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Replacing GB/T 19684-2005

Determination of chlortetracycline in feeds

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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 supersedes GB/T 19684-2005 "Determination of Chlortetracycline in Feed by High Performance Liquid Chromatography" and is consistent with GB/T 19684-2005. In comparison, aside from structural adjustments and editorial changes, the main technical changes are as follows:

a) The range and detection limit have been changed (see Chapter 1, Chapter 1 of the.2005 edition);

b) The principle has been changed (see 4.1, Chapter 3 of the.2005 edition);

c) The experimental procedures have been changed (see 4.5, Chapter 7 of the.2005 edition);

d) The precision was changed (see 4.7, Chapter 9 of the.2007 edition);

e) Liquid chromatography-tandem mass spectrometry has been added (see Chapter 5). Please note that some content in this document may involve patents. The issuing organization of this document assumes no responsibility for identifying patents. This document was proposed and is under the jurisdiction of the National Technical Committee

on Standardization of Feed Industry (SAC/TC76). This document was drafted by: China Agricultural University and Tangshan Food and Drug Comprehensive Inspection and Testing Center. The main drafters of this document are. Yang Wenjun, Xiao Jin, Tian Ying, Guo Liang, Chen Yiqiang, Zheng Baiqin, Li Mingyang, Zhang Zhenguo, Wang Lin, and Liang Shanshan. The release history of this document and the document it replaces is as follows:

---First published in.2005 as GB/T 19684-2005;

---This is the first revision. Determination of chlortetracycline in feed

1.Scope This document describes the high performance liquid chromatography and liquid chromatography-tandem mass spectrometry methods for the determination of chlortetracycline in feed. This document applies to compound feed, concentrated feed, concentrate supplements, additive premixes, mixed feed additives, and animal-derived feeds. Determination of chlortetracycline in feed ingredients. The detection limit for compound feed and animal-derived feed ingredients in this document is 2 mg/kg, and the quantitation limit is 4 mg/kg; (The text abruptly ends here, so the translation stops as well.) The detection limit for feed, concentrate supplement, additive premixed feed, and mixed feed additives is 5 mg/kg, and the quantification limit is 10 mg/kg. The detection limit for compound feed and animal-derived feed ingredients by liquid chromatography-tandem mass spectrometry was

0.01 mg/kg, and the quantitation limit was

0.05 mg/kg; The detection limit for saturated feed, concentrate supplements, additive premixed feed, and mixed feed additives is

0.025 mg/kg, and the quantitation limit is... The concentration is

0.1 mg/kg.

4 High Performance Liquid Chromatography

4.1 Principle The chlortetracycline in the sample was extracted with Na2EDTA-Mclvaine-methanol solution, determined by high performance liquid chromatography, and quantified by external standard method.

4.2 Reagents or Materials Unless otherwise specified, use only analytical grade reagents.

4.2.1 Water. GB/T 6682, Grade I.

4.2.2 Methanol. chromatographic grade.

4.2.3 Acetonitrile. chromatographic grade.

4.2.4 Sodium hydroxide solution (1 mol/L). Weigh 4 g of sodium hydroxide, dissolve it in water, transfer it to a 100 mL volumetric flask, make up to volume, and mix well.

4.2.5 Hydrochloric acid solution (1 mol/L). Transfer 9 mL of hydrochloric acid to a 100 mL

volumetric flask, dilute with water to the final volume, and mix well.

4.2.6 Na2EDTA-McIvaine buffer solution (pH=6). Weigh

7.1 g citric acid monohydrate and

17.9 g dihydrogen phosphate dodecahydrate. Sodium,

37.2 g disodium ethylenediaminetetraacetate dihydrate (Na2EDTA·2H2O), dissolved in water, transferred to a 1000 mL volumetric flask, and diluted. Mix well and adjust the pH to 6 with sodium hydroxide solution (4.2.4) or hydrochloric acid solution (4.2.5).

4.2.7 Extraction solution. Take 900 mL of Na2EDTA-McIvaine buffer solution (4.2.6) and 100 mL of methanol, and mix well.

4.2.8 Oxalic acid solution (10 mmol/L). Weigh

1.26 g of oxalic acid dihydrate, dissolve in water, and dilute to 1000 mL. Mix well.

4.2.9 Standard stock solution (1 mg/mL). Accurately weigh an appropriate amount of chlortetracycline hydrochloride standard (calculated as chlortetracycline, accurate to

0.01 mg). CAS No.. 64-72-2 (purity >=98%) was dissolved in a 10 mL volumetric flask with methanol (4.2.2), diluted to volume, and mixed well. Store below -18°C. Stored, valid for 6 months.

4.2.10 Standard Intermediate Solution (100 μg/mL). Accurately transfer 1 mL of the standard stock solution (4.2.9) into a 10 mL volumetric flask, and use [method name missing] to [previous preparation]. Dilute and bring to volume with alcohol (4.2.2), mix well. Store below -18°C, shelf life 7 days.

4.2.11 Standard series solutions. Accurately transfer an appropriate amount of the standard intermediate solution (4.2.10), dilute it with the extract (4.2.7), and prepare it into a series of solutions with different mass concentrations. Prepare a series of standard solutions with concentrations of 0.4 μg/mL, 1 μg/mL, 2 μg/mL, 5 μg/mL, 10 μg/mL, and 20 μg/mL. Prepare fresh before use.

4.2.12 Microporous filter membrane. 0.45μm, aqueous system.

4.3 Instruments and Equipment

4.3.1 High-performance liquid chromatograph. equipped with an ultraviolet detector or a diode array detector.

4.3.2 Analytical balance. accuracy 0.1mg, 0.01mg.

4.3.3 Centrifuge. The rotation speed shall not be less than 10,000 r/min.

4.3.4 pH meter. accuracy ±0.1.

4.3.5 Vortex mixer.

4.3.6 Ultrasonic cleaner.

4.4 Samples Prepare the sample according to GB/T 20195, at least.200g, crush it so that it all passes through a 0.425mm test sieve, mix it evenly, and pack it into a dense container. Store in a closed container for later use.

4.5 Test Procedure

4.5.1 Extraction Perform two parallel tests. Weigh 2g of the sample (5g of compound feed), accurate to 0.1mg, and place it in a 50mL centrifuge tube. Accurately add... 20 mL of extraction buffer (4.2.7) was vortexed and shaken for 10 min, followed by low-temperature ultrasonic extraction in an ice-water bath for 10 min, and then centrifuged at 10000 r/min. After 5 minutes, collect the supernatant in a centrifuge tube. Repeat the extraction twice with 15 mL of extract each time (4.2.7). Combine the supernatants (V), mix well, and centrifuge. Filtration was performed using a porous membrane (4.2.12). The filtrate was analyzed within 12 hours.

4.5.2 Measurement

4.5.2.1 Reference conditions for liquid chromatography The reference conditions for liquid chromatography are as follows:

a) Chromatographic column. C18 column, 250 mm in length,

4.6 mm in inner diameter, 5 μm in particle size, or equivalent;

b) Detection wavelength. 375nm;

c) Column temperature. 30°C;

e) Injection volume. 20 μL;

f) Mobile phase. Phase A is oxalic acid solution (4.2.8), Phase B is acetonitrile (4.2.3). methanol (4.2.2) = 3. 2 (volume ratio), gradient elution. The procedure is shown in Table 1.

4.5.2.2 Determination of Standard Series Solutions and Sample Solutions Under optimal instrument conditions, standard series solutions (4.2.11) and sample solutions (4.5.1) were tested using the instrument. Chlortetracycline standard solution... See Appendix A for the liquid chromatogram.

4.5.2.3 Qualitative Under the same test conditions, the retention time of chlortetracycline in the sample solution should be the same as that of chlortetracycline in the standard series solutions (with equivalent mass concentration). The retention time is consistent, with a relative deviation within ±2.5%.

4.5.2.4 Quantitative analysis A standard curve was plotted with the mass concentration of