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

GB 31658.1-2021

**National food safety standard - Determination of ceftiofur
residues in animal tissues by high performance liquid
chromatography method**

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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:

National food safety standards

Determination of ceftiofur residues in animal foods by high performance liquid chromatography

Method 1 Determination of ceftiofur residues in edible animal tissues

HPLC

1 Scope

This document specifies the sample preparation and HPLC determination methods for the detection of ceftiofur residues in edible animal tissues:

This document is applicable to the determination of ceftiofur residues in muscle, fat, liver and kidney of pigs and cattle:

2 Normative application documents

The contents of the following documents constitute essential provisions of this document

through normative citations in the text: Among them, the dated citations

For undated referenced documents, only the version corresponding to that 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 residual ceftiofur in the sample is related to ceftiofur and desfuranoylceftiofur (DFC) through the action of dithioerythritol (DTE):

Metabolites are separated from proteins or sulfur-containing compounds and react with iodoacetamide to generate stable acetamide derivatives (DCA), which are extracted, purified and purified:

chemical, high performance liquid chromatography, and external standard method for quantitation:

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 (CH_3CN): chromatographically pure:

5:1:2 Methanol (CH_3OH):

5:1:3 Dithioerythritol ($\text{C}_4\text{H}_{10}\text{O}_2\text{S}_2$, DTE):

5:1:4 Iodoacetamide ($\text{C}_2\text{H}_4\text{INO}$):

5:1:5 Anhydrous calcium chloride (CaCl_2):

5:1:6 Glacial acetic acid (CH_3COOH):

5:1:7 Phosphoric acid (H_3PO_4): 85%:

5:1:8 Potassium chloride (KCl):

5:1:9 Potassium hydroxide (NaOH):

5:1:10 Potassium dihydrogen phosphate (KH_2PO_4):

5:1:1 Sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$):

5:1:12 Sodium chloride (NaCl):

5:1:13 Sodium hydroxide (NaOH):

5:1:14 Trifluoroacetic acid (CF_3COOH , TFA):

5:2 Standard products

or equivalent:

5:3 Solution preparation

5:3:1 Boric acid buffer (0:05mol/L): Take 19g sodium tetraborate and 3:7g potassium chloride, add water to dissolve and dilute to 1000mL:

5:3:2 Phosphate buffer (0:025mol/L): Take 3:04g of potassium dihydrogen phosphate, add water to dissolve and dilute to 1000mL, and oxidize with 45% hydrogen phosphate:

Adjust the pH of the potassium solution to 7:0:

5:3 Extraction solution (0:4% DTE): Take 1g of DTE, add an appropriate amount of boric acid buffer to dissolve it and dilute it to 250mL: Prepare it now:

5:3:4 Iodoacetamide solution (14%): Dissolve 7g of iodoacetamide in 50mL of phosphate buffer and prepare it immediately:

5:3:5 Sodium hydroxide solution (0:01mol/L): Take 0:4g of sodium hydroxide, add water to dissolve and dilute to 1000mL:

5:3:6 Sodium chloride solution (0:1mol/L): Take 5:9g of sodium chloride, dissolve it in water and dilute it to 1000mL:

5:3:7 Calcium chloride solution (0:1mol/L): Take 11:1g of anhydrous calcium chloride, dissolve it in water and dilute it to 1000mL:

5:3:8 Phosphoric acid solution (25%): Take 25mL of phosphoric acid, add water to dissolve and dilute to 100mL:

5:3:9 Acetic acid solution (5%): Take 5mL of glacial acetic acid, add water to dissolve and dilute to 100mL:

5:3:10 C18 eluent: acetonitrile-water (15:85)

5:3:11 SAX pre-wash solution: methanol-sodium chloride solution (25:75):

5:3:12 SAX eluent: acetonitrile-acetic acid solution (5:95):

5:3:13 SCX pre-wash solution: methanol-calcium chloride solution (25:75):

5:3:14 SCX eluent: acetonitrile-sodium chloride solution (5:95):

5:3:15 Mobile phase:

A (0:1% TFA aqueous solution): Take 1mL of trifluoroacetic acid and add water to make it 1000mL:

B (0:1% TFA acetonitrile solution): Take 1mL of trifluoroacetic acid and add acetonitrile to 1000mL:

5:4 Preparation of standard solution

5:4:1 Standard stock solution (100µg/mL): Take about 11mg of ceftiofur hydrochloride standard, weigh it accurately, add methanol to dissolve and dilute to volume

into a 100mL volumetric flask, shake well, and you have it: Store at -18°C and is valid for 6 months:

5:4:2 Standard working solution (10 µg/mL): Precisely measure 5 mL of the standard stock solution into a 50 mL volumetric flask, and add phosphate buffer solution to the mark:

Shake well and get it: Store at 2 degrees C~8 degrees C, valid for 1 month:

5:5 Materials

5:5:1 C18 solid phase extraction column: 1g/6mL, or equivalent:

5:5:2 SAX solid phase extraction column: 500mg/10mL, or equivalent:

5:5:3 SCX solid phase extraction column: 100mg/10mL, or equivalent:

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 Constant temperature water bath oscillator:

6: Homogenizer:

6: Vortex mixer:

6: Refrigerated centrifuge

6:7 Solid phase extraction device:

6:8 pH meter:

6: Centrifuge tube: 50mL:

7 Preparation and preservation of specimens