BTS 286: XXXX IS 5400: 1969

BHUTAN STANDARD

METHODS FOR ESTIMATION OF NICOTINIC ACID (NIACIN) IN FOODSTUFFS

UDC 664:543.867:577.164.15

XXXX, 2019 Price Group B

BTS 286: XXXX IS 5400: 1969

NATIONAL FOREWORD

This Bhutan Standard which is identical with IS 5400: 1969 METHODS FOR ESTIMATION OF NICOTINIC ACID (NIACIN) 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 : 5400 - 1969
(Reaffirmed 2005)
(Reaffirmed 2015)

Indian Standard

METHODS FOR ESTIMATION OF NICOTINIC ACID (NIACIN) IN FOODSTUFFS

UDC 664 : 543·867 : 577·164·15

INDIAN STANDARDS INSTITUTION
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 1

Price Rs 5·00

May 1970

IS : 5400 – 1969

Indian Standard

METHODS FOR ESTIMATION OF NICOTINIC ACID (NIACIN) IN FOODSTUFFS

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Indian Standard

METHODS FOR ESTIMATION OF NICOTINIC ACID (NIACIN) IN FOODSTUFFS

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 18 November 1969, 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 dairy products, animal feeds, processed cereals and other foodstuffs. Moreover, different methods of vitamin assay 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 vitamin assays. These would include chemical and microbiological methods, wherever applicable.

0.3 This standard covers two methods, namely, chemical method and microbiological method, commonly used for estimation of niacin in foodstuffs. Depending upon the available facilities and applicability, either of the methods may be used. It is desirable that the method used is stated in the test report.

0.4 In the preparation of this standard, considerable assistance has been derived from a number of standard books and publications. However, the methods included in this standard are predominantly those which have been tried in various laboratories in the country. Thus the methods prescribed in this standard are mainly based on practical experience within the country.

0.5 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*.

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1. SCOPE

1.1 This standard specifies chemical as well as microbiological methods for estimation of nicotinic acid (niacin) in foodstuffs.

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

2. QUALITY OF REAGENTS

2.1 Unless specified otherwise, pure chemicals shall be employed in tests and distilled water (*see* IS: 1070-1960*) shall be used where the use of water as a reagent is intended.

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

3. PREPARATION OF ASSAY SAMPLE

3.0 The technique used for preparing the material for the analysis is mostly common to every vitamin determination. 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.

3.1 Powders and liquids should be mixed thoroughly until homogeneity is achieved. Dry materials, such as bread, biscuits and grains, should be ground to produce a fine powder.

3.2 Butter should be melted under constant stirring. Samples from margarine or cheese or other such foods should contain portions of the surface as well as of the interior.

3.3 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. CHEMICAL METHOD

4.0 Principle — Nicotinic acid reacts with cyanogen bromide to give a pyridinium compound which undergoes rearrangement yielding derivatives that couple with aromatic amines, giving coloured compounds which may be quantitatively estimated photometrically.

4.1 Apparatus — photoelectric colorimeter.

4.2 Reagents

4.2.1 *Standard Sulphuric Acid* — 1 N.

4.2.2 *Standard Sodium Hydroxide Solution* — 10 N.

4.2.3 *Ammonium Sulphate* — solid.

4.2.4 *Nicotinic Acid Stock Solution* — Dissolve 50 mg of USP nicotinic acid reference standard (or equivalent IP standard), previously dried and stored in dark in a desiccator over phosphorus pentoxide, in alcohol

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

to make 500 ml. Store at about 10°C. One millilitre of this solution contains 100 μg of nicotinic acid. Keep the solution in a dark bottle.

4.2.5 *Nicotinic Acid Standard Solution* — Take a small portion from the stock solution (*see* **4.2.4**), and bring to room temperature. Dilute 2 ml of this solution to 50 ml with water so that each millilitre of the resultant solution contains 4 μg of nicotinic acid.

4.2.6 *Sulphanilic Acid Solution* — 10 percent (*w/v*). Add ammonium hydroxide solution in one-millilitre portions to a mixture of 20 g of sulphanilic acid and 170 ml of water until the acid dissolves. Adjust the *p*H to 4·5 with dilute hydrochloric acid 1:1 (*v/v*), using bromocresol green as indicator and dilute to 200 ml. This solution should be almost colourless.

4.2.7 *Sulphanilic Acid Solution* — 55 percent (*w/v*). Add 27 ml of water and 27 ml of ammonium hydroxide solution to 55 g of sulphanilic acid and shake until dissolved. Warm, if necessary. Adjust the *p*H to 7 with a few drops of ammonium hydroxide or dilute hydrochloric acid. Dilute to 100 ml and store in dark.

4.2.8 *Cyanogen Bromide Solution* — 10 percent (*w/v*). Warm 370 ml of water to 40°C in a large flask and add 40 g of freshly prepared cyanogen bromide (CNBr). Shake until dissolved, cool and dilute to 400 ml taking care not to allow the cyanogen bromide or its solution to come in contact with the skin. Prepare the solution under a hood.

NOTE — Cyanogen bromide may be prepared by mixing bromine water and potassium cyanide solution, taking care that there is a slight excess of potassium cyanide.

4.2.9 *Dilute Ammonium Hydroxide Solution* — Dilute 5 ml of 28 percent ammonium hydroxide to 250 ml with water.

4.2.10 *Dilute Hydrochloric or Hydrobromic Acid* — 1: 5 (*v/v*).

4.2.11 *Phosphate Buffer Solution* — Dissolve 60 g of disodium hydrogen phosphate (Na_2HPO_4, $7H_2O$) and 10 g of monopotassium phosphate (KH_2PO_4) in warm water and dilute to 200 ml.

4.3 Procedure

4.3.1 *Extraction of Niacin from Non-cereal Foods and Feeds and Preparation of Sample Solution* — Weigh 40 g of sample into a one-litre Erlenmeyer flask, add 200 ml of 1 N sulphuric acid, mix and heat for 30 minutes in an autoclave at 120° to 123°C. Cool, adjust to *p*H 4·5 with standard sodium hydroxide solution, using bromocresol green as external indicator, dilute to 250 ml with water, and filter. Weigh 17 g of ammonium sulphate into a 50-ml volumetric flask, pipette in 40-ml aliquot sample solution, dilute to mark with water, and shake

vigorously. Filter, mix well and use (a) 1-ml aliquot, and (b) 0·5-ml aliquot with 0·5 ml of water for colour development.

4.3.1.1 *Preparation of standard solution* — Take a 40-ml aliquot of working standard (1 ml = 4 μg) and 17 g ammonium sulphate in a 50-ml volumetric flask and dilute to mark with water. Now one millilitre of standard contains 3·2 μg of niacin.

4.3.1.2 *Preparation of standard curve with standard vitamin*

	Standard Blank (A)	*Standard Solution (B)*
1. Standard solution	1 ml = 3·2 μg	1 ml = 3·2 μg
2. Water	5·0 ml	Nil
3. Dilute ammonium hydroxide	0·5 ml	0·5 ml
4. Cyanogen bromide	Nil	5·0 ml
5. 10 percent sulphanilic acid	2·0 ml	2·0 ml
6. Dilute hydrochloric acid	0·5 ml	0·5 ml
Total:	9·0 ml	9·0 ml

Add all subsequent solutions, mentioned above, to a single tube and read the colour before proceeding with the next tube.

4.3.1.3 Adding the standard solution (in standard blank tube), swirl the tube to impart rotary motion in liquid, add dilute ammonium hydroxide, immediately swirl again, add sulphanilic acid and swirl. Immediately add 0·5 ml of dilute hydrochloric acid, mix again; place in the photoelectric colorimeter, and adjust the instrument at zero absorbence at any specific wave-length (usually at 420 mμ) within 30 seconds after addition of sulphanilic acid.

4.3.1.4 Treat standard solution (in standard solution tube) in the same way as standard blank tube with respect to addition of dilute ammonium hydroxide. Immediately shake the tube and add cyanogen bromide solution and shake again. After 30 seconds shake the tube and add sulphanilic acid and shake again. Immediately add 0·5 ml of dilute hydrochloric acid, mix again and stopper. Read absorbence of standard solution in the photoelectrical colorimeter (as in **4.3.1.3**).

4.3.1.5 *Determination of unknown samples*

	Sample Blank (C)	*Unknown Sample (D)*
1. Sample solution	1·0 ml	1·0 ml
2. Water	5·0 ml	Nil
3. Dilute ammonium hydroxide	0·5 ml	0·5 ml
4. Cyanogen bromide	Nil	5·0 ml
5. 10 percent sulphanilic acid	2·0 ml	2·0 ml
6. Dilute hydrochloric acid	0·5 ml	0·5 ml
Total:	9·0 ml	9·0 ml

Develop the colour of sample blank (*C*) and unknown sample (*D*) in the same way as given in **4.3.1.3** and **4.3.1.4**. With sample blank set at zero absorbence, determine absorbence of sample solution in the same way.

4.3.1.6 *Calculation*

$$\mu g \text{ of Niacin/g of food sample (on dry basis or wet basis as the case may be)} = \frac{3{\cdot}2}{A} \times \frac{B}{W} \times C$$

where

B = absorbence of 1 ml of sample solution,

C = total volume of sample extract,

A = absorbence for 3·2 μg of niacin, and

W = weight in g of the sample taken for vitamin extraction.

4.3.2 *Preparation of Samples and Extraction of Nicotinic Acid (Niacin) from Cereal Products*

a) Take 2·5 g of sample containing about 100 μg niacin in a 250-ml Erlenmeyer flask containing 1·5 g of calcium hydroxide. Add about 90 ml of distilled water and mix thoroughly.

b) Autoclave the flask for 2 hours at 120° to 123°C.

c) After autoclaving, mix the hot mixture thoroughly and make the total volume to 100 ml when the contents attain 40°C.

d) Take 50 ml of supernatant [from (c)] and keep in an ice-bath for 15 minutes. Centrifuge and collect 20 ml of supernatant in a centrifuge tube containing 8 g of ammonium sulphate and 2 ml of phosphate buffer. Shake to dissolve and warm to 55° to 60°C. Centrifuge for 5 minutes and filter through Whatman No. 12 filter paper or equivalent.

e) For the preparation of standard curve, take only 0, 50, 100, 150, 200 and 250 μg of niacin and proceed according to (a) to (d).

4.3.2.1 *Preparation of standard curve*

Sl No.	*Reagent*	*Standard Blank*	*Reagent Blank*	*Standard Solution*
1.	Standard solution	5·0	Nil	5·0 ml
2.	Cyanogen bromide	Nil	10·0	10·0 ml
3.	Water	10·0	5·0	Nil
4.	55 percent sulphanilic acid	1·0	1·0	1·0 ml
	Total:	16·0 ml	16·0 ml	16·0 ml

a) After adding No. 1 and 3 reagents, keep all tubes in ice-salt bath for 30 minutes.

b) Add 10 ml of cold cyanogen bromide to reagent blank and standard solution and within 30 seconds, add 1 ml, 55 percent sulphanilic acid to all the tubes.

c) Mix thoroughly and keep the tubes in ice-salt bath.

d) Set colorimeter with 470 mμ wave-length at 100 percent transmittance with standard blank; and read optical density of the other tubes within 12 to 15 minutes after addition of sulphanilic acid.
(Let optical density for reagent blank be X and standard solution Y).

e) Plot concentration of standard niacin *versus* optical density $(Y-X.)$

4.3.2.2 *Determination of niacin content of unknown cereal samples*

Sl No.	*Reagent*	*Sample Blank*	*Reagent Blank*	*Sample Solution*
1.	Sample solution	5·0	Nil	5·0
2.	Cyanogen bromide	Nil	10·0	10·0
3.	Water	10·0	5·0	Nil
4.	55 percent sulphanilic acid	1·0	1·0	1·0
	Total:	16·0 ml	16·0 ml	16·0 ml

Repeat process (a) to (d) in **4.3.2.1** with sample blank, reagent blank and sample solution.

(Let optical density of reagent blank be X and optical density of sample solution be Z.)

From the standard curve, find out the concentration of niacin corresponding to $(Z-X)$ optical density. Let this be K.

4.3.2.3 *Calculation*

$$\mu g \text{ of niacin per g of cereal product} = K \times \frac{100}{5 \times 2{\cdot}5}$$

5. MICROBIOLOGICAL METHOD

5.0 Principle — The microbiological method is based upon the observation that *Lactobacillus arabinosus* 17-5; NCTC* No. 6376 (ATCC† No. 8014) required nicotinic acid (niacin) for growth. Using niacin-free but otherwise

*National Collection of Type Cultures.
†American Type Culture Collection.

complete basal medium growth responses of the organism are compared quantitatively in standard and unknown solutions. Either the acid or the turbidity produced by the organism is measured to determine the extent of growth and thereby the amount of niacin in the test solution.

5.1 Reagents

5.1.1 *Acid-Hydrolyzed Casein* — Take 50 g of vitamin-free casein in 500 ml of ground joint flask and add 250 ml of 1 N hydrochloric acid to it. Reflux the resulting mixture on a heating mantle for 12 hours. Concentrate the solution very slowly under reduced pressure. A thick paste of casein hydrolysate would be left. Dissolve the thick paste in distilled water (about 200 ml) and again concentrate under vacuum. Dissolve the black residue in distilled water (300 ml) and adjust the *p*H to 3·5 using 40 percent sodium hydroxide solution. Decolorize the hydrolysate with activated charcoal, warm and filter. Repeat the decolorization procedure 3 to 4 times (till a straw coloured hydrolysate is obtained). Then adjust the *p*H of the hydrolysate to 6·8 and dilute to 500 ml and determine the solid weight (1 ml hydrolysate 90 mg of solid).

5.1.2 *Cystine-Tryptophan Solution* — Suspend 4·0 g of *l*(−) cystine and 1·0 g of *l*(−) tryptophan (or 2·0 g of *dl*-tryptophan) in 700 to 800 ml of water, heat to 70° to 80°C and add 20 percent hydrochloric acid dropwise with stirring until the solids are dissolved. Approximately 12 ml of 20 percent hydrochloric acid are required. Cool to room temperature and make to 1 litre with water.

5.1.3 *Adenine-Guanine-Uracil Solution* — Heat 0·1 g each of adenine sulphate, guanine hydrochloride, and uracil in a 250-ml Erlenmeyer flask containing about 75 ml of water and 2 ml of concentrated hydrochloric acid. Cool when all solids have gone into solution. If a precipitate forms, add a few drops of concentrated hydrochloric acid and heat. Repeat until no precipitate forms on cooling and then transfer to a 100-ml volumetric flask and make to volume with water.

5.1.4 *d-Calcium Pantothenate-p-Aminobenzoic Acid-Pyridoxine Hydrochloride Solution* — Weigh 10·0 mg each of *d*-calcium pantothenate-*p*-aminobenzoic acid and pyridoxine hydrochloride. Transfer to a 1-litre volumetric flask and dilute to volume with water. Store the solution in the dark to protect pyridoxine from light.

5.1.5 *Riboflavin-Thiamine Hydrochloride-Biotin Solution* — Dissolve 1 mg of crystalline biotin (free acid) in 100 ml of 0·02 N acetic acid. Add 4·0 ml (equivalent to 40 μg) of this solution to a 1-litre volumetric flask. Add 20 mg of riboflavin and 10 mg of thiamine hydrochloride to the flask and make up to volume with 0·02 N acetic acid. Store in the dark to prevent destruction of riboflavin by light.

5.1.6 *Salt Solution A* — Dissolve 25 g of dipotassium hydrogen phosphate (K_2HPO_4) and 25 g of monopotassium phosphate (KH_2PO_4) and dilute to 500 ml with water. Store under toluene.

5.1.7 *Salt Solution B* — Dissolve the following amounts of salts and dilute to 500 ml with water:

Magnesium sulphate ($MgSO_4,7H_2O$)	10·0 g
Sodium chloride (NaCl)	0·5 g
Ferrous sulphate ($FeSO_4,7H_2O$)	0·5 g
Manganese sulphate ($MnSO_4,4H_2O$)	0·5 g

5.1.7.1 Add 5 drops of concentrated hydrochloric acid and store under toluene. Sodium chloride may be omitted when hydrochloric acid-hydrolyzed casein is used.

5.1.8 *Niacin Stock Solution* —(a) Weigh 50 g of USP or equivalent IP. Niacin in 500 ml 50 percent ethyl alcohol. Store this stock solution in refrigerator (1 ml = 100 μg). (b) Working standard of niacin: 1 ml of standard niacin/1 litre of distilled water (1 ml = 0·1 μg).

5.1.9 *Basal Medium Stock Solution* — In order to make up this basal medium stock solution for 100 assay tubes, mix the following ingredients in a 500-ml Erlenmeyer flask marked at 450 ml:

Sl No.	*Reagent*	*Quantity*
1.	Casein hydrolysate	50 ml
2.	Cystine-tryptophan	50 ml
3.	Adenine-guanine-uracil	10 ml
4.	*d*-Ca pantothenate-*p*-aminobenzoic acid-pyridoxine	10 ml
5.	Riboflavin-thiamine hydrochloride-biotin	10 ml
6.	Salt Solution A	10 ml
7.	Salt Solution B	10 ml
8.	Anhydrous glucose	10 g
9.	Anhydrous sodium acetate	10 g
10.	Water	250 ml

5.1.9.1 Mix the ingredients thoroughly, dilute to 450 ml and adjust the *p*H to 6·8 with 40 percent sodium hydroxide using a *p*H meter or bromothymol blue as an external indicator. Approximately 1 ml of alkali is necessary for the adjustment. Transfer to a 500-ml volumetric flask or a graduated cylinder and make up to volume with water. In the assay procedure one volume of basal medium stock solution is diluted to two volumes.

5.1.10 *Agar Medium for Stock Culture* — Dissolve 5·0 g of Difco/Bacto or equivalent to yeast extract in 200 ml of water, add 1 g anhydrous

glucose, 1 g of anhydrous sodium acetate (or 1·7 g $NaC_2H_3O_2$, $3H_2O$) and 3 g of agar and heat the mixture on a steam-bath with occasional stirring until the ingredients are dissolved. While the solution is still hot, filter it through cotton or cloth and add 10 ml to each of 20 culture tubes. Plug the tubes with cotton and sterilize in the autoclave for 20 minutes at 120° to 123°C. Cool in an upright position and store in the refrigerator.

5.1.11 *Culture Medium for Growing Inoculum* — To each of a series of culture tubes containing 5·0 ml of basal medium stock solution, add 5 ml of a solution containing 0·2 µg of nicotinic acid (niacin) per millilitre. Plug the tubes with cotton and sterilize in the autoclave at 120° to 123°C for 15 minutes. The tubes may be stored for several weeks provided evaporation, contamination, etc, do not take place.

5.1.12 *Isotonic Salt Solution* — Weigh 0·9 g of sodium chloride and transfer to a 100-ml volumetric flask. Dilute to volume with water and shake until salt has dissolved. Transfer 10-ml quantities of this solution to culture tubes, plug with cotton and sterilize in the autoclave at 120° to 123°C for 20 minutes.

5.1.13 *Sulphuric Acid* — 1 N.

5.1.14 *Sodium Hydroxide* — 1 N.

5.1.15 *Sodium Hydroxide* — 0·1 N.

5.1.16 *Bromothymol Blue Indicator Solution* — Weigh 0·1 g of bromothymol blue indicator into a small beaker. Add 1·6 ml of 0·10 N sodium hydroxide and triturate with a stirring rod until the powder has dissolved. Dilute with water to 250 ml. The solution may also be made up by dissolving in a few millilitres of 95 percent ethanol, adding 1·6 ml of 0·1 N sodium hydroxide, and diluting to 250 ml with water.

5.2 Procedure

5.2.1 *Preparation of the Stock Culture*

a) Prepare stab cultures in two or more agar stock culture tubes (*see* **5.1.10**) using a pure culture.

b) Incubate for 16 to 24 hours at 37·0° ± 0·5°C.

c) Store in the refrigerator under aseptic conditions not longer than one week, preferably only one week, before transferring to new stab.

5.2.2 *Preparation of the Inoculum*

a) On the day prior to use, transfer cells from the stock culture to a sterile tube of inoculum culture medium (**5.1.11**).

b) Incubate this culture for 6 to 18 hours at 37°C.

c) Secure cotton plug with a rubber band, adhesive tape or a pin, and centrifuge.

d) Decant the supernatant liquid and resuspend the cells in 20 ml of sterile isotonic salt solution (**5.1.12**). This should be done aseptically.

e) Fill the sterile syringe with the resuspended cells and use at once. (Sterile pipette may be used.)

5.2.3 *Preparation of Samples*

a) Weigh sufficient material to contain approximately 0·1 mg of niacin into a 250-ml flask.

b) Add 100 ml of 1 N sulphuric acid and mix thoroughly.

c) Autoclave the mixture at 120° to 123°C for 30 minutes.

d) Add 1 N sodium hydroxide to produce a *p*H of 6·8 using a *p*H meter or bromothymol blue as an external indicator.

e) Quantitatively transfer the solution to a 1-litre volumetric flask, dilute to volume with water and mix thoroughly.

f) Filter the solution through Whatman No. 40 or equivalent filter paper or its equivalent. This is the test solution used in the preparation of the assay tubes. The niacin concentration should be 0·1 to 0·2 µg per millilitre to obtain valid assays.

5.2.4 *Preparation of Standard Tubes for Titrometric Method*

a) To duplicate tubes add 0·0, 0·5, 1·0, 1·5, 2·0, 2·5, 3·0 and 4·0 ml of the working standard niacin solution (**5.1.8**).

b) Add sufficient water to bring the volume in each tube to 5 ml.

c) To each of these tubes add 5·0 ml of basal medium stock solution (**5.1.9**).

5.2.5 *Preparation of Assay Tubes*

a) To duplicate tubes add 0·5-, 1·0-, 2·0- and 3·0-ml aliquots of the test solution.

b) Add sufficient water to bring the volume to 5 ml.

c) To each of these tubes add 5·0 ml of basal medium stock solution.

5.2.6 *Sterilization*

a) Mix the contents of each tube thoroughly by rotating the tube vigorously in the palm of the hand.

b) Plug with cotton or cover with caps.

c) Autoclave at 120° to 123°C for 12 to 15 minutes.

5.2.7 *Inoculation and Incubation*

a) Cool all the tubes to the incubation temperature or below.

b) Aseptically inoculate each tube with one drop of inoculum.

c) Incubate for 72 hours at 37°C.

5.2.8 *Titration*

a) Transfer the contents of each tube, successively to a 50-ml Erlenmeyer flask and rinse with 10 ml of water.

b) Titrate the contents of the flask with 0·1 N sodium hydroxide (**5.1.15**) to a *p*H of 6·8 using bromothymol blue (**5.1.16**) as an internal indicator.

5.3 Calculation

a) Draw a standard curve for the assay by plotting the number of millilitres of 0·1 N sodium hydroxide used in titrating the standard tubes against μg niacin per tube in the standard series.

b) Determine the niacin content of the tubes in the unknown series by interpolation of the titer values on the standard curve.

c) Discard any values which show more than 0·4 or less than 0·05 μg of niacin per tube. Calculate the niacin content of each millilitre of test solution for each of the duplicate sets of tubes.

d) Calculate the niacin content of the test material from the average of the values for 1 ml of test solution obtained from not less than three sets of these tubes which do not vary by more than 10 percent from the average, using the following formula:

$$\mu g \text{ Nicotinic acid (niacin)/g of food sample (on dry or wet basis as the case may be)} = \frac{\mu g \text{ Niacin per ml of solution} \times \text{Volume}}{\text{Weight of sample in g}} \times \text{Dilution factor}$$

INDIAN STANDARDS

ON

Vitamin Assay of Foodstuffs

IS:		Rs
5398-1969	Methods for estimation of thiamine (vitamin B_1) in foodstuffs	5·00
5399-1969	Methods for estimation of riboflavin (vitamin B_2) in foodstuffs	5·00

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INDIAN STANDARDS

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Printed at the Catholic Press, Ranchi, India

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