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NATIONAL STANDARD OF THE
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ICS 65.120

B 46

GB 7300.203-2020

Replacing GB/T 21515-2008

**Feed additive - Part 2: Vitamins, provitamins and chemically
well-defined substances having similar effect - Betaine**

饲料添加剂 第2部分: 维生素及类维生素 甜菜碱

Issued on: May 29, 2020

Implemented on: December 1, 2020

**Issued by: State Administration for Market Regulation;
Standardization Administration of PRC.**

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Feed additive - Part 2: Vitamins, provitamins and chemically well-defined substances having similar effect - Betaine

1 Scope

This part of GB 7300 specifies the requirements, test methods, inspection rules, labeling, packaging, transportation, storage, and shelf life of the feed additive betaine.

This part applies to natural betaine products that are separated from beet molasses and processed, and feed additive betaine products that are chemically synthesized from sodium chloroacetate and trimethylamine.

2 Normative references

The following documents are essential to the application of this document. For the dated referenced documents, only the versions with the indicated dates are applicable to this document; for the undated referenced documents, only the latest version (including all the amendments) is applicable to this document.

GB/T 601 Chemical reagent - Preparations of reference titration solutions

GB/T 602 Chemical reagent - Preparations of standard solutions for impurity

GB/T 603 Chemical reagent - Preparations of reagent solutions for use in test methods

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

GB 10648 Feed label

GB/T 13079-2006 Determination of total arsenic in feeds

GB/T 14699.1 Feeding stuffs - Sampling

3 Chemical name, molecular formula, relative molecular mass, and structural formula

Chemical name: Trimethylglycine

Molecular formula: $C_5H_{11}NO_2$

5.1 Appearance characteristics

Take an appropriate amount of the sample, place it on a white porcelain plate, and visually inspect its color and shape under natural light.

5.2 Identification test

5.2.1 Reagents

5.2.1.1 Hydrochloric acid solution: hydrochloric acid + water = 1 + 4 (volume ratio).

5.2.1.2 Bismuth potassium iodide solution: Take 0.85 g of bismuth subnitrate, dissolve it in 10 mL of acetic acid, and add 40 mL of water. Take 40 g of potassium iodide, dissolve it in water, and dilute the solution to 100 mL. Mix the above two solutions in equal volumes and store the mixture in a brown glass container.

5.2.1.3 Bismuth potassium iodide hydrochloric acid solution: Take 1 mL of bismuth potassium iodide solution (5.2.1.2), add 2 mL of hydrochloric acid solution (5.2.1.1), and add water to 10 mL (prepare the solution newly when it will be used).

5.2.2 Identification method

5.2.2.1 Weigh 0.5 g of the sample, add 1 mL of water to dissolve, and add 2 mL of bismuth potassium iodide hydrochloric acid solution (5.2.1.3). Shake the mixture to produce an orange-red precipitate.

5.2.2.2 Take the solution in 5.3.1.4.1 and measure it with the instrument according to 5.3.1.4.3.3. The retention time is consistent with that of the standard solution.

5.3 Determination of betaine content

5.3.1 Ion chromatography (the arbitration method)

5.3.1.1 Method principle

The sample is dissolved in water, the solution is diluted to an appropriate concentration, and a cation exchange column and a non-suppressed conductivity detector are used for separation and determination. The quantitative analysis is carried out by the external standard method.

5.3.1.2 Reagents and materials

5.3.1.2.1 Standard betaine ($\geq 99\%$).

5.3.1.2.2 Methanesulfonic acid stock solution: Accurately pipette 2 mL of methanesulfonic acid into a 100 mL volumetric flask, dilute to volume with water, and shake well. The concentration is 300 mmol/L.

5.3.1.2.3 Mobile phase: Accurately pipette 10 mL of methanesulfonic acid stock solution (5.3.1.2.2) into a 1000 mL volumetric flask, dilute to volume with water, and shake well. The concentration is 3.0 mmol/L. Ultrasonic degassing for 5 min~10 min. Prepare the solution newly when it will be used.

5.3.1.2.4 Betaine standard stock solution: Weigh 0.10 g (accurate to 0.0001 g) of standard betaine (5.3.1.2.1) that has been dried at $105\text{ }^{\circ}\text{C}\pm 2\text{ }^{\circ}\text{C}$ for 4 h; put it in a 100 mL volumetric flask, make up to volume with water and shake well. The solution concentration is 1000 $\mu\text{g/mL}$.

5.3.1.2.5 Filter membrane: 0.45 μm , hydrophilic.

5.3.1.3 Instruments and equipment

5.3.1.3.1 Ion chromatograph: It has a cation exchange analytical column or another analytical column with comparable performance and a conductivity detector.

5.3.1.3.2 Analytical balance: The sense quantity is 0.1 mg.

5.3.1.3.3 Electrically heated drying oven: The temperature can be controlled at $105\text{ }^{\circ}\text{C}\pm 2\text{ }^{\circ}\text{C}$.

5.3.1.4 Analysis steps

5.3.1.4.1 Extraction

Weigh about 0.25 g (accurate to 0.0001 g) of the sample that has been dried in an oven at $105\text{ }^{\circ}\text{C}$ to a constant weight, place it in a 100 mL volumetric flask, add about 70 mL of water, and make up the volume to the mark after the sample dissolved. Take 2 mL of the solution to a 50 mL volumetric flask, make up the volume to the mark, shake well, and pass it through a 0.45 μm filter membrane; let the filtrate stand for later test.

5.3.1.4.2 Preparation of a series of standard solutions

Accurately pipette 1.00 mL, 5.00 mL, 10.00 mL, 20.00 mL, and 50.00 mL of betaine standard stock solution (5.3.1.2.4) into 100 mL volumetric flasks respectively, dilute to volume with water, and shake well. The concentrations of the series of standard solutions are 10.0 $\mu\text{g/mL}$, 50.0 $\mu\text{g/mL}$, 100.0 $\mu\text{g/mL}$, 200.0 $\mu\text{g/mL}$, and 500.0 $\mu\text{g/mL}$. Prepare these solutions newly when they will be used soon.

5.3.1.4.3 Determination

5.3.1.4.3.1 Reference conditions for ion chromatograph

Chromatographic column: a cation exchange analytical column with 4 mm of inner diameter and 250 mm of length, and 4.5 μm of packing particle size; or other chromatographic columns with equivalent performance.

5.3.2 Perchloric acid titration method

5.3.2.1 Method principle

The sample that has been dried to constant weight is dissolved in glacial acetic acid, and the perchloric acid is used as the standard titration solution; the crystal violet is used as the indicator for titration, and when the color changes from purple to blue-green, it is the end point of the titration.

5.3.2.2 Reagents or materials

5.3.2.2.1 Glacial acetic acid.

5.3.2.2.2 Acetic anhydride.

5.3.2.2.3 Crystal violet indicator solution: 5 g/L.

5.3.2.2.4 Perchloric acid standard titration solution: $c(\text{HClO}_4) = 0.1 \text{ mol/L}$.

5.3.2.3 Instruments and equipment

5.3.2.3.1 Acid burette: 25 mL.

5.3.2.3.2 Analytical balance: The sense quantity is 0.1 mg.

5.3.2.4 Test procedure

Weigh about 0.2 g (accurate to 0.0001 g) of the sample that has been dried in an oven at 105 °C to constant weight, add 20 mL of glacial acetic acid (5.3.2.2.1) to dissolve the sample, add 10 mL of acetic anhydride (5.3.2.2.2) and shake well; then, add 2 drops of crystal violet indicator solution (5.3.2.2.3), and titrate the mixture with perchloric acid standard titration solution (5.3.2.2.4) until the solution turns blue-green; do a blank test at the same time.

5.3.2.5 Test data processing

The content w_2 of betaine in the sample is expressed in mass fraction (%) and calculated according to formula (2):

$$w_2 = \frac{c_2 (V_2 - V_{20}) M_2}{m_2 \times 1\,000} \times 100 \quad \dots\dots\dots (2)$$

where:

c_2 -- the concentration of perchloric acid standard titration solution, in moles per liter (mol/L);

V_2 -- the volume of perchloric acid standard titration solution consumed when the

sample is titrated, in milliliters (mL);

V_{20} -- the volume of perchloric acid standard titration solution consumed by the blank test, in milliliters (mL);

m_2 -- the mass of the sample, in grams (g);

M_2 -- the molar mass of betaine, in grams per mole (g/mol) ($M=117.15$).

5.3.2.6 Result presentation

The measurement result is expressed as the arithmetic mean of parallel determinations, and the result is rounded to three significant figures.

5.3.2.7 Repeatability

The absolute difference between two independent determination results obtained under repeatability conditions is not more than 0.2%.

5.4 Loss on drying

5.4.1 Instruments and equipment

5.4.1.1 Electrically heated drying oven: The temperature can be controlled at $105\text{ }^{\circ}\text{C}\pm 2\text{ }^{\circ}\text{C}$.

5.4.1.2 Desiccator: The calcium chloride or color-changing silica gel is used as the desiccant.

5.4.2 Determination steps

Weigh about 1 g of the sample (accurate to 0.0001 g) into a weighing dish with constant weight, put it in an electrically heated drying oven (5.4.1.1) at $105\text{ }^{\circ}\text{C}\pm 2\text{ }^{\circ}\text{C}$, open the cover of the weighing dish, and dry the sample for 3 h. Take the weighing dish out and cover it, put it in a desiccator (5.4.1.2), cool it to room temperature, and weigh it. Repeat the drying for 1 h, and weigh it until the weight is constant.

5.4.3 Test data processing

The loss on drying w_3 of the sample is expressed in mass fraction (%) and calculated according to formula (3):

$$w_3 = \frac{m_{30} - m_3}{m_{30}} \times 100 \quad \dots\dots\dots (3)$$

where:

m_{30} -- the mass of the sample before drying, in grams (g);

gram (g).

5.5.4 Result presentation

The measurement result is expressed as the arithmetic mean of parallel determinations, and the result is rounded to two significant figures.

5.5.5 Repeatability

The absolute difference between two independent determination results obtained under repeatability conditions is not more than 0.1%.

5.6 Determination of total arsenic content (in As)

The determination shall be carried out according to the provisions of 5.4.1.3 in GB/T 13079-2006.

5.7 Determination of heavy metal content (in Pb)

5.7.1 Principle

Heavy metal ions and sulfide ions that have a charge of minus two form colored sulfide precipitates in a weak acid medium. When the content of heavy metal elements is low, a stable tan suspension is formed, which can be used for the visual colorimetric determination of heavy metals.

5.7.2 Reagents or materials

5.7.2.1 Hydrochloric acid.

5.7.2.2 Nitric acid.

5.7.2.3 Ammonium acetate.

5.7.2.4 Glycerol.

5.7.2.5 Thioacetamide.

5.7.2.6 Ammonia.

5.7.2.7 Lead nitrate.

5.7.2.8 Phenolphthalein indicator solution: 10 g/L.

5.7.2.9 Hydrochloric acid solution: 7 mol/L.

5.7.2.10 Ammonia test solution: Take 400 mL of ammonia water, and add water to dilute it to 1000 mL.

5.7.2.11 Acetate buffer solution (pH 3.5): Take 25 g of ammonium acetate (5.7.2.3), add 25 mL of water to dissolve it, add 38 mL of 7 mol/L hydrochloric acid solution (5.7.2.9), adjust the pH to 3.5, and add water to volume to 1000 mL.

5.7.2.12 Sodium hydroxide solution: $c(\text{NaOH}) = 1 \text{ mol/L}$.

5.7.2.13 Thioacetamide test solution: Weigh 4 g of thioacetamide (5.7.2.5), add water to dissolve it to 100 mL, and store it in a refrigerator. Before use, take 1.0 mL and add it to 5.0 mL of the mixed solution, which is composed of 15 mL of sodium hydroxide solution (5.7.2.12), 5.0 mL of water and 20 mL of glycerol (5.7.2.4); heat the mixture on a water bath for 20 s, cool, and immediately use.

5.7.2.14 Lead standard stock solution: Weigh 0.160 g of lead nitrate (5.7.2.7), put it in a 1000 mL volumetric flask, add 5 mL of nitric acid (5.7.2.2) and 50 mL of water to dissolve it, dilute to the mark with water, and shake well. The solution concentration is 100 $\mu\text{g/mL}$. Store at 2 °C~8 °C after sealing, and the validity period is 3 months. Or directly purchase lead standard solution with a concentration of 100 $\mu\text{g/mL}$.

5.7.2.15 Lead standard working solution: Accurately pipette 10 mL of lead standard stock solution (5.7.2.14) into a 100 mL volumetric flask, dilute with water to the mark, and shake well. The solution concentration is 10 $\mu\text{g/mL}$. Prepare the solution newly when it will be used soon.

5.7.3 Test procedure

5.7.3.1 Preparation of test solution

Weigh 1.00 $\text{g} \pm 0.01 \text{ g}$ of the sample in a crucible, carefully carbonize it in an electric furnace, and then put it into a high-temperature furnace and burn it at 550 °C for 3 h. After cooling, add 0.5 mL of nitric acid and evaporate to dryness. After the nitrogen oxide vapor is completely removed, let it cool, add 2 mL of hydrochloric acid, and place it on a water bath to evaporate to dryness; add 15 mL of water, add 2 drops of phenolphthalein indicator solution (5.7.2.8), and then add dropwise the ammonia test solution (5.7.2.10) until it reacts with the phenolphthalein indicator solution and the solution turns neutral; add 2 mL of acetate buffer (pH 3.5) (5.7.2.11), and dissolve it with slight heat.

5.7.3.2 Determination

Filter the test solution and transfer it to a Nessler colorimetric tube, add water to dilute it to 25 mL, then add 2 mL of thioacetamide test solution (5.7.2.13), and shake well; place it in the dark for 2 min, and then see through from top to bottom; the color of the test solution shall not be darker than the standard colorimetric solution.

Standard colorimetric solution: Pipette 1.00 mL lead standard working solution, and at the same time, treat it in the same way as the processing to the sample.

The measurement result is expressed as the arithmetic mean of parallel determinations, and the result is rounded to three significant figures.

5.8.7 Repeatability

The absolute difference between the two independent determination results obtained under repeatability conditions is not more than 30 mg/kg.

5.9 Determination of sodium chloride (NaCl) content

5.9.1 Synthetic betaine

5.9.1.1 Method principle

The chloride ions in the sample are dissolved in the aqueous solution, and the silver nitrate standard solution is added to react with the chloride to form a silver chloride precipitate, and the excess silver nitrate solution is titrated with the ammonium thiocyanate or potassium thiocyanate standard solution.

5.9.1.2 Reagents or materials

5.9.1.2.1 Nitric acid.

5.9.1.2.2 Ferric ammonium sulfate saturated solution: It is prepared with ferric ammonium sulfate $[\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}]$.

5.9.1.2.3 Silver nitrate standard titration solution: $c(\text{AgNO}_3) = 0.1 \text{ mol/L}$.

5.9.1.2.4 Ammonium thiocyanate or potassium thiocyanate standard titration solution: $c(\text{NH}_4\text{SCN}) = 0.1 \text{ mol/L}$, $c(\text{KSCN}) = 0.1 \text{ mol/L}$.

5.9.1.3 Instruments and equipment

5.9.1.3.1 Analytical balance: The sense quantity is 0.1 mg.

5.9.1.3.2 Acid burette: 25 mL.

5.9.1.4 Test procedure

Weigh about 10 g (accurate to 0.0001 g) of the sample that has been dried at 105 °C in an oven to constant weight in advance, and dissolve it in 100 mL of water in a conical flask. Add 5 mL of nitric acid (5.9.1.2.1), 2 mL of ferric ammonium sulfate saturated solution (5.9.1.2.2), and add 2 drops of ammonium thiocyanate or potassium thiocyanate standard titration solution dropwise from a burette that has been filled up to the scale 0 with ammonium thiocyanate or potassium thiocyanate standard titration solution (5.9.1.2.4).

The absolute difference between two independent determination results obtained under repeatability conditions is not more than 0.1%.

5.9.2 Natural Betaine

5.9.2.1 Instruments and equipment

5.9.2.1.1 Analytical balance: The sense quantity is 0.1 mg.

5.9.2.2 Reagents

5.9.2.2.1 Chloride stock solution (1 mL of solution contains 0.1 mg of Cl^-): Weigh 0.165 g of sodium chloride that has been burned to constant weight at 500 °C~600 °C, dissolve it in ultrapure water, and dilute to 1000 mL.

5.9.2.2.2 Chloride standard solution (1 mL of solution contains 0.01 mg of Cl^-): Accurately pipette 10 mL of chloride stock solution (5.9.2.2.1) into a 100 mL volumetric flask, dilute with ultrapure water to volume.

5.9.2.2.3 Nitric acid solution: 25%. Measure 308 mL of nitric acid and dilute it to 1000 mL with ultrapure water.

5.9.2.2.4 Silver nitrate solution: 17 g/L. Weigh 1.7 g of silver nitrate, dissolve it in ultrapure water, and dilute the solution to 100 mL.

5.9.2.3 Test procedure

Weigh 5 g of the sample (accurate to 0.01 g) in a beaker, dissolve it with ultrapure water, and dilute the solution to 100 mL. After filtration, take 10 mL of the filtrate into a 25 mL Nessler colorimetric tube.

Accurately pipette 5 mL of chloride standard solution (5.9.2.2.2) into another 25 mL Nessler colorimetric tube.

Add 1 mL of nitric acid solution (5.9.2.2.3) and 1 mL of silver nitrate solution (5.9.2.2.4) into the above-mentioned two Nessler colorimetric tubes, and add ultrapure water to dilute to 25 mL. Shake well and let them stand for 10 min.

The turbidity of the sample solution shall not be greater than that of the standard solution.

5.10 Determination of sulfate content (in SO_4^{2-})

5.10.1 Method principle

According to the principle that barium ions and sulfate ions form barium sulfate precipitates, the content of sulfate can be judged by comparing the turbidity of the

sample solution and the standard solution.

5.10.2 Instruments and equipment

Electronic balance: The sense quantity is 0.01 g.

5.10.3 Reagents

5.10.3.1 Sulfate standard solution: The concentration of SO_4^{2-} is 1000 mg/L, which can be purchased directly.

5.10.3.2 Hydrochloric acid solution: 10%.

5.10.3.3 Barium chloride solution: 120 g/L.

5.10.4 Test procedure

Weigh about 10 g of the sample, accurate to 0.01 g, put it into a 200 mL volumetric flask, dissolve it in water, and make up to the mark; shake well and filter it. Accurately pipette 20 mL of filtrate in a 50 mL beaker.

In addition, pipette 19 mL of water into another 50 mL beaker, and add 1 mL of sulfate standard solution (5.10.3.1). According to the sulfate content (in SO_4^{2-}) contained in the sample, different volumes of sulfate standard solution can be added.

Add 1 mL of hydrochloric acid solution (5.10.3.2) and 3 mL of barium chloride solution (5.10.3.3) into the above-mentioned two 50 mL beakers respectively, mix well, and let them stand for 10 min; compare the turbidity between the sample solution and the standard solution.

The turbidity of the sample solution shall not be greater than that of the standard solution.

6 Inspection rules

6.1 Batch grouping

Products that are produced continuously or in the same shift with the same raw materials and the same production process are regarded as a batch.

6.2 Sampling

It shall be carried out according to the provisions of GB/T 14699.1.

6.3 Factory inspection

The factory inspection items include betaine content, appearance and characteristics,

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