



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

GB 31657.2-2021

**National Food Safety Standard Determination of Multiple
Residues of Quinolones in Bee Products Liquid
Chromatography-Tandem Mass Spectrometry**

食品安全国家标准 蜂产品中喹诺酮类药物多残留的测定 液相色谱-串联质谱法
(English Translation)

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Table of Contents

- 1 Scope
- 2 Normative reference documents
- 3 Terms and Definitions
- 4 Principles
- 5 Reagents and Materials
- 6 Instruments and equipment
- 7 Preparation and preservation of samples
- 8 Measurement steps
- 9 Result calculation and presentation
- 10 Sensitivity, accuracy and precision of detection methods

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry detection methods for the detection of quinolone residues in honey and royal jelly: This document applies to oxalic acid, nalidixic acid, flumequine, ciprofloxacin, difloxacin, sarafloxacin, ofloxacin, enrofloxacin, lomefloxacin, and pefloxacin in honey and royal jelly single or Determination of multiple drug residues:

2 Normative reference documents

The contents of the following documents constitute essential provisions of this document through normative references in the text: Among them, for dated reference documents, only the version corresponding to the date applies to this document; for undated reference 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 to be defined in this document:

4 Principles

The remaining quinolones in the sample were extracted with phosphate buffer, purified with HLB solid phase extraction column, measured by liquid chromatography-tandem mass spectrometry, calibrated by matrix matching standard curve, and quantified by external

standard method:

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

Acetonitrile (CH_3CN)

Chromatographically pure:

5.1.2

Methanol (CH_3OH)

Chromatographically pure:

5.1.3

Formic acid (CHOOH)

Chromatographically pure:

5.1.4

Ethyl acetate ($\text{C}_4\text{H}_8\text{O}_2$):

5.1.5

Disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$):

5.1.6

Sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$):

5.1.7

Phosphoric acid (H_3PO_4):

5.2

Solution preparation

5.2.1

Phosphate buffer

Take 7.16 g of disodium hydrogen phosphate and 3.12 g of sodium dihydrogen phosphate, add 800 mL of water to dissolve, and adjust the pH to 3.0 ± 0.05 , dilute with water to 1000 mL:

5:2:2 5% formic acid in methanol solution: Take 5 mL of formic acid, add 95 mL of methanol, and mix:

5:2:30.1% formic acid solution: Take 0.5 mL of formic acid, add water to dilute to 500 mL, and mix well:

5:2:4 20% acetonitrile formic acid solution: Take 20 mL of acetonitrile, add 80 mL of 0.1% formic acid solution, and mix well:

5.3

Standard products Oxalinic acid, flumequine, cinoxacin, difloxacin, sarafloxacin, ofloxacin, enrofloxacin, lomefloxacin, pefloxacin, norfloxacin, Ciprofloxacin, nalidixic acid, enoxacin, danofloxacin, marbofloxacin, fleroxacin, gatifloxacin, orbifloxacin, pipemidic acid and sparfloxacin, the contents are all $\geq 95\%$, See Appendix A for specific information:

5.4

Preparation of standard solution

5.4.1

Standard stock solution (1 mg/mL)

Take oxalinic acid, flumequine, cinoxacin, difloxacin, sarafloxacin, ofloxacin, enrofloxacin, lomefloxacin, and Floxacin, norfloxacin, ciprofloxacin, nalidixic acid, enoxacin, danofloxacin, marbofloxacin, fleroxacin, gatifloxacin, orbifloxacin, piperonic acid, and difloxacin Pafloxacin reference substance is 10 mg each, weighed accurately, dissolved in 0.5 mL ammonia water, diluted with methanol to a 10 mL volumetric flask, and prepared into a standard stock solution with a concentration of 1 mg/mL: Store at -18°C away from light, valid for 6 months:

5.4.2

Mixed standard working solution (10 $\mu\text{g/mL}$)

Accurately measure 1 mL of each of the above standard stock solutions in a 100 mL volumetric flask, dilute to the mark with methanol, and prepare a mixed standard working solution with a concentration of 10 $\mu\text{g/mL}$: Store at 4°C away from light, valid for 1 month:

5.5

Materials

5.5.1

HLB solid phase extraction column::200 mg/6 mL, or equivalent:

5.5.2

Microporous nylon filter membrane

0.22 μm :

5.5.3

Glass fiber filter paper

1.6 μm :

6 Instruments and equipment

6.1

Liquid chromatography-tandem mass spectrometer

Equipped with electrospray ion source:

6.2

Analytical balance

Sensitive to 0.00001g and 0.01g:

6.3

Vortex mixer:

6.4

Vortex oscillator:

6.5

High-speed refrigerated centrifuge

The speed can reach:20000 r/min:

6.6

Ultrasonic cleaning machine:

6.7

Solid phase extraction device:

6.8

Nitrogen blower,

7 Preparation and preservation of samples

7.1

Preparation of samples Take an appropriate amount of fresh or thawed blank or test bee products and mix them evenly: For honey samples with crystallization, place them in a water bath not exceeding 60°C under closed conditions to decrystallize them: After the samples are completely melted, stir well and cool to room temperature: The prepared sample is placed in a sample bottle, sealed, and marked:

- 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: