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Determination of thiamine in feed

饲料中维生素 B_1 的测定

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Determination of thiamine in feed

1 Scope

This Standard specifies two methods for the determination of thiamine B_1 in feed, including fluorescence spectrophotometry and high performance liquid chromatography.

Method I of this Standard applies to the determination of thiamine B_1 in feed material, compound feed and concentrated feed. The quantitation limit of this method is 1 mg/kg (in case of the existence of any interfering substance which absorbs thiamine or influences thiamine fluorescence, this method is not applicable). Thiamine B_1 determined in this Method includes the total of intrinsic and additive amounts.

Method II of this Standard specifies the determination of compound premixed feed and thiamine premixed feed. The detection limit of method II is 3 mg/kg; the quantitation limit is 15 mg/kg.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition dated applies to this document. For undated references, the latest edition of the referenced documents (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 I: fluorescence spectrophotometry

3.1 Principle

After thiamine B_1 in sample is digested by diluted acid and digestive enzyme and absorbed-separated-purified by adsorbent, it is oxidized by potassium ferricyanide under alkaline conditions to generate fluorochrome-thiochrome and then extracted by normal butanol. The fluorescence intensity of thiochrome in normal butanol is in direct

for use within 6 months.

Before use, check the recovery of thiamine B_1 standard solution by zeolite; if it is less than 92%, re-activate zeolite.

NOTE: Check of the recovery of thiamine B_1 by zeolite: transfer 2 mL of thiamine B_1 standard medium solution (3.2.13.2) and add acidic potassium chloride solution (3.2.6) to make up to 100 mL. Carry out oxidization in accordance with the procedures specified in 3.5.4.1 ~ 3.5.4.3 as the external standard. Transfer 25 mL of thiamine B_1 standard working solution (3.2.13.3) to repeat the operation of column chromatography isolation specified in 3.5.3.1 ~ 3.5.3.3; carry out oxidization in accordance with the procedures specified in 3.5.4.1 ~ 3.5.4.3. Measure the fluorescence intensity of both solutions at the same time; carry out calculation in accordance with Equation (1); convert the result into a percentage, which is the recovery value of thiamine B_1 by zeolite.

3.2.13 Thiamine B_1 standard solution

3.2.13.1 Thiamine B_1 standard stock solution: take the standard substance of thiamine nitrate (with purity greater than 99%) and dry in a phosphorus pentoxide desiccator for 24 h. Weigh 0.01 g (accurate to 0.0001 g); dissolve in acidic 20% ethanol solution (3.2.11) and make up to 100 mL; load in a brown bottle; store in a refrigerator at 2°C ~ 8°C for use within 3 months. This solution contains 0.1 mg/mL of thiamine B_1.

3.2.13.2 Thiamine B_1 standard medium solution: take 10 mL of thiamine B_1 standard stock solution (3.2.13.1) before adding acidic 20% ethanol solution (3.2.11) to make up to 100 mL; load in a brown bottle; store in a refrigerator at 2°C ~ 8°C for use within 48 h. The solution contains 10 μg/mL of thiamine B_1.

3.2.13.3 Thiamine B_1 standard working solution: take 2 mL of thiamine B_1 medium solution (3.2.13.2) to mix with 65 mL of hydrochloric acid solution (3.2.1) and 5 mL of sodium acetate solution (3.2.3); make up to 100 mL; prepare before analysis. The solution contains 0.2 μg/mL of thiamine B_1.

3.2.14 Quinine sulfate solution

3.2.14.1 Quinine sulfate stock solution: weigh 0.1 g of quinine sulfate (accurate to 0.001 g); use sulfuric acid solution (3.2.2) to dissolve and make up to 1,000 mL. Store in a brown bottle for cold storage. If the solution is turbid, then prepare it once again.

3.2.14.2 Quinine sulfate working solution: take 3 mL of stock solution (3.2.13.1); use sulfuric acid solution (3.2.2) to make up to 1,000 mL. Store in a brown bottle for cold storage. The solution contains 0.3 μg/mL of quinine sulfate.

3.2.15 Normal butanol: the fluorescence intensity is not greater than that of 4% of quinine sulfate working solution (3.2.14.2). Or else, it shall be re-distilled using distilling glassware; take the fraction at 114°C ~ 118°C.

a brown volumetric flask of 100 mL; use water to make up to 100 mL; shake up.

3.5.2.3 Filtration: filter all preparation through ashless filter paper; discard 5 mL of primary filtrate; collect the filtrate as sample solution.

3.5.3 Purification of sample solution

3.5.3.1 Preparation of absorption column: weigh 1.5 g of activated artificial zeolite (3.2.11) to place in a small beaker of 50 mL; add 3% glacial acetic acid solution (3.2.10) for soaking, with the liquid level of the solution above zeolite. Place degreasing cotton at the bottom of the absorption-separation column (3.3.5); use a glass rod to press gently. Then wash all zeolite soaked in acetic acid into the column (do not let the absorption column be dehydrated). The flow rate passing the column is preferably controlled at 1 mL/min. Then use 10 mL of nearly-boiling water to wash the column once.

3.5.3.2 Absorb 25 mL of sample solution (3.5.2.3); add slowly into the absorption column prepared; discard the filtrate; use 5 mL of nearly-boiling water each time to wash the column for 3 times; discard the washings. Meanwhile, prepare parallel sample.

3.5.3.3 Use 25 mL of acidic potassium solution (3.2.6) at 60°C ~ 70°C to add into the absorption column continuously three times; collect the eluant in a volumetric flask of 25 mL; use acidic potassium chloride solution to make up after cooling; mix up.

3.5.3.4 Meanwhile, use 25 mL of thiamine B_1 standard working solution (3.2.13.3). Repeat the operation specified in 3.5.3.1 ~ 3.5.3.3 as the external standard.

3.5.4 Oxidization and extraction

Caution – The following operation is carried out in a dark place.

3.5.4.1 Add respectively 5 mL of eluant (3.5.3.3) into two centrifugal tubes with stopper (3.3.6); mark them as A and B.

3.5.4.2 Add 3 mL of sodium hydroxide solution (3.2.7) into tube B; add 3 mL of alkaline potassium ferricyanide solution (3.2.9) into tube A; rotate to shake gently. In succession, immediately add 15 mL of normal butanol (3.2.15) into tube A and put on stopper; vibrate to shake violently for 15 s; add 15 mL of normal butanol into tube B and put on stopper; vibrate to shake for 90 s together; place aside for layering.

3.5.4.3 Use an injection syringe (3.3.8) to remove the lower water phase; add about 2 g of anhydrous sodium sulfate into all reaction tubes; rotate to shake as sample to be tested.

3.5.4.4 Meanwhile, pour 5 mL of eluant (3.5.3.4) as the external standard into another

3.7 Repeatability

For feed whose thiamine B_1 content is less than 5 mg/kg, under repeatable conditions, the difference between the results measured independently two times and their arithmetical mean value shall not be greater than 15% of the arithmetical mean value of the two values measured;

For feed whose thiamine B_1 content is greater than 5 mg/kg and less than 50 mg/kg, under repeatable conditions, the difference between the results measured independently two times and their arithmetical mean value shall not be greater than 10% of the arithmetical mean value of the two values measured;

For feed whose thiamine B_1 content is greater than 50 mg/kg, under repeatable conditions, the difference between the results measured independently two times and their arithmetical mean value shall not be greater than 5% of the arithmetical mean value of the two values measured.

4 Method II: high performance liquid chromatography

4.1 Principle

After the ultrasonic extraction of sample by acidic extracting solution, inject the test solution filtered and centrifuged into the inverse phase of the high performance liquid chromatographic system for separation; use ultraviolet (or diode matrix detector) for testing; calculate the content of thiamine B_1 using the external standard method.

4.2 Reagents or solutions

Unless specified otherwise, all reagents used are analytically pure and the water for chromatography shall comply with the specification for grade one water as specified in GB/T 6682.

4.2.1 Ammonium chloride: guaranteed reagent.

4.2.2 Sodium heptanesulfonate ($PICB_7$): guaranteed reagent.

4.2.3 Glacial acetic acid: guaranteed reagent.

4.2.4 Triethylamine: chromatographically pure.

4.2.5 Methanol: chromatographically pure.

4.2.6 Acidic ethanol solution: 20%, see 3.2.11 for its preparation.

4.2.7 Ethylenediaminetetraacetic acid disodium (EDTA): guaranteed reagent.

4.3.5 High performance liquid chromatograph which is equipped with an ultraviolet or diode matrix detector.

4.4 Sample

Take representative feed sample as specified in GB/T 14699.1; reduce sample by quartering. Prepare sample as specified in GB/T 20195; fully mix up.

4.5 Test procedures

Caution – Protect from exposure to direct strong light.

4.5.1 Extraction

4.5.1.1 Extraction of thiamine premixed feed

Weigh 0.25 g ~ 0.5 g (accurate to 0.0001 g) of sample to place in a brown volumetric flask of 100 mL; add about 70 mL of extracting solution (4.2.8); add while shaking up; place on an ultrasonic water bath for ultrasonic extraction for 15 min; shake twice during the period; cool; use extracting solution to make up to scale; shake up. Take a small amount of solution on the centrifuge machine for centrifuge for 5 min at 8,000 r/min; pass the supernatant through micro-pore filter membrane of 0.45 μm; place on the HPLC for determination.

4.5.1.2 Extraction of compound prefixed feed

Weigh about 3.0 g (accurate to 0.001 g) of sample; place in a brown volumetric flask of 100 mL; add about 70 mL of extracting solution (4.2.9); add while shaking up before placing on an ultrasonic water bath for ultrasonic extraction for 30 min; shake twice during the period; use extracting solution to make up to scale; shake up. Take a small amount of solution on the centrifuge machine for centrifuge for 5 min at 8,000 r/min; pass the supernatant through Millipore filter membrane of 0.45 μm; place on the HPLC for determination.

4.5.2 Reference chromatographic conditions

Chromatographic column: C_{18} column, of length 250 mm, internal diameter 4.6 mm and granularity 5 μm (or analytical columns of equivalent performance).

Moving phase: 4.2.10.

Flow rate: 1.0 mL/min.

Temperature: 25°C ~ 28°C.

Detection wavelength: 242 nm.

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