BHUTAN STANDARD

METHODS FOR ESTIMATION OF PYRIDOXINE (VITAMIN B_6) IN FOODSTUFFS

UDC 664:543.867

BHUTAN STANDARD BUREAU
The National Standards Body of Bhutan
THIMPHU

XXXX, 2019 Price Group B

BTS 287: XXXX IS 7530: 1975

NATIONAL FOREWORD

This Bhutan Standard which is identical with IS 7530: 1975 METHODS FOR ESTIMATION OF PYRIDOXINE (VITAMIN B6) IN FOODSTUFFS Standard issued by the Bureau of Indian Standards was adopted by Bhutan Standards Bureau by Food and Agriculture technical committee (TC 02) and approved by the Bhutan Standards Bureau Board (BSB Board) on xxxx, 2019.

The text of the IS Standard has been approved as suitable for publication as Bhutan Standard without deviation. Certain conventions are however, not identical to those used in Bhutan Standard.

Attention is particularly drawn to the following:

a) Where the words “IS Standard” appear referring to this standard, they should be read as “Bhutan Standard”.

b) Wherever page numbers are quoted, they are “IS Standard” page numbers.

IS : 7530 - 1975
(Reaffirmed 2015)

Indian Standard

METHOD FOR ESTIMATION OF PYRIDOXINE (VITAMIN B_6) IN FOODSTUFFS

UDC 664 : 543·867 B_6

INDIAN STANDARDS INSTITUTION
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110001

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May 1975

Indian Standard

METHOD FOR ESTIMATION OF PYRIDOXINE (VITAMIN B_6) IN FOODSTUFFS

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Indian Standard

METHOD FOR ESTIMATION OF PYRIDOXINE (VITAMIN B_6) IN FOODSTUFFS

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 16 January 1975, after the draft finalized by the Food Hygiene, Sampling and Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Vitamins are required to be assessed in a large number of foodstuffs, such as processed cereals, dairy products, animal feeds, and other natural or manufactured foodstuffs. Moreover, different methods of vitamin assays are used in different laboratories. Therefore, with a view to establishing uniform procedures and also for facilitating a comparative study of results, ISI is bringing out a series of standards on vitaminas say. These would include chemical as well as microbiological methods, where applicable.

0.3 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

1. SCOPE

1.1 This standard specifies a microbiological method for estimation of pyridoxine (Vitamin B_6) in foodstuffs.

2. PRINCIPLE

2.1 This method is based on the observation that *Saccharomyces carlsbergensis* requires specified vitamins for growth using a basal medium complete in all respects except for vitamin B_6, and growth responses of the organism are comparable quantitatively in standard and unknown solutions.

3. REAGENTS AND MEDIA

3.1 Quality of Reagents — Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1960†) shall be employed in tests.

NOTE — ' Pure chemicals ' shall mean chemicals that do not contain impurities which affect the results of analysis.

*Rules for rounding off numerical values (*revised*).

†Specification for water, distilled quality (*revised*).

3.2 Stock Solutions

3.2.1 Dissolve the following in water and make up to 100 ml:

Monobasic potassium phosphate (KH_2PO_4)	1·1 g
Potassium chloride (KCl)	0·85 g
Calcium chloride ($CaCl_2$)	0·25 g
Magnesium sulphate ($MgSO_4$)	0·25 g
Manganese sulphate ($MnSO_4$)	0·005 g
Ferric chloride ($FeCl_3$)	0·005 g

If a precipitate forms, dissolve it by adding a few drops of hydrochloric acid.

3.2.2 *Casein Hydrolysate*

3.2.2.1 Stir 100 g of 'Vitamin-free' casein or casein hydrolysate (*see* IS : 7203-1973*) with 250 ml of 95 percent ethyl alcohol for 15 minutes in an 800-ml beaker and filter with suction. Repeat using another 250-ml portion of alcohol. Transfer the alcohol-washed casein into a round-bottom flask of at least one-litre capacity, and preferably one having two necks ground to standard taper. Mix well with 500 ml of constant boiling hydrochloric acid (HCl). Fit the flask with a glass stopper and a water-cooled condenser and reflux over a low flame or hot plate for 8 to 12 hours. Use HCl solution (1 : 1) for the hydrolysis. Heat carefully and gradually to avoid frothing during the initial stages of hydrolysis. Mix the contents of the flask occasionally by shaking and keep wet towel ready to cool the flask if the reaction becomes too vigorous.

3.2.2.2 After refluxing, fit the flask with a condenser and receiving flask suitable for vacuum distillation and remove as much HCl as possible by concentrating the hydrolysate to a thick paste under reduced pressure. Introduce air through a bleeder tube placed well into the bottom of the flask to minimize bumping during the final stages of the concentration. The temperature at which the distillation is carried out should not exceed that of a boiling water-bath, that is, 70 to 80°C. To get complete distillation rapidly at this low temperature, reduce the pressure considerably. This may not be possible with a water aspirator unless high water pressure is available. A steam aspirator or a vacuum pump may be used. Care should be taken to trap HCl fumes effectively, especially with a vacuum pump.

3.2.2.3 Re-dissolve the paste in approximately 200 ml of water and repeat the concentration to remove additional amounts of HCl, if necessary. The acid concentration should be sufficiently low so that subsequent neutralization will not yield salt which might retard bacterial growth on the basal medium. Dissolve the hydrolysate paste in about 700 ml of water and adjust the *p*H to 3·5 with 40 percent sodium hydroxide. Decolourize by stirring

*Specification for casein hydrolysate (acid digested), microbiological grade.

with 20 g of activated charcoal at room temperature. Stir until a small test filtrate is light straw colour. The decolourization may be complete in 5 minutes or may require more than an hour depending upon the charcoal used. This step removes any niacin which may have remained in the alcohol-washed casein. Filter through a large fluted filter or by suction.

3.2.2.4 Adjust the *p*H of the filtrate to 6·8, dilute to one litre, and store under toluene and over chloroform in a refrigerator. Occasionally, a precipitate will form in this solution on standing. This is mainly tyrosine. It is a good practice to shake the solution and use the suspended material as well as the fluid portion. The insoluble material will dissolve when the entire medium is prepared.

3.2.3 *Biotin Solution* — Dissolve 4 mg of biotin in 100 ml of distilled water by warming, if necessary. Further, dilute one millilitre of the resulting solution to 10 ml with distilled water. Prepare fresh.

3.2.4 *Calcium Pantothenate Solution* — Dissolve by warming 5 mg Ca-D-Pantothenate, 50 mg meso-inositol and 5 mg niacin in 100 ml of distilled water.

3.2.5 *Thiamine Solution* — Dissolve 5 mg thiamine hydrochloride in 25 ml of distilled water.

3.2.6 *Potassium Citrate Buffer* — Dissolve 10 g potassium citrate and 2 g citric acid in 100 ml of distilled water.

3.3 Basal Medium

Stock solution (**3.2.1**)	10 ml
Stock solution (**3.2.2**)	8 ml
Stock solution (**3.2.3**)	0·4 ml
Stock solution (**3.2.4**)	10 ml
Stock solution (**3.2.5**)	0·25 ml
Stock solution (**3.2.6**)	10 ml

3.3.1 Mix the above solutions, adjust the *p*H to 5·5 and make up to 100 ml. To 100 ml of this medium, add 60 ml of glucose solution (100 g in 600 ml water).

3.4 Medium for Stock Culture

Malt extract (*see* IS : 7591-1975*)	0·3 g
Glucose	1·0 g
Yeast extract (*see* IS : 7004-1973†)	0·3 g
Peptone (*see* IS : 6853-1973‡)	0·5 g
Agar (*see* IS : 6850-1973§)	2·1 g

*Specification for malt extract, microbiological grade.

†Specification for yeast extract, microbiological grade.

‡Specification for peptone, microbiological grade.

§Specification for agar, microbiological grade.

3.4.1 Dissolve by heating the above ingredients in 100 ml of water. Dispense 8-ml portions into test tubes while the solution is hot. Plug the tubes with cotton and sterilize at 121 to 123°C for 15 minutes. Allow the tubes to cool in slanting position. Subculture *Saccharomyces carlsbergensis* every fortnight in slope cultures.

3.5 Inoculum — Wash freshly grown agar slope (after 20 hours), culture with 10 ml saline and transfer under sterile conditions into a sterile tube. As this solution is very rich in organisms, prepare a suitably diluted inoculum of 15 percent turbidity and use in inoculating the standard and samples.

3.5.1 A 15 percent turbidity or 85 percent transmission corresponds to about 0·071 optical density.

3.6 Stock Standard — Dissolve 50 mg of pyridoxine in 100 ml of distilled water. Store in a refrigerator.

3.7 Working Standards — Dilute one ml of the stock standard (**3.6**) to 100 ml so that it contains 5 μg/ml. Dilute this further hundred fold so that it contains mμg/ml. Dispense standards containing 0, 5, 10, 15, 20, 25, 30, 40 and 50 mμg of pyridoxine into volumetric flasks and make up to 100 ml. Prepare working standard daily.

4. PREPARATION OF SAMPLES

4.0 It should be ensured that the sample taken for the assay is representative of the whole, and any deterioration of the vitamin to be examined is prevented. Powders and liquids should be mixed thoroughly until homogeneity is achieved. Dry materials, such as bread, biscuits and grains shall be ground and mixed thoroughly. Wet or fresh material may be minced with a knife or scissors, or homogenized in a blender, if necessary, in the presence of the extracting solvent.

4.1 Foods of Vegetable Origin — Powder about 10 g of dry foods. In the case of vegetables and fruits, prepare a representative sample of cut material. Suspend two grams of fresh material or dry powder in 20 ml of water, add 20 ml of 0·5 N sulphuric acid and autoclave for 4 hours at 120 to 123°C. Cool the mixture and adjust the *p*H to 5·5, dilute with water to 100 ml and filter. Take suitable aliquots from the filtrate in triplicate at 3 levels for vitamin B_6 assay.

4.2 Flesh Foods — Prepare 10 percent aqueous homogenates of the flesh foods in a waring blender. Dilute one ml of 10 percent homogenate up to 5 ml with water, add 5 ml 1 N hydrochloric acid and autoclave for 4 hours at 120 to 123°C. Cool the mixture, adjust the *p*H to 5·5 and dilute to 50 ml and filter. Take suitable aliquots, in triplicate at 3 levels for vitamin B_6 assay.

5. PROCEDURE

5.1 Inoculation and Incubation — Dispense the standards or samples into 50 ml corning conical flasks and make up the volume to 1·0 ml. Add 8 ml of the basal medium and sterilize for 12 minutes at 120 to 123°C. Add one ml of the inoculum (turbidity, 15 percent) under sterile conditions and incubate at 37°C for 20 hours. Measure the turbidity in a colorimeter 660 nm using the uninoculated blank tube to set the instrument at zero.

6. CALCULATION

6.1 Draw a standard curve for the assay by plotting optical density of turbidity reading on the X-axis against concentration of the vitamin on the Y-axis. Determine the vitamin content of the tubes in the unknown series by interpolation of the colorimeter reading on the standard curve. Calculate the average for one ml of test solution from values obtained from not less than three tubes which do not vary more than 10 percent on the average. Calculate the vitamin content by the following formula:

$$\text{Vitamin } B_6 \text{ in mg per g of sample} = \frac{\text{Average mg/ml} \times \text{diluting factor}}{\text{mass of the sample}}$$

7. REPEATABILITY

7.1 The results should be repeatable within the range of $\pm$ 5 percent.

INDIAN STANDARDS

ON

FOOD ANALYSIS

IS:

5398-1968	Methods for estimation of thiamine (vitamin B_1) in foodstuffs
5399-1969	Methods for estimation of riboflavin (vitamin B_2) in foodstuffs
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5835-1970	Method for estimation of vitamin D in foodstuffs
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5886-1970	Methods for estimation of carotenes and vitamin A (retinol) in foodstuffs
5887-1970	Method for detection of bacteria responsible for food poisoning and foodborne diseases
7219-1973	Method for determination of protein in foods and feeds
7234-1974	Method for estimation of folic acid in foodstuffs
7235-1974	Method for estimation of tocopherol (vitamin E) in foodstuffs
7529-1975	Method for estimation of vitamin B_{12} in foodstuffs

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INDIAN STANDARDS INSTITUTION

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110001

Telephone : 27 01 31 (20 lines) **Telegrams : Manaksanstha**

Branch Offices :	Telephone
'Sadhna', Nurmohamed Shaikh Marg, Khanpur, AHMEDABAD 380001	2 03 91
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