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

GB 31659.2-2022

**National Food Safety Standard Determination of Doxycycline
Residues in Poultry Eggs, Milk and Milk Powder by Liquid
Chromatography-Tandem Mass Spectrometry**

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foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1. Structure and Drafting Rules for Standardization Documents" drafting.

This document is published for the first time.

National Food Safety Standards

Determination of doxycycline residues in poultry eggs, milk and milk powder by liquid chromatography-tandem mass spectrometry

1 Scope

This document specifies the sample preparation and liquid chromatography-tandem mass spectrometry method for the detection of doxycycline residues in poultry eggs, milk and milk powder.

This document is applicable to the determination of doxycycline residues in eggs, duck eggs, goose eggs, milk powder, goat milk powder, milk and goat milk.

2 Normative references

The content in the following documents constitutes the essential provisions of this document through normative references in the text. Among them, the dated reference documents,

Only the version corresponding to the date applies to this document; for undated references, the latest version (including all amendments) applies to this document document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Terms and Definitions

This document does not have terms and definitions that need to be defined.

4 principles

The residual doxycycline in the sample was extracted by McIlvaine-Na₂EDTA buffer, purified

by HLB column, and detected by liquid chromatography-tandem mass spectrometry. determined, and quantified by the external standard method.

5.1 Reagents

Unless otherwise specified, all reagents are of analytical grade, and the water is first-class water in accordance with GB/T 6682.

5.2 Solution preparation

5.2.1 Sodium hydroxide solution (1mol/L). Take 4g of sodium hydroxide, add water to dissolve and dilute to 100mL, mix well.

5.2.2 Mclvaine-Na₂EDTA buffer solution. take 12.9 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate, and 27.6 g of ethylenediaminetetraethyl dihydrate

Add 900 mL of water to dissolve 37.2 g of disodium diacetate, adjust the pH to 4.0 +/- 0.5 with sodium hydroxide solution, add water to dilute to 1000 mL, and mix well.

5.2.3 0.1% formic acid solution. Take 500 µL of formic acid, dilute it with water to 500 mL, and mix well.

5.2.4 5% methanol solution. Take 5mL of methanol, dilute to 100mL with water, and mix well.

5.2.5 30% methanol solution. Take 30mL of methanol, dilute to 100mL with water, and mix well.

5.3 Standards

Doxycycline hydrochloride (doxycyclinehydrochloride, C₂₂H₂₄N₂O₈.HCl, CAS number. 10592-13-9), content >=

98.7%.

5.4 Preparation of standard solution

5.4.1 Standard stock solution. Take an appropriate amount of doxycycline hydrochloride (equivalent to 10 mg of doxycycline), weigh it accurately, add an appropriate amount of methanol to dissolve and constant volume

to a 10mL volumetric flask, and prepared into a standard stock solution with a concentration of 1mg/mL. Store below -18°C, and the validity period is 1 month.

5.4.2 Standard intermediate solution I. Accurately measure 0.1mL of standard stock solution, put it into a 10mL volumetric flask, dilute to the mark with 30% methanol, mix well,

and prepare

The standard intermediate solution I with a concentration of 10 µg/mL was prepared.

5.4.3 Standard intermediate solution II. Accurately measure 1.0mL of intermediate solution I, put it in a 10mL volumetric flask, dilute it to the mark with 30% methanol, mix well, and prepare

A standard intermediate solution II with a concentration of 1000ng/mL was prepared.

5.4.4 series of standard working solutions. Accurately measure 0.1mL, 0.2mL, 0.5mL, 1.0mL and 2.0mL of the standard intermediate solution II at

In a 10mL volumetric flask, dilute to the mark with 30% methanol, mix well, and prepare concentrations of 10ng/mL, 20ng/mL, 50ng/mL,

100ng/mL, 200ng/mL series of standard working solutions. Ready to use.

5.5 Materials

5.5.1 Solid-phase extraction column. hydrophilic-lipophilic balanced solid-phase extraction column 60mg/3mL.

7.1 Preparation of samples

Take an appropriate amount of fresh blank or test poultry eggs, shell them and homogenize them.

Take an appropriate amount of fresh or thawed blank or test milk, and mix well.

Take an appropriate amount of fresh blank or test milk powder and mix well.

- a) Take the homogeneous test sample as the test sample;
- b) Take a homogeneous blank sample as a blank sample;
- c) Take a homogeneous blank sample, add a standard solution of appropriate concentration, and add a sample as a blank.

7.2 Storage of samples

Store poultry eggs and milk below -18°C, store milk powder at room temperature and avoid light.

8.1 Extraction

Poultry eggs and milk. Take 2g of the test material (accurate to +/-0.05g), put it in a 50mL polypropylene centrifuge tube, add McIlvaine-Na₂EDTA buffer

8 mL of solution, shaken for 10 min, centrifuged at 14000 r/min for 10 min at 4 °C, and