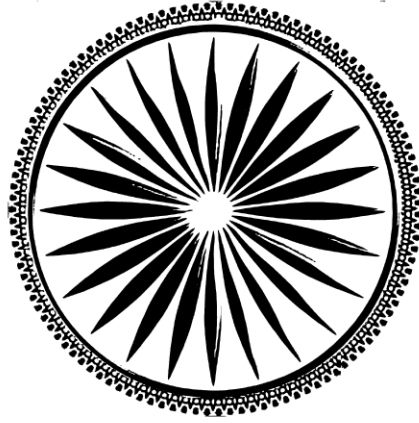


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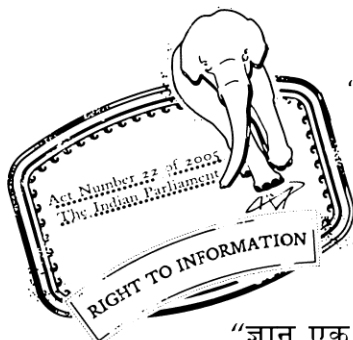
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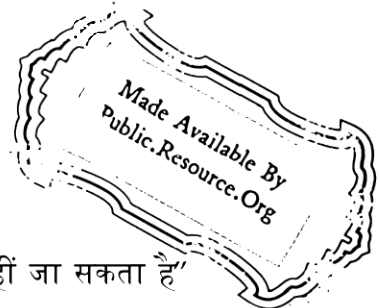
Mazdoor Kisan Shakti Sangathan
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Jawaharlal Nehru
"Step Out From the Old to the New"

**IS 3752 (2005) : Alcoholic drinks— Methods of test [FAD 14:
Drinks and Carbonated Beverages]**



Satyanarayan Gangaram Pitroda
"Inve



"ज्ञान एक ऐसा

नहीं जा सकता है"

Bhartrhari—Nitisatakam

"Knowled be is such a treasure w'hich can not be stolen"

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भारतीय मानक
एल्कोहलीय पेय — परीक्षण पद्धतियाँ
दूसरा पुनरीक्षण

Indian Standard
ALCOHOLIC DRINKS — METHODS OF TEST
(*Second Revision*)

ICS 67.160.10

O 8IS 2005

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

FOREWORD

This Indian Standard (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Drinks and Carbonated Beverages Sectional Committee had been approved by the Food and Agriculture Division Council.

This standard was originally issued in 1967 and revised in 1988.

This revision has been taken up in order to update the methods of test in the light of experience gained in its usage and align it with the latest practices being followed in the field in the country.

In the preparation of this standard, due consideration has been **given to** the : (a) **Prevention of Food Adulteration Act**, 1954 and the Rules framed thereunder; (b) **Standards of heights and Measures (Packaged Commodities) Rules**, 1977; and (c) **State Excise Duty Rules** which permit the withdrawal **of duty free samples for testing**. It is recommended that sample for testing by Bureau of Indian Standards, whenever called for, may also be exempted from excise duty. The standard is subject to restrictions imposed under these Rules, wherever applicable.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values *{revised}*'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

ALCOHOLIC DRINKS — METHODS OF TEST

(Second Revision)

1 SCOPE

This standard prescribes the methods of test for alcoholic drinks. For the estimation of esters, higher alcohols, aldehydes, furfural and methanol, gas chromatographic (GC) method is provided in Annex A as an alternative method.

2 REFERENCES

The standards listed in Annex B 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 indicated in Annex B.

3 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals shall be employed in tests and distilled water {see IS 1070} shall be used where use of water as reagent is intended.

4 DETERMINATION OF ETHYL ALCOHOL CONTENT

Three methods have been prescribed, namely, Method 1, Method 2 and Method 3. Method 1 shall be used as routine method for apparent strength, Method 2 as referee method for real strength, and Method 3 in case of those alcoholic drinks which yield volatile oils on distillation.

4.1 Method 1, Hydrometer Method

4.1.1 See IS 2302 and express the strength as percent by volume at 20/20°C.

4.2 Method 2, Pycnometer Method

4.2.1 Apparatus

- a) *Distillation assembly*, the delivery end of the condenser is attached to a glass tube with a bulb by means of a ground glass joint. The lower part of this tube should reach the bottom of the receiver and dip into the minimum quantity of distilled water.
- b) *Pycnometer*, 25 to 50-ml capacity.
- c) *Thermometer*, 0 to 50°C.
- d) *Measuring flask*, 200-ml capacity.

4.2.2 Procedure

4.2.2.1 Take 200 ml of sample in a 500-ml distillation flask containing about 25 ml of distilled water and a few pieces of pumice stone. Complete the distillation in about 35 min and collect the distillate in a 200 ml measuring flask till the volume in the flask nears the mark. Allow the distillate to come to room temperature, make up the volume to 200 ml with distilled water and mix thoroughly.

4.2.2.2 Find out the specific gravity of the distillate at the required temperature with the help of a Pycnometer. Obtain corresponding alcohol, percent by volume, from the tables given in IS 3506.

4.3 Method 3, Distillation Method

4.3.1 Distillation Assembly

4.3.2 Apparatus

- a) *Measuring flask*, 200-ml capacity.
- b) *Separating funnels*, 500-ml capacity.

4.3.3 Reagents

- 4.3.3.1 *Sodium chloride*, powder.
- 4.3.3.2 *Petroleum ether*, 40 to 60°C.
- 4.3.3.3 *Sodium hydroxide*, 0.1 N.
- 4.3.3.4 *Phenolphthalein*, powder.

4.3.4 Procedure

4.3.4.1 Measure 200 ml of the liquor sample in a measuring flask. Transfer to a separating funnel, wash, the measuring flask with about 100 ml of water, add the washings to the content of the separating funnel and add sufficient powdered sodium chloride to saturate the liquid. Add about 100 ml of petroleum ether and shake vigorously for 2 to 3 min. Allow the mixture to stand for 15 to 30 min and run the lower layer into a distillation flask. Wash the petroleum layer twice with about 20 ml of the saturated solution of sodium chloride. Add these washings to the distillation flask. Make the mixed solutions just alkaline with sodium hydroxide solution using phenolphthalein powder as indicator, add a little pumice powder and distil. Collect the distillate in the measuring flask till it nears the mark. Bring the distillate to room temperature, make up the

volume to 200 ml with distilled water and mix thoroughly.

4.3.4.2 Find out the specific gravity of the distillate at a part ten far temperature with the help of a Pyknometer. Obtain corresponding alcohol, percent by

volume from the tables given in IS 3506.

S DET ERMI NATION OF RESIDUE ON EVAPORATION

5.1 Apparatus

5.1.1 Hot Air Oven

5.1.2 Water Bath

5.1.3 Measuring Flask, 200-ml capacity.

5.1.4 Dish, 150-ml capacity, silica or platinum.

5.1.5 Desiccator

5.2 Procedure

Evaporate 200 ml of the sample in a dried, tared dish on a water bath. Dry the dish in an air oven at 110 + 2°C. Cool in desiccator and weigh the dish. Repeat till constant weight is obtained. Calculate the dissolved solids to fourth decimal places.

5.3 Calculation

Calculate residue on evaporation as follows:

$$\text{Residue on evaporation, percent (Iv)} = \frac{100 (m_2 - m_1)}{v}$$

where

m_2 , -- weight of the dish with dry material, in

m_1 - weight of the empty dish, in g; and

v volume of liquor taken for estimation, in ml.

fi DETERMINATION OF ASH

6.1 Apparatus

6.1.1 Muffle Furnace

6.1.2 Desiccator

6.1.3 Water Bath

6.1.4 Dish, 150-ml capacity, silica or platinum.

6.2 Procedure

Shake the content of the container. Evaporate 100 ml of the sample in a dried, tared dish on a water bath. Place the dish with the residue on a hot plate or on a low flame; heat the contents at low temperature until thoroughly charred. Place the dish in the muffle furnace maintained at 450° to 500°C for about an hour. Cool the dish in a desiccator and weigh. Express the result to four decimal places.

6.3 Calculation

Calculate ash as follows:

$$\text{Ash content, percent (m/v)} = \frac{100 (m_2 - m_1)}{v}$$

where

m_2 -- weight of the dish with ash, in g;

m_1 = weight of the empty dish, in g; and

v = volume of liquor taken for estimation, in ml.

7 DETERMINATION OF TOTAL ACIDITY

Two methods, have been prescribed, namely, Method 1 and Method 2. Method 1 shall be used for the products which are colorless like Vodka and Gin. Method 2 shall be used for the products which are colored.

7.1 Method 1

7.1.1 Reagents

7.1.1.1 ' Standard sodium hydroxide, 0.05 N.

7.1.1.2 Phenolphthalein indicator, as prescribed in IS 2263.

7.1.2 Procedure

Take 50 ml of sample and add about 200 ml of neutralized distilled water. Titrate against standard sodium hydroxide solution using phenolphthalein as indicator.

7.1.3 Calculation

Calculate the total acidity as follows:

Total acidity expressed as tartaric acid, grams per 100 litres of absolute alcohol =

$$\frac{V \times 0.00375 \times 100 \times 1000 \times 2}{V_1}$$

where

V = volume of standard sodium hydroxide used for titration, in ml; and

V_1 -- alcohol, percent by volume.

7.2 Method 2

7.2.1 Apparatus

7.2.1.* pH meter

7.2.1.2 Magnetic stirrer

7.2.2 Reagents

7.2.2.1 Standard sodium hydroxide, 0.05 N.

7.2.2.2 Buffer solutions, of pH = 4.0, 7.0 and 9.2.

7.2.3 Procedure

Calibrate and standardize the pH meter using the buffer

solutions of pH = 4.0, 7.0 and 9.2. Add approximately 100 ml of distilled water to 250-ml beaker. Place one stirrer in the beaker and place it on the magnetic stirrer. Carefully immerse the electrode into the beaker. Add approximately 50 ml of sample and rapidly titrate with standard sodium hydroxide solution to pH 8.2. Add 50 ml of sample into the resulting solution and titrate to pH 8.2. Record the volume of standard sodium hydroxide solution consumed for the second titration.

7.2.4 Calculation

Calculate the total acidity as follows:

$$\text{Total acidity expressed as tartaric acid, grams per 100 litres of absolute alcohol} = \frac{V \times 0.00375 \times 100 \times 1000 \times 2}{V_1}$$

where

V = volume of standard sodium hydroxide used for titration, in ml; and

V_1 — alcohol, percent by volume.

NOTE — One millilitre of standard sodium hydroxide is equivalent to 0.00375 g of tartaric acid.

8 DETERMINATION OF VOLATILE ACIDITY

8.1 Reagents

8.1.1 *Standard Sodium Hydroxide*, 0.05 N.

8.1.2 *Phenolphthalein Indicator*, as prescribed in IS 2263.

8.2 Procedure

Take 50 ml of the distillate obtained from the determination of ethyl alcohol (see 4.2.2.1) and titrate against standard sodium hydroxide solution using phenolphthalein as indicator.

8.3 Calculation

Calculate the volatile acidity as follows:

$$\text{Volatile acidity expressed as acetic acid, grams per 100 litres of absolute alcohol} = \frac{V \times 0.003 \times 100 \times 1000 \times 2}{V_1}$$

where

V = volume of standard sodium hydroxide used for titration, in ml; and

V_1 = alcohol, percent by volume.

NOTE — One milliliter of standard sodium hydroxide is equivalent to 0.003 g of acetic acid.

9 DETERMINATION OF FIXED ACIDITY

9.1 Reagents

9.1.1 *Standard Sodium Hydroxide*, 0.05 N.

9.1.2 *Phenolphthalein Indicator*, as prescribed in IS 2263.

9.2 Procedure

Take 50 ml of sample and evaporate to near dryness. Dilute with neutralized distilled water to 100 ml and again evaporate to dryness. Dilute and titrate against standard sodium hydroxide solution using phenolphthalein as indicator.

9.3 Calculation

Calculate fixed acidity as follows:

Fixed acidity expressed as tartaric acid, grams per 100 litres of absolute alcohol =

$$\frac{V \times 0.0088 \times 100 \times 1000 \times 2}{V_1}$$

where

V = volume of standard sodium hydroxide used for titration, in ml; and

V_1 = alcohol, percent by volume.

NOTE — One millilitre of standard sodium hydroxide is equivalent to 0.0088 g of tartaric acid.

10 DETERMINATION OF ESTERS

10.1 Reagents

10.1.1 *Standard Sodium Hydroxide*, 0.1 N.

10.1.2 *Standard Sulphuric Acid*, 0.1 N.

10.2 Procedure

10.2.1 To the neutralized distillate from the volatile acidity determination (see 7.2), add 10 ml of standard alkali and reflux it on a steam bath for an hour. Cool and back titrate the excess of alkali with standard sulphuric acid.

10.2.2 Simultaneously run a blank taking 50 ml of distilled water in place of the distillate of the sample in the same way. The difference in titration value in millilitres of standard acid solution gives the equivalent ester.

10.3 Calculation

Calculate the esters as follows:

Esters expressed as ethyl acetate, grams/100 litres of absolute alcohol =

$$\frac{V \times 0.0088 \times 100 \times 1000 \times 2}{V_1}$$

where

V = difference of standard acid used for blank and sample, in ml; and

V_1 — alcohol, percent by volume.

NOTE — One milliliter of standard alkali is equivalent to 0.0088 g of ethyl acetate.

11 DETERMINATION OF HIGHER ALCOHOLS

Three methods have been prescribed, namely, Method 1, Method 2 and Method 3. Method 1 may be used to determine approximately the quantity of higher alcohols in process control. Method 2 shall be employed for accurate determination, while Method 3 shall be used as referee method.

11.1 Method 1 (Komarowski Method)

11.1.1 Reagents

- Salicylic aldehyde*, 1 percent {mlv} — Dissolve 1 g salicylic aldehyde in 95 percent alcohol (free of higher alcohol) to make 100 ml.
- Sulphuric acid*, conforming to IS 266.

11.1.2 Procedure

11.1.2.1 Take a clean glass-stoppered bottle and wash it twice with the sample to be tested. Similarly, wash a small graduated cylinder or a 100-ml pipette.

11.1.2.2 Take 10 ml of sample in the bottle and add 1 ml of 1 percent salicylic aldehyde and 20 ml of concentrated sulphuric acid. Allow to stand at room temperature for over 12 h. Note the colour change (For quick routine analysis, the colour change may be noted after a shorter interval of about 15 to 20 min at 15° to 20°C).

The colours developed after the reaction indicate the amount of higher alcohol as follows:

Colour	Amount of Higher Alcohol
Light yellow	Only traces
Yellow to brownish	About 0.01 percent (v/v)
Brown	0.02 to 0.03 percent (v/v)
Red	About 0.05 to 0.1 percent (v/v)
Dark red to black	Above 0.1 percent (r/v)

11.2 Method 2

11.2.1 Apparatus

It consists of an 800-ml capacity Kjeldahl flask fitted with splash head connected to distillation head. The other end of distillation head is connected to a Liebig condenser, which opens into a receiver having a capacity of 250 ml. All joints are made of ground glass.

11.2.2 Reagents

- Sulphuric acid*, conforming to IS 266.
- Oxidizing mixture*, Dissolve 100 g of potassium dichromate in 500-ml distilled water and add 100 ml of sulphuric acid and make the volume to 1 litre with distilled water.
- Standard sodium hydroxide*, 0.1 N.

d) *Carbon tetrachloride*, distilled.

e) *Sodium chloride*, AR grade.

f) *Sodium sulphate*, AR grade.

g) *Phenolphthalein indicator*, as prescribed in IS 2263.

11.2.3 Procedure

11.2.3.1 Add 50 ml of water to the solution resulting from the determination of esters {see 10.2.1}. Saturate it with sodium chloride; extract the saturated solution four times using 40, 30, 20 and 10 ml, respectively of carbon tetrachloride. Combine the extracts and wash three times with saturated sodium chloride solution and twice with saturated sodium sulphate solution.

11.2.3.2 Filter the extract and add 50 ml of oxidizing mixture. Reflux for 2 h. Cool, wash the reflux condenser with 50 ml of water and transfer it to distillation flask using about 50 ml of water. Distil till about 50 ml is left over in the flask. Avoid charring.

11.2.3.3 Titrate the distillate against standard sodium hydroxide, using phenolphthalein as indicator.

11.2.3.4 Run a blank in the same way taking 50 ml of distilled water in place of the distillate of the liquor.

11.2.4 Calculation

Calculate higher alcohol as follows:

Higher alcohol expressed as amyl alcohol, grams per 100 litres of absolute alcohol =

$$\frac{V \times 0.0088 \times 100 \times 1000 \times 2}{V_1 \times V_2}$$

where

V = difference of standard acid used for blank and sample,

V_1 = volume of sample taken; and

$\%$ = alcohol, percent by volume.

NOTE — One milliliter of standard alkali is equivalent to 0.0088 g of amyl alcohol.

11.3 Method 5

11.3.1 Apparatus Spectrophotometer.

11.3.2 Reagents

- p*-dimethylaminobenzaldehyde **solution** — Dissolve 1 g *p*-dimethylaminobenzaldehyde in mixture of 5 ml sulphuric acid and 90 ml water in a 100-ml volumetric flask and dilute to mark with wster.
- Isobutyl alcohol*, AR grade.
- isoamyl alcohol*, AR grade.

- d) *Ethyl alcohol*, redistilled, middle 50 percent fraction.
- e) *Synthetic standard higher alcohol*, weigh 2 g isobutyl alcohol and 8 g isoamyl alcohol into 1 litre volumetric flask and dilute to mark with water. Pipette two 10 ml portions into 100-ml volumetric flasks and dilute to mark, one with water and other with ethanol. Prepare working standards for products in the range of 0 to 170 proof containing 1.0 to 6.0 g synthetic higher alcohol per 100 litres by diluting 1.0 to 6.0 ml aliquots of aqueous standard solution to 100 ml with alcoholic solution of proof expected for diluted sample when pipetted into analysis tube. Prepare similar working standards for products in range of 170 to 190 proof by diluting 1.0 to 6.0 ml aliquots of alcohol standards solution to 100 ml with alcohol solution of number of samples or its dilution. When 6 ml synthetic standard diluted with 190 proof alcohol is carried through analysis, absorbance shall be 0.83 ± 0.03 at 530 nm.

11.3.3 Procedure

11.3.3.1 Preparation of sample

- a) Take 200 ml of sample (liquor) in 500-ml Erlenmeyer flask, add about 33 ml water and few grains of carbonindum. Distil slowly into 200-ml **volumetric** flask until distillate is nearly at mark. Make up to mark with distilled water and mix.
- b) For samples containing 6 g fusel oil per 100 litres, dilute the distilled sample with water to concentration of 2.0 to 5.0 g fusel oil per 100 litres (dilute 5 ml brandy, rum, or blended whisky to 100 ml; dilute 5 ml blended brandy, rum or straight whisky to 250 ml).

Pipette 2 ml of aliquot of sample or diluted sample, 2 ml of water (for reagent blank), and 2 ml aliquot of standards into 15 mm 150 mm stoppered or covered test tubes. Stopper or cover tubes, place in rack, and then in ice-bath. Pipette 1 ml y-dimethylaminobenzaldehyde solution into each tube; shake, and replace in ice-bath for 3 min. With tubes still in ice-bath, add 10 ml sulphuric acid from burette down the side of the each tube. Shake tubes individually and replace in ice-bath for 3 min. Transfer rack of tubes from ice-bath to boiling water-bath for 3 to 5 min and then to room-temperature-bath. Read percentage transmittance of developed colour of samples and standards on spectrophotometer at 530 to 535 nm against reagent blank as reference (use same wavelength for both standards and unknowns). Plot *g* higher alcohol per 100 litres on linear scale of as abscissa against percentage *T* as ordinate on log scale of Semilog paper. Convert percentage transmittance of samples to *g* higher alcohol per 100 litres from

standard curve. If dilution was used, multiply *g* higher alcohol per 100 litres in original sample. Analyze two levels of standards with each series of unknowns.

12 DETERMINATION OF ALDEHYDES

12.1 Apparatus

12.1.1 *Iodine Flask*, of 250-ml capacity.

12.2 Reagents

12.2.1 *Sodium Bisulphite Solution*, approximately 0.05 N.

12.2.2 *Standard Iodine Solution*, 0.05 N.

12.2.3 *Standard Sodium Thiosulphate Solution*, 0.05 N.

12.2.4 *Starch Indicator*, as prescribed in IS 2263.

12.3 Procedure

Take 50 ml of distillate of liquor {see 4.2.2.1} in a 250-ml iodine flask and add 10 ml of bisulphite solution. Keep the flask in a dark place for 30 min with occasional shaking. Add 25 ml of standard iodine solution and back titrate excess iodine against standard sodium thiosulphate solution using starch indicator. Run a blank taking 50 ml of distilled water in place of distillate of the liquor in the same way. The difference in titration value, in millilitres, of sodium thiosulphate solution gives the equivalent aldehyde.

12.4 Calculation

Calculate aldehydes as follows:

Aldehydes expressed as acetaldehyde, grams per 100 litres of absolute alcohol =

$$\frac{V \times 0.001 \times 1 \times 100 \times 1\,000 \times 2}{V_1}$$

where

P = difference of standard sodium thiosulphate solution used for blank and sample, in ml; and

P, = alcohol, percent by volume.

NOTE — One millilitre of standard sodium thiosulphate solution is equivalent to 0.001 g of acetaldehyde.

13 DETERMINATION OF FURFURAL

13.1 Apparatus

Nessler tubes with flat bottom tubes of thin colourless glass 25 mm in diameter, about 150 mm in length and graduated at 50 ml. The depth measured internally from graduation mark to the bottom shall not vary by more than 2 mm.

13.2 Reagents

13.2.1 *Aniline*, distilled and colourless.

13.2.2 *Hydrochloric Acid*, conforming to IS 265.

13.2.3 *furfural Free Alcohol* — Let alcohol containing 5 g of m-phenylene diamine hydrochloride per litre stand at least for 24 h with frequent shaking (previous treatment with potassium hydroxide is not necessary). Ret)ux for at least 8 h, longer if necessary. Let stand overnight and distil, rejecting the first 100 ml and the last 200 ml of the distillate. If this gives colouration with outline hydrochloride, repeat the treatment:

I 3.2.4 *Standard Furfural Solution* — Dissolve 1 g of redistilled, colourless furfural in 100 ml of furfural free alcohol (see 13.2.3). Prepare standard furfural solution by diluting 1 ml of this solution to 100 ml with 50 percent furfural free alcohol. One millilitre of this diluted solution contains 0.1 mg of furfural (strong furfural solution will retain its strength but the diluted standard solutions should be prepared afresh every time).

13.3 Procedure

13.3.1 Take 5 ml of distillate {see 4.2.2.1), add 1 ml of colourless aniline and 0.5 ml of hydrochloric acid, and keep for 15 min. Red colour indicates the presence of furfural. Proceed for quantitative estimation as in 13.3.2, if colour develops.

13.3.2 Dilute a measured portion of the distillate with 50 percent furfural-free alcohol to 50 ml. First add 2 ml of colourless aniline and then 0.5 ml of hydrochloric acid. Mix and keep at 15°C for 15 min. Compare the colour developed with standard furfural solution.

13.4 Calculation

Calculate furfural as follows:

Furfural, grams per 100 litres of absolute alcohol =

$$\frac{w \cdot 1\,000 \times 100 \times 100}{V_1 \times V_2}$$

where

w — weight of furfural present in volume used for matching the experimental solution, in g;

V_1 — volume of experimental solution used for estimation, in ml; and

V_2 — alcohol, percent by volume.

14 DETERMINATION OF COLIFORM COUNT

Two methods are prescribed, namely, Method 1 and Method 2. Method 1 may be used as routine method and Method 2 as referee method.

14.1 Method 1

Coliform bacteria include all aerobic and facultative anaerobic Gram-negative non-spore-forming bacteria, which ferment lactose with the production of acid and

gas. A positive presumptive test is indicated by the formation of acid and gas within 48 h at 35 to 37°C in a fermentation tube containing lactose bile salt broth. Alternatively, the development of dark red colonies approximately 0.5 mm in diameter in a solid medium (violet red bile agar) within 20 to 24 h at 35 to 37°C may be considered as a positive evidence for the presence of coliform bacteria. Violet red bile agar is one of the standard media used for the determination of general types of coliform organisms, including those of faecal origin in water, milk and other materials of sanitary importance.

14.1.1 Apparatus

- a) Weighing scoop, sterile, with counter-weight.
- b) Bacteriological pipettes, sterile **accurately** graduated with cotton plug in the upper orifice.
- c) Dilution bottles, sterile, made of heat-resistant glass (preferably borosilicate glass), closed with rubber stoppers (preferably screw caps) with new friction-fit liners for making them leak-proof and of the following capacities:
 - 1) 150 ml, with mark at 99 ml level; and
 - 2) 25 ml, with mark at 9 ml level.
- d) Petri dishes, with outside dish diameter 100 mm, inside dish diameter 91 mm and depth 15 mm. The exterior and interior surface of the bottom should be flat and free from bubbles, scratches or other defects, which would interfere with the counting of the colonies.
- e) Bacteriological tubes, **sterile**, 15-ml **capacity** with a mark at 10-ml level and with cotton plugs.
- f) Durham fermentation tubes.

14.1.2 Reagents

- a) Dissolve 34 g of potassium dihydrogen phosphate in 500 ml of distilled water, adjust the pH to 7.4 with 1 N sodium **hydroxide** solution and make up to 1 litre with distilled water. Dilute 1.25 ml of this stock phosphate buffer solution with distilled water to 1 litre to obtain dilution water.
- b) *Medium* — Violet red bile agar shall be the medium, the composition of which shall be as follows, its final pH being 7.4 ± 0.1:

Yeast extract	3.0 g	Lactose	10.0 g
Peptone	7.0 g	Agar-agar	20.0 g
Sodium taurocholate	1.5 g	Sodium chloride	5.3 g
Water	1 000 ml		

Indicator :

- 1) Neutral red 0.03 g
- 2) Crystal violet 0.002 g

Peptone	20.0 g	Sodium taurocholate	0.5 g
Lactose	10.0 g	Sodium chloride	5.0 g
Water	1 000 ml		

Dissolve the above ingredients in distilled water. Add 5 ml of 0.04 percent bromocresol purple per 95 ml of the medium. Fill 10 ml in each of the bacteriological tubes. Sterilize in an autoclave for 15 min at 1 kgf/cm² pressure (121°C).

- c) *Brilliant green lactose bile broth*. Composition of brilliant green lactose bile broth medium shall be as follows, its final pH being 7.2 ± 0.1.

Peptone	10.0 g	Bacto oxgall	20.0 g
Lactose	10.0 g	Brilliant green	0.0133g
Water	1000 ml		

Dissolve the above ingredients in distilled water. Sterilise in an autoclave for 15 min at 1 kgf/cm² pressure (121°C).

14.2.3 Procedure

Inoculate the beverage into the tubes containing MacConkey broth at 35° to 37°C. If gas is produced within 48 h, transfer the broth to the tubes containing brilliant green lactose bile broth *see* 14.2.2(c)] for further confirmation of the presence of coliform bacteria. The latter medium inhibits the growth of the lactose fermenting bacteria other than coliforms and hence the production of gas in it positively shows the presence of coliform bacteria.

15 DETERMINATION OF COPPER

Two methods, namely, diethyldithiocarbamate method and potassium ferrocyanidemetho4 are employed. The potassium ferrocyanide method is easier to perform and sufficiently sensitive and accurate for routine type of analysis. The diethyldithiocarbainate method{see 15.3) is more sensitive and shall serve as a referee method in case of dispute or where zinc is present.

15.1 Apparatus

15.1.1 Nessler Tubes — Flat bottom tubes of thin, colourless glass, about 25 nun in diameter and about 150 mm in length and graduated at 50 ml.' The depth measured internally from graduation mark to the bottom shall not vary by more than 2 mm in the tubes used for the test.

15.2 Reagents

15.2.1 Dilute Sulphuric Acid, approximately 10 percent (v/v)

15.2.2 Aqua begin, a mixture of one volume of

14.1.3 Procedure

- a) *Dilution* — Measure 1 ml of the material from the individual samples using a sterile pipette and transfer into 99 ml of dilute water. Mix the contents properly. Prepare serial dilutions of this and add 1 ml of suitable dilutions in triplicate to the sterile petri dishes.
- b) *Pouring plates* — Melt the medium *see* 14.1.2 (b)] in bacteriological tubes and keep at 48• to 50•U. Introduce this medium aseptically at 42° to 44°C into the petri dishes and mix by rotating and tilting dishes without spreading over the edges. Spread the mixture evenly over the bottom of the plate. Allow to solidify. After solidification of medium in plate, add cover layer of medium.
- c) *Incubation* — Invert the plates and incubate at 35° to 37•C for 24 h.
- d) *Counting* — Count the dark red colonies with diameter of approximately 0.5 mm.
- e) *Computation* — Compute the coliform count per millilitre from the dilutions used [*see* 14.1.3 (a)].

NOTES

1 In case of doubt regarding the colonics developed on violet red bile agar, representative colonies are picked and transferred to lactose bile salt broth in Durham fermentation tubes. Production of acid and gas is confirmatory for coliform organisms.

2 All precautions shall be observed to prevent microbiological contamination throughout the test.

14.2 Method 2

This method consists of inoculating the beverage into MacConkey broth-medium. A positive presumptive test is indicated by the formation of gas within 48 h at 37°C in the fermentation tube containing the above medium. Subsequently, the production of gas in brilliant green lactose bile broth **may be considered as** a positive evidence for the presence of coliform bacteria.

14.2.1 Apparatus, *See* 14.1.1.

14.2.2 Reagents

- a) Water, distilled.
- b) *MacConkey booth*: Composition of MacConkey broth medium shall be as follows, its final EH being 7.2 ± 0.1:

concentrated nitric acid, and three volumes of concentrated hydrochloric acid. Nitric acid shall be conforming to IS 264 and hydrochloric acid shall be conforming to IS 265.

15.2.3 *Citric Acid*, AR grade.

15.2.4 *Dilute Ammonium Hydroxide*, approximately 10 percent (v/v).

15.2.5 *Standard Copper Solution* — Dissolve 1.119 g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in water and dilute to one litre. Dilute 10 ml of this solution to 100 ml. One millilitre of the diluted solution contains

0.028 45 mg of copper. The diluted solution shall always be prepared immediately before use.

15.2.6 *Sodium Diethyldithiocarbamate* — Prepare 0.1 percent by weight solution of sodium diethyldithiocarbamate in water. Sometimes diethyl dithiocarbamate available may not be completely soluble in water, in which case the insoluble material may be removed by filtration through an ash less filter paper. The **reagent** is best prepared just before use but may stand for one or two weeks in amber coloured bottle without appreciable deterioration.

15.2.7 *Carbon Tetrachloride*, AR grade.

15.2.8 *Ammonium Chloride*, AR grade.

15.2.9 *Acetic Acid*, approximately 5 percent by weight.

15.2.10 *Potassium Ferrocyanide Solution*, approximately 4 percent by weight.

15.3 Diethyldithiocarbamate **Method**

15.3.1 In the presence of copper, an aqueous solution of sodium (or zinc) diethyldithiocarbamate gives a golden brown colour in acid or ammoniacal or neutral solution. The diethyldithiocarbamate method has advantages over the ferrocyanide method, which is in vogue in some laboratories since it is more sensitive and is free from interference by iron and zinc. This method is suitable when the copper content ranges from 0.01 to 0.15 mg of copper in the quantity of the material taken. With larger quantities of copper, the mixture of the test solution and reagent rapidly becomes cloudy and any observance of this in the prescribed test is sufficient for condemning the sample as containing excessive quantities of copper. If a quantitative determination is required, the test should be repeated by using proportionately smaller quantities of sample for test.

15.3.2 There are two variations of the method, that is: (a) without extraction, and (b) with extraction. Ordinarily, it is not necessary to resort to the procedure of extraction, which shall be obligatory only when a referee method is required in case of dispute between the purchaser and the supplier.

15.3.5 *Preparation of Test Solution* — Transfer 20 ml of the material into silica evaporating dish and add 1 ml of dilute sulphuric acid. Heat gently in the beginning and then evaporate almost to dryness on a water-bath. Ignite the residue over a smokeless flame to eliminate sulphuric acid. **Cool**, dissolve the residue in 2 ml of water, add three drops of aqua regia and evaporate to dryness on a water bath. Dissolve the residue in water, neutralize, if required, with dilute ammonium hydroxide and make up the volume to 25 ml.

To detect copper contamination, if any, in any of the reagents, blank experiment shall be carried out using the same quantities of the reagents.

15.3.3.1 *Procedure {without extraction}*

Take in 50-ml Nessler tube, 10 ml of the test solution prepared as described in 15.3.3. Add 2 g of citric acid and 10 ml of dilute ammonium hydroxide. Make up to 50 ml with water.

Prepare a series of control solutions, each containing in 50 ml, 2 g of citric acid and 10 ml of dilute ammonium hydroxide together with an increasing amount of copper, namely, 0.1 ml, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml of standard copper solution (see 15.2.5).

The test solution and controls should be free from any turbidity.

Cool all solutions to 20°C, add 2 ml of diethyldithiocarbamate solution to each and match the test solution against the control solution. Note the number of millilitres of standard copper solution added in the control of the test solution having, as nearly as possible, the same intensity of colour as that of the test solution.

15.3.3.2 *Procedure {with extraction}*

Extract immediately the copper organometallic compound **produced as** described in the last paragraph under 15.3J.1 with four successive portions, 2.5 ml each, of carbon tetrachloride and compare the colour of the solution so obtained in a colorimeter with the extracts of control solution similarly **prepared**.

Chloroform may be used but carbon tetrachloride is better as it is almost insoluble in water and forms clearer solution, which **separates** quickly.

15.3.4 *Calculation*

Calculate copper as follows:

Copper (as Cu), in ppm = $0.2845 \cdot 12.5V$

where

V = volume of standard copper solution in the control solution which gives the closest match, in ml.

15.4 Potassium **Ferrocyanide Method**

15.4.1 Procedure

Transfer 20 ml of the material into silica evaporating dish and add 1 ml of dilute sulphuric acid. Heat gently in the beginning and then evaporate almost to dryness on a water-bath. Ignite the residue over a smokeless flame to eliminate sulphuric acid. Cool, dissolve the residue in 2 ml of water, add three drops of aqua regia and evaporate to dryness on a water bath. Dissolve the residue in 2 ml of dilute hydrochloric acid and warm gently till the residue is dissolved. Add 0.5 g of ammonium chloride and dilute to 15 ml with water distilled in an all-glass apparatus. Add dilute ammonium hydroxide till alkaline. Boil off excess of ammonia and filter into a clean Nessler tube. Cool and then render the solution acidic with **acetic** acid (3 to 5 drops are usually sufficient). Dilute to 40 ml. Add 0.5 ml of potassium ferrocyanide solution, stir and make up the volume to 50 ml.

NOTE — If the copper is more, a lesser amount, say 10 ml of the material may be taken for the test.

15.4.1.1 Prepare a series of control solutions each containing in 50 ml, 0.5 g of ammonium chloride, 3 to 5 drops of acetic acid and 0.5 ml of potassium ferrocyanide solution together with an increasing amount of copper, namely, 2 ml, 4 ml, 6 ml, 8 ml and 10 ml of the standard copper solution.

Compare the test solution (see 15.4.1) with control solutions and note the number of millilitres of standard copper solution added in the control of the test solution having, as nearly as possible, the same intensity of colour as that of the test solution.

15.4.2 Calculation

Calculate copper as follows:

Copper (as Cu), in ppm = 0.2845×12.5

where

- k volume of standard copper solution in the control solution which gives the closest match, in ml.

16 DETERMINATION OF METHYL ALCOHOL

Two methods, namely, spectrophotometric method and gas chromatography method are employed. The spectrophotometric method is sufficiently sensitive for routine type of analysis. The gas chromatography method is more sensitive and shall serve as an alternative method.

16.1 Spectrophotometric Method

16.1.1 Apparatus

16.1.1.1 *Spectrophotometer*, of any make with

wavelength range from 350 to 700 nm and a maximum band width of 5 nm.

16.1.2 Reagents

16.1.2.1 *Sodium bisulphite*, AR grade.

16.1.2.2 *Potassium permanganate solution* — Dissolve 3.0 g of potassium permanganate and 15.0 ml of phosphoric acid in 100 ml distilled water. The solution shall be prepared monthly.

16.1.2.3 Sodium salt of chromotropic acid solution (Sodium 1,8-dihydroxynaphthalene-3,6-disulphonate), 5 percent aqueous solution (m/v).

- Prepare fresh solution every week of either acid or salt and filter, if not clear.
- If necessary, prepare purified chromotropic acid by dissolving 10 g of chromotropic acid or its sodium salt in 25 ml water (add 2 ml sulphuric acid to aqueous solution of salt to convert it to free acid). Add 50 ml of methyl **alcohol** heat just to boiling and filter. Add 100 ml isopropyl alcohol to precipitate free acid chromotropic acid. Add more isopropyl alcohol to increase yield of purified acid.

16.1.2.4 *Methanol stock solution* — Dilute 1.0 g of methanol (99.9 percent, v/v) to 100 ml with 40 percent (v/v) ethanol (methanol free). Dilute 10 ml of this solution to 100 ml with 40 percent ethanol (methanol free).

16.1.2.5 *Methanol standard solution* — Dilute appropriate volume of methanol stock solution (see 16.1.2.4) to 100-ml volumetric flasks with 40 percent (v/v) ethanol (**methanol free**) to get final concentration 20, 40, 60, 80 and 100 ppm of methanol.

16.1.3 Procedure

Take 50 ml of sample in a simple still and distil, collecting about 40 ml of distillate. **Dilute** 1 ml of distillate to 5 ml with distilled water and shake well. Take 1 ml of this solution, 1 ml of distilled water (for blank) and 1 ml of each of methanol standard solutions in to 50-ml stoppered test tubes and keep them in an ice-cold water-bath. Add to each test tube 2 ml of potassium permanganate reagent and keep aside for 30 min. Decolorize the solution by adding a little sodium bisulphite and add 1 ml of chromotropic acid solution. Mix well and add 15 ml of sulphuric acid slowly with swirling and place in hot water bath maintaining at 80°C for 20 min. Observe the colour development from violet to red. Cool the reaction mixture and measure the absorbance at 575 nm using 1 cm path length cell.

16.1.4 Calculation

Calculate methanol content in g/100 litres of absolute ethanol as follows:

$$\text{Methanol} = \frac{A_2 \times C \times D \times 1000 \times 100 \times 100}{A_1 \times S}$$

where

A_2 = absorbance for sample standard solution;

C = concentration of methanol standard solution, g/ml;

D = dilution factor for sample solution;

A_1 = absorbance for methanol standard solution; and

S = ethanol content of liquor sample in percent (v/v).

16.2 Gas Chromatographic method

16.2.1 Apparatus

a) *Gas chromatograph and operating parameters* — Gas chromatograph equipped with flame ionization detector and split injection port and fixed with a capillary column of HP Carbowax 20M or equivalent having the dimensions of 25 m length, 0.32 mm ID and 0.30 µ film thickness. The split ratio will be approximately 1:40 with nitrogen or helium as a carrier gas at the flow rate of about 1.7 ml/min. The detector and injector port temperatures may be maintained at about 250°C. Keep the oven temperature at 45°C for 4 min, raise to 100°C/min at the rate of 10°C/min and finally to 200°C for 10 min at the rate of 15°C

NOTE — Optimum operating conditions may vary with column and instrument used and must be determined by using standard solutions. Adjust the parameters for maximum peak sharpness and optimum separation. With high level standard, n-propanol should give almost complete baseline separation from ethanol.

b) *Syringe* — 10 µl, Hamilton Co. No 701, or equivalent.

16.2.2 Reagents

a) *Ethanol* — Methanol-free.

b) *N-Pentanol internal standard* — 0.05 percent v/v n-pentanol in 40 percent v/v ethanol (methanol-free).

c) *Methanol stock solution* — Dilute 1.0 g of methanol (99.9 percent, v/v) to 100 ml with 40 percent (v/v) ethanol (methanol-free).

d) *Methanol stock solution* — Dilute 10 ml of methanol stock solution see 16.2.2(c)] to 100 ml with 40 percent (v/v) ethanol (methanol-free). Dilute 10 ml of this solution to 100 ml with 40 percent (v/v) ethanol (methanol-free). Transfer 5 ml of the resulting solution into a 10 ml-stoppered test tube, add 1 ml of n-pentanol internal standard solution and mix well.

16.2.3 Procedure

Transfer 5 ml of sample into a 10-ml stoppered test tube, add 1 ml of n-pentanol internal standard solution and mix well. Inject 2 µl of methanol standard solution into chromatograph and record the chromatogram. Adjust the operating parameters and attenuation to obtain measurable peaks (at least 25 percent of full-scale deflection). Determine the retention time of methanol and n-pentanol ethanol. Inject 2 µl sample solution into chromatograph and record the chromatogram (adjust attenuation, if necessary).

16.2.4 Calculation

Calculate methanol content in grams per 100 liters of absolute alcohol as follows:

$$\text{Methanol} = \frac{R_2 \times C \times D \times 1000 \times 100 \times 100}{R_1 \times S}$$

$$\text{Methanol} = \frac{R_2 \times C}{R_1}$$

where

R_2 = peak ratio of methanol to n-pentanol ethanol for sample solution;

C = concentration of methanol standard solution, in g/ml; in g per 100 liters of absolute alcohol;

D = dilution factor for sample solution;

R_1 = peak ratio of methanol to n-pentanol ethanol for standard solution; and

S = ethanol content of liquor sample in percent (v/v).

ANNEX A

Clause 1)

ESTIMATION OF ESTERS, HIGHER ALCOHOLS, ALDEHYDES, FURFURAL AND METHANOL BY GAS CHROMATOGRAPHIC METHOD

A-1 DETAILED GAS CHROMATOGRAPHIC METHOD**A-1.1 Apparatus**

A-1.1.1 Gas chromatograph and operating parameters — Gas chromatograph equipped with flame ionization detector and split injection port and fixed with a capillary column of HP Carbowax 20M or equivalent having the dimensions of 25 m length, 0.32 mm ID and 0.30 µm film thickness. The split ratio will be approximately 1:40 with nitrogen or helium as a carrier gas at the flow rate of about 1.7 ml/min. The detector and injector port temperatures may be maintained at about 250°C. Keep the oven temperature at 45°C for 4 min, raise to 100°C/min at the rate of 10°C/min and finally to 200°C for 10 min at the rate of 15°C/min.

NOTE — Optimum operating conditions may vary with column and instrument used and must be determined by using standard solutions. Adjust the parameters for maximum peak sharpness and optimum separation. With high level standard, n-propanol should give almost complete baseline separation from ethanol.

A-1.1.2 Syringe — 10 µl, Hamilton Co. No 701, or equivalent.

A-1.1.3 Reagents

1) Internal standard: 0.5 percent (v/v) n-pentanol in 40 percent (v/v) ethanol (methanol-free).

- 2) Ethanol — Methanol-free.
- 3) Methanol
- 4) Acetaldehyde
- 5) Isobutyraldehyde
- 6) Methyl acetate
- 7) Ethyl acetate
- 8) Iso-valeraldehyde
- 9) n-Propyl acetate
- 10) Diacetyl
- 11) t-Amyl alcohol
- 12) n-Butyl acetate
- 13) Ethyl propionate
- 14) n-Propanol
- 15) Iso-butanol
- 16) Iso-amyl acetate
- 17) n-Butanol
- 18) Iso-amyl alcohol

- 19) Ethyl caprylate
- 20) Furfural
- 21) Ethyl caprate
- 22) Ethyl laurate
- 23) Phenethyl acetate
- 24) Phenethyl alcohol
- 25) Ethyl palmitate
- 26) Isovaleric acid
- 27) Ethyl caproate
- 28) Ethyl lactate
- 29) Acetic acid
- 30) Isobutyric acid
- 31) Phenyl acetate
- 32) Ethyl myristate
- 33) Caprylic acid
- 34) Pelargonic acid
- 35) Capric acid

A-1.1.4 Preparation of Standard Mixture

Transfer accurately a known quantity of about 5.0 g of the reagents listed from **A-1.1.3(3)** to **A-1.1.3(35)** into different 100-ml volumetric flasks and dilute to 100 ml with 40 percent (v/v) ethanol (methanol-free). Transfer 1.0 ml of each of the resulting solutions into a 100-ml volumetric flask and dilute to volume with 40 percent (v/v) ethanol (methanol-free). This solution will give approximately 500 ppm of each of component listed above.

A-1.1.4.1 Preparation of working standard mixture

Transfer 5 ml of standard mixture (see **A-1.1.4**) into a 10 ml-stoppered test tube, add 1 ml of internal standard solution see **A-1.1.3 (1)** and mix well.

A-1.1.5 Procedure

Transfer 5 ml of sample into a 10-ml stoppered test tube, add 1 ml of n-pentanol internal standard solution and mix well. Inject 2 µl of working standard mixture solution into chromatograph and record the chromatogram. Adjust the operating parameters and attenuation to obtain measurable

peaks (at least 25 percent of full-scale deflection). Determine the retention time of methanol and n-pentanol ethanol. Inject 2 µl sample solution into chromatograph and record the chromatogram (adjust attenuation, if necessary).

NOTE — Identify the individual components by injecting respective component standard solutions to the gas chromatography and record the retention times.

A-1.1.6 Calculation

Calculate the individual component in grams per 100 liters of absolute alcohol as follows:

$$\text{Individual component} = \frac{R_2 \times C \times D \times 1000 \times 100 \times 100}{R_1 \times S}$$

$$\text{Individual component} = \frac{R_2 \times C}{R_1}$$

where

R_2 = peak ratio of respective individual component (with respect to standard) to n-pentanol ethanol for sample solution;

C = concentration of respective individual component in standard solution, in g/ml; in g per 100 liters of absolute alcohol;

D = dilution factor for sample solution;

R_1 = peak ratio of respective individual component to n-pentanol ethanol for standard solution; and

S = ethanol content of liquor sample in percent (v/v).

A-2 DETAILED GAS CHROMATOGRAPHIC METHOD

A-2.1 Apparatus

A-2.1.1 Gas chromatograph and operating parameters — Gas chromatography equipped with flame

ionization detector and packed inlet and fixed with a glass column packed with 5 percent Carbowax 20M on carbowax B, 80/120 mesh or equivalent packed columns like Porapak - Q having the dimensions of 2 m in length and 4 mm in ID. Nitrogen or helium may be used as carrier gas at suitable flow rate. The detector and injector port temperatures may be maintained at about 250°C. Keep the oven temperature at 45°C for 4 min, raise to 100°C/min at the rate of 10°C/min and finally to 200°C for 10 min at the rate of 15°C/min.

NOTE — Optimum operating conditions may vary with column and instrument used and must be determined by using standard solutions. Adjust the parameters for maximum peak sharpness and optimum separation. With high level standard, n-propanol should give almost complete baseline separation from ethanol.

A-2.1.2 Syringe — 10 µl, Hamilton Co. No 701, or equivalent.

A-2.1.3 Reagents

- 1) Internal standard: 0.5 percent (v/v) n-pentanol in 40 percent (v/v) ethanol (methanol-free).
- 2) Ethanol — Methanol-free.
- 3) Methanol
- 4) Acetaldehyde
- 5) Ethyl acetate
- 6) n-Propanol
- 7) Iso-butanol
- 8) Iso-amyl acetate
- 9) Iso-amyl alcohol
- 10) Ethyl caprylate
- 11) Furfural
- 12) Ethyl caprate
- 13) Ethyl laurate
- 14) Phenethyl alcohol
- 15) Ethyl caproate
- 16) Ethyl lactate
- 17) Acetic acid

A-2.1.4 Preparation of Standard Mixture

Transfer accurately a known quantity of about 5.0 g of the reagents listed from A-2.1.3(3) to A-2.1.3(17) in to different 100 ml volumetric flasks and dilute to 100-ml with 40 percent (v/v) ethanol (methanol-free). Transfer 1.0 ml of each of the resulting solutions into a 100-ml volumetric flask and dilute to volume with 40 percent (v/v) ethanol (methanol-free). This solution will give approximately 500 ppm of each of component listed above.

A-2.1.4.1 Preparation of working standard mixture

Transfer 5 ml of standard mixture {see A-2.1.4} into a 10-ml stoppered test tube, add 1 ml of internal standard solution [see A-2.1.3(1)] and mix well.

A-2.1.5 Procedure

Transfer 5 ml of sample into a 10-ml stoppered

test tube, add 1 ml of n-pentanol internal standard solution and mix well. Inject 2µl of working standard mixture solution into chromatograph and record the chromatogram. Adjust the operating parameters and attenuation to obtain measurable peaks (at least 25 percent of full-scale deflection). Determine the retention time of methanol and n-pentanol ethanol. Inject 2µl sample solution into chromatograph and record the chromatogram (adjust attenuation, if necessary).

NOTE — Identify the individual components by injecting respective component standard solutions to the gas chromatograph and record the retention times.

A-2.1.6 Calculation

Calculate the individual component in grams per 100 liters of absolute alcohol as follows:

$$\text{Individual component} = \frac{R_2 \times C \times D \times 1000 \times 100 \times 100}{R_1 \times S}$$

$$\text{Individual component} = \frac{R_2 \times C}{R_1}$$

where

R_2 = peak ratio of respective individual component (with respect to standard) to n-pentanol ethanol for sample solution;

C = concentration of respective individual component in standard solution, in g/ml; in g per 100 liters of absolute alcohol;

D = dilution factor for sample solution;

R_1 = peak ratio of respective individual component to n-pentanol ethanol for standard solution; and

S = ethanol content of liquor sample in percent (v/v).

ANNEX B

{Clause 2}

LIST OF REFERRED INDIAN STANDARDS

<i>IS No.</i>	<i>Title</i>	<i>IS No.</i>	<i>Title</i>
264 : 1976	Nitric acid — Specification <i>[second revision)</i>	2263 : 1979	Methods of preparation of indicator solutions <i>first</i> <i>revision)</i>
265 : 1987	Hydrochloric acid — Specification <i>[fourth revision)</i>	2302 : 1989	Tables for alcoholometry <i>rsf</i> <i>revisions</i>
266 : 1976	Specification 'for sulphuric acid <i>{third revision)</i>	3506 : 1989	Tables for alcoholometry (by Pyknometer method) <i>first</i> revirfon)
1070 : 1992	Reagent grade water — Specification <i>{third revision)</i>		

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Amendments Issued Since Publication

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