BHUTAN STANDARD

METHODS FOR ESTIMATION OF CAROTENES AND VITAMIN A (RETINOL) IN FOODSTUFFS

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NATIONAL FOREWORD

This Bhutan Standard which is identical with IS 5886: 1970 METHODS FOR ESTIMATION OF CAROTENES AND VITAMIN A (RETINOL) 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: 5886 - 1970
(Reaffirmed 2005)
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Indian Standard

METHODS FOR ESTIMATION OF CAROTENES AND VITAMIN A (RETINOL) IN FOODSTUFFS

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

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Indian Standard

METHODS FOR ESTIMATION OF CAROTENES AND VITAMIN A (RETINOL) IN FOODSTUFFS

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Indian Standard

METHODS FOR ESTIMATION OF CAROTENES AND VITAMIN A (RETINOL) IN FOODSTUFFS

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 30 November 1970, 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 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 prescribes the methods for estimation of carotenes and vitamin A in foodstuffs. Two methods have been prescribed, namely, spectrophotometric method and Carr-Price method. The spectrophotometric method is simpler than the colorimetric procedure. In case of spectrophotometric method, it is essential that the interfering substances should be removed by purification using column chromatography.

0.3.1 Carr-Price method is generally used for routine estimations, whereas the spectrophotometric method is used as a reference method.

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*.

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

1. SCOPE

1.1 This standard prescribes methods for the estimation of carotenes and vitamin A (retinol) in foodstuffs.

2. QUALITY OF REAGENTS

2.1 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 test results.

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 determination 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 and mixed thoroughly.

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. ESTIMATION OF CAROTENES

4.0 Principle — Carotenes are extracted from the moist pulverized or homogenized assay sample with hexane, petroleum ether or other suitable solvent mixed with acetone or alcohol. In case of fatty materials, preliminary saponification is necessary. The extract is purified from non-carotene pigments by chromatography. The carotene content of aliquot from the elute in hexane or petroleum ether is estimated in spectrophotometer at 450 mμ or in a photo-electric colorimeter with a 440 mμ filter.

4.1 Apparatus

4.1.1 *Spectrophotometer*

or

Photo-electric Colorimeter — 440 mμ filter.

4.1.2 *Chromatographic Tube* — 180 to 220 mm long and 20 mm diameter.

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

4.1.3 *Extraction Apparatus* — Depending on the nature of assay sample, a glass mortar for moist, homogenized food material, a beaker with glass rod with a flat end for maceration and Bailey-Walker type or similar hot extraction apparatus for dried leaves and powdered beans shall be selected.

4.1.4 *Beaker* — 150 ml capacity.

4.2 Reagents

4.2.1 *n-Hexane or Petroleum Ether* — It may be purified by shaking with concentrated sulphuric acid and collecting the fraction distilling between 67°C and 70°C.

4.2.2 *Petroleum Ether Acetone Mixture*

a) For extraction — 1 : 1 (*v/v*), and

b) For elution — 9 : 1 (*v/v*).

4.2.3 *Diethyl Ether* — purified. This may be washed with saturated ferrous sulphate solution and then distilled over sodium hydroxide.

4.2.4 *Absorbent* — Mix equal proportion by weight of activated magnesia and hyflo-super cel or equivalent and keep it in the dry state till used.

4.2.5 *Granular Anhydrous Sodium Sulphate* — conforming to IS : 255-1967*.

4.2.6 *Glass Wool or Fat-Free Cotton*

4.3 Procedure

4.3.1 *Extraction* — Weigh accurately a quantity of the assay sample containing 0·05 to 0·10 mg of carotene and transfer it to the extraction vessel. Add equal volume of quartz powder or acid-washed sand and moisten the mixture with a little water. Then add 10 ml of petroleum ether-acetone mixture (1 : 1). Grind and decant the solvent, when the particles have settled, to a separating funnel containing water. Repeat the grinding and decanting until no more carotene is left. Normally 4 to 5 extractions are adequate.

4.3.1.1 Wash the combined petroleum ether acetone extract with five 100-ml portions of water to remove acetone. The acetone shall be completely removed to avoid any interference with chromatography. The extract, containing carotenoids and chlorophylls shall be preserved for chromatography.

NOTE 1 — Carotenes are extracted much more conveniently from wet materials. In course of extraction when acetone takes away the moisture, the material may be kept moist by adding a few drops of water between the extractions.

NOTE 2 — By the cold extraction process, it is difficult to extract carotenes from dry leafy materials or beans, where hot extraction is necessary. With fatty materials, when the ratio of fat to carotene is very large, for example 1 000 : 1, the fat may have to be hydrolyzed as in estimation of vitamin A and then taking up the non-saponifiable matter in petroleum ether or hexane.

*Specification for sodium sulphate, anhydrous (*first revision*).

4.3.2 *Purification*

4.3.2.1 *Preparation of column* — Connect the chromatographic tube to a Witt's or equivalent filtering apparatus with a beaker (150 ml) as receiver or to a suction flask of 250 ml capacity, through a rubber stopper. Loosely plug the lower end of the chromatographic tube with glass wool or cotton and turn on the suction. Add adsorption mixture through a funnel in small amounts to a height of 10 to 12 cm and pack the column by pressing down with a cork-stopper, just fitting the tube and attached to a glass rod. Place on the top of the column 2 to 3 cm layer of anhydrous sodium sulphate.

4.3.2.2 *Chromatography* — Pour slowly the petroleum ether or hexane extract into the absorption tube and rinse the vessel carefully with petroleum ether, to transfer all colouring matter to the column. Finally, rinse down sides of the tube with a few millilitre portion of petroleum ether or hexane, taking particular care that the chromatographic column containing carotenes remains always covered with solvent. When a few millilitre of petroleum ether or hexane comes through the column, change the receiver of the suction flask. When petroleum ether or hexane layer in the column has come down to the sodium sulphate layer, add slowly 50 ml of petroleum ether acetone mixture (9 : 1) to elute the carotene. When this mixed solvent has passed through the sodium sulphate layer, stop the suction, transfer the elute to 10 or 25 ml volumetric flask as required and dilute to volume with petroleum ether.

4.3.3 *Preparation of Standard Curve*

4.3.3.0 The standard curve is necessary only when the final estimation is carried out in a colorimeter or when the true optical density cannot be obtained from the spectrophotometric readings.

4.3.3.1 Weigh accurately about 10 to 20 mg of purified crystals of β-carotene and disssolve in about 20 ml of diethyl ether. Transfer this into a volumetric flask of 1 litre with about 20 ml of diethyl ether and make the volume to the mark with *n*-hexane or petroleum ether. From this stock solution, prepare eight concentrations of β-carotene solution containing 0·25, 0·50, 0·75, 1·00, 1·25, 1·50, 1·75 and 2·00 mg per litre.

4.3.3.2 Find out the absorbances of these solutions in the instrument and with the same cuvettes, to be used for final estimation, at 450 mμ in case of spectrophotometer or with 440 mμ narrow band filter in case of colorimeters. Plot the absorbence against concentration of carotene per litre. This relationship should be almost linear.

4.3.4 *Determination of Carotenes* — Find the absorbence of the hexane or petroleum ether extract of the assay sample as in **4.3.3.2.** From the observed absorbence, calculate the concentration of carotenes in milligrams

per litre. In case the absorbance is more than 0·50, make suitable dilution, so that the absorbance lies below 0·5.

4.3.5 *Calculation*

4.3.5.1 *Content of carotenes, in mg per 100 g of assay sample* (A)

a) From standard curve:

$$A = \frac{C \times V}{10 \times g}$$

where

C = concentration of carotenes in mg per litre from the standard curve,

V = final volume of extract, and

g = weight of assay sample.

b) From optical density (E) at 450 mμ :

$$A = \frac{E \times 100}{196 \times L \times W}$$

where

E = optical density (absorbance),

L = length of light path in the cuvette in cm, and

W = weight in g of the sample per ml of final extract

NOTE 1 — In case carotene has to be calculated directly without the use of standard curve, the spectrophotometer should be calibrated for wave-length and absorption by standard procedure.

NOTE 2 — The factor ' 196 ' is for average mixture of carotenes in food samples.

$E^{1\%}_{1cm}$ = 1 960, in petroleum ether for mixture of carotenes commonly present in foodstuffs; and for pure all trans β-carotenes

$E^{1\%}_{1cm}$ = 2 550, in petroleum ether.

NOTE 3 — When the carotene mixture is further purified by chromatography and all trans β-carotenes is isolated and estimated, the following formula should be used:

$$A = \frac{E \times 100}{255 \times L \times W}$$

5. ESTIMATION OF VITAMIN A (RETINOL)

5.1 Spectrophotometric Method

5.1.0 *Principle* — The vitamin A is extracted from the assay sample, purified by saponification or chromatography and dissolved in optically pure isopropanol, hexane or cyclohexane. This method is based on the measurement of light absorption of the vitamin solution. This light

absorption is proportional to the concentration of the vitamin at a wave length of 325 mμ where maximum absorption occurs. To correct for extraneous materials which also absorb in this region, readings are also made at 310 mμ and 334 mμ followed by appropriate mathematical correction, in case of samples having an absorption maxima for the unsaponifiable matter lying within the region 323 mμ and 327 mμ and extinction ratio E_{300} mμ/E_{325} mμ, not exceeding 0·73. But for those samples having an absorption maxima for the unsaponifiable matter lying outside the region 323 mμ to 327 mμ or an extinction ratio E_{300} mμ/E_{325} mμ, exceeding 0·73, further purification by chromatography is necessary.

5.1.1 *Apparatus*

5.1.1.1 *Spectrophotometer* — with suitable source of ultraviolet light.

5.1.1.2 *Chromatography apparatus* — The chromatography tube has three sections fused together. At the upper part, it has a wider section, 80 mm length and 16 mm internal diameter, with a side tube attached at 45 to 50 mm below the top; this side tube has a three-way cock. The central part of the tube is 180 to 200 mm long and has 10 mm internal diameter; at the lower end it has a stem, 50 mm long and 5 mm diameter. The side-tube at the top is connected through a rubber tubing to a pressure system consisting of a Buchner Flask and a rubber hand-bellows with a non-return valve, so that pressure inside the tube may be applied or released as and when required. A 50-ml separatory funnel is fitted at the top with a wooden cork (not rubber) to serve as a reservoir for solvents.

5.1.1.3 *Ultraviolet lamp for detection of vitamin A zone* — A mercury vapour lamp with a light filter to transmit 320 to 340 mμ

5.1.2 *Reagents*

5.1.2.1 *Ethanol and isopropanol*

a) *Ethanol* — Ethanol may be purified as follows:

Reflux absolute ethanol for 30 minutes in presence of 10 g caustic potash and 10 g aluminium powder per litre of ethanol. The mixture is then distilled in an all-glass apparatus with a fractionating column of 50 to 60 cm length. Collect only middle fraction of the distillate, discarding the first and the last fraction, each amounting to 5 percent of the total volume. The ethanol thus obtained should have the same optical absorption as glass distilled water in ultraviolet region.

b) *Isopropanol* — Purify isopropanol by redistillation in an all-glass apparatus with a fractionating column of 40 cm length and collecting the fraction distilling at 82·3 ± 0·2°C.

5.1.2.2 *n-Hexane or petroleum ether* — Purify as given in **4.2.1.**

5.1.2.3 *Potassium hydroxide solution* — 50 percent (*w/v*).

5.1.2.4 *Ether* — peroxide-free, redistilled (*see* IS : 336-1964*). Maintain ether free from peroxide by the method given in **5.2.2.4.**

5.1.2.5 *Sodium sulphate* — anhydrous and granular. It shall not absorb vitamin A under conditions of use, and 10 percent solution shall not be acid to methyl red indicator solution.

5.1.2.6 *Aluminium oxide* — Heat neutral aluminium oxide [which passes through 125- to 75- micron IS Sieve (*see* IS : 460-1962†)] for chromatography at 500 ± 50°C for 2 to 3 hours and protect from moisture by cooling it under vacuum and storing it in an air-tight bottle. Shake 50 g of this dehydrated alumina very thoroughly with 2·0 to 2·5 ml of distilled water till a uniform lump-free mass is obtained. Allow to stand for 2 to 3 hours before chromatography.

5.1.2.7 *Solvents for chromatography*

a) *Petroleum Ether* — Boiling point 67 to 70°C. Purify as given in **4.2.1.**

b) *Elution mixture* — Petroleum ether containing 15 to 24 percent (*v/v*) diethyl ether. The amount of diethyl ether necessary, depends on the activity of alumina; this may be found out by occasionally checking the vitamin A zone with a weak ultraviolet lamp (**5.1.1.3**); the vitamin A zone would reveal itself by greenish yellow fluorescence.

5.1.3 *Procedure*

5.1.3.0 Adequate precautions should be taken to protect the vitamin A content in the flask from direct actinic light.

5.1.3.1 Weigh accurately a quantity of the material (not more than seven grams) containing 30 to 50 I. U. of vitamin A. Transfer the material quantitatively to an all glass saponification flask (150 ml), if necessary with a little alcohol. Add 30 ml of ethanol [95 percent (*v/v*)] and 5 ml of potassium hydroxide solution. To replace the air, pass a stream of nitrogen gas through the solution with a jet-tube for 5 minutes. Then in a dark corner, saponify the contents under reflux in an atmosphere of nitrogen, over an electrically heated water-bath for 30 to 40 minutes. Cool and then add 30 ml of water. Transfer the contents quantitatively to a separating funnel (conical, Squibb shaped, 250 ml capacity). Wash the flask twice with 10 ml of water and add the washings to the separating funnel.

*Specification for ether (*revised*).
†Specification for test sieves (*revised*).

Extract the saponified solution thrice, respectively with 50 ml, 20 ml and 20 ml ether; combine the ether extract in another separating funnel and wash this with 50 ml ice cold water containing a little sodium acid sulphate ($NaHSO_4$) or potassium acid phosphate (KH_2PO_4), just enough to neutralise the alkalinity of the extract. Repeat the washing with only ice cold water, till the washings are neutral to phenolphthalein.

5.1.3.2 Transfer the ethereal extract to a stoppered measuring cylinder (100 ml), rinse the separating funnel with a little ether and then add this to the cylinder. Add more ether to make the volume to 100 ml mark. Then add 15 g of anhydrous sodium sulphate; mildly shake a little and then allow it to settle in a dark cool place.

5.1.3.3 *Chromatography* — Evaporate an aliquot of the ethereal extract of the unsaponifiable matter in a stream of nitrogen. Take up the residue in 5 ml of petroleum ether. Place a wad of fat-free cotton in the lower stem of the chromatographic tube and set it up in a stand. Pour in petroleum ether upto the middle of the 10 mm section, and then add sufficient alumina absorbent, with continuous tapping, to a height of 100 mm in the column; then add anhydrous sodium sulphate to make a layer of 1 cm. Attach the separatory funnel at the top and apply pressure to drain off excess solvent, but taking particular care that at no time, the level of solvent falls below the alumina surface in the column. Release the pressure, remove the funnel and transfer the petroleum ether extract of the unsaponifiable matter quantitatively to the column, using more petroleum ether, if necessary. Replace the funnel and add gradually 30 ml of petroleum ether, 10 ml at a time, through the funnel, and regulate the pressure to obtain a flow rate of approximately 60 drops per minute. This washing would contain carotene fraction, which may be collected and estimated, if necessary. Replace the receiver and gradually add 30 ml of the petroleum ether-diethyl ether elution mixture of appropriate composition, as determined by a pilot test, described in **5.1.2.7** (b). Elute carefully and preserve for estimation.

5.1.3.4 Evaporate the ethereal extract of the unsaponifiable residue or the elute from the chromatographic procedure, using moderate heat and in a dark place away from actinic light in a stream of nitrogen. Take up the residue in sufficient isopropanol or hexane to give the concentration, expected to yield absorbance reading of 0·2 to 0·5 at 325 mμ. Determine absorbance (E) of this solution at 310, 325 and 334 mμ, in the spectrophotometer.

5.1.4 *Calculation*

a) Determine the corrected absorbance at 325 mμ
[E_{325} (corrected) = $6{\cdot}815E_{325} - 2{\cdot}555E_{310} - 4{\cdot}260E_{334}$].

h) Vitamin A content in I.U. per 100 g of sample

$$= \frac{E_{325}\ (\text{Corrected}) \times 1\,830 \times 100}{L \times C}$$

where

L = length of light path in absorption cell in cm, and

C = amount of assay sample, in g per 100 ml, of isopropyl alcohol solution.

NOTE — This formula is applicable only when isopropanol or hexane is used. In case cyclohexane is used, then the factor 1 737 should be used instead of 1 830.

5.2 Carr-Price Method

5.2.0 Principle the assay sample is saponified with ethanolic potassium hydroxide and vitamin A is extracted with petroleum ether. After reaction with antimony trichloride, the blue colour formed is measured in a colorimeter at 610 to 620 mμ. Calculation of vitamin A content is carried out with reference to a calibration curve.

5.2.1 *Apparatus*

5.2.1.1 *Photo-electric colorimeter* — with a direct reading deflection type galvanometer, suitable for measuring transmittance or absorbance at 610 to 620 mμ.

5.2.2 *Reagents*

5.2.2.1 *Vitamin A reference standard* — a solution of crystalline vitamin A of accurately known strength.

5.2.2.2 *Absolute ethanol* — Purified as given in **5.1.2.1** (a).

5.2.2.3 *Potassium hydroxide solution* — 50 percent (*w/v*).

5.2.2.4 *Ether* — peroxide-free, redistilled (*see* IS : 336-1964*).

Ether may be maintained free from peroxides by adding wet zinc foil approximately 80 cm² per litre, cut in strips long enough to reach at least half way up the container; the zinc strips previously should have been completely immersed in dilute acidified copper sulphate solution for one minute and subsequently washed with water.

5.2.2.5 *Sodium sulphate* — anhydrous, granular. It shall not absorb vitamin A under conditions of use, and 10 percent solution shall not be acid to methyl red indicator solution.

*Specification for ether (*revised*).

5.2.2.6 *Chloroform* — This may be purified as follows:

Wash the chloroform thrice with fresh 10 percent aqueous solution of sodium thiosulphate in a separatory funnel. Dry the chloroform with anhydrous calcium chloride and filter. Distill the chloroform over anhydrous sodium thiosulphate in an all-glass apparatus with a fractionating column of 70 cm length. Collect only middle fraction of the distillate, discarding the first 10 percent and last 10 percent of the distillate.

Anhydrous sodium thiosulphate may be prepared from crystalline thiosulphate by careful heating between 105 to 110°C, and stored in a desiccator.

5.2.2.7 *Antimony trichloride solution* — Prepare by dissolving 113·4 g antimony trichloride in 300 to 400 ml of chloroform. Add 5 g of anhydrous calcium chloride and filter while hot. Dilute the filtrate to 500 ml with chloroform.

5.2.3 *Procedure*

5.2.3.0 Adequate precautions should be taken to protect the vitamin A content in the flask from direct actinic light.

5.2.3.1 *Saponification* — Weigh accurately pure vitamin A reference standard capsules 0·5 to 1·0 g. Transfer the material quantitatively to an all glass saponification flask (150 ml), if necessary with a little alcohol. Add 30 ml of ethanol (95 percent *v/v*), and 5 ml of potassium hydroxide solution. Pass a stream of nitrogen gas through the solution with a jet tube for 5 minutes to replace the air. Then in a dark corner, saponify the contents under reflux in an atmosphere of nitrogen, over an electrically heated water-bath for 30 to 40 minutes. Cool and then add 30 ml of water. Transfer the contents quantitatively to a separating funnel (conical, Squibb shaped, 250 ml capacity). Wash the flask twice, with 10 ml of water and add the washings to the separating funnel. Extract the saponified solution thrice, respectively with 50 ml, 20 ml and 20 ml ether; combine the ether extract in another separating funnel and wash this with 50 ml of ice cold water containing a little sodium acid sulphate ($NaHSO_4$) or potassium acid phosphate (KH_2PO_4) just enough to neutralise the alkalinity of the extract. Repeat the washing with only ice cold water, till the washings are neutral to phenolphthalein.

5.2.3.2 Transfer the ethereal extract to a stoppered measuring cylinder (100 ml), rinse the separating funnel with a little ether and then add this to the cylinder. Add more ether to make the volume to 100 ml mark. Then add anhydrous sodium sulphate 15 grams; mildly shake a little and then allow it to settle in a dark cool place.

5.2.3.3 *Preparation of the calibration curve* — Evaporate a suitable aliquot of the ether solution of the unsaponifiable extract to about 5 ml. Evaporate off the remaining ether at low heat under reduced pressure. Take up the residue in sufficient chloroform so that after addition of antimony trichloride solution an absorbance of about 0·8 in the photo-electric colorimeter is obtained. From this stock solution of the standard, make a series of dilutions in chloroform to give absorbance values of 80, 60, 40 and 20 percent of the original absorbance. Determine absorbances of the blue colour formed when one millilitre aliquot of each of these five solutions plus one millilitre of chloroform with a drop of acetic anhydride is treated with the volume of the antimony trichloride solution, that is, suitable for the operation and hereinafter referred to as the 'fixed volume'. The blank is adjusted to 100 percent transmittance using a tube containing 2 ml of chloroform and the fixed volume of the antimony trichloride solution.

Using a rectangular coordinate paper, plot the five absorbances obtained against known quantities of vitamin A and draw up the best smooth curve from the origin through these points. Do not attempt to draw straight line unless the curve is in fact a straight line with the origin at zero. For those instruments that provide other than straight line curve, check this curve at frequent intervals. For those instruments that do provide straight line calibration curve, make reading of the reference solution with each set of sample readings to establish the curve. In the latter case re-establish the calibration curve whenever variation in the reagent or other variables in procedure occurs.

5.2.3.4 *Determination* — Weigh accurately a quantity of the material (not more than 5 g) containing 20 to 45 I.U. of vitamin A, then proceed as in **5.2.3.1** and **5.2.3.2** and obtain the residue after evaporating the ether under moderate heat and reduced pressure. Dissolve the residue in a definite volume of chloroform so that 2 ml of the chloroform solution with the fixed volume of the antimony trichloride solution would give an absorbance of about 0·5 to 0·2. Set the instrument at 100 percent transmittance with 2 ml of chloroform and the fixed volume of the antimony trichloride as blank. Place the tube containing 2 ml of the chloroform solution of the residue, add a drop of acetic anhydride and then add rapidly the 'fixed volume' of antimony trichloride solution with the help of vacqupet or a similar device. Record the maximum colorimetric reading. Determine vitamin A from the standard curve and calculate units of vitamin A per 100 g of the sample.

NOTE — Care should be taken that the readings are noted within 15 seconds after the addition of antimony trichloride solution.

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'Pushpak', Nurmohamed Shaikh Marg, Khanpur, AHMADABAD 380001	2 63 48 2 63 49
‡Peenya Industrial Area 1st Stage, Bangalore Tumkur Road BANGALORE 560058	38 49 55 38 49 56
Gangotri Complex, 5th Floor, Bhadbhada Road, T. T. Nagar, BHOPAL 462003	6 67 16
Plot No. 82/83, Lewis Road, BHUBANESHWAR 751002	5 36 27
53/5, Ward No. 29, R.G. Barua Road, 5th Byelane, GUWAHATI 781003	3 31 77
5-8-56C L. N. Gupta Marg (Nampally Station Road), HYDERABAD 500001	23 10 83
R14 Yudhister Marg, C Scheme, JAIPUR 302005	6 34 71 6 98 32
117/418 B Sarvodaya Nagar, KANPUR 208005	21 68 76 21 82 92
Patliputra Industrial Estate, PATNA 800013	6 23 05
T.C. No. 14/1421, University P.O., Palayam TRIVANDRUM 695035	6 21 04 6 21 17
Inspection Offices (With Sale Point):	
Pushpanjali, First Floor, 205-A West High Court Road, Shankar Nagar Square, NAGPUR 440010	2 51 71
Institution of Engineers (India) Building, 1332 Shivaji Nagar, PUNE 411005	5 24 35

*Sales Office in Calcutta is at 5 Chowringhee Approach, P. O. Princep Street, Calcutta 700072 — 27 68 00

†Sales Office in Bombay is at Novelty Chambers, Grant Road, Bombay 400007 — 89 65 28

‡Sales Office in Bangalore is at Unity Building, Narasimharaja Square, Bangalore 560002 — 22 36 71

Reprography Unit, BIS, New Delhi, India

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(TC 02/SC 06)

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Bhutan Agriculture and Food Regulatory Authority	Mr. Pasang Wangdi
Department of School Education	Ms. Kunzang Deki
Department of School Education	Mr. Sangay Tenzin
Food Corporation of Bhutan limited	Mr. Dinesh Subba
Food Corporation of Bhutan limited	Mr. Ugyen Tenzin
Ministry of Health	Mr.Laigden Dzed
Ministry of Health	Mr. Loday Zangpo
UNICEF	Dr. Chandralal Mongar
WHO	Mr. Kencho Wangdi
WFP	Mrs. Kencho Wangmo
Bhutan Standards Bureau	Mr. Sonam Phuntsho, Director General (Ex-officio member)

Member Secretary
Ms. Tashi Choden
Standardization Division
Bhutan Standards Bureau

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(TC 02)

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Bhutan Agriculture and Food Regulatory Authority	Mrs. Gyem Bidha
Bhutan Agro Industry Ltd	Mrs. Nim Dem Hingmang
Bhutan Agro Industry Ltd	Mrs. Jigme Wangmo
Bhutan Exporters Association	Mr. Dorji Tshering
Bhutan Livestock Development Corporation Limited	Mr. Sithar Dorji
Bhutan Livestock Development Corporation Limited	Mr. Pema Khandu
Department of Agriculture, MoAF	Mrs.Pema Choden
Department of Agriculture, MoAF	Mr. Jimba Rabgyel
Department of Agriculture and Marketing Cooperatives	Mr. Dawa Tshering
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Department of Industry, MoEA	Mr.Tashi Dorji
National Post Harvest Center, Paro	Mr.Dechen Tshering
Office of Consumer Protection, MoEA	Mr. Jigme Dorji
Office of Consumer Protection, MoEA	Mrs. Chencho Zangmo
Bhutan Standards Bureau	Mr. Sonam Phuntsho, Director General (Ex-officio member)

Member Secretary
Ms. Tashi Choden
Standardization Division
Bhutan Standards Bureau