



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 14752-2010

**National food safety standards -- Food additives -- Vitamin B2
(riboflavin)**

食品安全国家标准 食品添加剂 维生素 B 2（核黄素）

(English Translation)

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1 Scope

This standard is applicable to the food additive - vitamin B2 (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

C₁₇ H₂₀ N₄ O₆

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 B₂ (riboflavin) contains aromatic ring and heterocyclic ring, and has transition of $\pi \rightarrow \pi^*$ of long conjugated structure and fluorescence. Vitamin B₂ (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 B₂ (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 B₂ (riboflavin) is identified according to the ratio of

A444nm/A267nm and A375nm/A267nm.

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 $\pm$ 1nm, 375nm $\pm$ 1nm and 267nm $\pm$ 1nm; and determine the absorbance (A), and calculate the ratio of A375nm/A267nm to be 0.31~0.33 and according to Formula (A.1):

Where,

5000 - the dilution volume of laboratory sample, mL;

323 - the percent absorption coefficient of vitamin B₂ (riboflavin), E

1%

1cm ;

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

m - the mass of laboratory sample, g;

w₂ - 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 B2 (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 B2 (riboflavin), determination is made at this wavelength and a limitation inspection is conducted. But vitamin B2 (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.