

BUREAU OF INDIAN STANDARDS
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भारतीय मानक मसौदा

तम्बाकू एवं तम्बाकू उत्पाद — तम्बाकू की परीक्षण पद्धतियाँ
(आई एस 5643 का चौथा पुनरीक्षण)

Draft Indian Standard

TOBACCO AND TOBACCO PRODUCTS — METHODS OF TEST FOR TOBACCO
(Fourth Revision of IS 5643)

ICS 65.160

Tobacco and Tobacco Products Sectional
Committee, FAD 04

Last date for Comments: **31 March 2025**

FOREWORD

(Formal clauses would be added later)

The methods of test prescribed in this standard are based on the methods followed by the manufacturers, consumers and testing authorities concerned in the country. The application of this standard will ensure uniformity in test methodology for assessing the quality of raw tobacco as well as processed tobacco used in tobacco products.

This standard was originally published in 1970, and subsequently revised in 1989, 1999 and 2019. In the second revision, provisions of conditioning of atmosphere as given in ISO 3402 : 1991 'Tobacco and tobacco products — Atmosphere for conditioning and testing' were included and provisions of IS 7753 : 1989 'Method for expression of analytical test results of tobacco (first revision)' were merged. Consequently, with the publication of second revision of this standard, IS 7753 was withdrawn. In the third revision, various test methods were updated in light of new scientific knowledge and advances in analytical techniques.

In this fourth revision, the standard has been updated considering latest technological developments at national and international level. Following major changes have been made in this revision:

- a) Scope of the standard has been expanded to cover test methods for both unmanufactured and manufactured tobacco used in tobacco products
- b) New clauses on determination of organochlorine pesticide residues; ammonia; reducing carbohydrates; residual stem content in threshed tobacco; strip particle size of leaf tobacco; width of the strands of cut tobacco; nitrate content; reducing and total sugars; and tobacco-specific nitrosamines by relevant Indian standards have been added.
- c) New clause on replication of analysis has been added to ensure reliable test results.

- d) Provision of conditioning of samples has been removed considering that the tobacco samples do not require conditioning and the results are reported on dry mass basis.
- e) For determination of total alkaloids by continuous flow analysis, the earlier test method has been replaced with IS 16832 eliminating the use of potassium cyanide.
- f) For determination of acid-insoluble ash, the earlier test method has been replaced with IS 16236 for determination of silicated residues insoluble in hydrochloric acid.

In reporting the results 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: 2022 'Rules for rounding off numerical values (*second revision*)'.

Draft Indian Standard
TOBACCO AND TOBACCO PRODUCTS — METHODS OF TEST FOR TOBACCO
(*Fourth Revision*)

1 SCOPE

The standard prescribes methods of test for unmanufactured tobacco and manufactured tobacco used in tobacco products or extracted from tobacco products.

2 REFERENCES

The standards listed below contain provisions which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards listed below:

<i>IS No.</i>	<i>Title</i>
IS 5194 : 1969	Method of determination of nitrogen - Kjeldahl method
IS 9379 : 2023 / ISO 6488 : 2021	Tobacco and tobacco products — Determination of water content — Karl Fischer method (<i>fourth revision</i>)
IS 11820: 2004 / ISO 4389 : 2000	Tobacco and tobacco products — Determination of organochlorine pesticide residues — Gas chromatographic method (<i>first revision</i>)
IS 16832 : 2018	Tobacco and tobacco products — Determination of total alkaloids as nicotine by continuous-flow analysis — An alternate method eliminating use of potassium cyanide
IS 16132 : 2014 / ISO 2881 : 1992	Tobacco and tobacco products — Determination alkaloid content — Spectrometric method
IS 16236 : 2015 / 2015/ISO 2871: 1999	Tobacco and tobacco products — Determination of silicated residues insoluble in hydrochloric acid
IS 16262 : 2015 / ISO 12195 : 1995	Threshed tobacco — Determination of residual stem content
IS 16263 : 2017 / ISO 12194 : 1995	Leaf tobacco — Determination of strip particle size
IS 16308 : 2022 / ISO 16632 : 2021	Tobacco and tobacco products — Determination of water content — Gas-chromatographic method (<i>first revision</i>)
IS 16779 : 2021 / ISO 20193 : 2012	Tobacco and tobacco products — Determination of the width of the strands of cut tobacco (<i>first revision</i>)
IS 16780 : 2018 / ISO 15517 : 2003	Tobacco — Determination of nitrate content — Continuous - flow analysis method
IS 17010 : 2018	Tobacco and tobacco products — Determination of reducing and total sugars

IS 17199 : 2019 / Tobacco — Determination of the content of reducing carbohydrates -
ISO 15154 : 2003 Continuous - flow analysis method
IS 18499 : 2023 / Tobacco and tobacco products Determination of tobacco-specific
ISO 21766 : 2021 nitrosamines in tobacco products — Method using LC-MS/MS

3 QUALITY OF REAGENTS

Only analytical grade quality reagents and distilled water shall be used.

4 SAMPLE PREPARATION

4.1 Draw sufficient amount of the test sample for the tests to be carried out. Keep aside 50 g of the sample for the tests given under **6** and **7**. If required, reduce the sample particle size to about 1 mm by grinding.

NOTES

1 Grinding, cutting or undue handling of the sample can cause change in the moisture content of the sample. Moisture content should be carried out on sample as received as far as possible.

2 If the test sample has higher moisture than 15 percent, it cannot be ground and should be dried in air or at 40°C to reduce the moisture to about 10 percent or less.

3 Grinding can also cause changes in the sample due to heat generated. Hence, due care should be taken while grinding not to allow unnecessary high heat. In case of doubt, grinding should be done using cryogenic mill.

4.2 Storage

Sample as received and/or after particle size reduction and homogenization, should be packed in air tight containers and stored in conditions that will minimize deterioration and maintain sample integrity for any follow up analytical work. For long term storage, sample containers shall be stored below 4°C.

5 REPLICATION OF ANALYSIS

Analysis of samples will be replicated. At least two test portions from each sample shall be drawn from the prepared sample for testing. Mean of the values along with the range shall be reported.

6 DETERMINATION OF OVEN MOISTURE

6.1 Principle

Tobacco loses mass on heating due to the loss of moisture and some volatiles. If the heating is done under strictly controlled set of parameters, this loss of mass is termed oven moisture in common tobacco terminology. This is also referred to as oven volatiles and loss on heating in some tobacco circles

NOTE — For the determination of exact water content in tobacco ~~and tobacco products~~, see IS 9379 and IS 16308.

6.2 Apparatus

Usual laboratory apparatus and, in particular, the following:

6.2.1 Dish, made of aluminium, stainless steel, silica or porcelain and provided with a perforated cover. The diameter of the dish shall be at least 50 mm and the depth not more than 40 mm.

6.2.2 Oven, fitted with a ventilator and means for forced internal circulation of air and maintained at a temperature of $100 \pm 0.5^\circ\text{C}$.

6.2.3 Analytical Balance, with a resolution of 0.000 1 g.

6.3 Procedure

Place about 10 g of the material in a tared dish, close it with the perforated cover, weigh, and place it in the oven which shall previously have been brought to a temperature of $100 \pm 0.5^\circ\text{C}$. Maintain this temperature in the oven for 15 h. Remove the dish and allow it to cool in a desiccator. Weigh the dish with cover and the contents and note the weight of the material.

6.4 Calculation

$$\text{Oven moisture, percent by mass} = \frac{100 (W_1 - W_2)}{W_1}$$

Where,

W_1 = mass in g, of the material taken for the test; and

W_2 = mass in g, of the material after heating.

7 EXAMINATION FOR FREEDOM FROM WEEVIL ATTACK

7.1 Principle

Many methods are available for the detection of live weevil infestation, most satisfactory being the use of pheromone trap. However, for the detection of weevils in laboratory samples, the visual examination usually with magnification is still prevalent. The sample as received is visually examined for live or dead infestation.

7.2 Procedure

Take about 10 g of the material (*see 4*) on a large, clean sheet of paper. Loosen the lumps of the material and examine for the presence or absence of weevil (dead and alive) by naked eye (corrected for abnormal vision). A hand lens (magnification 10 X) may also be used. In case a larger magnification is used, this shall be stated in the Pass/Fail test report. The sample will fail the test if even one weevil, dead or alive, is found on examination.

8 DETERMINATION OF TOTAL ALKALOIDS AS NICOTINE

8.1 Principle

Determination of total alkaloids may be carried out either gravimetrically by silico-tungstic acid method (*see* 8.2), or by spectrophotometric method (*see* 8.3), or by continuous flow analysis method (*see* 8.4).

In silico-tungstic acid method, nicotine alkaloids form a complex with silico-tungstic acid in acidic medium to form insoluble nicotine silico-tungstate. The mass of the precipitate is determined by filtration using an ash less filter paper in combination with incineration or a sintered glass crucible in combination with oven-drying.

For spectrometric analysis, the aqueous extract of tobacco is treated with alkaloid specific reagent/reagents. Nicotine alkaloids form a colour complex with the reagent. The colour intensity is measured on a spectrophotometer and the total alkaloid content is calculated from a calibration graph prepared using pure nicotine salt as the calibrating standard.

The same principle is applicable to continuous-flow analysis method where sulphanilic acid and cyanogen chloride are used to develop colour with nicotine alkaloids. So far, cyanogen chloride was generated in situ by reacting chloramine T with highly toxic potassium cyanide. This method (*see* IS 16026) has been replaced by KCN-free method IS 16832 where cyanogen chloride is generated by reacting potassium thiocyanate with dichloroisocyanate.

8.2 Silica-Tungstic Acid Method

8.2.1 Apparatus — Normal laboratory apparatus in particular the following:

8.2.1.1 Kjeldahl flask, 500 ml capacity.

8.2.1.2 Distillation assembly — The assembly apparatus consists of a distillation flask 500 ml (Kjeldahl flask or a round-bottom flask) of suitable capacity, having a provision for fitting a splash head and an inlet tube for steam reaching the bottom of the flask. The free outlet of the splash head is connected to a well cooled condenser. The delivery tube of which dips below the surface of dilute hydrochloric acid contained in a receiving Erlenmeyer flask. It should also be possible to heat the distillation flask, using a burner, heating mantle or some such heating device, to maintain a constant liquid level during distillation.

8.2.1.3 Crucible, silica or platinum for incineration.

8.2.1.4 Laboratory oven, capable of maintaining $120 \pm 5^\circ\text{C}$.

8.2.1.5 Sintered glass crucible Gooch type, of porosity 2 (40 μm to 100 μm).

8.2.1.6 Filter flask (Buchner flask.)

8.2.1.7 Vacuum source.

8.2.1.8 Desiccator, containing an effective drying agent.

8.2.1.9 *Analytical balance*, with a resolution of 0.0001 g.

8.2.1.10 *Bunsen burner*

8.2.2 *Reagents*

8.2.2.1 *Liquid paraffin*

8.2.2.2 *Sodium hydroxide solution*, aqueous 30 percent (m/v).

8.2.2.3 *Sodium chloride*

8.2.2.4 *Phenolphthalein indicator solution*, 0.2 percent (m/v) — Dissolve 0.2 g of phenolphthalein in 60 ml of rectified spirit and dilute to 100 ml with water.

8.2.2.5 *Dilute hydrochloric acid*, 1 : 4 and 1 : 1 000 (v/v).

8.2.2.6 *Silico-tungstic acid solution* — Dissolve 120 g of silico-tungstic acid ($4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 12\text{WO}_3 \cdot 22\text{H}_2\text{O}$) in 100 ml water and dilute to one litre. The solution should be free from cloudiness and green colour.

NOTE — The silico-tungstic acid should be white or pale yellow crystals free from green colour. Of the silico-tungstic acids, $4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 10\text{WO}_3 \cdot 3 \text{H}_2\text{O}$, and $4\text{H}_2\text{O} \cdot \text{SiO}_2 \cdot 12\text{WO}_3 \cdot 20\text{H}_2\text{O}$ should not be used as these do not give crystalline precipitate with nicotine.

8.2.2.7 *Methyl orange indicator solution*, 0.04 percent (m/v) in aqueous ethyl alcohol (20 percent by volume).

8.2.3 *Procedure*

8.2.3.1 Weigh accurately a quantity of the material that shall contain preferably a minimum of 0.1 to 1.0 g of nicotine in a suitable tared vessel. Quantitatively, transfer the weighed material to the distillation flask (a 500 ml Kjeldahl flask or a round bottom flask) using water to wash the last traces of the material into the flask. If necessary, add to the contents of Kjeldahl flask or a round bottom distillation flask, a few drops of liquid paraffin to prevent frothing during distillation and a few small pieces of pumice stone to prevent bumping. Add 10 ml of sodium hydroxide solution (*see* **8.2.2.2**) and a few drops of phenolphthalein as indicator. Fit the mouth of the Kjeldahl flask with a two holed, rubber stopper through which pass the stem of a trap bulb and an inlet tube for steam. Connect the free end of the trap bulb to a condenser. Assemble the distillation unit with the receiving flask containing 10 ml of dilute hydrochloric acid (1 : 4), the delivery end of the condenser dipping well below the surface of the liquid in the receiving flask.

Connect the steam inlet tube to the source of steam and pass steam in the distillation flask to start distillation. Distil rapidly with the current of steam. When the distillation is well under way, heat the distillation flask using Bunsen burner to reduce the volume of contents of the flask as far as practicable without causing bumping or undue separation of insoluble matter externally to maintain constant liquid level in the flask during distillation. Continue the distillation until a small quantity of the distillate shows no cloudiness or opalescence when

treated with a drop of silico-tungstic acid and a drop of dilute hydrochloric acid (1 : 4). Confirm the alkalinity of the residue in the distillation flask with phenolphthalein indicator solution.

Reduce the volume of the distillate by concentrating it on a steam boiling water bath (*see Note*) and make up the volume of the concentrated distillate to a convenient volume in a graduated flask with water at room temperature. Mix the contents of the graduated flask thoroughly and filter through a dry filter paper if it is not clear. Collect the filtrate in a convenient flask. Test a portion of this filtrate with methyl orange indicator to confirm its acidity.

NOTE — By heating on a steam-boiling water bath, the nicotine content of the distillate is not affected.

8.2.3.2 Pipette an aliquot (*see Note 1*) of the filtrate, containing about 0.1 g of nicotine into a beaker and add at the rate of 3 ml of dilute hydrochloric acid (1 : 4) for each 100 ml of the aliquot and one ml of silico-tungstic acid solution for every 0.01 g of nicotine supposed to be present in the aliquot. Stir the contents of the beaker thoroughly, heat it on a water bath for about 30 min till the precipitate settles down and allow to stand overnight in a refrigerator. Before filtering, stir the contents of the beaker to see that the precipitate settles down quickly and is in a crystalline form.

NOTES

1 If the nicotine content of the material is very low, an aliquot containing at least 0.01 g of nicotine should be used.

2 The mass of the precipitate can be determined by collecting the precipitate on a filter paper followed by incineration (*see 8.2.4*) or by collecting on a sintered glass crucible and drying (*see 8.2.5*).

8.2.4 *Filter Paper Incineration Method*

8.2.4.1 Filter the contents of the beaker (*see 8.2.3.2*) through an ash less filter paper. Wash all the residue remaining in the beaker on to the filter using dilute hydrochloric acid (1 : 1000) at room temperature. Wash the filter paper with dilute hydrochloric acid (1 : 1000) until a few millilitres of the filtrate do not produce a precipitate or opalescence when tested with a few drops of the distillate containing nicotine.

8.2.4.2 Dry the crucible (*see 8.2.1.3*) in the laboratory oven until it shows constant weight (± 1 mg). Store the crucible in a desiccator (*see 8.2.1.8*).

8.2.4.3 Transfer the filter paper containing the precipitate to the tared crucible (*see 8.2.4.2*). Place the crucible on a silica triangle resting on a tripod, heat gently at first to dry and then ignite the filter paper with a Bunsen burner completely to ash until all carbon is oxidized. Finally, heat the platinum crucible over a Bunsen burner for not more than 10 min. Cool the crucible in a desiccator (*see 8.2.1.8*) and weigh. Repeat the heating process until a constant weight (± 1 mg) is obtained.

8.2.4.4 *Calculation*

Total alkaloid as nicotine (on dry basis),

$$\text{Percent by mass} = \frac{0.1141(W_1 \times V_1)}{(W_2 \times V_2)(100 - M)}$$

where,

W_1 = mass in g, of the residue in the crucible;

V_1 = total volume in ml, of the concentrated distillate after making up the volume (*see 8.2.3.1*);

W_2 = mass in g, of the material taken for steam distillation;

V_2 = volume in ml, of the aliquot of the filtrate taken for the precipitation (*see 8.2.3.2*);
and

M = moisture, percent by mass (*see 6.4*).

8.2.5 Sintered Glass Crucible Method

8.2.5.1 Dry the sintered glass filter crucible in a laboratory oven to constant mass (± 1 mg). Store in a desiccator. Weigh to the nearest 0.0001 g, the dried sintered glass crucible and filter the precipitate directly into the glass filter using a Buchner flask connected to a vacuum source. Ensure that all the precipitate is removed from the sides of the beaker and the glass stirring rod by rinsing three times with hydrochloric acid solution using 15 ml each time and transferring the rinse to the crucible. Discard the washings.

Rinse with a further aliquot of hydrochloric acid (up to 400 ml may be required) which should be collected and tested with a few drops of nicotine solution to ensure that no opalescence occurs that is, all silico-tungstic acid has been removed. Dry the sintered glass crucible with the precipitate in the laboratory oven, cool in the desiccator and weigh to the nearest 0.0001 g. Place the crucible back in the oven for 1 h, allow to cool and reweigh. Repeat until a constant weight ± 1 mg is obtained.

8.2.5.2 Calculation

$$\text{Total alkaloid as nicotine (on dry basis), percent by mass} = \frac{0.1012(W_1 \times V_1)}{(W_2 \times V_2)(100 - M)}$$

where,

W_1 = mass in g, of the residue in the crucible;

V_1 = total volume in ml, of the concentrated distillate after making up the volume (*see 8.2.3.1*);

W_2 = mass in g, of the material taken for steam distillation;

V_2 = volume in ml, of the aliquot of the filtrate taken for the precipitation (*see 8.2.3.2*);
and

M = moisture, percent by mass (*see* 6.4).

8.3 Spectrophotometric Method

Total nicotine alkaloids can be determined by a spectrometric method prescribed in IS 16132.

8.4 Continuous Flow Analysis Method

Total nicotine alkaloids can also be determined by continuous flow analysis as prescribed in IS 16832.

8.5 Repeatability

Duplicate determination should agree within 0.05 percent by mass of nicotine. If not, further duplicate determinations should be made until this requirement is fulfilled.

9 DETERMINATION OF TOTAL NITROGEN

9.1 Principle

Total nitrogen is the amount of both organic and inorganic nitrogen present in the tobacco sample. The sample is first pre-digested with reducing agents to convert inorganic nitrogen (nitrate and nitrite) into ammonium salt followed by digesting with oxidizing agents to convert all nitrogenous matter into ammonium sulphate. Ammonia in the Kjeldhal digest is quantitatively liberated by steam distillation, analyzed and expressed as total nitrogen. The ammonia concentration in the distillate can be determined using titrimetric, colorimetric or specific ion electrode determination methods. The method prescribed here employs the titrimetric method.

9.2 Apparatus — Usual laboratory apparatus and, in particular, the following:

9.2.1 Kjeldahl digestion flask, 500 ml or of suitable capacity.

9.2.2 Kjeldahl distillation apparatus with distillation flask of suitable capacity (*see* IS 5194)

NOTE — Kjeldahl apparatus of micro scale consisting of digestion and distillation in one unit are available commercially. These units work satisfactorily and can be used following the user instructions including the oxidizing mixture formulation provided by the supplier.

9.3 Procedure

9.3.1 Digestion

Transfer about 1 g of sample (*see* 4) accurately weighed to 0.0001 g, in a 500 ml Kjeldahl digestion flask containing 1 g of salicylic acid and add 30 ml of concentrated sulphuric acid rinsing the neck of the flask. Shake until thoroughly mixed and allow to stand, shaking frequently, for at least 30 min. Add 5 g of sodium thiosulphate and heat the solution for 5 min. Cool and add a mixture of 10 g potassium sulphate or anhydrous sodium sulphate and 1 g copper sulphate carefully to avoid sticking of the mixture in the neck of the flask. Heat very gently keeping the flask in an inclined position until foaming ceases. Increase the heat till acid

boils briskly, continue boiling till the digest becomes clear and green and digest for a few more minutes (total digestion time is approximately two hours).

NOTES

1 Readymade salt catalyst mixtures available commercially can also be used following the instructions provided by the supplier.

2 This digesting operation shall be carried out in a fume hood

9.3.2 Distillation

After cooling, dilute with 200 ml of water. Transfer the contents quantitatively to the distillation flask by rinsing the Kjeldhal flask repeatedly. Add a few pieces of granulated zinc or pumice stone to prevent bumping. Add a few drops of phenolphthalein indicator. Assemble the Kjeldahl distillation unit. Connect the receiving Erlenmeyer flask or beaker containing 50 ml of 4 percent boric acid solution and a few drops of 0.5 ml mixed indicator, ensuring that the delivery end of the condenser is below the level of the liquid in the receiving flask. Add gradually 100 ml of sodium hydroxide solution through the funnel to make the contents thoroughly alkaline as indicated by the phenolphthalein indicator. Heat the distillation flask to boiling and distil about 150 ml of the distillate in the receiving flask. Lower the receiving flask to raise the delivery tube above the liquid, distil further for a few seconds. Rinse the tube with water into the flask. Remove the flask.

9.3.3 Titration

The content in the receiving flask is titrated with 0.1 N sulphuric acid (*see 9.3.2*), the end point in case of the mixed indicator, being the colour turning from green to pink and in the case of Sher indicator from blue to pale pink/yellow.

9.3.4 Carry out a blank determination using all reagents in the same quantities but without the material to be tested.

9.4 Calculation

$$\text{Total nitrogen, percent by mass (on dry basis)} = \frac{(V_1 - V_0) \times N \times 140}{W(100 - M)}$$

where,

V_0 = volume in ml, of standard 0.1N sulphuric acid solution used to titrate the blank distillate;

V_1 = volume in ml, of standard 0.1N sulphuric acid solution used to titrate the sample distillate;

N = normality of standard sulphuric acid solution;

W = mass in g, of the material taken for the test; and

M = moisture, percent by mass (*see* 6.4).

10 DETERMINATION OF TOTAL ASH

10.1 Principle

The test portion is first charred and then incinerated in a muffle furnace under the conditions specified below and the ash weighed. If complete combustion is realised, ash contains only inorganic, non-combustible components.

10.2 Procedure

10.2.1 Accurately weigh about 10 g of the material (*see* 4) into a tared 9 cm diameter platinum, porcelain or silica dish. Carefully dry the material on a burner flame and char it completely until all organic matter is destroyed. Ignite the charred material by placing the dish in a muffle furnace maintained at a temperature of $550 \pm 25^\circ\text{C}$ for 2 h. Cool the dish and weigh. Note the mass of the ash contained in the dish.

10.2.2 Preserve the ash for the determination of acid insoluble ash (*see* 11).

10.2.3 Calculation

$$\text{Total ash content of the material (on dry basis), percent by mass} = \frac{10000 W_1}{W_2(100 - M)}$$

where,

W_1 = mass in g, of the ash;

W_2 = mass, in g, of the material taken for the test; and

M = moisture content, percent by mass (*see* 6.4).

11 DETERMINATION OF SILICATED RESIDUES INSOLUBLE IN HYDROCHLORIC ACID

Acid-insoluble ash will be determined by the method prescribed in IS 16236.

NOTE — If total ash of the sample is also being determined by the method prescribed in 10 of this standard, the same ash can be used for determining silicated residues of the sample.

12 DETERMINATION OF TOTAL CHLORIDES

12.1 Principle

Chloride reacts with silver nitrate to precipitate silver chloride stoichiometrically. The quantity of silver nitrate exhausted in the reaction is a direct measure of total chlorides present in the sample. The analysis can be done using potentiometric, iodometric titration or colorometry. The method prescribed here is based on potentiometric titration.

12.2 Apparatus

12.2.1 *pH* Meter, equipped with silver and glass electrodes.

12.2.2 *Burette*, 10 ml capacity, graduated in 0.05 or 0.02 ml, units.

12.3 Reagents

12.3.1 *Standard Silver Nitrate Solution, 0.1 N* — Standardize against a standard solution of potassium chloride as per the potentiometric titration procedure prescribed in 12.4.

12.3.2 *Dilute Nitric Acid, 1 : 9 (v/v)*.

12.4 Procedure

Weigh accurately about 2 g of tobacco sample (*see 4*) into a 250 ml beaker. Add 100 ml of water, a small amount in the first instance to wet the tobacco thoroughly and then the remainder. Allow it to stand for at least 5 min at room temperature, stirring intermittently. Add 5 ml of dilute nitric acid into the mixture and insert the clean electrodes. Start magnetic stirrer and continue stirring throughout titration at a rate sufficient to produce vigorous agitation without sputtering. Titrate with standard silver nitrate solution to the potential previously established as equivalent point. Determine equivalence point graphically by making several titrations on one or more tobacco samples. Recheck occasionally and determine when either electrode is replaced. Record the volume of the titrant.

12.5 Calculation

$$\text{Total chlorides (on dry basis), percent by mass} = \frac{V \times N \times 354.533}{W(100 - M)}$$

where,

V= volume ml, of silver nitrate solution required for the test;

N= normality of silver nitrate solution;

W= mass in g, of the sample taken for the test; and

M= percent moisture content (*see 6.4*).

13 DETERMINATION OF ORGANOCHLORINE PESTICIDE RESIDUES

Pesticide residues in tobacco will be determined by using gas chromatography as prescribed in IS 11820.

14 DETERMINATION OF AMMONIA

Ammonia in tobacco will be determined by using ion chromatographic analysis as prescribed in IS 18499.

15 DETERMINATION OF REDUCING CARBOHYDRATES

The reducing carbohydrates content in tobacco will be determined by using the continuous flow analysis method prescribed in IS 17199.

16 DETERMINATION OF RESIDUAL STEM CONTENT IN THRESHED TOBACCO

Stem content in threshed tobacco will be determined by the method prescribed in IS 16262.

17 DETERMINATION OF STRIP PARTICLE SIZE OF LEAF TOBACCO

Leaf particle size in threshed tobacco will be determined by the method prescribed in IS 16263.

18 DETERMINATION OF THE WIDTH OF THE STRANDS OF CUT TOBACCO

The width of cut tobacco will be determined by the method prescribed in IS 16779.

19 DETERMINATION OF NITRATE CONTENT

Nitrate content will be determined by continuous-flow analysis as prescribed in IS 16780.

20 DETERMINATION OF REDUCING AND TOTAL SUGARS

Reducing and total sugars will be determined by the method prescribed in IS 17010.

21 DETERMINATION OF TOBACCO-SPECIFIC NITROSAMINES

Tobacco specific nitrosamines (TSNAs) will be determined by the method prescribed in IS 18499.

22 REPORTING

22.1 Basis of Expression of Results

The test results shall be reported as a percentage of the moisture free mass of the sample unless mentioned otherwise in any method.