except that no standard solution is added:

9Result calculation and presentation The residual amount of the substance to be tested 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 oxytetracycline and tetracycline in the edible tissues of aquatic products by this method is 10:0 ug/kg, and the detection limit of doxycycline and chlortetracycline is 10:0 ug/kg:

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