BUREAU OF INDIAN STANDARDS
DRAFT FOR COMMENTS ONLY

भारतीय मानक *मसौदा*

चीनी - परीक्षण की पद्धति

(*आई एस* 15279 *का पहला पुनरीक्षण*)

Draft ***Indian Standard***

SUGARS - METHODS OF TEST

(*First revision of IS 15279*)

ICS No 67.180.10

Sugar Industry Sectional Committee, FAD 02	Last date of comments: 31 August 2025

FOREWORD

(*Formal clauses will be added later*)

Sugar and sugar products form a crucial part of the food and beverage industry in India, with their quality and safety being of paramount importance. To ensure that sugar consistently meet the highest standards of purity, quality, and safety, it is essential to have standardized and reliable test methods. This standard provides a comprehensive framework for testing various sugars, including refined sugar, plantation white sugar, and other sugar derivatives.

The standard was originally published in 2003 with a title "*Sugar and Sugar Products – Methods of Test*". The standard has been revised to incorporate advancements in testing methodologies, improved precision, and alignment with international practices. Reference was drawn from the sugar analysis methods by ICUMSA (International Commission for Uniform Methods of Sugar Analysis) and Food Safety and Standards Authority of India for the revision of this standard.

In this revision, the title of the standard has been modified and following changes have been made:

(a) Test methods of the following parameters have been modified
   i. Loss of drying;
   ii. Polarization of plantation white sugar and refined sugar;

iii. Polarization of raw sugar;
iv. Reducing sugar
v. Colour of crystalline white or refined sugar;
vi. Conductivity ash;
vii. Starch in icing sugar;
viii. Sulphur dioxide;
ix. Insoluble matter.

(b) For determination of colour of plantation white sugar, new method has been introduced;

(c) The method for determination of lead by colorimetric method and chromium content has been deleted and instrumentation method has been introduced for the determination of heavy metals like lead, arsenic, cadmium, copper, iron, chromium, mercury.

In reporting the result of a test or analysis made in accordance with this standard, is to be rounded off, it shall be done in accordance with IS 2: 2022 'Rules for rounding off numerical values (second revision)'.

The draft of this standard was issued for wide circulation (WC) for 60 days on 19 February 2025 for comments. No comment was received on the first WC draft. Considering the technical complexity and length of the draft, the draft is being sent for second wide circulation for 30 days to allow thorough review and input from all relevant stakeholders.

TABLE OF CONTENTS

1 SCOPE

This standard prescribes the methods of test for different types of sugars.

2 REFERENCES

The following standards contain provisions, which through reference in this text constitute provisions 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 indicated below:

IS No.	*Title*
915:2012	Laboratory glassware - One - Mark volumetric flasks (*third revision)*
1070:2023	Reagent Grade Water Specification (*fourth revision*)

3 QUALITY OF REAGENTS

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

NOTE — Pure chemicals shall mean chemicals that do not contain impurities that affect the analysis result.

4 DETERMINATION OF LOSS ON DRYING

4.1 Scope of Application

The method is applicable to various types of sugar including refined sugar, plantation white sugar, raw sugar and any other forms of solid crystalline sugar.

4.2 Definitions

4.2.1 *Loss on Drying*

Also called ‘moisture’ or ‘water content’, water represents the primary heat-volatile liquid hence it is the main volatile component in sugarcane processing which is lost on drying sugar. Moisture in sugar exists in three forms which are free moisture (**4.2.2**), bound moisture (**4.2.3**) and inherent moisture (**4.2.4**).

4.2.2 *Free moisture* — Moisture found adhered on the surface of the crystal which is easily and quickly removed on drying.

4.2.3 *Bound moisture* — Moisture contained in the glassy layer on the surface and in the re-entrant angles which is released slowly only when glass crystallizes.

4.2.4 *Inherent moisture* — Moisture trapped in crystal which is released by grinding in general.

4.3 Principle

The moisture which is found adhered to the sugar crystal surface is removed during oven drying (105 ± 1^{o} C) under atmospheric pressure followed by standard cooling conditions. Thus, the free moisture is estimated by this method.

4.4 Apparatus

4.4.1 *Atmospheric Pressure Oven* — Capable of maintaining temperature of 105 ± 1^{o} C.

4.4.2 *Desiccator* — Containing self- indicating silica gel.

4.4.3 *Dishes with Fitting Lids* — Glass, platinum, nickel or aluminum dishes with 6 cm to 11 cm diameter and 2 cm to 4 cm depth.

4.4.4 *Clean Dry Cloth/Tongs*

4.4.5 *Surface Pattern Dial Thermometer* — An electronic thermometer fitted with a surface probe may be used.

4.4.6 *Analytical Balance* — with a least count nearest to 0.1 mg.

4.5 Procedure

4.5.1 Carry out the estimation in duplicate. Firstly, preheat the oven (**4.4.1**) to 105 ± 1^{o}C. Then place the open empty dishes and lids in the oven for 30 minutes. Remove the dishes and lids from the oven using clean dry cloth/tongs (**4.4.4**) and place in desiccator (**4.4.2**). Place the surface pattern dial thermometer (**4.4.5**) on top of the one of the dishes. When temperature falls to ambient ± 2 ^{o}C, rapidly remove the dishes with lids and weigh to an accuracy of ± 0.1mg (M_1).

4.5.2 Now, quickly transfer about 20 to 30 g of sugar sample to each dish, place lid and weigh with an accuracy of ± 0.1mg (M_2). Ensure that there is no other material in the oven. Return the open dishes with lids to the oven. Heat for exactly 3 hrs at 105 ± 1 ^{o}C. Remove the dishes with lids from the oven using a clean dry cloth/tongs and place them in the desiccator. A contact thermometer is placed on top of one of the dishes. When the temperature of the dishes falls to ambient ±2 ^{o}C, remove the dishes with lids from the desiccator and weigh them as rapidly as possible to an accuracy of 1 mg (M_3).

4.6 Calculation and Expression of Results

4.6.1 Loss in mass is expressed as a percentage of the original mass of the sample:

$$\% \text{ loss on drying} = \frac{(M_2 - M_3)}{(M_2 - M_1)} \times 100$$

Where,

M_1 = mass of dish with lid in g;
M_2 = mass of dish with lid + sugar sample before drying, in g;
M_3 = mass of dish with lid + sugar sample after drying, in g.

4.6.2 The mean of duplicate results should be within ± 10% of the two individual results. If any of the duplicate crosses this limit, the test shall be repeated.

5 DETERMINATION OF POLARIZATION OF REFINED SUGAR AND PLANTATION WHITE SUGAR

5.1 Scope of Application

The method is applicable to refined sugar and plantation white sugar having a loss on drying of not more than 0.1 percent and not needing clarification. The solutions of sugar samples are prepared by weight/ weight method. This method is also applicable for sucrose content if the purity of sugar is more than 99.5%.

5.2 Definition

5.2.1 *Normal sugar solution*

It is defined as 26.00 g of pure sucrose weighed in air under normal condition (101.3 kPa, 20°C & 50% relative humidity) and dissolved in water to a final volume of 100 cm^3. The practical alternative procedure for making normal sugar solution is gravimetry method by preparing the solution weight/weight to achieve the same results with greater precision.

5.3 Principle

5.3.1 The pol (polarization) of a solution is known as the concentration (in grams of solute consistent with 100 g of solution) of a solution of pure sucrose in water having the identical optical rotation as the sample at a specified temperature.

5.3.2 Sucrose percent is determined by measuring pol of solutions containing only pure sucrose in water; for solutions containing sucrose and different optically active substances, pol represents the sum of the rotations of the materials present and is therefore known

as “apparent sucrose”. In case of sugar, the contribution of sucrose to this sum far exceeds than that of other parts. Pol is expressed in °Z at 20 °C, according to the International Sugar Scale.

5.4 Apparatus

5.4.1 *Polarimeter (visual/photoelectric)* — Equipped with international sugar scale with a visible light source at 589 nm (circular polarimeter/saccharimeters) or 587 nm (quartz-wedge saccharimeters) having reading accuracy ± 0.01 °Z.

5.4.2 *Polarising tube with or without thermostatic jacketing* - Made up of borosilicate/normal glass having following specification:

a**)** nominal length - 200 mm with maximum tolerance acceptance value 0.2%.

b) end faces - with maximum roughness 4.0 μm; flatness-0.8 μm; parallelism- 8.0 μm; perpendicularity to tube axis- 8 min of the arc.

c) cover glass having thickness in between 1 nm to 2 nm and flatness maximum 0.2 mm with a good optical finish, and parallel within 5 min of arc. They shall be sufficiently free from birefringence that, when the polarimeter tube is rotated through 180° about its own axis, the variation in polarimetric reading shall not vary ±0.01 °Z.

5.4.3 *Quartz control plate* — Certified value as close as possible to 100 °Z at wavelength 589 nm & 20.0 °C.

5.4.4 *Analytical balance* — Readable to 0.0001 g.

5.4.5 *Thermometer* — Readable to 0.1^0C over the range 0-50 ^{o}C.

5.4.3 *Water Bath* — Maintained at 20.0 ± 0.5 °C.

5.5 Procedure

5.5.1 *Preparation of the sample solution*

The preparation of the normal solution of sugar shall be carried out at 20 ^{o}C as far as possible. Weigh 23.7018 g of the sugar sample accurately as rapidly as possible in a weighing dish. Record the actual weight of the sugar sample taken in gram. Add about 50 g water to dissolve the sugar by swirling or by use of a mechanical shaker. Hold the flask to the light and check that all sugar is dissolved. Finally add rest amount of water and make the solution to final mass value 100 g.

5.5.2 *Checking the calibration of the polarimeter*

Zero the polarimeter on air with the all the sample compartment empty. Record the reading of certified quartz plate. When using quartz-wedge saccharimeter, measurement of temperature is

not required. The difference between the observed quartz plate reading and the certified quartz plate value shall be subtracted from any subsequent sample reading. When using a circular polarimeter, if the quartz plate is not equipped with a temperature sensor and the temperature of the quartz plate is other than 20 ± 0.5°C, a temperature correction must be applied by using formula:

$$Q_R = Q_T - 0.000144 \times (T\text{-}20) \times Q_{20}$$

Where,

T = temperature of the quartz plate in °C
Q_T = quartz plate reading at temperature T
$Q_{\underline{20}}$ = certified quartz plate value at 20.0°C
Q_R = quartz plate reading corrected to 20.0°C

Calibrate the polarimeter to show the correct adjusted value for the quartz plate.

5.5.3 *Reading of the sample*

The determination of polarization for sugar sample shall be carried out at 20 °C as far as possible. Fill the sample into the polarizing tube carefully without leaving any air bubble. Record the temperature of the polarizing sample. Place the polarizing tube on the sample compartment of the polarimeter and note the reading for polarization value. Reading should be taken within 1 min of placing the tube in the sample compartment while using the tube without thermostatic temperature control.

5.6 Calculation and Reporting of Results

5.6.1 Note all polarimeter readings in °Z to two decimal places, all temperatures in ^oC to one decimal place. Calculate the polarization, corrected to 20 ^oC by the formula given below:

(i) For circular polarimeters/saccharimeter-

$$P_{20} = P_T / (1 - 0.00046\ (T\text{-}20))$$

(ii) For quartz-wedge saccharimeters-

$$P_{20} = P_T / (1 - 0.0006\ (T - 20))$$

Where,

P_{20} = Polarization reading at 20^oC.
T = Temperature of solution in ^oC.
P_T = Polarization reading at temperature T.

5.6.2 The final polarization value is calculated (in ^oZ at 20 ^oC) by making weight correction adjustment (if any actual weight of sugar sample taken other than 23.7018g) by the use of following equation:

Polarization = $23.7018/(Actual\ weight\ sugar\ sample\ taken\ in\ g) \times P_{20}$

Where,

P_{20} = polarization reading at 20^0C.

5.6.3 Report the result to the nearest 0.01°Z.

6 METHOD FOR DETERMINATION OF POLARIZATION OF RAW SUGAR

6.1 Scope

This method is applicable to all raw sugars and specialty sugars requiring clarification. The solutions of sugar samples are prepared by weight/ weight method.

6.2 Definitions

6.2.1 *Normal sugar solution*

Same as under **5.2.1**.

6.2.2 *Basis of calibration of 100°Z Point of International Sugar Scale*

Basis of calibration of 100°Z point of the International Sugar Scale is polarization of normal solution of pure sucrose (26.00g/100 ml) at 20 °C in 20 mm tubes. This solution, polarized at 20 °C, must give a saccharimeter reading of exactly 100°S.

6.3 Principle

The determination of pol (polarization) is a physical analysis involving three steps. Firstly, normal solution of the requisite sugars is prepared by weight/ weight method. Then, the solution is clarified by adding a solution of basic lead acetate, (which coagulates colloidal impurities and removes some colorant matter), followed by filtration. The polarization is then determined by measurement of the optical rotation of the clarified solution. The optical rotation is the algebraic summation of the predominant effects of the sucrose content of the sample, modified by the presence of other optically active constituents and by the clarification procedure.

6.4 Apparatus

6.4.1 *Polarimeter (visual/photoelectric)* —Equipped with international sugar scale with a visible light source at 589 nm (circular polarimeter/saccharimeters) or 587 nm (quartz-wedge saccharimeters) having reading accuracy ± 0.01 °Z.

6.4.2 *Polarising tube with or without thermostatic jacketing* — Made up of borosilicate/normal glass having following specification:

a) Nominal length-200 mm with maximum tolerance acceptance value 0.2%.
b) End faces- with maximum roughness 4.0 μm; flatness 0.8 μm; parallelism 8.0 μm; perpendicularity to tube axis- 8 min of the arc.
c) Cover glass having thickness- in between 1 nm to 2 nm; flatness- maximum 0.2 mm with a good optical finish, and parallel within 5 min of arc. They shall be sufficiently free from birefringence that, when the polarimeter tube is rotated through 180° about its own axis, the variation in polarimetric reading shall not vary ±0.01 °Z.

6.4.3 *Quartz control plate* — Certified value as close as possible to 100 °Z at wavelength 589 nm & 20.0 °C. The specification for the quartz control plate is given under Annex A.

6.4.4 *Analytical balance* — Readable to 0.0001 g.

6.4.5 *Thermometer* — Readable to 0.1^0C over the range 0 to 50 ^{o}C.

6.4.6 *Water Bath* — Maintained at 20 ± 0.5°C.

6.4.7 *Automatic dispenser* — Auto dispenser 0-2 ml or safety burette, 50 ml capacity, fitted with CO2 absorption tubes for Lead Acetate, to avoid contact with CO_2 in the air, fit a cartridge containing a mixture of calcium and sodium hydroxides, or equivalent, to the air inlet of the dispenser.

6.4.8 *Whatman No. 91 filter paper* — Whatman No. 91 filter paper (15 cm) or equivalent with moisture content in range 6-8% water, determined by drying it for 3 hours at 100°C. If necessary, store the papers in containers, in which the relative humidity is such that the equilibrium moisture content of the papers is in the range 6 to 8%.

6.4.9 *Filtration apparatus* — Consisting of 100 mm stemless funnel; three 150ml beakers; 100 mm watch/cover glass.

6.5 Reagents

6.5.1 *Acetic acid* — Concentrated or glacial.

NOTE — Acetic acid (CH3COOH) is a corrosive acid and contact with the skin, eyes and through inhalation must be avoided. Work in a fume cupboard while wearing gloves and safety glasses.

6.5.2 *Nitrogen Gas*

6.5.3 *Lead sub-acetate solution [$Pb(OAc)_2 \cdot 3H_2O$]*
The lead sub-acetate should conform to the following specifications:

Basic lead (as PbO)	> 33%
Moisture at 105°C	< 1.5%
Insoluble in dilute acetic acid	< 0.02%
Insoluble in water	< 1.0%
Chloride (Cl)	< 0.003%
Nitrate and nitrite (NO3)	< 0.003%
Copper (Cu)	< 0.002%
Substances not precipitated by H_2S (as sulphates)	< 0.30%
Iron	< 0.002%

NOTE — Lead sub-acetate trihydrate likewise called basic lead acetic acid is harmful chemical. Thus, it should be kept away from direct contact through the skin, inward breath (powder dust) or gulping. Wear a dust mask, safety glasses and gloves during use.

6.5.3.1 *Preparation of lead acetate solution*

Weigh 280 g of lead sub-acetate trihydrate powder into a 1 L conical flask and add 500 ml of freshly boiled reagent grade water. Boil for 30 minutes in a fume cupboard, cover the beaker and let it stand overnight. Decant the supernatant liquor and filter through a Whatman No. 540 or equivalent filter paper using a Buchner funnel and flask. Use vacuum to aid filtration if necessary. Dilute the filtrate with recently boiled reagent grade water to a density of 1.24 ± 0.01 g/cm3 or a total lead content of 24.4 ± 1.0 g PbO/100 cm^3. The basic lead content should be between 9.5 and 10.5 g PbO/100 cm^3. Assuming the lead content is lower than the necessary reach, heat up the solution for vanish some of the water. Assuming the lead content is higher than the necessary reach, lessen the fixation by adding glacial acetic acid. After making the adjustment, recheck both the total lead and basic lead contents. Store the solution in a vessel equipped with an airlock mechanism to prevent exposure to carbon dioxide from the air. Before sealing, flush the vessel with nitrogen gas.

6.6 Procedure

6.6.1 *Preparation of the sample solution*

The preparation of the normal solution of sugar shall be carried out at 20 °C, as far as possible. Weigh 23.7018 g of the sugar accurately as rapidly as possible in a weighing dish. Record the actual weight of the sugar sample taken in gram. Add about 50 g water to dissolve the sugar by swirling or by use of a mechanical shaker. Hold the flask to the light and check that all sugar is dissolved. Add basic lead acetate solution according to the expected polarisation of the raw sugar using automatic dispenser. Add 1 ml basic lead acetate solution for an expected pol of 99.00°Z and below and use 0.5 ml for an expected pol of more than 99.00°Z. Finally add rest amount of water and make the solution to final mass value 100 g.

6.6.2 *Filtration*

Fold a Whatman 91 filter paper (or equivalent) and place in a clean, dry, stemless glass or plastic funnel. Do not use a fluted paper or filter aid. Place the funnel containing the filter paper on a waste filter glass. Fill the filter paper by decanting the solution, ensuring that the paper is fully wetted but not overfilled. Immediately cover the filter funnel with a cover slip. Allow approximately 10 ml of solution to run into the waste filter glass and then transfer the filter funnel to a clean, dry filter glass. The filtrate should be clear and bright. If the filtrate in the filter glass is not clear and bright, allow a further 10 ml to filter, then transfer the funnel to another clean dry filter glass. If the filtrate is still not clear, discard and repeat the preparation. (To minimize evaporation, the solution in the filter paper must not be replenished, and must be kept out of draughts and direct sunlight). Collect approximately 50 ml of the filtrate (sufficient to rinse and fill a polarimeter tube), remove the filter funnel and place the cover slip on the filter glass. (To minimise evaporation the solution must be read as soon as possible after sufficient filtrate has been collected).

6.6.3 *Checking the calibration of the polarimeter*

6.6.3.1 Zero the polarimeter on air with the all-sample compartment empty. Record the reading of certified quartz plate. When using quartz-wedge saccharimeter, measurement of temperature is not required. The difference between the observed quartz plate reading and the certified quartz plate value shall be subtracted from any subsequent sample reading. When using a circular polarimeter, if the quartz plate is not equipped with a temperature sensor and the temperature of the quartz plate is other than 20 ± 0.5°C, a temperature correction must be applied by using formula:

$$Q_R = Q_T - 0.000144 \times (T-20) \times Q_{20}$$

Where,

T = temperature of the quartz plate in °C;
Q_T = quartz plate reading at temperature T;
Q_{20} = certified quartz plate value at 20.0°C;
Q_R = quartz plate reading corrected to 20.0°C.

6.6.3.2 Calibrate the polarimeter to show the correct adjusted value for the quartz plate.

NOTE — An alternate method for standardization of polarimeter and application of temperature corrections is given under Annex B.

6.7 Reading of the Sample

The determination of polarization for sugar sample shall be carried out at 20°C as far as possible. Fill the sample into the polarizing tube carefully without leaving any air bubble. Record the temperature of the polarizing sample. Place the polarizing tube on the sample compartment of the polarimeter and note the reading for polarization value. Reading should be taken within 1

min of placing the tube in the sample compartment while using the tube without thermostatic temperature control.

6.8 Calculation and Expression of Results

6.8.1 Note all polarimeter readings in °Z to two decimal places, all temperatures in ^{o}C to one decimal place.

Calculate the polarization, corrected to 20 ^{0}C by the formula given below:

(i) For circular polarimeters/saccharimeter-

$$P_{20} = P_T / (1 - 0.00046 (T\text{-}20))$$

(ii) For quartz-wedge saccharimeters-

$$P_{20} = P_T / (1 - 0.0006 (T - 20))$$

Where

P_{20} = polarization reading at 20^{o}C;
T = temperature of solution in ^{o}C;
P_T = polarization reading at temperature T.

6.8.2 The final polarization value is calculated (in ^{o}Z at 20^{o} C) by making weight correction adjustment (if any actual weight of sugar sample taken other than 23.7018 g) by the use of following equation:

$$\text{Polarization} = 23.7018/(Actual\ weight\ sugar\ sample\ taken\ in\ g) \times P_{20}$$

Where,
P_{20}= polarization reading at 20^{o}C.

6.8.3 Report the result to the nearest 0.01°Z.

7 DETERMINATION OF REDUCING SUGAR BY KNIGHT AND ALLEN METHOD

7.1 Scope of Application

The method is applicable to refined sugar and plantation white sugar in which reducing sugar content does not exceed 0.05%.

7.2 Definition

7.2.1. *Reducing sugar*

Reducing sugar consists primarily but not exclusively of glucose and fructose and is obtained through the hydrolysis of sucrose.

7.3 Principle

A mixture of alkaline copper reagent and sugar solution is heated in boiling water bath where cupric ions are reduced to insoluble cupric oxide by reducing sugars present in sugar solution, there after cooling the solution, the remaining cupric ion is titrated with EDTA using murexide indicator.

7.4 Apparatus:

7.4.1 *Balance* — Least count 0.0001g

7.4.2 *Test tube* — 30ml; graduated.

7.4.3 *Porciline dish* — 100ml capacity.

7.4.4 *Water bath* — Maintained at 100^0C.

7.4.5 *Burette* — 50ml capacity.

7.4.6 *Pipette* — 5ml capacity.

7.4.7 *Mortar and pestle*

7.4.8 *Small spatula*

7.5 Reagents

7.5.1 *Alkaline copper reagent* — Dissolve 12.5 g of sodium carbonate and 12.5 g of rochelle salt (potassium sodium tartrate) in about 500ml of water. Add 20 ml of 1 mol/L sodium hydroxide in a 500 ml volumetric flask. Dissolve 3 g of cupric sulphate pentahydrate in about 100 ml of water separately and add it all to alkaline tartrate solution and make up volume 500 ml.

7.5.2 *0.005 mol/L EDTA solution* — Dissolve 1.860 g EDTA (ethylenediamine tera acetic acid disodium salt) in 1 L volumetric flask.

7.5.3 *Murexide indicator* — Mix 0.5 g ammonium perpurate (Murexide), 0.15 g methylene blue and 40g sodium chloride and grind with a mortar and pestle. Store in a desiccator.

7.5.4 *Invert sugar solution (10 g/L)* — Weigh 4.75 g of sucrose and dissolve in 100ml of water in 500ml volumetric flask, add 2.5 ml concentrated HCl and stand for 3 days for inversion of

sugar. After 3 days add 1 g benzoic acid with 40 ml hot water to completely dissolve benzoic acid. Make up the solution up to the mark 500ml.

7.5.5 *Reagent grade water*, Grade 3 or better as per IS 1070.

7.6 Procedure

7.6.1 Dissolve 5 g of a sample of refined or plantation white sugar in 5 ml of water in a graduated test tube.

7.6.2 Add 5 ml of alkaline copper regent solution and mix the contents of the graduated tube. Immerse the tube in boiling water bath for 5 minutes and cool in the running water.

7.6.3 Transfer the contents of the graduated test tube into a white porcelain dish. Add approximately 0.1 g of the indicator with a small spatula. Titrate this solution rapidly with the EDTA solution with continuous stirring using a glass rod. The colour will change from green to gray to purple. Titrate until the purple colour is sustained. Read the titre, T ml.

7.6.4 *Standard curve*

7.6.4.1 Prepare a 0.5g/L invert sugar solution by diluting 10g/l invert sugar solution (**7.5.4**). Pipette aliquots of this diluted invert sugar solution (0,1,2,3,4,5 ml) to a clean and dry graduated test tube and making up to 5 ml corresponding to standards of 0.00, 0.01, 0.02, 0.03, 0.04 ,0.05% invert. To each, add 5 g of low invert sucrose and carry out the procedure mentioned in **7.6.2** and **7.6.3**. Plot the result on a graph.

7.6.4.2 Use this standard curve to derive the reducing sugar % of the test sample corresponding to titer T ml (**7.6.3**). The graphical method exhibits the linearity up to 0.05 % of reducing sugar present in sucrose sample.

7.7 Calculation and Expression of Results

Obtain the reducing sugar concentration in sugar sample directly from calibration graph. Express the result to three decimal place in % on sample.

8 DETERMINATION OF SUGAR SOLUTION COLOUR BY MOPS BUFFER AT *p*H 7

8.1 Scope of Application

This method can be used for determination of colour in solution phase for refined sugar and plantation white sugar and raw sugar.

8.2 Principle

The spectrophotometric technique is based on the Beer-Lambert Law which states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution and thickness of the solution under analysis.
The colour of plantation white sugar is measured at *p*H 7 using MOPS buffer solution. Sugar is dissolved in reagent grade water in which MOPS buffer is added. The solution is filtered through 0.45 µm membrane. The absorbance is measured on a spectrophotometer at 420 nm. The colour value in IU is calculated using given formula.

8.3 Definition

8.3.1 *Absorbance (extinction)* (A)

A measure of the quantity of light absorbed by a sample at given wavelength.

8.3.2 *Absorbancy index (extinction index) (a_s)*

If b is the length of the optical cell in cm and c is the concentration of sugar solution in g/ml, then

a_s (absorbancy index of the solution**)** = A / b x c

8.3.3 *ICUMSA color*

ICUMSA Colour = Absorbancy index x 1000

The unit of ICUMSA color is IU (ICUMSA Units).

8.4 Apparatus

8.4.1 *Spectrophotometer* — Capable of measuring the absorbance at wavelength of 420 nm with the narrowest practical bandwidth.

8.4.2 *Matched optical cells* — Use optical cells of 1cm, 5 cm and 10 cm depending upon colour of sugar. Generally, for raw sugar 1cm optical cell is used whereas for plantation white and refined sugar 5 cm and 10 cm are used respectively.

8.4.3 *Membrane filters* — Membrane filter of pore size 0.45 µm and diameter 50mm of cellulose based material.

8.4.4 *Vacuum filtration system* — Comprising of an oil free vacuum pump, funnel, 47mm sintered base, clamp and 1L conical flask.

8.4.5 *Laboratory balance* — With a least count of 0.01 g.

8.4.6 *pH meter* — With least count of 0.01 and inbuilt magnetic stirrer. It should be calibrated by suitable CRM used for *p*H metry.

8.4.7 *Volumetric flasks* — 1000 ml and 100 ml capacity.

8.4.8 *Beaker* — 1L capacity.

8.4.9 *Pipettes* — 10 ml capacity.

8.5 Reagents

All reagents should be of AR grade.

8.5.1 *Reagent grade water***,** Grade 3 or better as per IS 1070.

8.5.2 *1 M Sodium hydroxide*

8.5.3 *MOPS Buffer solution*

Dissolve 41.8 g MOPS [3-(N-morpholino) propane sulphonic acid] in reagent grade water in a beaker. Make volume approximately 800 ml . Keep the beaker on a magnetic stirrer (**8.4.6**). Add NaOH solution (**8.5.2**) while continuous stirring. Adjust the *p*H of solution to 7±0.01 (about 80 ml NaOH will be required). Keep the *p*H meter electrodeimmersed in the solution till a constant *p*H value is shown on the *p*H meter. Remove the electrode. Make the volume of solution to 1L with reagent grade water.

8.6 Procedure

8.6.1 *Sample Preparation*

Mix the dry sample of sugar thoroughly. Weigh the quantity of sugar as per Table 1 into a 100 ml volumetric flask and add about 50.0 ml of reagent grade water. Dissolve the sample. Add 10 ± 0.1 ml of MOPS buffer solution (*see* **8.5.3**), mix well and dilute up to the mark with reagent grade water. Filter the solution using 0.45 µm membrane and vacuum filtration system (**8.4.4**).

Table 1 Sugar aliquot to be taken based on the color range

(Cl 8.6.1 and 8.6.3)

Colour range (IU) (1)	Sugar aliquot (g) (2)	Cell length (b) (cm) (3)	Concentration (c) (g/ml) (4)
Upto 50	50± 0.1	10	0.5

Upto 800 200-1600	20± 0.04 10± 0.02	5 5	0.2 0.1
500-4000 1000-8000	20± 0.04 10± 0.02	1 1	0.2 0.1

8.6.2 *Reference/blank solution*

Take 10 ± 0.1 ml of MOPS buffer (*see* **8.5.2**) in a 100 ml volumetric flask and dilute up to the mark with reagent grade water. Filter the sample using a membrane filter and holder (**8.4.3** and **8.4.4**) in a clean and dry conical flask.

8.6.3 *Absorbance measurement*

Measure the absorbance of the filtered test solution using the reference (**8.6.2**) at 420 nm with optical cells as per Table 1.

8.7 Calculations and Expression of Results

8.7.1 Calculate colour value in ICUMSA units as follows:

$$\text{Colour} = \frac{A}{b \times c} \times 1000$$

Where,

A = Absorbance;

b = Cell length in cm;

c = concentration of sugar solution in g/ml.

8.7.2 Express the results in integral number.

9 DETERMINATION OF SUGAR SOLUTION COLOUR USING DISTILLED WATER

9.1 Scope of application

This method can be used for estimation of color in solution phase for crystalline white or refined sugar provided that the color values are less than 60 IU.

9.2 Principle

The spectrophotometric technique is based on the Beer-Lambert Law which states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution and thickness of the solution under analysis. The color of refined and white sugar is measured using distilled water as a solvent. The solution is filtered through 0.45 µm membrane. The

absorbance is measured on a spectrophotometer at 420nm. The color value in IU is calculated using given formula.

9.3 Definition

The definitions as described in **8.3** shall apply.

9.4 Reagents

The Grade 3 or better reagent grade water as per IS 1070 shall be used for testing.

9.5 Apparatus

9.5.1 *Spectrophotometer* — Capable of measuring the absorbance at wavelength of 420 nm withthe narrowest practical bandwidth.

9.5.2 Matched *Optical Cells* — Use optical cells of minimum 5 cm in length. For sugar having very low color value, 10cm optical cells are preferred for better accuracy.

9.5.3 *Membrane Filters* —Membrane filter of pore size 0.45 and diameter 50mm, made up of cellulose based material.

9.5.4 Vacuum filtration system — Comprising of an oil free vacuum pump, funnel, 47mm sintered base, clamp and 1L conical flask.

9.5.5 *Refractometer* — Refractometer capable of measuring 50 % Refractometric Dry Substance (RDS) shall be used.

9.5.6 *Laboratory Balance* — With a least count of 0.1 g.

9.6 Procedure

9.6.1 *Sample Preparation*

Mix the dry sample of sugar thoroughly. Weigh 50.0 ± 0.1 g of the sample into a 250 ml conical flask and add 50.0 ± 0.1 g of reagent grade water. Dissolve the sample by swirling method. The homogeneous sugar solution is filtered under vacuum through 0.45 µm membrane filter into a clean dry flask. Filter the reagent grade water using separate 0.45 µm membrane and use as a blank. Measure the Refractometric Dry Substance (RDS) of the solution to an accuracy of ± 0.1 g/100 g by the method given in Annex C.

9.6.2 *Colour Measurement*

Measure the absorbance (A) of filtered solution on a spectrophotometer at 420 nm using optical

cells with 5cm length. Use 10 cm cell length if the color of sugar is below 20 IU. Use filtered reagent grade water as a blank.

9.7 Calculations and Expression of Results

9.7.1 Solids concentration 'c' (g/cm^3) resent in sample is calculated from the RDS by using Table 2.

ICUMSA Color = (1000 x *A*) / *b* x *c* IU

Where,

A = absorbancy;
b = cell length in cm;
c = concentration in g/cm^3.

Express results to the nearest whole number.

Table 2 Concentration of Sample Solids from the RDS Percentage
(Clause 9.7.1)

RDS Percent (1)	c (g/cm^3) (2)	RDS Percent (3)	c (g/cm^3) (4)
48.0	0.584 989	49.5	0.607 320
48.1	0.586 469	49.6	0.608 819
48.2	0.587 950	49.7	0.610 319
48.3	0.569 432	49.8	0.611 821
48.4	0.590 916	49.9	0.613 324
48.5	0.592 401	50.0	0.614 828
48.6	0.593 887	50.1	0.616 333
48.7	0.595 374	50.3	0.619 348
48.9	0.598 353	50.4	0.920 857
49.0	0.599 844	50.5	0.622 368
49.1	0.601 337	50.6	0.623 880
49.2	0.602 831	50.7	0.625 393
49.3	0.604 326	50.8	0.626 907
49.4	0.605 822	50.9	0.628 423

10 DETERMINATION OF CONDUCTIVITY ASH

10.1 Scope of application

This is the method applied for measurement of concentration of ionized soluble salt present in sugar solutions in terms of conductivity ash. This method is applicable to plantation white sugar and refined sugar.

10.2 Principle

The specific conductivity of sugar solution is determined by preparing 28% weight/weight solution of sugar in deionized water. The conductivity ash is then calculated using given formula. Conductivity ash is not directly correlated to gravimetric ash determined by incineration.

10.3 Definitions

10.3.1 *Conductivity*

Conductivity is the ability of water or a solution prepared by dissolving salts or compounds in water to conduct electric current.

10.3.2 C*onductivity meter*

An instrument that measures the electrical conductivity in a solution.

10.3.3 *Cell constant*

The ratio of separation between the two electrodes divided by the area of cross section of the electrode is called the cell constant.

10.4 Apparatus

10.4.1 *Conductivity meter*

10.4.2 *Volumetric flasks* — Capacities 100ml, 500ml, 1000 ml.

10.4.3 *Beakers* — Capacity 100 ml.

10.4.4 *Pipettes* — A class having capacity 10 ml.

10.4.5 *Analytical balance* — Readable to 0.01 mg.

10.5 Reagents

10.5.1 *Reagent grade water* — Grade 1 or Grade 2 as per IS 1070. Use this water for the preparation of samples and KCl solutions.

10.5.2 *0.01 M Potassium Chloride* — Dry potassium chloride (AR grade) at 500°C for 1 hour. Weigh 745.56 mg in a 1L volumetric flask , dissolve in water and makeup to the mark.

10.5.3 *0.0002 M Potassium chloride* — Dilute 10ml of 0.01 mol/L solution to 500 ml. This solution has conductivity of 26.6± 0.03µS/cm at 20°C after deduction of the specific conductivity of the solvent water.

10.6 Procedure

10.6.1 *Determination of cell constant*

10.6.1.1 Use 0.0002 mol/L potassium chloride solution for determining the cell constant.

10.6.1.2 Measure the conductivity of reagent grade water at 20°C (**10.5.1**)

10.6.1.3 Calculate rough conductivity of water as follows:

Rough conductivity of water (A) = Cell constant given by manufacturer x conductivity of reagent grade water at 20°C.

10.6.1.4 Measure the conductivity of 0.0002 mol/L potassium chloride solution at 20° C (B). The rough cell constant is:

$$K = \frac{\text{Conductivity of standard KCl solution + A}}{\text{B}}$$

NOTE — If measurement is not made at standard temperature of 20°C ,then conductivity of KCl is determined by formula:

Conductivity of KCl at T°C =26.6 [1+0.021(T-20)] in range 20±5°C

10.6.1.5 The corrected water conductivity is

C_{water} = K x reading of water conductivity

10.6.1.6 The corrected cell constant,

K' = Conductivity of standard KCl solution + C_{water} / Reading of KCl solution at 20^0C

Conductivity of 0.0002 mol/L is 26.6µS/cm.

Either use this value in conductivity meter or multiply the conductivity readings by this value.

10.6.2 Measurement of Sample

Dissolve 28 ± 0.1g of sugar in water to give a solution of 100.0.g at 20° C. Transfer the solution into a100 ml beaker. Measure the conductivity at 20° C.

10.6.3 Calculations and Expression of Results

10.6.3.1 If C_1 is the measured conductivity in µS/cm at 20°C and if C_2 is the specific conductivity of the water at 20^0 C, the corrected conductivity (C_{28}) of 28g/100g solution is

$$C_{28} = C_1 - 0.35\ C_2$$

10.6.3.2 Conductivity ash % = $6 \times 10^{-4} \times C_{28}$

NOTE — Temperature Correction

In the absence of standard 20° C make a temperature correction as follows

$$C_{28} = \frac{C_T}{1 + 0.026(T-20)}$$ *where T should not exceed ± 5°C*

Where, CT is the conductivity at temperature T °C

11 DETERMINATION OF SULPHATED ASH

11.1 Field of Application

This standard specifies a method for the determination of sulphated ash in sugar.

11.2 Definition

11.2.1 *Sulphated Ash*

The residue obtained after incineration of the product according to the method specified in this standard is expressed as a percentage by mass, either of the product as received or on the dry basis.

11.3 Principle

Incineration of a test portion is carried out in the presence of sulphuric acid at a temperature of 525 ± 25°C. The sulphuric acid facilitates the destruction of the organic matter and avoids losses by converting the volatile chlorides into non-volatile sulphates.

11.4 Apparatus

11.4.1 *Incineration Dish* — Made up of platinum or any other material which does not deteriorate under the test conditions (for example, a silica incineration dish), of capacity 100 to 200 ml and with a minimum useful surface of 15 cm^2.

11.4.2 *Electric Furnace with Air Circulation* — Capable of being controlled at 60 to 70°C.

11.4.3 *Electric Hot Plate or Gas Burner or Heating Lamp*

11.4.4 *Desiccator*— Provided with an efficient desiccant.

11.4.5 *Water Bath* — Capable of being controlled at 60 to 70°C.

11.4.6 *Analytical Balance* — Capacity 0.0001g.

11.5 Reagents

11.5.1 *Sulphuric Acid Solution*, Add carefully 100 ml of concentrated sulphuric acid having density (ρ_{20}) 1.83 g/ml to 500 ml of water and mix.

11.5.2 *Hydrochloric acid solution*

11.6 Procedure

11.6.1 *Preparation of the incineration dish*

Clean the incineration dish (**11.4.1**), whether it is new or used, with boiling hydrochloric acid solution (**11.5.2**), then rinse generously with water. Calcinate the incineration dish for 30 min in the furnace (**11.4.2**) controlled at 525 ± 25°C. Allow to cool to ambient temperature in the desiccator (**11.4.4**) and weigh to the nearest 0.0002 g (the incineration dish should be calcinated to constant mass).

11.6.2 *Preparation of the test sample*

11.6.2.1 Mix the sample carefully and quickly by stirring (for a powder) or by mixing with a spatula (for a liquid) in a sample container. If the volume of the container is insufficient for this, quickly transfer the whole sample to another, previously dried container of a suitable size. Take care to avoid any change in the moisture content of the sample.

11.6.2.2 The drawl of representative sample of approximately 5 g can be difficult (for example, glucose in lumps). In this case, use one of the procedures described below:

a) Weigh carefully to the nearest 0.01 g approximately 100 g of the sample into a dry container, provided with a lid and previously tared with the lid. Add approximately 100 ml of water at 90°C, place the lid on the container and stir until the sample is completely dissolved. Allow to cool to ambient temperature and weigh to the nearest 0.01 g.

b) Melt the sample in solid from by immersing it, in a container, provided with lid, in the water bath (**11.4.5**), controlled at 60 to 70°C, and placing the lid on the container. Remove the container from the water bath and allow it to cool to ambient temperature, agitating frequently but without removing the lid, and then mix the condensed water with the sample.

11.6.3 *Test Portion*

If a dilution has been carried out, take an aliquot portion of the solution obtained (**11.6.2**), so as to obtain a mass of sample corresponding to amass of test portion as given below.

In all other cases, weigh, to the nearest 0.001g, in the incineration dish (**11.4.1**), previously weighed to the nearest 0.0002 g, a mass of test sample (**11.6.2**) in accordance with the following:

Sulphated Ash Percent (m/m)	*Mass of Test Portion (g)*
<5	10
>5 <10	5
>10	2

11.6.4 *Preincineration*

Add 5 ml of the sulphuric acid solution (**11.5.1**) to the test portion or the aliquot portion (**11.6.3**). Mix with a glass stirring rod and rinse with a little water, collecting the rinsings in the incineration dish. Heat the incineration dish slowly and carefully, over the electric hot-plate or gas burner or using the heating lamp (**11.4.3**), until completely carbonized (it is recommended that this be carried out under an extraction hood).

11.6.5 *Incineration*

11.6.5.1 Place the incineration dish in the oven (**11.4.2**), controlled at 525 ± 25 °C, and maintain this temperature until the carbon residue has disappeared. A period of 2 h is usually sufficient.

11.6.5.2 Allow to cool. Take up the residue with several drops of the sulphuric acid solution (**11.4.1**), evaporate on the edge of the oven (**11.4.2**) and incinerate again for 0.5 h. Place the incineration dish in the desiccator (**11.4.4**) and allow it to cool to ambient temperature. Weigh the dish and contents to the nearest 0.0002 g. The incineration should be continued until constant mass is attained. Do not put more than four incineration dishes in the desiccator at any one time.

11.7 Calculation

11.7.1 The sulphated ash, expressed as a percentage by mass of product as received, is given by the following formula:

$$\frac{(m_2 \times m_1) \times 100}{m_0}$$

11.7.2 The sulphated ash, expressed as a percentage in mass on the dry basis, is given by the following formula:

$$\frac{(m_2 \times m_1) \times 100}{m_0} \times \frac{100}{100 - H}$$

Where,

m_0 = mass, in g, of the test portion, taking into account any dilution (**11.6.3**);

m_1 = mass, in g, of the incineration dish before incineration (**11.6.1**);

m_2 = mass, in g, of the incineration residue and incineration dish after incineration (*see* **11.6.5.2**); and

H = moisture content of the product.

12 DETERMINATION OF STARCH IN ICING SUGAR

12.1 Principle

In this method the starch is precipitated using alcohol and isolated from sugar. Then it is converted to glucose which is then estimated by Lane and Eynon method.

12.2 Reagents

12.2.1 *Molisch Reagent* – Prepared by dissolving 3.75 g of alpha naphthol in 25ml of 99% ethanol.

NOTE — This reagent should be prepared fresh.

12.2.2 *Fehling's solution*

12.2.2.1 *Solution A* — Dissolve 69.28 g of copper sulphate ($CuSO_4.5H_2O$) in 1000 ml reagent grade water. Filter and store in amber colored bottle.

12.2.2.2 *Solution B* — Dissolve 346 g of Rochelle salt (potassium sodium tartrate, $KNaC_4,H_4,O_6$, $4H_2O$) and 100 g of sodium hydroxide, in reagent grade water and dilute to 1000 ml. Filter and store in an amber colour bottle.

12.2.3 *Methylene Blue Indicator* — Dissolve 1.0 g methylene blue in reagent grade water and dilute to 100 ml.

12.2.4 *Concentrated Sulphuric Acid*

12.2.5 *Concentrated Hydrochloric Acid* — sp gr 1.16.

12.2.6 *AR Grade Sucrose*

12.2.7 *Ethanol,* 50 % strength.

12.2.8 *Reagent Grade Water,* Grade 3 or better as per IS 1070.

12.3 Procedure

12.3.1 *Sample Preparation*

12.3.1.1 Weigh 20g of sample and dissolve in 100 ml of hot water. After the sample solution is cool down, add equal volume of ethanol, stir and let it stand for 2 hours. Filter the solution through Whatman No. 1 filter paper. Wash the precipitate with 50% ethanol until the washings tests negative for Molisch test (**12.3.1.2**).

12.3.1.2 *Molisch Test*

Take 2ml of sample (washings) in dry test tube. Take 2ml of reagent grade water in another tube as control. Add 2-3 drops of molisch reagent to (see **12.1.1**) the solution. Gently pipette 1ml Conc. H_2SO_4 along the side of the tube so that two distinct layers are formed. Observe colour change at the junction of two layers. Appearance of purple colour indicates positive test (presence of sugar in the washings).

12.3.1.3 When the Molisch test is negative, transfer the precipitate with 200ml hot water into a flask. Add 20ml hydrochloric acid and connect the reflux condenser and heat in boiling water bath for 2.5 hour. Cool, neutralize with NaOH and dilute to 500ml. Determine % of glucose by Lane and Eynon method.

12.3.2 *Lane and Eynon Method*

12.3.2.1 *Primary titration*
In a flask, pipette 5 ml of each Fehling solution A and solution B and mix. To this, add 10ml water and a few boiling chips or glass beads. Heat the flask to boiling. Fill the burette with sample

solution and beads and dispense solution. Add 3 drops of methylene blue indicator. Continue the addition of sample solution dropwise until the blue colour disappears to a brick red end point. (The concentration of the sample solution should be such that the titre value is between 15 and 50 ml). Note down the titre value.

12.3.2.2 *Final Titration*

Pipette 5 ml each of Fehling A and B (**12.2.2.1** and **12.2.2.2**). Add sample solution about 2 ml less than titre value of the preliminary titration. Heat the flask to boiling within 3 minutes and complete the titration. Perform the titration in duplicate and take the average titre value.

12.3.3 *Determination of Fehling factor*

12.3.3.1 Accurately weigh about 4.75 g of AR grade sucrose and transfer to 500 ml volumetric flask with 50 ml reagent grade water. To this, add 5 ml hydrochloric acid (**12.2.5**) and allow to stand for 24 hours . Neutralize with NaOH solution and make up to volume. Mix well and transfer 50 ml to a 100ml volumetric flask and make up to volume. Transfer this solution to a burette and perform the titration as described in **12.3.2**.

12.3.3.2 The Fehling factor is calculated as follows

$$\text{Fehling Factor (f)} = \frac{\textit{Titre (T) x weight of sucrose (g)}}{\textit{500}}$$

12.4 Calculation

12.4.1 The glucose percentage is calculated as follows:

$$\text{Glucose \%} = \frac{\text{Dilution x Factor of Fehling (f) x 100}}{\text{Weight of sample (g) x Titre value}}$$

12.4.2 Calculate starch % as shown below:

Starch % = Glucose % x 0.90

13 DETERMINATION OF SUCROSE IN ICING SUGAR

13.1 Scope of Application

The method measures the sucrose content of icing sugar.

13.2 Reagents

The following reagents along with those given under **12.2.2** to **12.2.8** shall be used.

13.2.1 *6.34 M Hydrochloric Acid (HCl)* — 630 ml HCL dilute to 1000 ml.

13.2.2 *2M Sodium Hydroxide (NaOH)* — 80 g NaOH dilute to 1000 ml.

13.3 Procedure

13.3.1 *Sample Preparation*

Weigh accurately 5.00 g of the sample in a beaker. Add about 50ml of reagent grade water and warm the mixture in a water bath at 50°C to 60°C for about 5 min to dissolve the sample. Cool and filter through a Whatmann filter paper No. 40. Collect the filtrate carefully in a 100 ml volumetric flask. Wash the beaker and the insoluble residue of starch on filter paper using water. Make up the volume of filtrate to 200 ml.

13.3.2 *Inversion of sucrose solution*

Take 25ml of prepared sample solution (**13.3.1**) in a conical flask and add 5ml of 6.34 M HCl. Heat the flask at 60-70°C for 15 min in a water bath. Swirl gently for 3 min to raise temperature and allow to stand in water bath for 12 min. Cool immediately and transfer quantitatively the inverted solution in 250 ml volumetric flask. Neutralize with 2M NaOH and make up the volume. Transfer this solution to 50ml burette having an offset tip. Perform the steps described in **13.3.3**.

13.3.3 *Primary titration*

In a flask, pipette 5 ml of each Fehling solution A (**12.2.2.1)** and solution B (**12.2.2.2)** and mix. To this, add 10ml reagent grade water and a few boiling chips or glass beads. Heat the flask to boiling. Fill the burette with sample solution and beads and dispense solution. Add 3 drops of methylene blue indicator (**12.2.3**). Continue the addition of sample solution dropwise until the blue colour disappears to a brick red end point. (The concentration of the sample solution should be such that the titre value is between 15 and 50 ml). Note down the titre value.

13.3.4 *Final titration*

13.3.4.1 Pipette 5 ml each of Fehling A and B (**12.2.2.1** and **12.2.2.2**). Add sample solution about 2 ml less than titre value of the preliminary titration. Heat the flask to boiling within 3 minutes and complete the titration. Perform the titration in duplicate and take the average titre value (t).

13.3.4.2 Determine the Fehling Factor as described in **12.3.3**.

13.4 Calculations

13.4.1 The percentage of total reducing sugars is calculated as

$$\% \text{ Total reducing sugars} = \frac{2000 \times \text{Fehling Factor} \times 100}{\text{Mass of sample} \times t}$$

13.4.2 The percentage sucrose is calculated as

$$\% \text{ Sucrose} = (\% \text{ Total Reducing sugars}) \times 0.95$$

14 DETERMINATION OF SULPHUR DIOXIDE BY THE ROSANILINE SPECTROPHOTOMETRIC METHOD

14.1 Scope of Application

This given colorimetric method is applicable to plantation white sugar and refined sugar for the determination of SO_2 using rosaniline hydrochloride reagent.

14.2 Principle

The pink to purple color of a sulphite/ rosaniline complex is measured using spectrophotometer, at a wavelength of 560 nm, after reaction with formaldehyde.

14.3 Reagents

14.3.1 *Rosaniline Hydrochloric Solution (saturated)* — Weigh accurately 1g of rosaniline hydrochloride in a beaker and add 100 ml of reagent grade water. Heat the solution to 50º C and cool with shaking. Let the solution get stabilized for 48 h. Shake well and filter.

14.3.2 *Decolourized Rosaniline Solution* — Take 4 ml of filtered saturated rosaniline hydrochloride solution to a 100 ml volumetric flask and add about 6ml of concentrated hydrochloric acid. Make the volume. Allow the solution to stand for minimum 1 h before use.

14.3.3 *Formaldehyde Solution (approximately 0.2 g/ 100 ml)* — Dilute 2.5 ml of analytical reagent grade formaldehyde solution.

14.3.4 *Pure Sucrose Solution* — Dissolve 100 g of analytical reagent grade sulphite-free sucrose in water and dilute to 1L.

14.3.5 *Sodium Hydroxide Solution*, 0.1 mol/L.

14.3.6 *0.05 mol/l Iodine Solution* — Take 5 g of analytical grade iodate-free potassium iodide in 10 ml of reagent grade water in a 250 ml volumetric flask and add 3.17 g of analytical grade iodine to it, shake the flask until all the iodine is dissolved and make up the volume with reagent grade water.

14.3.7 *Concentrated Hydrochloric Acid*

14.3.8 1M *Hydrochloric Acid Solution*

14.3.9 *Iodine (starch) Indicator* — Readymade or a starch solution.

14.3.10 *0.1 M Sodium thiosulphate solution* — Take 6.20 g of analytical grade sodium thiosulphate pentahydrate and dissolve in 50 ml of reagent grade water in a 250 ml volumetric flask and then dilute to the mark.

14.3.11 *Standard Sulphite Solution* — Dissolve about 2.5 g of sodium sulphite heptahydrate in sucrose solution (**14.3.4**) and make up the volume to 500 ml with same solution (**14.3.4**). To standardize this solution, take 25 ml of the 0.05 mol/L iodine solution (**14.3.6**) in a 250 ml conical flask and add 10 ml of 1 mol/L hydrochloric acid solution (**14.3.8**) and approximately 100 ml of reagent grade water. Pipette 25 ml of standard sulphite solution into this flask and excess iodine is titrated with the 0.1 mol/L sodium thiosulphate solution (**14.3.10**) until the pale straw colour appears. Add 0.2 to 0.5 g of the iodine (starch) indicator (**14.3.9**) to the flask and continue the titration until the blue colour disappears. This is the titre value as "t".

14.3.12 *Dilute Standard Sulphite Solution* — Dilute 5 ml of standard sulphite solution(**14.3.11**) to exactly 100 ml with pure sucrose solution (**14.3.4**). The exact value of the sulphite content, c, is calculated as follows from the titre, t, found in **14.3.11**:

$$c = (25 - t) \times 3.203 \times 2 \text{ mg } SO_2 \text{ /ml}$$

14.4 Apparatus

14.4.1 *Spectrophotometer* — With minimum spectral bandwidth and 1cm matched optical cells.

14.4.2 *Volumetric Flasks* — Capacities 100 ml, 250 ml and 500 ml and conforming to Class A of IS 915.

14.4.3 *Graduated Pipette* — 10 ml and Class A.

14.4.4 *Pipettes* — 2, 10 and 25 ml capacities.

14.4.5 *Burette* — 10 ml and *graduated* by 0.05 ml.

14.4.6 *Test Tubes*

14.4.7 *Analytical Balance* — Capable of weighing to the nearest 0.1 mg

14.5 Procedure

14.5.1 *Colour Development*

For determination of color due to SO_2 present in sugar, dissolve a sample of sugar in reagent grade water in a 100 ml volumetric flask, add 0.1 mol/L sodium hydroxide (**14.3.5**) solution (4 ml) , make up the solution up to mark and mix. Use the sample quantities as below

Range of SO_2 mg/kg	*Sample weight (g)*
Upto 5	*40*
5 to 15	*20*
15 to 30	*10*

14.5.2 *Test solution*

Take 10 ml of sugar aliquot prepared as 14.5.1 in a test tube. Add 2ml of decolorized rosaniline solution (**14.3.2**)followed by 2ml of formaldehyde solution **(14.3.3**). Measure the absorbance of test solutionat 560 nm on a spectrophotometer after exactly 30 min using reagent grade water as a blank. Measure the absorbance of test solution at 560 nm after exactly 30 min using the respective blank.

14.5.3 Preparation of *Standard Curve*

14.5.3.1 Pipette out aliquots of dilute sulphite solution (**14.3.12**) 0.0, 0.4, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml each in series of 100 ml volumetric flasks. To each flask add 4 ml of 0.1mol/L sodium hydroxide and make the volume to 100 ml with10% pure sucrose solution and mix.

14.5.3.2 From each flask transfer 10 ml of aliquot into clean and dry test tubes. Add 2ml of decolorized rosanilie solution and 2 ml of formaldehyde solution. Allow to standfor exactly 30min.

14.5.3.3 Measure the absorbance using 1cm cell on spectrophotometer at 560 nm using reagent

grade water as blank.

14.5.3.4 The amount of SO_2 in each test tube is –

$$SO_2 \text{ content } \mu g \text{ , } = (c \times n) / 10$$

Where,

"n" is the number of ml of dilute sulphite added to each 100 ml flask and c is concentration from **14.3.12**.

14.6 Expression of Results

14.6.1 *Calculation*

Calculate the concentration of sulphite by reference to the standard curve and express the result as mg SO_2/kg sugar as follows:

$(SO)_2$ mg/kg = (µg SO_2 from graph) x10)/ Mass of sugar used in **14.5.1.**

15 DETERMINATION OF INSOLUBLE MATTER IN REFINED AND PLANTATION WHITE SUGAR

15.1 Scope of Application

The method is applicable to refined sugar and plantation white sugar.

15.2 Definition

Insoluble matter is a non-sucrose water insoluble impurities present in sugar having particle size more than 8µm.

15.3 Principle

A specific mass of sugar is dissolved in water. The solution is passed through 8µm membrane. The water insoluble impurities are separated on membrane and measured gravimetrically.

15.4 Apparatus

15.4.1 *Weighing Balance* — Least count 0.0001g.

15.4.2 *Laboratory balance* — Capacity 5 kg.

15.4.3 *Filtration apparatus* — Comprising a holder for the membrane filter into a conical flask (1L) connected to a vacuum pump.

15.4.4 *Beakers* — 250 ml.

15.4.5 *Burette* — 50 ml capacity.

15.4.6 *Glass petri dishes*

15.4.7 *Drying oven* — maintained at 60-65° C.

15.4.8 *Membrane filter* — 47mm, pore size 8µm.

15.4.9 *Tweezer*

15.4.10 *Stainless steel jug (1.5 L) and stainless steel spoon*

15.5 Procedure

15.5.1 *Preparation of membrane*

Wash the membrane filter by immersing in boiling water for 5-6 min, drain the excess water and transfer to a petri plate using tweezer. Dry in oven at 60-65 °C for an hour and cool in desiccator for 30 min.

15.5.2 *Preparation of water*

Use membrane filtered water (8 µm) for dissolving sugar. Rinse filtration flask using filtered water before starting filtration. For all analytical steps only this water is used.

15.5.3 *Procedure for refined sugar*

15.5.3.1 Weigh 500 ± 1g (M) sample in a beaker and transfer carefully in stainless steel jug. Rinse the beaker with about 100mL of hot water and transfer into stainless steel jug. Further add more hot water (temperature 45-50 ° C) so that the total volume of water including rinsing water will be about 900 ml. Heat the solution up to 45 ° C while stirring with stainless steel spoon.

15.5.3.2 Weigh the prepared membrane (M_1) and dip in hot water using tweezer. Place the membrane in filter holder. Pass the hot sugar solution through the membrane using vacuum pump. After passing all solution rinse the jug and spoon with hot water. Wash the insoluble matter retained on the membrane thoroughly with hot water using a wash bottle. With wash bottle transfer the insoluble particles sticking inside and bottom portion of funnel on the membrane. Use about 500-600 ml hot water for washing purpose to remove traces of sugar from the membrane.

15.5.3.3 Remove the membrane and place in petri dish. Dry in an oven at 60-65 ° C for one hour. Cover the dish and transfer in desiccator. Cool for 30 min. Weigh the membrane (M_2)

15.5.3.4 *Calculation*

Insoluble matter (mg/kg) = $\left(\frac{M2-M1}{M}\right) \times 10^6$

15.5.4 *Procedure for plantation white sugar and other difficult to filter samples*

15.5.4.1 Weigh 500 ± 1 g (M) of the sugar sample in a clean stainless-steel jug (stock). Take 500 mL of reagent grade water of temperature 45 °C in another container.

15.5.4.2 In a second clean stainless-steel container take a small portion (about 30 g) of sugar from the stock. Add a suitable volume of reagent grade water at about 45-50 ^{o} C and stir the mixture with stainless-steel rod to dissolve sugar completely.

15.5.4.3 Pass the sugar solution through the pre weighed (M_1) membrane under vacuum. Observe the rate of filtration. Continue to dissolve small portions of sugar from stock in hot reagent grade water and filtering the solution through the membrane. Each portion or lot should be prepared only after the previous one has completely passed through. If the sample is easy to dissolve, filtration will be fast, and the solution will not stay in the funnel holding the membrane. If the sample is difficult to dissolve, then the solution will tend to stagnate in the funnel and filtration will be slow. The higher the degree of stagnation (and hence slower the filtration) the more difficult to dissolve the sample.

15.5.4.4 When the filtration becomes slow, let the solution in the funnel pass through the membrane completely and stop dissolving further lots of sugar. Follow the procedure of washing and drying of membrane as given in **15.5.3.2** and **15.5.3.3**.

15.5.4.5 Weigh the remnant undissolved sugar in original stainless-steel jug (stock) as M_3 (g). Calculate the mass of sugar passed through the membrane (g) as $M - M_3$.

15.6 Calculation

Insoluble matter (mg/kg) = $\left(\frac{M2-M1}{M-M3}\right) \times 10^6$

16 DETERMINATION OF LEAD, ARSENIC, CADMIUM, COPPER, IRON CHROMIUM AND MERCURY IN SUGAR

16.1 Scope of Application

The method is applicable to all types of sugar for determination of heavy metals like lead,arsenic, cadmium, copper, iron, chromium, mercury and any other as per requirement.

16.2 Principle

16.2.1 The samples are digested with a suitable technique like wet ashing or microwave digestion system. Once suitable sample solution is prepared, the heavy metals are determined using a

suitable analytical instrumentation like Flame Atomic Absorption Spectroscopy (FAAS), ICP-AES and ICP-MS.

16.2.2 AAS is the most extensively used technique for determination of metals in different sample matrices.The flame-AAS (FAAS) is used when concentration of the analyte is high whereas the graphite furnace AAS is used (GF-AAS) when it is at low level. Highly specific hollow cathode lamps are used for determination of each metal.

16.2.3 For analysis of mercury AAS with VGA (vapor generation accessory) is used. ICP-AES/OES and ICP MS techniques are capable of handling the analytes upto ppb or ppt level.

16.3 Reagents

All the reagents used in analysis should be of purest quality available. The water, acids, oxidizing agents, must be used with extremely low level contamination of respected analyses. The reagents should be checked previously for presence of concentrations of elements under test so that their presence will not affect the ultimate results. For very low level concentration of elements proper quality control attempts must be followed.

16.4 Apparatus

A class glassware should be used for all volumetric work. Glassware washing procedures should be followed meticulously involving stepwise washing with clean water, soaking in 2% nitric acid and then with reagent grade water.

16.5 Instrument

Any instrument mentioned in **16.2** is appropriate.

16.6 Standard Solutions and calibration curves

16.6.1 All standards of elements should be NIST traceable or standardized by an authorized national/international body. Pure metal salts or metals may be used after ensuring their purities by proper means.

16.6.2 The concentrations of calibration solutions should be prepared considering the capacity of instrument with respect to lower and higher detection limits and required testing limits.

16.6.3 The standard solutions should be prepared as stock, intermediate and working. The working standards should be prepared on dailybasis. Any standard procedure may be followed for preparation of calibration solutions.

16.7 Procedure

16.7.1 Sample *Preparation*

Preparation of the sample is done by wet ash method. Mix the sample properly to get uniform and representative sample. Weigh the sample accurately considering the approximate range of the analyte in sample and lower detection limits of the instrument selected. Use purest grade of nitric acid, hydrogen peroxide or any other reagent selected for digestion. A standard digestion procedure should be followed. Microwave digestion system can be usedwith proper temperature programming. Prepare a reagent blank exactly same manner as preparation of sample but without any sample.

16.7.2 *Standard /calibration solutions*

Multi-elemental standards may be prepared by selection of appropriate concentrations. Calibration standards should be selected considering the range of instruments selected and the expected levels of analytes in a sample. Working standards should be freshly prepared. A reagent blank should be run with standards. Initially, six level calibration curve should be constructed. For daily purposes three standard points and one blank may be used. Standards shall be prepared exactly in the same manner as the preparation of reagent blank but with the addition of elements to be analyzed.

16.8 Calibration Curve

Set and adjust the instrument as per instructions given by the manufacturer. Select the necessary parameters of the analyte as wavelength, mass numbers for optimization of responses. Run 6 or 7 calibration standards in duplicate and confirm the constant and linear readings. Carry out the analysis of samples when calibration is valid for the selected range of analysis.

16.9 Sample Analysis

16.9.1 Relative deviation D is calculated as

$$D = \frac{(R - R_{avg}) \times 100}{R_{avg}}$$

Where,

D = Relative deviation

R = Observed reading

R_{avg} = Average of two readings

16.9.2 Run the samples in duplicate. The relative deviation between two replicates should not be more than 10% on either side.

16.10 Quality Control

Run the reagent blank intermittently. The traces of analytes in reagent blanks should be below LOQ of specific analyte. Run the sample in duplicate and follow the requirement of relative deviation (**16.9**). A standard solution having concentration within the range of calibration curve but different from standard points should be used as an internal check. It should be prepared exactly as calibration standard. It should be injected after interval of certain samples (for example say 5). The deviation between actual concentration and the observed concentration should meet **16.9**.

16.11 Calculations

16.11.1 The modern instruments are operated with dedicated software. When parameters like sample mass, calibration detail and dilution factors are fed properly, results are automatically calculated.

16.11.2 Calculation by formula is as follows

$$A \times V \times C = M \text{ mg/kg}$$

Where,

C = mass of analyte in sample, (mg/kg)
A= mass of analyte by calibration curve (mg/kg)
V = volume of test solution, (ml)
M= mass of sample (g)

ANNEX A

(Clause 6.4.3)

SPECIFICATION FOR QUARTZ CONTROL PLATE

A-1 DEFINITION OF NORMAL QUARTZCONTROL PLATE

The normal quartz control plate is the quartz plate which shows the rotation value 40.765 $+0.001^0$ C of arc at 20^0C and the wavelength of the green line of the isotope 198 Hg = 546.227 1 nm. The sugar value of this plate is 100.000^0S which is to corrected in terms of ^{0}Z.

A-2 MATERIAL AND CONSTRUCTION

A-2.1 Quartz, including all parts covered by the mounting, should be completely free from all non-homogeneities such as twinning, striate and inclusions. No defects shall be visible on the surface of the plate when examined interferometrically.

A-2.2 A quartz double plate from dextrorotatory and levorotatory quartz shall be used for values less than 24°Z. The thickness of each plate shall be not less than 0.4 mm. The thickness of both plates together shall not exceed 1.6 mm.

A-2.3 The thickness of the plate shall not vary by more than 150 nm over the whole area of the plate.

A-2.4 Each surface of the plate shall be between two imaginary parallel planes 500 nm apart.

A-2.5 Squareness of the plate to the axis of the mounting tube should be within 10 min of arc.

A-2.6 The overall diameter of the plate must not be smaller than 15 mm, nor greater than 17 mm, and at least 4 mm greater than the clear aperture.

A-2.7 The aperture of the plate shall be at least 10 mm in diameter.

A-2.8 The angle between the optic axis of the plate and that normal to the faces of the plate must be less than 10 min of arc.

A-2.9 The axial play of the plate in its cell must be between 5 µm and 30 µm.

A-3 INSCRIPTIONS

Each quartz control plate shall have permanently and legible marked on it:

a) Nominal Value in ^{0}Z,
b) Corresponding wavelength,
c) Maker's or Vendor's name or mark, and
d) Identification number of the plate.

ANNEX B

(Clause 6.6.3.2*)*

ALTERNATE METHOD FOR STANDARDIZATION OF POLARIMETER AND APPLICATION OF TEMPERATURE CORRECTIONS

B-1 PROCEDURE

B-1 As per this alternate method quartz plate reading be carried out periodically but not necessarily at the same time as test solutions are read. Standardization of the polarimeter is achieved by application of a scale correction derived by this procedure.

B-1.1 To carry out this following parameters are required.

a) Following parameters are to be recorded at the time of periodic check:

1) Polarimeter zero, that is, air reading, P_a

2) Quartz plate reading, Q_{tq} at temperature t_q of quartz plate at time of reading.

3) The temperature of the Polarimeter, t_p ,if it is of a type for which a temperature coefficient is prescribed, for example, quartz wedge instrument.

b) Following parameters are to be recorded at the time of reading test solutions:

1) Reading of a Polarimeter tube filled with water, P_w.

2) Readings of test solution, P_{tr} , at temperature t_r.

3) The temperature of the Polarimeter, t_{pr} if it is of a type for which a temperature coefficient is prescribed.

4) The temperature of making up to the mark. t_m will already have been recorded.

B-2 EXPRESSION OF RESULTS

B-2.1 Prior to calculation of corrected polarization at 20^0C, it is necessary to determine the scale error derived from the quartz plate standardization data and recorded and kept safely for further use. Determine the scale error Δ, by correcting the observed quartz plate value to 20^0 C and comparing it with the certified value. In practice, calculate by adding to or subtracting from the observed quartz plate value, Q_{tq}, the quantities given below:

a) Subtract Polarimeter zero, P_a,

b) Subtract quartz plate temperature correction = $0.000\,144 \times Q_{20} \times (t_p - 20)$

c) Add Polarimeter temperature correction

Quartz wedge: $0.000\,144 \times Q_{20} \times (t_p - 20)$
Circular : No corrections is needed unless indicated by manufacturer's specifications.

d) Subtract certified quartz plate value at 20^0C, $Q_{20.}$

B-2.2 Polarization corrected at 20^0C, $P_{20,}$ is calculated by applying the corrections given below to the observed polarization, P_{tr}:

a) Subtract water and Polarimeter tube correction, P_w

b) Add temperature of reading, t_r correction

t_r correction = $c \times P_{tr} \times (t_r - 20) - 0.004 \times R \times (t_r - 20)$,

where

C = coefficient in Table 1, and
R = reducing sugars, percent on sample

c) Subtract temperature of making to mark, t_m correction

t_m correction = $f \times P_{tr} \times (t_m - 20)$

Where f = coefficient in Table 1.

d) Add Polarimeter temperature, t_p correction

Quartz wedge: $t_m$correction= 0.000 144 × p_{tr}× (t_m -20)
Circular: No corrections are needed unless indicated by manufacturer's specifications.

e) Subtract scale correction which is the scale error, Δ calculated above (see D-2.1)
Thus,

$P_{20} = P_{tr} - P_w + t_r$ corr. – t_m corr. (if applicable) -Δ
Express the results to 2 decimal places as ^{0}Z. Indicate in the result the corrections that have been made to the result observed without corrections (for example, corrected for instrument standardization and to a temperature of 20 ^{0}C).

ANNEX C
(Clause 9.6.1)

DETERMINATION OF REFRACTOMETRIC DRY SUBSTANCE (RDS Percent)

C-1 SCOPE OF APPLICATION

The method is applied to determination of the refractometric ^{o}Brix or the refractometric dry substance (RDS percent) of sugar solution.

C-2 PRINCIPLE

The refractive index of aqueous sugar solutions depends upon the density of dissolved material and can therefore serve as a measure of the sugar content. This is mainly valid for pure sugar solutions; however, the non-sugars present in sugar products influence the refractive index in a similar way to sucrose as they contributes to total solid content. For these reasons, the measurement of refractive index can be utilized for an approximate determination of the dry substance content of solutions containing mainly sucrose.
Measurements are generally carried out with sugar refractometers graduated in percent sucrose (g/l 00 g); alternatively this result may be obtained from refractive index tables for pure sucrose solutions.

C-3 APPARATUS

C-3.1 *Refractometer*, calibrated at 20^0C and having a water-jacketed prism.

C-3.2 *Light Source*

C-3.3 *Plastic Rod*, approximately 3 mm diameter.

C-3.4 *Thermometer*, 150 mm, range 0-50^0C.

C-3.5 *Beaker*, capacity 50 ml.

C-3.6 *Water Bath and Pump*, Thermos tatted generally at 20°C.

C-4 PROCEDURE

C-4.1 *Reading the Refractometer*

Calibration of instrument as per manufacturer's instructions is essential and that the prism faces are clean and dry. The following apply to the Abbe type. With the prisms closed, allow temperature-controlled water (20°C) to flow through the prisms jacket for a period long enough for equilibrium to be reached; 5 min is usually sufficient.

NOTE — When operating at temperature other than 20^0C refer Table 4 for corrections to be applied.

Table 4 Temperature Correction
(*Clause* C- 4.1)

Temperature	**Measured Sucrose (Mass Fraction)**		
	45	50	55
(1)	(2)	(3)	(4)
15	-0.38	-0.38	-0.38
16	-0.30	-0.31	-0.31
17	-0.23	-0.23	-0.23
18	-0.15	-0.15	-0.15
19	-0.08	-0.08	-0.08
20	0.00	0.00	0.00
21	+0.08	+0.08	+0.08
22	+0.16	+0.16	+0.16
23	+0.24	+0.24	+0.24
24	+0.32	+0.32	+0.32
25	+0.40	+0.40	+0.40

26	+0.48	+0.48	+0.48
27	+0.56	+0.56	+0.56
28	+0.65	+0.65	+0.64
29	+0.73	+0.73	+0.72
30	+0.82	+0.81	+0.80
31	+0.90	+0.90	+0.89
32	+0.99	+0.99	+0.98
33	+1.08	+1.07	+1.07
34	+1.16	+1.16	+1.15
35	+1.25	+1.25	+1.24
36	+1.34	+1.34	+1.33
37	+1.43	+1.43	+1.41
38	+1.53	+1.52	+1.50
39	+1.62	+1.61	+1.59
40	+1.71	+1.70	+1.68

C-4.2 Pre check of instrument can made by transferring a drop of water to the refractometer prism to first determine whether a reading of zero is obtained or if a correction needs to be applied. Transfer a small amount of sugar solution from the container to the beaker and adjust the sugar solution temperature to approximately that of the instrument, 18 – 28°C is suitable.

C-4.3 Open the refractometer prism and apply a drop of sugar solution to the fixed prism face by means of the plastic rod. Extend the sugar solution quickly as a line along the face without touching the prism surface with the rod, taking care to avoid the formation of air bubbles. Close the prisms quickly. Take the refractometer reading according to the instrument manufacturer's handbook. Apply any scale correction to the reading to obtain a corrected reading.

C-5 EXPRESSION OF RESULTS

C-5.1 Express results to the nearest 0.1^0 Brix (0.1 percent RDS).

C-5.1.1 *Calculation*

Where the Refractometer is calibrated in refractive index, read the nearest 0.00005 units and determine the ^{0}Brix (RDS percent) from Table 5.

Table 5 International Refractive Index Scale for Pure Sucrose Solution at 20^0C

(*Clause* C-5.1.1)

This table give values of refractive index against air with sucrose mass fraction

Sucrose	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9

G/100 g										
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
46	1.411808	1.412011	1.412215	1.412420	1.412624	1.412828	1.413033	1.412238	1.413443	1.413648
47	1.413853	1.414059	1.414265	1.414470	1.414676	1.414822	1.415089	1.415295	1.415502	1.415708
48	1.415915	1.416122	1.416330	1.416537	1.416744	1.416952	1.417060	1.417368	1.417576	1.417785
49	1.417993	1.418202	1.418411	1.418620	1.418829	1.419038	1.419247	1.419457	1.419667	1.419877
50	1.420087	1.420297	1.420508	1.420718	1.420929	1.421140	1.421351	1.421562	1.421774	1.421985
51	1.422197	1.422409	1.422621	1.422833	1.423046	1.423258	1.423471	1.423684	1.423897	1.424110
52	1.424323	1.424537	1.424750	1.424964	1.425178	1.425393	1.425607	1.425821	1.426036	1.426251
53	1.426466	1.426896	1.426896	1.427112	1.427328	1.427543	1.427759	1.427975	1.428192	1.428428
54	1.428625	1.428842	1.426059	1.429276	1.429493	1.429711	1.429928	1.430146	1.430364	1.430582
55	1.403800	1.431019	1.431238	1.431456	1.434675	1.431894	1.432114	1.432333	1.432553	1.432773