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Determination of vitamin B2 in feeds

饲料中维生素 B_2 的测定

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Determination of Vitamin B_2 in feeds

1 Scope

This Standard specifies the fluorescence spectrophotometry and high-performance liquid chromatography for the determination of vitamin B_2 (namely riboflavin $C_{17}H_{20}N_{10}$) in feeds.

Method 1 of this Standard is applicable to the determination of vitamin B_2 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 B_2 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 B_2 in concentrated feed, method 1 is the arbitration method; for the determination of vitamin B_2 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 B_2 (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 Fluorescein standard solution

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 B_2 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 $\frac{T1-T3}{T2-T1}$ 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 B_2 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 B_2 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 B_2 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 B_2.

4.2 Reagents or solutions

4.2.10.2 Vitamin B_2 standard working solution: for the determination of vitamin premix feed sample, the standard solution in the standard stock solution of vitamin B_2 (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 B_2 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 B_2 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.

4.5.2 Determination

4.5.2.1 High-performance liquid chromatography reference conditions I

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