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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 22259-2025

Replacing GB/T 22259-2008

Determination of oxytetracycline in feeds

饲料中土霉素的测定

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1 Scope

This document describes methods for the determination of oxytetracycline in feeds using liquid chromatography-tandem mass spectrometry and high-performance liquid chromatography.

This document is applicable to the determination of oxytetracycline in compound feeds, concentrate feeds, concentrate supplements, premixes, feed ingredients, and mixed feed additives.

In this document, the detection limit for the liquid chromatography-tandem mass spectrometry method is 0.02 mg/kg, and the quantification limit is 0.05 mg/kg. For the high-performance liquid chromatography method, the detection limit for compound feeds (excluding aquatic compound feeds), concentrate feeds, concentrate supplements, and feed ingredients is 1.0 mg/kg, with a quantification limit of 2.0 mg/kg; for aquatic compound feeds, the detection limit is 5.0 mg/kg, with a quantification limit of 10.0 mg/kg; and for additive premixes and mixed-type feed additives, the detection limit is 2.5 mg/kg, with a quantification limit of 5.0 mg/kg.

2 Normative references

GB/T 6682

GB/T 20195

3 Terms and definitions

This document contains no terms or definitions requiring definition.

4 Liquid chromatography-tandem mass spectrometry (LC-

MS/MS)

4.1 Principle

Oxytetracycline in the specimen is extracted using a methanolic hydrochloric acid solution or a buffer solution. The extract is subsequently diluted or purified using an HLB solid-phase extraction column. Determination is performed using liquid chromatography-tandem mass spectrometry. Correction is applied using a matrix- matched standard curve, and quantification is carried out using the external standard method.

4.2 Reagents or materials

Unless otherwise specified, only analytically pure reagents shall be used.

4.2.1 Water. GB/T 6682, Grade 1.

4.2.2 Acetonitrile. chromatographically pure.

4.2.3 Methanol. chromatographically pure.

4.2.4 Formic acid. chromatographically pure.

4.2.5 Methanolic hydrochloric acid solution. pipette 20 mL of hydrochloric acid into

1000 mL of methanol, and mix thoroughly.

4.2.6 Hydrochloric acid solution (1 mol/L). pipette 9 mL of hydrochloric acid, dilute

with water to 100 mL, and mix thoroughly.

4.2.7 Sodium hydroxide solution (1 mol/L). weigh 4 g of sodium hydroxide, dissolve it in water, dilute to 100 mL, and mix thoroughly.

4.2.8 Buffer solution I. weigh out 12.9 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate, and 37.2 g of disodium ethylenediaminetetraacetate dihydrate, respectively. Add 900 mL of water to dissolve, and mix thoroughly. Adjust the pH to 4.0 using hydrochloric acid solution (4.2.6) and sodium hydroxide solution (4.2.7). Dilute with water to a final volume of 1000 mL, and mix thoroughly.

4.2.9 10% methanol solution. pipette 10 mL of methanol (4.2.3), dilute with water to 100 mL, and mix well.

4.2.10 20% methanol solution. pipette 20 mL of methanol (4.2.3), dilute with water to 100 mL, and mix well.

4.2.11 0.1% formic acid solution. pipette 1 mL of formic acid (4.2.4), dilute with water to 1,000 mL, and mix well.

4.2.12 Standard stock solution (1 mg/mL). accurately weigh an appropriate amount of

the oxytetracycline reference standard (CAS. 79-57-2; purity >= 88%) - weighed to the nearest 0.01 mg and equivalent to 10 mg of oxytetracycline - into a 10 mL volumetric flask. Add methanol (4.2.3) to dissolve the substance and bring the solution to the set volume. Mix thoroughly. Store at a temperature of -18°C or below. The solution is valid for 1 month.

4.2.13 Standard working solution (10 μg/mL). accurately transfer 1 mL of the standard

stock solution (4.2.12) into a 100 mL volumetric flask. Dilute to volume with methanol and mix thoroughly. Prepare immediately before use.

4.2.14 Standard series solution I. accurately pipette an appropriate volume of the

standard working solution (4.2.13). Dilute with 20% methanol solution (4.2.10) to prepare a series of standard solutions with mass concentrations of 20 ng/mL, 40 ng/mL, 100 ng/mL, 200 ng/mL, 1000 ng/mL, and 2000 ng/mL, respectively. Prepare when needed.

4.2.15 Standard series solution II. accurately pipette an appropriate volume of the

standard working solution (4.2.13). Dilute with 20% methanol solution (4.2.10) to prepare a series of standard solutions with mass concentrations of 1 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/mL, 50 ng/mL, and 100 ng/mL, respectively. Prepare when needed.

4.2.16 Hydrophilic-lipophilic balance (HLB) solid-phase extraction column. 60 mg/3

mL, or equivalent performance.

4.2.17 Microporous filter membrane. nylon, 0.22 μm.

4.3.1 Liquid chromatography-tandem mass spectrometer. equipped with an

electrospray ionization source.

4.3.2 Analytical balance. with a precision of 0.01 g and 0.01 mg. 4.3.3 Vortex mixer. 4.3.4 Vortex shaker. 4.3.5 Ultrasonic cleaner.

4.3.6 Centrifuge. with a rotation speed of not less than 10000 r/min.

4.3.7 Solid-phase extraction device. 4.3.8 Nitrogen evaporator.

4.4 Sample

Prepare a specimen in accordance with GB/T 20195, weighing at least 200 g. Pulverize the specimen until it passes entirely through a 0.425 mm test sieve. Mix thoroughly, then transfer the specimen into a sealed container for future use. Select a feed sample of the same type - one that is homogeneous and consistent, and for which the instrument response at the retention time of the analyte is less than 30% of the method's limit of quantification - to serve as the blank sample.

4.5.1 Compound feeds, concentrated feeds, concentrate supplements, and feed

ingredients Perform the test in duplicate. Weigh 5 g (accurate to 0.01 g) of the specimen into a 50 mL centrifuge tube. Accurately add 25 mL of the hydrochloric acid-methanol solution (4.2.5). Vortex to mix. Shake to extract for 10 min. Centrifuge at 10000 r/min for 5 min.

Accurately transfer 0.2 mL of the supernatant into another centrifuge tube. Accurately add 0.8 mL of water. Vortex to mix. Centrifuge at 10000 r/min for 5 min. Filter the supernatant through a microporous membrane (4.2.17). The filtrate is ready for analysis.

4.5.2.1 Extraction

Perform the test in duplicate. Weigh 2 g (accurate to 0.01 g) of the specimen into a 50 mL centrifuge tube. Add 20 mL of buffer solution I (4.2.8). Vortex to mix. Shake for extraction for 10 min. Perform ultrasonic extraction for 10 min. Centrifuge at 10000 r/min for 10 min. Transfer the supernatant to another centrifuge tube. Re-extract the residue successively with 20 mL and 10 mL of buffer solution I (4.2.8). Combine the supernatants. Dilute to a final volume of 50 mL using buffer solution I (4.2.8). Mix thoroughly.

4.5.2.2 Purification

Activate the solid-phase extraction column (4.2.16) sequentially with 3 mL of methanol, 3 mL of water, and 3 mL of buffer solution I (4.2.8). Accurately transfer 1 mL of the extraction solution onto the column. Wash the column successively with 3 mL of water and 3 mL of 10% methanol solution (4.2.9). Draw to dryness. Elute with 3 mL of methanol. Collect the eluate and dry it under a stream of nitrogen at 40°C. Accurately add 1 mL of 20% methanol solution (4.2.10) and vortex to dissolve the residue. Filter through a microporous membrane (4.2.17) and submit for analysis.

4.5.3.1 Compound feeds, concentrated feeds, concentrate supplements, and feed

ingredients Take a blank specimen. Process it in accordance with 4.5.1 to obtain a blank matrix solution. Accurately pipette 50 µL aliquots of standard series solution I (4.2.14) into separate vessels. Dilute each aliquot to a final volume of 1.0 mL using the blank matrix