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

GB/T 14701-2019

Replacing GB/T 14701-2002

Determination of vitamin B2 in feeds

饲料中维生素B2的测定

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Table of Contents

- 1 Scope
- 2 Normative references
- 3 Method 1: Fluorescence spectrophotometry
 - 3.2.11 Fluorescein standard solution
 - 3.2.11.1 Fluorescein stock solution: weigh
 - 3.2.12 Bromocresol green pH indicator Take
- 3.3 Instruments and apparatuses
- 3.5.1 Preparation of sample solution Weigh 1 g ~ 2 g (accurate to
- 4 Method 2: High-performance liquid chromatography
 - 4.2 Reagents or solutions
 - 4.3 Instruments and apparatuses
 - 4.5.1 Preparation of sample solution Weigh

1 Scope

GB/T 14701-2019 is the Chinese national standard on determination of vitamin b2 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 4 June 2019 by the State Administration for Market Regulation; Standardization Administration of China, and has been in force since 1 January 2020. 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 Standard specifies the fluorescence spectrophotometry and high- performance liquid chromatography for the determination of vitamin B2 (namely riboflavin C₁₇H₂₀N₁₀) in feeds. Method 1 of this Standard is applicable to the determination of vitamin B2 in animal and plant feed materials, compound feed and concentrated feed; the quantitation-limit is 0.25 mg/kg. Method 2 of this Standard is applicable to the determination of vitamin B2 in vitamin premix feed, compound premix feed, and concentrated feed. When it is detected by a fluorescence detector, the quantitation-limit is 5 mg/kg; when it is detected by an ultraviolet detector, the quantitation-limit is 10 mg/kg. For the determination of vitamin B2 in concentrated feed, method 1 is the arbitration method; for the determination of vitamin B2 in

vitamin premix feed and additive premix feed, the high-performance liquid chromatography fluorescence detector method of method 2 is the arbitration method.

2 Normative references

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

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

GB/T 14699.1, Feeding stuffs. Sampling

GB/T 20195, Animal feeding stuffs. Preparation of test samples

3 Method 1: Fluorescence spectrophotometry

3.1 Principle Vitamin B₂ (riboflavin) produces green fluorescence under excitation of 440 nm ultraviolet light; its fluorescence intensity is proportional to the riboflavin content in a certain concentration range. Use sodium dithionite to reduce riboflavin to a non-fluorescent substance; use the ratio OF the difference between the

3.2.11.1 Fluorescein stock solution: weigh

0.050 g of fluorescein; use water to dilute to 1 000 mL; place it in a brown bottle in the refrigerator at 2°C ~ 8°C to store. The solution contains 50 µg/mL of fluorescein.

3.2.11.2 Fluorescein standard working solution: take 1 mL of fluorescein stock solution (3.2.11.1); use water to dilute to 1 000 mL; place in a brown bottle in a refrigerator at 2°C ~ 8°C to store. The solution contains 0.05 µg/mL of fluorescein.

3.2.12 Bromocresol green pH indicator Take

0.1 g of bromocresol green; add

2.8 mL of sodium hydroxide solution (3.2.1) to dissolve; then, add water to dilute to 200 mL. The color-changing range is pH 3.6~5.2.

3.3 Instruments and apparatuses

3.3.1 Analytical balance: sensitivity of 0.000 1 g.

3.3.2 Electric constant-temperature water bath.

3.3.3 Glass scale test tube with stopper: 15 mL. 3.3.4, Fluorescence spectrophotometer.

3.4 Sample preparation According to the provisions of GB/T 14699.1, select at least 500 g of representative sample; use quartering to reduce it to 100 g; prepare samples according

to GB/T 20195; grind; pass

0.425 mm hole sieve; mix well; place in a sealed container; keep out of the sun and store at low temperature for use.

3.5 Test steps The following operations shall be taken without strong light exposure.

3.5.1 Preparation of sample solution Weigh 1 g ~ 2 g (accurate to

0.001

g) of feed materials, compound feed and concentrated feed in a 100 mL brown conical flask. Add 65 mL of hydrochloric acid solution (3.2.3); boil for 30 min in a boiling water bath. At the beginning of the heating, shake the conical flask once every 5 min ~ 10 min, so as to prevent the sample from agglomerating. After it is cooled to room temperature, use sodium hydroxide solution (3.2.2) to adjust the pH to 6.0 ~ 6.5; immediately add hydrochloric acid solution (3.2.4) to adjust the pH to 4.5 (bromocresol green indicator becomes grass green). Transfer to a 100 mL brown volumetric flask; m_0 -- the amount of the added vitamin B2 standard, in micrograms (μg); V -- the initial volume of the test solution, in milliliters (mL); V_1 -- the volume of the taken test solution during the test, in milliliters (mL); m -- sample mass, in grams (g); n -- dilution ratio; The value of shall be 0.66 ~ 1.5; otherwise, the concentration of the sample solution shall be adjusted, and the sample volume or the dilution ratio shall be adjusted. The determination result is expressed in the arithmetic mean of the parallel determinations, with three significant figures retained.

3.7 Precision For feeds whose vitamin B2 content is less than 5 mg/kg, the difference BETWEEN the two independent determination results that are obtained under repeated conditions AND their arithmetic mean is not more than 15% of the arithmetic mean of the two determination values; For feeds whose vitamin B2 content is more than 5 mg/kg but less than 50 mg/kg, the difference BETWEEN the two independent determination results that are obtained under repeated conditions AND their arithmetic mean is not more than 10% of the arithmetic mean of the two determination values; For feeds whose vitamin B2 content is more than 50 mg/kg, the difference BETWEEN the two independent determination results that are obtained under repeated conditions AND their arithmetic mean is not more than 5% of the arithmetic mean of the two determination values;

4 Method 2: High-performance liquid chromatography

4.1 Principle After the riboflavin in the sample is ultrasonically extracted by an acidic solution, inject the centrifuged and filtered sample solution into a high-performance liquid chromatography reverse-phase chromatography system for separation; use a UV detector (diode matrix detector) or a fluorescence detector to detect; use the external standard method to calculate the content of vitamin B2.

4.2 Reagents or solutions

4.2.10.2 Vitamin B2 standard working solution: for the determination of vitamin premix feed sample, the standard solution in the standard stock solution of vitamin B2 (4.2.10.1) can be directly used as the standard solution of the upper machine; for the determination of compound premix feed, concentrated feed samples, accurately absorb 5 mL of vitamin B2 standard stock solution (4.2.10.1) in a 50 mL brown volumetric flask; use extract to dilute to the mark. The concentration of vitamin B2 in the standard working solution is 5 µg/mL; dilute before analysis.

4.3 Instruments and apparatuses

4.3.1 High-performance liquid chromatograph: with UV detector (diode matrix detector) or fluorescence detector.

4.3.2 pH meter: with temperature control; accuracy of 0.01.

4.3.3 Constant-temperature water bath: 0°C ~ 100°C.

4.3.4 Needle filter: with a 0.45 µm water filter.

4.4 Preparation of samples Sample according to the provisions of GB/T 14699.1; select at least 500 g of representative sample; use quartering to reduce it to 100 g; prepare samples according to GB/T 20195; grind; pass through

0.425 mm hole sieves; mix well; place in a sealed container; keep out of the sun and store at low temperature for use.

4.5 Test steps The following operations shall be taken without strong light exposure.

4.5.1 Preparation of sample solution Weigh

0.25 g ~

0.50 g (accurate to 0.000 1

g) of vitamin premix feed or 2 g ~ 3 g (accurate to

0.001

g) of compound premix feed, concentrated feed; add about 70 mL of extract (4.2.7) in a 100 mL brown conical flask; boil in a 100°C water bath for 30 min to 40 min; in the first few minutes, shake the conical flask to prevent solid agglomeration. After cooling, transfer to a 100 mL brown volumetric flask; use the extract to fix-volume to the mark; mix and filter. For vitamin premix feeds, it's necessary to use the extract to further dilute for 5 ~ 10 times. All test solutions need to be filtered through a 0.45 µm filter (4.3.4) before filtration by the liquid chromatography.