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# NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA



# GB 31658.4-2021

## National food safety standard - Determination of cephalosporins residues in animal derived food by liquid chromatography-tandem 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 cephalosporin residues in animal foods

Liquid chromatography-tandem mass spectrometry

## 1 Scope

This document specifies sample preparation and liquid chromatography G-string for detection of cephalosporin residues in pig, cattle muscle, liver, kidney, fat and milk:

Mass spectrometric determination method:

This document applies to cephalosporin drugs (cephalexin, cefradine, cefazolin,

Detection of residues of cefoperazone, cefacetonitrile, cefpirin, ceftronine, cefquinoxime, and cefotaxime):

## 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

## 3 Terms and definitions

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

## 4 Principles

The residual cephalosporins in the sample were extracted with acetonitrile-water and phosphate buffer, purified with a solid-phase extraction column, and measured by liquid chromatography-tandem mass spectrometry:

Determination, external standard method quantification:

## 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

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

5:1:2 Acetonitrile (CH3CN): chromatographically pure:

5:1:3 Formic acid (HCOOH): chromatographically pure:

5:1:4 Potassium dihydrogen phosphate (KH2PO4):

5:1:5 Sodium hydroxide (NaOH):

5:1:6 Sodium chloride (NaCl):

5:2 Solution preparation

5:2:1 Sodium hydroxide solution: Take 50g of sodium hydroxide, add water to dissolve and dilute to 500mL:

5:2:2 Acetonitrile-water solution (15:2, V/V):

5:2:3 30% acetonitrile solution: Take 30mL of acetonitrile and dilute to 100mL with water:

5:05mol/L phosphate buffer (pH=8:5): Take 6:8g of potassium dihydrogen phosphate, dissolve it in water and dilute it to 1000mL, add hydroxide

Adjust the pH of the sodium solution to 8:5+/-0:1:

5:2:5 0:1% formic acid solution: Take 1mL of formic acid, dissolve and dilute to 1000mL with water, and mix well:

5:3 Standard products

Cephalexin, cefradine, cefazolin, cefoperazone, cefacetonitrile, cefpirin, desacetylcefapirin, ceftronin, cefquine

Oxime and cefotaxime standard products, the contents are >=95:0%, see Appendix A:

5:4 Preparation of standard solution

5:4:1 Mixed standard stock solution: take cephalexin, cefradine, cefazolin, cefoperazone, cefacetonitrile, cefpirin, deacetylcephalosporin

Take 10 mg each of Pirin, ceftronine, cefquinoxime, and cefotaxime standard standards, weigh them accurately, dissolve them with 30% acetonitrile solution, and dilute to a constant volume:

25 mL volumetric flask, prepare a solution with a concentration of 400 μg/mL: Store in -18°C protected from light, valid for 1 month:

5:4:2 Mixed standard working solution: Precisely measure an appropriate amount of mixed standard stock solution, put it into a 10mL volumetric flask, and dilute it with water to the mark: Prepare

Mix standard working solutions with concentrations of 0:05 μg/mL, 0:50 μg/mL, 2:0 μg/mL and 20 μg/mL: Store in the dark at 4°C:

Validity period 7d:

5:5 Materials

5:5:1 HLB solid phase extraction column, 500mg/6mL, or equivalent:

5:5:2 Filter membrane: nylon material, pore size 0:22μm, or equivalent performance:

## 6 Instruments and equipment

6:1 Liquid chromatography-tandem mass spectrometer: equipped with electrospray ion source:

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

6:3 Rotary evaporator:

6:4 Solid phase extraction device:

6: Homogenizer:

6: Vortex mixer:

6:7 Polypropylene plastic centrifuge tubes: 10mL, 50mL:

6: Centrifuge: 5000r/min or above:

6:9 Chicken heart bottle: 50mL:

7: Preparation and preservation of samples

7:1 Preparation of samples

Take an appropriate amount of fresh or thawed blank or test tissue, mince it, and homogenize it:

a) Take the homogenized test sample as the test material;

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 Preservation of samples

Store below -18 degrees C and conduct analysis and testing within 3 months:

## 8 Measurement steps

8:1 Extraction

Take 2g of the sample (accurate to +/-0:02g), put it in a 50mL centrifuge tube, add 10mL of acetonitrile-G aqueous solution or 10mL of acetonitrile (only applicable to cattle

Milk), homogenize at 10000r/min for 1min, centrifuge at 5000r/min for 2min, collect the supernatant and put it in a chicken heart bottle: Use 10mL of the above solution for the residue

Repeat the extraction once, combine the extracts, and rotary evaporate in a 40°C water bath to remove acetonitrile (if there is foam, add 4 mL of saturated sodium chloride aqueous solution), and immediately

That is, add 25 mL of phosphate buffer solution and set aside:

8:2 Purification

Take the solid phase extraction column and activate it with 5 mL of methanol and 10 mL of phosphate buffer: Pass the backup solution through the column: When the liquid level reaches the surface of the column bed, use phosphorus:

Elute with 3 mL of acetonitrile buffer solution, rinse with 2 mL of water, elute with 3 mL of acetonitrile in a 10 mL centrifuge tube, blow dry with nitrogen in a 40°C water bath, and add