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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31658.6-2021

National food safety standard - Determination of tetracylines residues in animal derived food by high performance liquid chromatography method

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

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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 tetracycline drug residues in animal foods

HPLC

1 Scope

This document specifies the sample preparation and high-performance liquid chromatography determination methods for the detection of tetracycline drug residues in animal foods:

This document is applicable to the muscles, livers and kidneys of pigs, cattle, sheep and chickens, the skin + fat of pigs and chickens, eggs, milk, fish skin + meat, and soil in shrimp muscles:

Detection of residual amounts of mycin, tetracycline, chlortetracycline and doxycycline:

2 Normative reference documents

The contents of the following documents constitute essential provisions of this document through normative citations in the text: Among them, referenced 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:

GB/T 6682 Specifications and test methods for water used in analytical laboratories

3 Terms and definitions

There are no terms or definitions that need to be defined in this document:

4 Principles

The remaining tetracyclines in the sample were extracted with EDTA.2Na-Mclvaine buffer solution, purified by solid phase extraction column, and analyzed by high performance liquid chromatography:

Determination by spectral UV method and quantification by external standard method:

5 Reagents and materials

The reagents used below are all analytically pure reagents 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 Acetonitrile (CH3CN): chromatographically pure:

5:1:3 Trifluoroacetic acid (CF3COOH):

5:1:4 Dichloromethane (CH2Cl2):

5:1:5 Disodium ethylenediaminetetraacetate (C10H14N2Na2O8):

5:1:6 Citric acid (C6H8O7:H2O):

5:1:7 Disodium hydrogen phosphate (NaH2PO4:12H2O):

5:1:8 Oxalic acid (H2C2O4:1:2H2O):

5:1:9 Sulfuric acid (H2SO4):

5:1:10 Sodium tungstate (Na2WO4):

5:2 Standard products

The content of oxytetracycline hydrochloride is >=97:0%, the content of tetracycline

hydrochloride is >=97:15%, the content of chlortetracycline hydrochloride is >=93:1%, and the content of doxycycline hydrochloride is

>=98:12%, see Appendix A:

5:3 Solution preparation

5:3:1 Citric acid solution: Take 21:01g of citric acid, dissolve it in water and dilute it to 1000mL:

5:3:2 Disodium hydrogen phosphate solution: Take 71:63g of disodium hydrogen phosphate, dissolve it in water and dilute it to 1000mL:

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5: Mclvain buffer solution (pH 4:0): Take 1000 mL of citric acid solution and 625 mL of disodium hydrogen phosphate solution, mix well, and add hydrochloric acid or hydrochloric acid to the solution:

Adjust the pH of the sodium hydroxide solution to 4:0+/-0:05:

5:EDTA:3:4 EDTA:2Na-Mclvaine buffer solution: Take 60:5g of disodium ethylenediaminetetraacetate and add Mclvaine buffer solution

1625mL, dissolve and mix:

5:3:5 Oxalic acid solution (0:01mol/L): Take 1:26g of oxalic acid, dissolve it in water and dilute it to 1000mL:

5:3:6 Trifluoroacetic acid solution: Take 0:8mL of trifluoroacetic acid, dissolve it in water and dilute it to 1000mL:

5:3:7 Sulfuric acid solution: Take 1:85mL of sulfuric acid, dissolve it in water and dilute it to 100mL:

5:3:8 Sodium tungstate solution: Take 7g of sodium tungstate, dissolve it in water and dilute it to 100mL:

5:3:9 Oxalic acid solution (1mol/L): Take 12:6g of oxalic acid, dissolve it in water and dilute it to 100mL:

5:3:10 Oxalic acid-acetonitrile solution: Take 20mL of oxalic acid solution (1mol/L), dissolve it in acetonitrile and dilute it to 100mL:

5:4 Preparation of standard solution

5:4:1 Standard stock solution: Take about 10 mg each of oxytetracycline hydrochloride, tetracycline hydrochloride, chlortetracycline hydrochloride and doxycycline hydrochloride standards and weigh them accurately:

Dissolve and dilute with methanol to a 10mL volumetric flask, and prepare oxytetracycline,

tetracycline, chlortetracycline and doxycycline at a concentration of 1 mg/mL:

Gluten standard stock solution: Store below -18 degrees C, valid for 1 month:

5:4:2 Mix standard working solution: Precisely measure 1 mL each of oxytetracycline, tetracycline, chlortetracycline and doxycycline standard stock solutions, and put it into a 100 mL volume:

In a measuring flask, dilute with methanol to the mark and prepare a mixed standard working solution with a concentration of 10 μg/mL: Store at 2°C ~ 8°C: Prepare ready for use:

5:5 Materials

5.

Polymer, or equivalent:

5:5:2 LCX solid phase extraction column 1): 500mg/6mL, the filler is phosphorylated polystyrene divinylbenzene polymer, or equivalent:

1) The LCX solid-phase extraction columns listed in this experiment are developed and provided by Agela Company: The solid-phase extraction columns listed here are for reference only and do not involve commercial purposes: Standard users are encouraged

Try using solid phase extraction columns from different manufacturers or models:

6 Instruments and equipment

6:1 High performance liquid chromatograph: equipped with UV detector:

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

6:3 Tissue homogenizer:

6: Vortex mixer: 3000r/min:

6: Low temperature centrifuge: the speed can reach 8500r/min:

6:6 Solid phase extraction device:

6:7 Nitrogen blower:

6:8 Nylon microporous filter membrane: 0:22μm:

6: Centrifuge tube: 50mL:

7: Preparation and preservation of samples

7:1 Preparation of samples

7:1 Organization