

# National Standard of the People's Republic of China

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Instead of GB/T 17810-1999

## Feed Grade DL-Methionine



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AND

National Standard Management Committee of the People's Republic of China

## Feed Grade DL-Methionine

### 1. Range

This Standard specifies the DL-Methionine's production requirements, test methods, inspection rules, label, package, storage and transportation.

This Standard is applicable to feed grade DL-Methionine, which made from methylthio propionaldehyde, chloride, sulfuric acid and sodium hydroxide.

Chemical name: 2-amino-4- methyl sulfenyl butyric acid

Molecular formula:  $\text{CH}_3\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$

Molecular weight: 149.2 (according to international relative atomic mass in 2007)

### 2. Normative references.

The clauses in the following documents were referenced as a part of clauses of this Standard. For dated references, all subsequent amended or revised content (except corrigendum) will not be applicable to this Standard. However, all parties authorized according to this Standard are encouraged to study if the latest edition of these documents can be used. For undated references, the latest edition of the normative document is applicable to these specifications.

GB/T 601 Chemical reagent-Preparations of standard volumetric solutions

GB/T 1250 Rules for expression and judgement of limiting values

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

GB 10648 Feed label

GB/T 14699.1 Feeding stuffs-Sampling

*Pharmacopoeia of the People's Republic of China, 2005 2<sup>nd</sup> part*

### 3. Requirements

#### 3.1 Appearance and Characters

The product should be white or light grey powder or crystal. Slightly dissolved in water, ethanol, diluted acid, sodium/potassium hydroxide solution.

#### 3.2 Technical Specification

The technical specification should comply with the standards in table 1.

Table 1 Technical Specification

Item	Specification
DL-Methionine (%)	$\geq 98.5$
Loss on drying (%)	$\leq 0.5$
Chloride (NaCl, %)	$\leq 0.2$
Heavy metal (Pb, mg/kg)	$\leq 20$
As (mg/kg)	$\leq 2$

## 4 Test methods

Reagents and water used in the standard are analytical grade reagents and three levels water according to GB/T 6682, except special annotation. Instruments and equipments are general laboratory equipments.

Warnings: Be cautious when using strong acid and avoid to splash into the skin. Test should be done in a fume hood when using volatile reagent.

### 4.1 Appearance and Characters

The appearance and characters should comply with the requirements of 3.1.

### 4.2 Identification

#### 4.2.1 Reagents and solutions

4.2.1.1. Saturated anhydrous copper sulfate solution:

4.2.1.2. Sodium hydroxide solution: 200g/L

4.2.1.3. Sodium nitroferricyanide solution 100g/L

4.2.1.4. Hydrochloric acid: 1+10(V+V)

4.2.1.5. Hydrochloric acid:  $c(\text{HCL})=0.02 \text{ mol/L}$

4.2.1.6. Sodium citrate buffer for elution of different pH and ionic strength: prepare it according to automatic amino acid analyzer manual.

4.2.1.7. Ninhydrin solution: prepare it according to automatic amino acid analyzer manual

4.2.1.8. Methionine standard stock solution  $c(\text{methionine})=2.5 \mu \text{ mol/mL}$ : Weigh 93.3g methionine in 100 ml beaker, add about 50 ml of water and several drops of concentrated hydrochloric acid, dissolved and quantitatively transferred to a 250 ml volumetric flask, dissolve with 50mL water and drops of concentrated hydrochloric acid. Transfer it to 250mL volumetric flask quantificationally, dilute with water to the mark.

4.2.1.9. Methionine standard solution  $c(\text{methionine})=100 \text{ nmol/mL}$ : draw 1.00mL methionine standard stock solution(4.2.1.8) to in 25mL volumetric flask, dilute with water to the mark.

#### 4.2.2 Equipments

Automatic amino acid analyzer, Post-column ninhydrin derivative ion exchange chromatograph, the resolution of the amino acids should be larger than 90%

#### 4.2.3 Procedures

4.2.3.1 Add 25 mg sample into 1ml saturated anhydrous copper sulfate solution (4.2.1.1), the color is yellow.

4.2.3.2 Add 5 mg of sample into 2 ml sodium hydroxide solution(4.2.1.2), mix by shaking, add 0.3 ml sodium nitroferricyanide solution(4.2.1.3), mix well, let it stand for 10 minutes at 35° C-40° C, and ice bathing for 2 minutes. Then add 10 ml hydrochloric acid (4.2.1.4), shake up. The color of the solution is red.

4.2.3.3 Weigh sample of approximately 0.15g(accurate to 0.0001g), dissolve in hydrochloric acid (4.2.1.5) and dilute the methionine to a concentration of 100nmol/ml, then determined by automatic amino acid analyzer(4.2.2) , the chromatogram should only have methionine peak, with legal retention time, and calculate the methionine standard solution (4.2.1.9) and peak area of sample methionine in the solution, using the external standard method to calculate the content of the sample methionine. Requiring the absolute difference of the arithmetic average value for parallel determination results and the labeled amount is not bigger than 2%.

### 4.3 Test of methionine content

#### 4.3.1 Principles

Add excessive iodine solution into in the neutral medium accurately, which would combine two iodine atom with sulfur atoms from methionine, then titrate the excessive iodine solution with sodium thiosulfate standard titration solution.

#### 4.3.2 Reagents and solutions

4.3.2.1 Dipotassium hydrogen phosphate solution: 500g/L

4.3.2.2 Potassium dihydrogen phosphate soluton: 200g/L

4.3.2.3 Potasshm iodide solution: 200g/L, stored in brown bottle

4.3.2.4 Iodide solution  $c(1/2 I_2)=0.1 \text{ mol/L}$ ; dissolve 13g iodide and 35g potassium iodide to a little water, then dilute to 1000 mL by water and stir. Store in brown bottle with plug

4.3.2.5 Sodium thiosulfate standard titration solution  $c(\text{Na}_2\text{S}_2\text{O}_3)=0.1000\text{mol/L}$ : prepare according to GB/T 601.

4.3.2.6 Starch soluton: 10g/L.

#### 4.3.3 Equipments

4.3.3.1 Analytical balance: sensitiveness 0.1mg

4.3.3.2 Magnetic stirrer

#### 4.3.4 Procedures

1.5.1 Add 0.23-0.25g sample (accurate to 0.0001g) into 50mL iodine flask, and dissolve by 70 mL deionized water. Then add 10mL dipotassium hydrogen phosphate solution (4.3.2.1), 10mL potassium dihydrogen phosphate solution (4.3.2.2), 10mL potassium iodide solution (4.3.2.3) . And add 50.00mL iodide solution (4.3.2.4) into the mixture after the sample is dissolved. Cover the bottle cap and water seal. Shake and place it in the dark for 30 minutes. Titrate the solution by the sodium thiosulfate titrant (4.3.2.5) with stiring by magnetic stirrer (4.3.3.2). Add 1mL starch solution (4.3.2.6) into the solution when approach to end point of titration. When the solution become colorless and sustain for 30 min as the end point. Blank test in the mean time.

#### 4.3.5 Result

The content of methionine,  $X_1$ , is calculated as formula(1)

$$X_1=c(V_0-V)\times 0.0746\times 100\%/m \text{ ——(1)}$$

In the formula:

c: Actual concentration of sodium thiosulfate titrant, mol/L

$V_0$ : Actual volume used in blank tests, mL

V: Actual volume used in sample titration, mL

m: Weight of sample, g

0.0746: the weight of methionine equivalents to the weight of 1.00mL sodium thiosulfate titrant [ $c(\text{Na}_2\text{S}_2\text{O}_3)=1.000\text{mol/L}$ ], which calculated as g.

Keep one decimal place for the result.

#### 4.3.6 Deviation

Take the arithmetic mean value of the parallel test as the test result. Absolute difference of two parallel tests is not bigger than 0.3%

### 4.4 Test of loss on drying

#### 4.4.1 Principles

Dry the sample to constant weight in drying oven at  $105\pm 2^\circ\text{C}$  under standard atmospheric pressure

#### 4.4.2 Procedures

Dry 10g sample (accurate to 0.0002g) in drying oven at  $105\pm 2^{\circ}\text{C}$  for 3h, then cool it in the desiccator for 30 minutes and weigh. Dry, cool and weigh again until the difference of two tests is not bigger than 0.002g.

#### 4.4.3 Result

Loss on drying,  $X_2$ , is calculated as formula(2)

$$X_2 = (m_2 - m_3) \times 100\% / (m_2 - m_1) \text{ —— (2)}$$

In the formula:

$m_1$ : Weight of glass dish, g

$m_2$ : Weight of glass dish and sample before drying, g

$m_3$ : Weight of glass dish and sample after drying, g

#### 4.4.4 Deviation

To take the arithmetic mean value of the parallel test as the test result. Absolute difference of two parallel tests is not bigger than 0.1%.

#### 4.5 Test of Chloride

##### 4.5.1 Principles

Add nitric acid and silver nitrate into dissolved sample, which reacts with chloridions get silver chloride muddy liquid, compare it with standard hue.

##### 4.5.2 Reagents and Solutions

4.5.2.1 Silver nitrate solution: 0.1mol/L

4.5.2.2 Nitric acid solution: 1+8 (V+V)

4.5.2.3 Sodium chloride solution: 1mg/mL

##### 4.5.3 Procedures

4.5.3.1 Preparation of Standard Comparison Solution

Add 40mL water, 2.0mL sodium chloride solution (4.5.2.3), 1.0mL nitric acid solution (4.5.2.2) and 1.0mL silver nitrate solution(4.5.2.1) into 50mL comparison tubes, then deliquate it to 50mL and stir until the solution become uniform.

4.5.3.2 Preparation of Sample Solution

Add 1g sample (accurate to 0.01g) and 40mL water into 50mL comparison tubes, then heat slowly until the sample dissolved. Then add 1.0mL nitric acid solution (4.5.2.2) and 1.0mL silver nitrate solution (4.5.2.1) into the above mixture after cooling. Deliquate the solution is deliquated to 50mL and stir until the solution become uniform.

4.5.3.3 Test

Compare the standard comparison solution and sample solution. The sample is qualified if the turbidity of the sample is not higher than the standard comparison solution.

#### 4.6 Test of Heavy Metal

##### 4.6.1 Principles

After the sample is dissolved, heavy metals react with sodium sulfide under experimental condition, which gets brown sulfide. Compare its color with reference solution.

##### 4.6.2 Reagents and solutions

4.6.2.1 Sodium Dydroxide Solution: 43g/l

4.6.2.2 Sodium Sulfide Solution: 100g/l

4.6.2.3 Pb standard reserve solution (100 $\mu\text{g}/\text{mL}$ ): dissolve 0.160g lead nitrate and 5mL concentrated nitric acid with 50mL water, then move it into 1000mL volumetric flask and

deliquate it to 1000mL.

4.6.2.4 Pb standard solution (10 $\mu$ g/mL): get 10mL Pb standard reserve solution (4.6.2.3) into 100mL volumetric flask, then deliquated to 100mL.

#### 4.6.3 Procedures

Mark two 25mL comparison tubes as A and B, add 2mL Pb standard solution (4.6.2.4) and 5mL Sodium Dydroxide Solution(4.6.2.1) to comparison tubes A, deliquate it to 25mL. Dissolve 1.0g sample (accurate to 0.01g) and 5mL Sodium Dydroxide Solution (4.6.2.1) with a little water in comparison tube B, deliquate it to 25mL. Add 5 drops of Sodium Sulfide Solution (4.6.2.2) to each comparison tube, shake and place it for 2 minutes. Observe the color of comparison tube A and B above white paper. The sample is qualified if the color is less brown than the standard comparison solution.

#### 4.7 Test of As

Dissolve 1.0g sample (accurate to 0.01g) and 5 mL concentrated hydrochloric acid in 23mL water, then test it according to *Pharmacopoeia of the People's Republic of China*, 2005 2<sup>nd</sup> part, appendix VIII J, the first process.

### 5 Inspection Rules

5.1 Product should be inspected by quality inspection department of the manufacturer. The manufacturer should guarantee that all products conform to Chapter 3 of this Standard, and accompany with formed Certificate of Quality.

5.2 It is regarded as the same batch if it is produced and packed with the same batch of raw material by the same working shift, with the same processing conditions, batch number, specification and certificate of quality.

5.3 The quantity of sample complies with the regulations in GB/T 14699.1. When sampling, insert the sampler at three-fourths depths of the material, mix the sample well, and quartering 500g each. Pack in two dry and clean bottles with a grinding mouth plug. Label on the bottle, which indicating the name of the manufacturer, product name, date of manufacture, batch number. One of the bottles is used for inspection, and one for reservation.

5.4 Routine inspection: The test items including sensory index, loss on drying and the content of DL-Methionine.

5.5 Type inspection: semiannually. The type inspection items including all regulations in Chapter 3 of this Standard. A type inspection should be done under any of the following situations:

- a) Any alteration of production technology and the main raw material.
- b) Resume production after shut down for more than 3 months.
- c) When statutory quality supervision department requests.
- d) Contract requirement.

5.6 Decision rules: It is proved as qualified product if all test results in compliance with the requirements in Chapter 3 of this Standard. The product should be resample and reinspect if there is an index does not conform to the requirements of this Standard. It is proved as disqualified product if the reinspection results do not conform to the Standard.

5.7 Data processing of this Standard is according to GB/T 1250, adopt rounding off comparison method.

## 6 Label, Package, Transportation and Storage

- 6.1 The content of label should comply with the regulations in GB 10648.
- 6.2 Products are packed with three layer bags, the outer layer is polyethylene composite fabric, the middle layer is a kraft paper, inner layer is low-density polyethylene film or non-toxic PVC film, or other packaging that ensure the product quality.
- 6.3 Avoid rain, moisture and direct sunshine during transportation. Transporting with toxic goods is prohibited.
- 6.4 The product should be stored in dry and clean warehouse. Avoid rain and moisture. Storing with toxic goods is prohibited.
- 6.5 The shelf life of original packaging is 36 months under the requested storage conditions.

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