# GB

National Standard of the People's Republic of China

# GB 14752-2010

## National Food Safety Standard - Food Additive - Vitamin $B_2$ (Riboflavin)

## 食品安全国家标准　食品添加剂　维生素 $B_2$（核黄素）

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# National Food Safety Standard - Food Additive - Vitamin $B_2$ (Riboflavin)

## 1 Scope

This standard is applicable to the food additive - vitamin $B_2$ (riboflavin) prepared by chemical synthesis method and bio-fermentation.

## 2 Normative References

The following referenced documents are indispensable for the application of this standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

## 3 Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

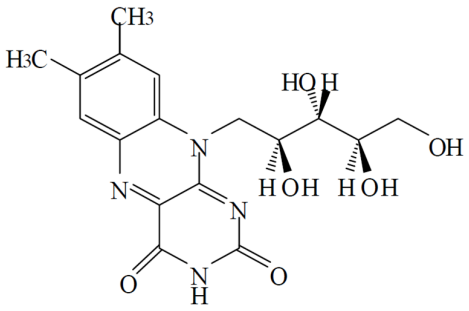
### 3.1 Chemical name

7,8-dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]-3,10-benzo[g]pteridine-2,4-dione

### 3.2 Molecular formula

$C_{17}H_{20}N_4O_6$

### 3.3 Structural formula




### 3.4 Relative molecular mass

# Annex A

## (Normative)

## Inspection Methods

### A.1 Safety warning

Some reagents used in the inspection methods of this standard are toxic or corrosive, therefore the operator shall operate according to the relevant requirements carefully. In case of splashing to skin, flush with water immediately, where the situation is serious, seek treatment immediately. Volatile acid, if required, shall be used in fume cupboard.

### A.2 General requirements

Unless otherwise specified, analytically pure reagents and Grade 3 water (specified in GB/T 6682-2008) shall be used in this standard. Unspecified solution is aqueous solution.

If there is no requirement, standard titration solution and other required solutions used in test shall be prepared according to those specified in GB/T 601, GB/T 602 and GB/T 603.

### A.3 Identification test

**A.3.1** Fluorescence test

**A.3.1.1** Method principle

Vitamin $B_2$ (riboflavin) contains aromatic ring and heterocyclic ring, and has transition of $\pi \rightarrow \pi^*$ of long conjugated structure and fluorescence. Vitamin $B_2$ (riboflavin) will be changed into non-fluorescent substance under acid and alkaline conditions and when any reducer is added.

**A.3.1.2** Reagents and materials

**A.3.1.2.1** Hydrochloric acid solution: 1+2.

**A.3.1.2.2** Sodium hydroxide solution: 40g/L.

**A.3.1.2.3** Sodium hydrosulfite.

**A.3.1.3** Analytical procedures

Take about 1mg of laboratory sample, dissolve it in 100mL of water, then the solution is yellowish green and has strong yellowish green fluorescence under transmission light; divide the solution into two portions: add hydrochloric acid solution or sodium hydroxide solution in one portion, then the fluorescence is disappeared; add a small amount of sodium hydrosulfite in the other portion and shake well, then the yellow fades and fluorescence disappears too.

**A.3.2** Identification by ultraviolet-visible absorption spectrum

**A.3.2.1** Method principle

Vitamin $B_2$ (riboflavin) has a benzene ring structure and multiple conjugated double bonds, in its ultraviolet - visible absorption spectrum, there are three absorption peaks (444nm, 375nm and 267nm), vitamin $B_2$ (riboflavin) is identified according to the ratio of $A_{444nm}/A_{267nm}$ and $A_{375nm}/A_{267nm}$.

**A.3.2.2** Reagents and materials

**A.3.2.2.1** Glacial acetic acid.

**A.3.2.2.2** Sodium acetate solution: 14g/L.

**A.3.2.2.3** Laboratory sample solution: operate in a dark place. Put about 0.075g (to the nearest 0.001g) of laboratory sample into a beaker, add 1mL of glacial acetic acid and 75mL of water, heat them until they are dissolved, dilute the solution with water and cool to room temperature, then transfer it into a 500mL brown volumetric flask, dilute it to the scale with water, and shake well; exactly measure 10mL and put it in a 100mL brown volumetric flask, add into 7mL of sodium acetate solution, dilute to the scale with water and shake well.

**A.3.2.3** Apparatuses

**A.3.2.3.1** Ultraviolet-visible spectrophotometer.

**A.3.2.3.2** Quartz cell (1cm).

**A.3.2.4** Analytical procedures

Scan the laboratory sample solution, it is found that the maximum absorptions exist at wavelengths of 444nm±1nm, 375nm±1nm and 267nm±1nm; and determine the absorbance (A), and calculate the ratio of $A_{375nm}/A_{267nm}$ to be 0.31~0.33 and

according to Formula (A.1):

$$w_1 = \frac{5000 \times A}{323 \times m \times (1 - w_2) \times 100} \times 100\% \quad \text{(A.1)}$$

Where,

5000 — the dilution volume of laboratory sample, mL;

323 — the percent absorption coefficient of vitamin $B_2$ (riboflavin), $E_{1cm}^{1\%}$;

$A$ — the absorbance of laboratory sample solution (A.3.2.2.3);

$m$ — the mass of laboratory sample, g;

$w_2$ — the value of loss on drying measured in A.7, %.

The absolute difference of two parallel determination results shall not be greater than 1.5%.

## A.5 Determination of specific rotation

### A.5.1 Method principle

Vitamin $B_2$ (riboflavin) ribitol has three asymmetric carbon atoms at positions-2,3,4 of the side chain, and it has optical activity and presents left optical property under alkaline condition, the specific rotation of sample is inspected accordingly.

### A.5.2 Reagents and materials

**A.5.2.1** Dinitrophenylhydrazine test solution: take 1.5g of 2,4-dinitrophenylhydrazine, add 20mL of sulfuric acid solution (1+1), after it is dissolved, add water to 100mL, then filter it.

**A.5.2.2** Non-aldehyde ethanol: put 2.5g of lead acetate into a conical flask with stopper, add 5mL of water to dissolve it, then add 1 000mL of ethanol, shake well, add 25mL of potassium hydroxide solution (1→5) prepared by ethanol slowly, place it for 1h, shake violently, then put it still for 12h, pour out the supernatant liquor and distill it, after that the solution is obtained.

Inspection: put 25mL of non-aldehyde ethanol into a conical flask, add 75mL of dinitrophenylhydrazine test solution, subject it to heating reflux for 24h in water bath, boil off the ethanol, add 200mL of sulfuric acid solution (2→100), then place for 24h, there shall be no crystal separation.

dimethylisoalloxazine, is soluble in trichloromethane and there is ultraviolet absorption at 440nm, so trichloromethane is used to extract dimethylisoalloxazine, after eliminating the interference of vitamin $B_2$ (riboflavin), determination is made at this wavelength and a limitation inspection is conducted. But vitamin $B_2$ (riboflavin) is slightly soluble in ethanol, to overcome the interference in determination, non-alcohol trichloromethane must be used.

**A.6.2** Reagents and materials

**A.6.2.1** Anhydrous sodium sulfate.

**A.6.2.2** Non-alcohol trichloromethane: take 500mL of trichloromethane, wash for three times with water (50mL per time), take trichloromethane layer and dry with anhydrous sodium sulfate for more than 12h, filter with degreasing cotton and distill it, then the solution is obtained. Prepare this standard solution immediately before use.

**A.6.3** Apparatuses

**A.6.3.1** Ultraviolet-visible spectrophotometer.

**A.6.3.2** Quartz cell (1cm).

**A.6.4** Analytical procedures

**A.6.4.1** Preparation of laboratory sample solution: weight about 0.025g (to the nearest 0.000 1g) of laboratory sample, add 10mL of non-alcohol trichloromethane, shake for 5min and filter it.

**A.6.4.2** Determination

Take laboratory sample solution and determine its absorbance ($A$) at the wave length of 440nm by taking non-alcohol trichloromethane as black control.

**A.7 Determination of loss on drying**

**A.7.1** Apparatuses

**A.7.1.1** Flat weighing bottle.

**A.7.1.2** Thermostatic drying oven.

**A.7.2** Analytical procedures

Put about 0.5g (to the nearest 0.000 1g) of laboratory sample into a flat weighing

**A.9.2.6** Lead nitrate.

**A.9.2.7** Thioacetamide.

**A.9.2.8** Ammonia test solution: 400→1 000.

**A.9.2.9** Sodium hydroxide solution: *c*(NaOH)=1mol/L.

**A.9.2.10** Hydrochloric acid solution: *c*(HCl)=2mol/L.

**A.9.2.11** Hydrochloric acid solution: *c*(HCl)=7mol/L.

**A.9.2.12** Ammonia solution: $c(NH_3 \cdot H_2O)$=5mol/L.

**A.9.2.13** Phenolphthalein indicator solution: 10g/L ethanol solution.

**A.9.2.14** Acetate buffer solution (pH3.5): take 25g of ammonium acetate, add 25mL of water to dissolve it, add 38mL of 7mol/L hydrochloric acid solution, accurately adjust the pH value to 3.5 (pH meter) with 2mol/L hydrochloric acid solution or ammonia solution and dilute it to 100 mL with water, then the solution is obtained.

**A.9.2.15** Thioacetamide test solution: weigh about 4g (to the nearest 0.01g) of thioacetamide , add water to dissolve it into 100mL, and keep the solution in refrigerator. Before use, take 5.0mL of mixed solution (composed of 15mL of 1mol/L sodium hydroxide solution, 5.0mL of water and 20mL of glycerol), add 1.0mL of the above thioacetamide solution, put it in water bath to heat for 20s, cool it, then use it immediately.

**A.9.2.16** Lead standard solution: weigh about 0.160g (to the nearest 0.000 2g) of lead nitrate, put it in a 1 000mL volumetric flask, add 5mL of nitric acid and 50mL of water to dissolve it, then dilute it to the scale with water and shake well to make it serve as stock solution. Before use, transfer 10mL±0.02mL of stock solution into a 100mL volumetric flask, add water to dilute it to the scale, shake well, after that the solution (containing 10μg of Pb per mL) is obtained. The glass apparatuses used for preparation and storage shall be free from lead.

**A.9.3** Analytical procedures

According to *Pharmacopeia of the People's Republic of China*, 2005 Edition, Annex VIII H, Method II of heavy metal inspection method, specific methods are as follows:

Take the remaining residue in A.8, add 0.5mL of nitric acid, evaporate them to dryness until nitrogen oxide steam vapor is completed removed (or take 1.0g of laboratory sample, burn it to completely carbonized slowly, then cool it to room

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