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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 22262-2025

Replacing GB/T 22262-2008

Determination of clopidol in feeds

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1 Scope

GB/T 22262-2025 is the Chinese national standard on determination of clopidol in feeds, in the field of agriculture. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 1 August 2025 by the State Administration for Market Regulation; Standardization Administration of China, and has been in force since 1 February 2026. Classification: ICS 65.120, CCS B46. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This document describes methods for the determination of clopidol in feeds using high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). This document is applicable to the determination of clopidol in compound feeds, concentrate feeds, protein supplements, premixes, mixed feed additives, and animal- derived feed ingredients. In this document, the detection limit for high-performance liquid chromatography is

0.5 mg/kg, and the quantification limit is 1 mg/kg. The detection limit for liquid chromatography-tandem mass spectrometry is

0.05 mg/kg, and the quantification limit is

0.1 mg/kg.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682, Water for analytical laboratory use -- Specification and test methods

GB/T 20195, Animal feed -- Preparation of test samples

3 Terms and definitions

This document contains no terms or definitions requiring definition.

4 High-performance liquid chromatography

4.1 Principle Clopidol in the specimen is extracted using an ammonia-methanol solution. It is then purified using an alkaline alumina solid-phase extraction column. Determination is performed using high-performance liquid chromatography, and quantification is carried out using the external standard method.

4.2 Reagents or materials WARNING. The organic reagents used in this test are irritating to the upper respiratory tract. For safety, appropriate protective measures must be taken, and operations shall be conducted within a fume hood. Unless otherwise specified, only analytically pure reagents shall be used.

4.2.1 Water. GB/T 6682, Grade 1.

4.2.2 Methanol. chromatographically pure.

4.2.3 n-Hexane. 4.2.4 0.2% ammonia solution in methanol. accurately transfer 2 mL of ammonia solution. Dilute to 1000 mL with methanol. Mix thoroughly.

4.2.5 Methanol solution. measure 70 mL of methanol (4.2.2) and 30 mL of water, and mix thoroughly.

4.2.6 Standard stock solution (200 μg/mL). accurately weigh 10 mg (weighed to the nearest

0.01 mg) of the clopidol standard (CAS No.. 2971-90-6; purity >= 98%) into a 50 mL amber volumetric flask. Dissolve using methanol (4.2.2) with sonication, dilute to the set volume, and mix thoroughly. Store at temperatures below -18°C. The shelf life is 6 months. Alternatively, a certified standard solution may be purchased.

4.2.7 Standard series solutions. accurately transfer appropriate volumes of the standard stock solution (4.2.6). Dilute to the set volume using the methanol solution (4.2.5) and mix thoroughly. Prepare a series of standard solutions with mass concentrations of 0.2 μg/mL, 0.5 μg/mL, 2 μg/mL, 5 μg/mL, 20 μg/mL, 50 μg/mL, and 100 μg/mL. Prepare when needed.

4.2.8 Basic alumina solid-phase extraction column. 10 g/20 mL, or equivalent.

4.2.9 Microporous filter membrane. 0.45 μm, organic-based.

4.3 Instruments and equipment

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

4.3.2 Analytical balance. with a precision of

0.01 g and

0.01 mg.

4.3.3 Vortex mixer.

4.3.4 Nitrogen evaporator. with temperature control at 50°C ± 2°C.

4.3.5 Reciprocal shaker.

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

4.3.7 Ultrasonic cleaner.

4.4 Sample Prepare a specimen of at least 200 g in accordance with GB/T 20195.Grind the specimen until it passes entirely through a

0.425 mm test sieve. Mix thoroughly. Store in a sealed container, protected from light, for future use.

4.5.1 Extraction Perform two parallel tests. Weigh 2 g of the specimen (accurate to

0.01

g) and place it in a 50 mL centrifuge tube. Accurately add 25 mL (V1) of the ammonia-methanol solution (4.2.4). Vortex for 1 min. Shake at 200 r/min for 20 min to extract. Centrifuge at 5000 r/min for 5 min, and set aside for purification.

4.5.2 Purification Activate the basic alumina solid-phase extraction column (4.2.8) beforehand with 15 mL of methanol (4.2.2). Accurately transfer 5 mL (V2) of the solution to be purified (4.5.1) into the solid-phase extraction column (4.2.8). Rinse with 5 mL of methanol (4.2.2) and discard the eluate. Elute with 20 mL of methanol (4.2.2) and collect the eluate. Evaporate to near dryness under a stream of nitrogen at 50°C. Accurately add 1 mL (V3) of the methanol solution (4.2.5) and 10 mL of n-hexane (4.2.3). Vortex for 1 min. Collect the lower layer and centrifuge at 10000 r/min for 5 min. Filter through a 0.45 μm microporous membrane, and it is ready for testing.

4.5.3 Determination

4.5.3.1 Reference conditions for high-performance liquid chromatography The reference conditions for high-performance liquid chromatography (HPLC) are as follows:

a) Chromatographic column. C18 column; column length is 250 mm, inner diameter is

4.6 mm, particle size is 5.0 μm, or an equivalent column;

c) Column temperature. 35°C;

d) Injection volume. 20 μL;

e) Detection wavelength. 270 nm;

f) Mobile phase. methanol (4.2.2). water (4.2.1) = 15.85 (by volume).

4.5.3.2 Determination of standard series solutions and specimen solutions Under optimal instrumental conditions, analyze the series of standard solutions (4.2.7) and the specimen solution (4.5.2) separately. The high-performance liquid chromatogram of the clopidol standard solution is shown in Figure A.1 in Annex A.

4.5.3.3 Qualitative determination Qualitative determination is performed based on retention time. The retention time of clopidol in the specimen solution must match that of clopidol in a standard series solution of comparable mass concentration. The relative deviation shall be within ±2.5%.

4.5.3.4 Quantification Construct a standard curve by plotting the mass concentration of clopidol on the x-axis and the chromatographic peak area on the y-axis. The correlation coefficient of the standard curve shall be no less than 0.99.The mass concentration of clopidol in the specimen solution must fall within the linear range of the standard curve. If it exceeds this linear range, the specimen solution shall be diluted with methanol solution (4.2.5) by a dilution factor (f), and the measurement repeated. When performing quantitative determination using single-point calibration, the mass concentration of clopidol in the specimen solution shall not differ from that of the standard solution by more than 30%.