MANUAL OF METHODS

OF

ANALYSIS OF FOODS

ALCOHOLIC BEVERAGES

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
MINISTRY OF HEALTH AND FAMILY WELFARE
GOVERNMENT OF INDIA
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MANUAL OF METHODS FOR ANALYSIS OF ALCOHOLIC BEVERAGES

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Note: The test methods given in the manuals are validated/ standardized test methods. However, it would be the responsibility of the respective testing laboratory to confirm that the above methods are validated in its laboratory and gives proper result in their laboratory.

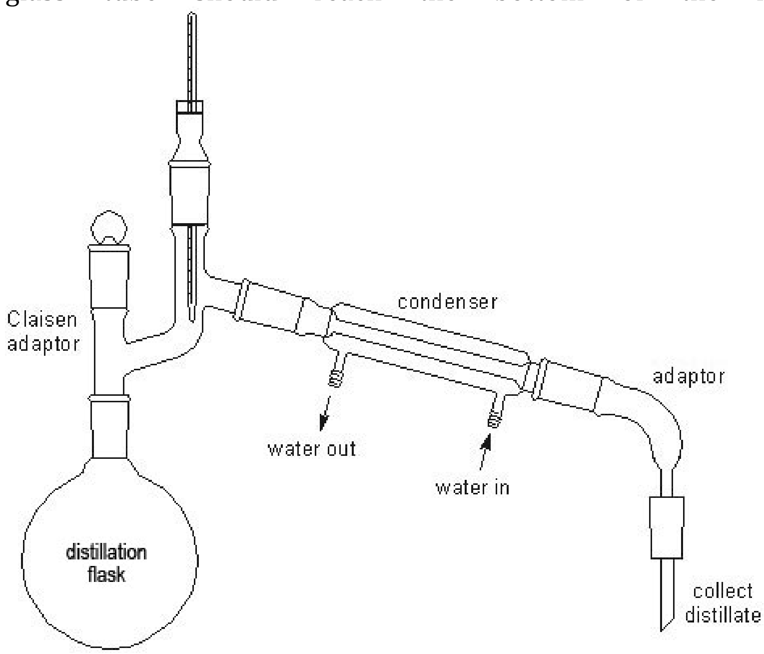
1.0 Types of Alcoholic Beverages

- Rum
- Gin
- Whisky
- Brandy
- Beer
- Vodka
- Wine
- Toddy
- Fenny (Cashew & Coconut) etc.

1.1 **General Apparatus and Glassware**

1. Beakers (different sizes)
2. Conical flasks with and without lids (different sizes)
3. Round bottom flasks (different sizes)
4. Pipettes (different sizes)
5. Burettes (different sizes)
6. Measuring cylinders (different sizes)
7. Buchner funnels (different sizes)
8. Air condensers
9. Water condensers
10. Distillation heads
11. Receiving adapters

12. Ground glass joints
13. Thermometers (different minimum and maximum temperatures in centigrade degrees)
14. Wash bottles (different sizes)
15. Separating funnels (different sizes)
16. Petri dishes (different sizes)
17. Weighing balances (upto milligram)
18. Weighing balances (upto gram)
19. Air Oven
20. Water bath
21. Whatman filter papers (different numbers)

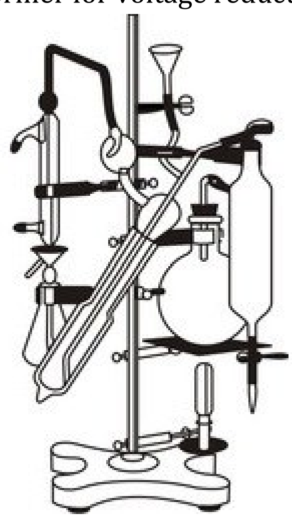
	2. Method for Determination of Ethyl alcohol content		
Method No.	**2.1**	**Revision No. & Date**	
Introduction/ Caution	Pycnometer Method or Hydrometer Method (after distillation)		
Principle	It is determined by distilling the alcoholic beverage and measuring the specific gravity of the distillate. Sp. gravity Vs Alcohol percent (Refer Annexure I).		
Apparatus	1. General Glass ware and apparatus (refer page 2). 2. Distillation Unit: Distillation flask of 500mL capacity is connected to water cooled condenser and the tip of the condenser is extended through a glass tube with a bulb by means of standard B14 joint. The other end of the glass tube should reach the bottom of the receiver flask.  3. Pycnometer: 50mL capacity/ SG Hydrometer, Short range (0.96 – 1.00). 4. Thermometer: 0-100 °C 5. Volumetric flask: 200mL capacity		
Chemicals	Alcoholic beverages		
Extraction/ Procedure	1. Transfer exactly 200mL of alcoholic drink into a 500mL distillation flask containing about 25mL of distilled water and a few pieces of pumice stone. 2. Distil the contents in about 35 min and collect the distillate in a 200mL volumetric flask till the volume almost reaches the mark. 3. Bring the distillate to room temperature 20°C and make up to volume with distilled water and mix thoroughly.		

	Find out the specific gravity of the distillate as follows: 4. Take a clean and dry pycnometer and weigh it empty along with the stopper at 20°C (W). 5. Fill it with the liquor sample distillate to the brim and insert the stopper gently. 6. Wipe the Liquid that spills out using water absorbing filter paper and weigh at 20°C (W1). 7. Next remove the liquor sample distillate and wash it with distilled water. 8. Fill the pycnometer with distilled water in the same manner as described above and at 20°C take the weight (W2).
Calculation	9. $$\text{Sp. gravity} = \frac{W1 - W}{W2 - W}$$ 10. Find out the corresponding alcohol percent by volume from the table showing Sp. gravity Vs Alcohol percent (Refer Annexure I). 11. Alternatively, use a SG hydrometer to find out the specific gravity (SG) and use the following equation to convert SG to % Alcohol. $$\%\text{Alcohol (v/v)} = 8610.6 - (16584 \times SG) + (7973.3 \times SG\ 2)$$ (One can use computer program to automate this process)
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of Ethyl alcohol content**		
Method No.	**2.2**	**Revision No. & Date**	
Introduction/ Caution	Distillation method (for products containing high volatile acids)		
Principle	Volatile acids were extracted into petroleum ether from the Sodium chloride saturated alcoholic beverage solution and aqueous alcoholic layer distilled and specific gravity of the distillate measured.		
Apparatus	1. General Glass ware and apparatus (refer page 2) 2. Volumetric flask, 200mL capacity 3. Separatory funnels, 500mL capacity 4. Distillation unit with assembly		
Chemicals	1. Alcoholic beverages 2. Sodium chloride 3. Petroleum ether 40- 60°C grade 4. Sodium hydroxide 5. Phenolphthalein indicator 6. Rectified spirit		
Preparation of reagents	1. Sodium hydroxide solution (0.1N): Sodium hydroxide (4gm) dissolved in 1 L water. 2. Phenolphthalein indicator solution - Dissolve 1.0 gm of phenolphthalein in 100mL rectified spirit.		
Procedure / Extraction	1. Measure 200mL of liquor sample in a volumetric flask. 2. Transfer to a separatory funnel and wash the volumetric flask with about 100mL water. 3. Add sodium chloride powder so that the solution becomes almost saturated with NaCl. 4. Add about 100 ml of petroleum ether and shake for 2-3 min. 5. Allow the layers to settle and transfer the lower layer to the distillation flask. 6. Add about 20-30mL of saturated sodium chloride solution to the petroleum ether layer and gently shake. 7. Allow again to settle and transfer the aqueous layer to the distillation flask. 8. Mix gently and make the solution just alkaline with NaOH solution using phenolphthalein indicator. 9. Add little pumice stone and connect the distillation assembly via condenser to the volumetric flask. 10. Distill gently and collect the distillate in the volumetric flask almost to the mark. 11. Bring the contents to room temperature and make up the volume with distilled water and mix well.		

Calculation	Determine the specific gravity of the distillate as described in earlier section and find out the corresponding alcohol percent by volume from the table showing Sp. gravity Vs Alcohol percent.
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

<table>
<tr><td></td><td colspan="3">Method for Determination of Ethyl alcohol content</td></tr>
<tr><td>Method No</td><td>2.3</td><td>Revision No. & Date</td><td></td></tr>
<tr><td>Introduction/ Caution</td><td colspan="3">Gas Chromatography-FID Method</td></tr>
<tr><td>Principle</td><td colspan="3">n-propanol internal standard is added to sample and ethanol is determined by GC - flame ionization detection.</td></tr>
<tr><td>Apparatus</td><td colspan="3">1. General Glass ware and apparatus (refer page 2).
2. Gas chromatograph - With the flame ionization detector and 6ft × 1/8in. (1.8m ×0.3cm) stainless steel or glass column containing 80-100 mesh chromosorb 103. He or N_2 carrier gas 20 ml/min; injector temperature 175^0C, column temperature 185^0C isothermal (adjust temperature so ethanol elutes in 1min, n-propanol in 1.6min); detector temperature 250^0C; chart speed and attenuation as required based on instrument used.</td></tr>
<tr><td>Chemicals</td><td colspan="3">1. Alcoholic beverages
2. n-Propanol
3. Ethanol</td></tr>
<tr><td>Preparation of reagents</td><td colspan="3">1. n-Propanol- Internal standard 5% aqueous stock solution. Refrigerate.
2. Ethanol standard solutions - 3, 4, 5, 6, 7, and 8% aqueous ethanol solutions. Determine exact % ethanol by pycnometer, hydrometer, or refractometer. Alternatively, prepare standard solutions by quantitative dilution of concentrated ethanol solution analyzed by one of above techniques. Keep solutions refrigerated.</td></tr>
<tr><td>Procedure / Extraction</td><td colspan="3">1. Pipet 5.0mL ethanol standard solutions into separate glass-stoppered flasks.
2. Add 5.0mL internal standard solution to each and mix well.
3. De-carbonate beer by filtering through S&S 560 or equivalent paper. Pipet 5.0mL into glass-stoppered flask. Add 5.0mL aqueous n-propanol internal standard solution. Mix thoroughly by swirling.</td></tr>
<tr><td>Column Chromatography</td><td colspan="3">1. Inject 0.2µL of each standard solution in duplicate and measure peak heights (integrator may be used). Calculate ratio of ethanol to n-propanol peaks and average for each concentration. Plot ratio against concentration and calculate slope of line (F). Repeat analysis of 5% ethanol standard solution each day.
2. Inject 0.2µL of beverage (prepared beer solution) onto GC column, and determine ratio of ethanol to n-propanol peaks.</td></tr>
<tr><td>Calculation</td><td colspan="3">Ethanol, % (v/v) = (peak height ethanol/ peak height n-propanol)</td></tr>
<tr><td>Reference</td><td colspan="3">AOAC 984.14</td></tr>
<tr><td>Approved by</td><td colspan="3">Food Authority based on recommendation of Scientific Panel</td></tr>
</table>

	Method for Determination of Ethyl alcohol content
Method No.	**2.4** | **Revision No. & Date** |
Introduction/ Caution	Dichromate oxidation method
Principle	Wine is steam distilled into acidified $K_2Cr_2O_7$ solution of known volume and concentration. Oxidation of ethyl alcohol to CH_3COOH is completed by heating. Unreacted dichromate is determined by titration with standard $Fe(NH_4)_2(SO_4)_2$ solution, using o-phenanthroline as indicator.
Apparatus	1. General Glass ware and apparatus (refer page 2). 2. Micro Kjeldahl apparatus with gas micro-burner. Alternatively, Kirk-type electric apparatus may be used. Apparatus must have 3 way stopcock or tee with pinch clamps attached to drain line of still to allow filling of outer chamber with distilled water. Connect electric outlet of still to variable transformer for voltage reduction. 
Chemicals	1. Alcoholic beverages. 2. Potassium dichromate. 3. Sulphuric acid. 4. Ferrous ammonium sulfate. 5. 1,10-Phenanthroline. 6. Ferrous sulfate
Preparation of reagents	1. Potassium dichromate solution-Add 325mL H_2SO_4 to ca 400mL H_2O in 1 L volumetric flask. Mix and cool to 80°- 90°C. Add 33.768gm $K_2Cr_2O_7$ (primary standard). Dissolve, cool, and dilute to volume with H_2O at 20°C. 2. Ferrous ammonium sulfate solution - Dissolve 135.5gm

	$FeSO_4(NH_4)_2SO_4\cdot6H_2O$ in ca 500 mL H_2O in 1 L volumetric flask. Add 30 mL H_2SO_4, Dilute to volume with H_2O at 20°C. 3. 1,10-Phenanthroline ferrous sulfate indicator.-Dissolve 0.695gm $FeSO_4.7H_2O$ in ca 50 mL H_2O, add 1.485gm o-phenanthroline·H_2O, and dilute to 100 mL with H_2O.
Procedure / Extraction	**By micro Kjeldahl apparatus** 1. To begin distillation, boil H_2O in steam generator. Open steam trap side tube. Turn 3-way stopcock so that steam from trap vents through side tube and distilling bulb is closed. 2. Place 25mL $K_2Cr_2O_7$ solution in 50mL Erlenmeyer under condenser with tip below surface of solution, Close stopcock and place small amount H_2O in funnel. Distilling bulb is empty and micro-burner is not lighted. Transfer 1 mL test portion as follows: Fill 1 mL pipet (class A) slightly over mark, and wipe excess wine from exterior. Hold pipet vertical with tip touching inside neck of test bottle, drain to mark. Drain pipet completely into funnel. Open stopcock to drain test portion into still then reclose. Add small amount H_2O to funnel, drain into still, and rinse with H_2O until distilling bulb is half filled. 3. Place H_2O in funnel to ensure seal. Close steam trap discharge with pinch clamp. Open 3-way stopcock, permitting steam to enter bulb while vent is closed. Light micro-burner. 4. Distil until receiving flask contains ca 40mL, lower flask, and rinse outside of condenser outlet into flask with H_2O. 5. Stopper flask and immerse to shoulder in 60°±2°C H_2O. Admit cold water into steam generator to flush contents of distilling bulb into steam trap. 6. Refill bulb with H_2O, flush again, open trap discharge, and vent 3-way stopcock. Apparatus is now ready for next test portion. **By electric apparatus** 1. Connect electric outlet of apparatus to variable transformer set at ca 60-70% line voltage. Open condenser stopcock to let cold water flow through condenser. 2. Fill outer chamber of still with distilled water to well above heating coil by opening 3-way stopcock or pinch clamp on drain line tee to distilled H_2O source. 3. Transfer 1mL test portion by filling 1mL pipet and place pipet tip in contact with inside of funnel with stopcock closed and with funnel containing small amount distilled H_2O so that pipet tip rests just above H_2O. Let pipet drain 15 sec after discharge of test portion. 4. Open stopcock and drain test portion-H_2O mixture into inner chamber of still then close stopcock. Add small amount H_2O to funnel, and then drain into inner chamber of still. 5. Close stopcock and add H_2O to funnel to ensure seal. Place 25mL $K_2Cr_2O_7$ solution in 50mL Erlenmeyer placed under condenser so that tip of condenser is below surface of solution. 6. Turn on variable transformer and steam distils until receiving flask

	contains ca 40mL. 7. Lower flask, and rinse outside of condenser outlet with distilled water, letting rinse drain into flask. Stopper flask and immerse to shoulder in 60° ± 2°C H_2O. 8. Turn off variable transformer. 9. Residue in inner chamber is flushed out to outer chamber automatically by vacuum action when current is shut off. 10. Open funnel stopcock and add distilled water; close to rinse inner chamber into outer chamber and drain line again by vacuum. Repeat with second rinse. 11. Open 3-way stopcock or pinch clamp on drain line tee to drain outer chamber. Close, then open to distilled water source and fill outer chamber as before. Apparatus is now ready for next test portion. **Titration** 1. Remove flask from bath after 20-25 min. 2. Rinse contents into 500mL flask with H_2O. 3. Titrate with $FeSO_4(NH_4)_2SO_4$ solution to almost clear green in front of daylight fluorescent light, add 3 drops indicator, and titrate to end point (change is from blue-green to brown) (*V* mL). 4. Since $FeSO_4(NH_4)_2SO_4$ solution is slowly oxidized by air, perform a blank determination daily by titrating 25mL $K_2Cr_2O_7$ (*V'* ml). Discard $FeSO_4(NH_4)_2SO_4$ solution that has been standing in buret >30 min.
Calculation	Calculate % alcohol by volume = 25.00 - (25 x *V/V'*).
Reference	AOAC 969.12
Approved by	Food Authority based on recommendation of Scientific Panel

<table>
<tr><td></td><td colspan="3">Method for Determination of Ethyl alcohol content</td></tr>
<tr><td>Method No.</td><td>2.5</td><td>Revision No. & Date</td><td></td></tr>
<tr><td>Introduction/ Caution</td><td colspan="3">Gas Chromatography</td></tr>
<tr><td>Principle</td><td colspan="3">Ethyl alcohol content is determined by mixing known internal standard and injecting to GC. Peak responses of ethyl alcohol and internal standard are compared and determined.</td></tr>
<tr><td>Apparatus</td><td colspan="3">1. General Glass ware and apparatus (refer page 2).
2. Gas chromatograph - With flame ionization detector, integrator, heated on-column injector, and 6 ft (1.8 m) x 2mm id glass column packed with 0.2% Carbowax 1500 on 80-100 mesh Carbopack C.
3. Diluter -Capable of ± 0.1% precision.</td></tr>
<tr><td>Chemicals</td><td colspan="3">1. Alocoholic beverages
2. 2-propanol
3. Ethanol</td></tr>
<tr><td>Preparation of reagents</td><td colspan="3">1. Internal standard solution - 0.2% (v/v) 2-propanol in H_2O.
2. Alcohol standard solution.-Prepare alcohol-H_20 solution containing approximate % alcohol expected in test portion. Determine exact % alcohol by pycnometer, refractometer, hydrometer or other appropriate AOAC method, or use Standard Reference Material 1590, Stabilized Wine (NIST).</td></tr>
<tr><td>Procedure / Extraction</td><td colspan="3">1. Dilute alcohol standard solution 1: 100 with internal standard solution.
2. Inject at least three 1.0 µL aliquots, after adjusting the air and carrier has flow rates as well as electrometer sensitivity as mentioned below and determine average response ratio of area of alcohol peak to area of 2-propanol peak (RR').
3. Dilute test portion 1: 100 with internal standard solution.
4. Inject 1.0 µL, and determine response ratio (RR).</td></tr>
<tr><td>Column Chromatography</td><td colspan="3">1. Adjust air and H_2 for flame detector to optimum for carrier gas flow of column used. Adjust electrometer sensitivity to provide ≥50,000 counts of integrator counts for internal standard peak.
2. Gas chromatograph specifications:
<table>
<tr><td>Carrier gas</td><td>N_2</td></tr>
<tr><td>Flow rate, ml/min</td><td>15</td></tr>
<tr><td>Oven temperature</td><td>105° C</td></tr>
<tr><td>Injector temperature</td><td>175° C</td></tr>
<tr><td>Detector temperature</td><td>175° C</td></tr>
</table></td></tr>
<tr><td>Calculation</td><td colspan="3">$$\text{Alcohol, \%} = \frac{RR \times \text{\%alcohol in standard}}{RR'}$$</td></tr>
<tr><td>Reference</td><td colspan="3">AOAC 983.13</td></tr>
<tr><td>Approved by</td><td colspan="3">Food Authority based on recommendation of Scientific Panel</td></tr>
</table>

	3. Method for Determination of Residue on evaporation		
Method No.	**3.0**	**Revision No. & Date**	
Introduction/ Caution	Organic or inorganic solids present in alcoholic beverages are residues. It may include high boiling liquids also.		
Principle	By evaporation of beverages on boiling water bath, residue is determined.		
Apparatus	1. General Glass ware and apparatus (refer page 2) 2. Hot Air oven 3. Water bath 4. Desiccator 5. Glass bowl, 250mL capacity 6. Volumetric flask, 200mL		
Procedure / Extraction	1. Transfer 200mL of alcoholic drink into a dried, weighed (W) glass bowl and evaporate on a water bath. 2. Wipe the external sides of the bowl and keep in an air oven maintained at 100 ± 10^{o}C for 2 hrs. 3. Cool in a desiccator and weigh the dish (W1). 4. Repeat till constant weight is obtained. Calculate the % residual solids.		
Calculation	Residue on evaporation % (w/v) = $\frac{W1 - W}{V} \times 100$ Where, W1 = weight of glass bowl with dry residue, in gm W = weight of empty glass bowl, in gm V = volume of liquor taken for the estimation, in mL		
Reference			
Approved by	Food Authority based on recommendation of Scientific Panel		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**4. Method for Determination of Total acidity**
Method No.	**4.1** **Revision No. & Date**
Introduction/ Caution	Method I (For Colourless Liquors)
Principle	Total acids present in alcoholic beverages are estimated using acid –base titration using phenolphthalein as indicator.
Apparatus	1. General Glass ware and apparatus (refer page 2)
Chemicals	1. Sodium hydroxide 2. Phenolphthalein indicator 3. Rectified spirit
Preparation of reagents	1. Sodium hydroxide solution (0.05N): Sodium hydroxide (2gm) dissolved in 1 L water. 2. Phenolphthalein indicator solution - Dissolve 1.0gm of phenolphthalein in 100mL rectified spirit.
Procedure / Extraction	1. Take 50mL of liquor sample and add about 200mL neutral distilled water. 2. Titrate against standard sodium hydroxide using Phenolphthalein indicator.
Calculation	Total acidity as tartaric acid, gms. per 100 liters of abs. alcohol = $\frac{V \times 0.00375 \times 100 \times 1000 \times 2}{V1}$ Where, V1 = alcohol % by volume V = volume of std. NaOH used for titration, in mL
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

	Method for Determination of Total acidity		
Method No.	4.2	Revision No. & Date	
Introduction/ Caution	Method II (For Coloured Liquors such as Wine, Toddy)		
Principle	Total acids present in alcoholic beverages are estimated using acid –base titration using pH meter.		
Apparatus	1. General Glass ware and apparatus (refer page 2) 2. pH Meter 3. Magnetic stirrer 4. Beaker 250 mL capacity		
Chemicals	1. Alcoholic beverages 2. Sodium Hydroxide (NaOH) 3. Buffer solutions of pH 4.0, 7.0 and 9.2		
Preparation of reagents	1. Sodium hydroxide solution (0.05N): Sodium hydroxide (2gm) dissolved in 1 L water.		
Procedure / Extraction	1. Calibrate and standardize the pH meter using the buffer solutions of pH 4.0, 7.0 and 9.2. 2. Take approximately 100mL of distilled water in a beaker and put a magnetic bead and place the beaker on a magnetic stirrer. 3. Carefully immerse the electrode of the pH meter into the water and titrate against standard NaOH solution to pH 8.2. Now add 50mL of liquor sample to the pH adjusted water and titrate to pH 8.2. Note down the volume of NaOH required (The wine sample may be initially degassed by stirring and heating to 90°C to remove carbon dioxide).		
Calculation	For wines: Total acidity as tartaric acid gms. per liter of wine / toddy $= \frac{V \times 0.00375 \times 1000}{V1}$ Where, V1 = Volume of wine taken for estimation V = Volume of std. NaOH used for titration, in ml **Note:** 1 mL of 0.05N NaOH is equivalent to 0.00375 gm of tartaric acid		
Reference			
Approved by	Food Authority based on recommendation of Scientific Panel		

fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**5. Method for Determination of Volatile acidity**		
Method No.	**5.0**	**Revision No. & Date**	
Introduction/ Caution	Volatile acids present in alcoholic beverages are estimated		
Principle	Alcoholic beverages are distilled and the volatile acids present, in the distillate are estimated.		
Apparatus	General Glass ware and apparatus (refer page 2)		
Chemicals	1. Sodium Hydroxide 2. Phenolphthalein indicator 3. Rectified spirit		
Preparation of reagents	1. Sodium hydroxide solution (0.05N): Sodium hydroxide (2gm) dissolved in 1 L water. 2. Phenolphthalein indicator solution - Dissolve 1.0gm of phenolphthalein in 100mL rectified spirit.		
Procedure / Extraction	1. Take 50mL distillate collected during the determination of ethyl alcohol for volatile acidity determination (Method 2.1) 2. Titrate against std. NaOH using phenolphthalein indicator		
Calculation	1. For liquors: Volatile acidity as acetic acid, gms. per 100 liters of abs. alcohol $= \frac{V \times 0.003 \times 100 \times 1000 \times 2}{V1}$ Where, V = volume of std. NaOH used for titration, in mL V1 = alcohol % by volume 2. For wines: Volatile acidity as acetic acid gms. per liter of wine $= \frac{V \times 0.003 \times 1000}{V1}$ Where, V1 = Volume of wine taken for estimation V = volume of std. NaOH used for titration, in mL **Note:** 1 mL of 0.05N NaOH is equivalent to 0.003gm of acetic acid.		
Reference			
Approved by	Food Authority based on recommendation of Scientific Panel		

	6. Method for Determination of Esters		
Method No.	**6.1**	**Revision No. & Date**	
Introduction/ Caution	Esters present in the alcoholic beverages are determined.		
Principle	Esters present in the neutralized alcoholic beverages are hydrolyzed and estimated.		
Apparatus	General Glass ware and apparatus (refer page 2)		
Chemicals	1. Alcoholic beverages 2. Sodium Hydroxide 3. Sulphuric acid		
Preparation of reagents	1. Sodium hydroxide solution (0.1N): Sodium hydroxide (4gm) dissolved in 1 L water. 2. Standard Sulphuric acid, 0.1N: Sulphuric acid (4.9gm) dissolved in 1L water.		
Procedure / Extraction	1. To the neutralized distillate from the volatile acidity determination (Sec. 5.0.), add 10ml of Std. NaOH and reflux on a steam bath for 1hour. 2. Cool and back titrate the unspent alkali against standard sulphuric acid. 3. Carry out a blank simultaneously taking 50ml of distilled water instead of distillate in the same way. 4. The difference in titer value in milliliters of standard sulphuric acid gives the equivalent ester.		
Calculation	Esters expressed as ethyl acetate, gms. per 100 liters of abs. alcohol $= \frac{V \times 0.0088 \times 100 \times 1000 \times 2}{V1}$ Where, V = difference of titer value of std.H_2SO_4 used for blank and sample, in ml V1 = alcohol % by volume. **Note:** 1 mL of 0.1N NaOH is equivalent to 0.0088gm of Ethyl acetate.		
Reference			
Approved by	Food Authority based on recommendation of Scientific Panel		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of Esters**

Method No.	**6.2**	**Revision No. & Date**	
Introduction/ Caution	By Gas chromatography		
Principle	Sample peak areas in GC are compared with that of standards and esters are determined.		
Apparatus	1. General Glass ware and apparatus (refer page 2). 2. **Gas chromatography -** Gas chromatography 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 25m length, 0.32mm ID and 0.30µ film thickness. 3. **Syringe -** 10µL; Hamilton Co. No. 701, or equivalent.		

Chemicals

S. No.	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	t-Amyl alcohol
11	n-Butyl acetate
12	Ethyl propionate
13	n-Proponol
14	Iso-butanol
15	Iso-amyl acetate
16	Phenyl acetate
17	Caprylic acid
18	n-Butanol
19	Iso-amyl alcohol
20	Ethyl caprylate
21	Furfural
22	Ethyl caprate
23	Ethyl laurate
24	Phenethyl alcohol
25	Ethyl palmitate
26	Isovaleric acid
27	Ethyl caproate

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<tr><td>29</td><td>Ethyl lactate</td></tr>
<tr><td>30</td><td>Acetic acid</td></tr>
<tr><td>31</td><td>Isobutyric acid</td></tr>
<tr><td>32</td><td>Ethyl myristate</td></tr>
<tr><td>33</td><td>Pelargonic acid</td></tr>
<tr><td>34</td><td>Capric acid</td></tr>
<tr><td>35</td><td>Diacetyl</td></tr>
</table>
</td></tr>
<tr><td>Preparation of reagents</td><td>Preparation of standard mixture
1. Transfer accurately a known quantity of about 5.0gm of reagents listed from (3) to (35) into different 100mL volumetric flasks and dilute to 100mL with 40 percent (v/v) ethanol (methanol-free).
2. Transfer 1.0mL of each of the resulting solutions into a 100mL volumetric flask and dilute to volume with 40percent (v/v) ethanol (methanol-free).
3. This solution will give approximately 500ppm of each of component listed above.
Preparation of working standard mixture
4. Transfer 5mL of standard mixture into a 10mL stoppered test tube. Add 1mL of internal standard solution (1) and mix well.</td></tr>
<tr><td>Preparation of Test Samples</td><td>Transfer 5mL of sample into a 10mL stoppered test tube, add 1mL of n-pentanol internal standard solution and mix well.</td></tr>
<tr><td>Column Chromatography</td><td>Gas chromatography and operating parameters.
1. The split ratio will be approximately 1:40 with nitrogen or helium as a carrier gas at the flow rate of about 1.7mL/min.
2. The detector and injector port temperatures may be maintained at about 250^{0}C.
3. Keep the oven temperature at 45^{0}C for 4min, raise to 100^{0}C at the rate of 10^{0}C/min and finally to 200^{0}C for 10min at the rate of 15^{0}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.
4. Inject 2µL of working standard mixture solution into chromatograph and record the chromatogram.
5. Adjust the operating parameters and attenuation to obtain measurable peaks (at least 25 percent of full-scale deflection).
6. Determine the retention time of methanol and n-pentanol.
7. 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.</td></tr>
<tr><td>Calculation</td><td>Calculate the individual component in gram per 100 litres of absolute alcohol as follows:
Individual component = $\frac{R_2 x C x D x 1000 x 100 x 100}{R_1 x S}$</td></tr>
</table>

	Where, R_2- peak ratio of respective individual component (with respect to standard) to n-pentanol for sample solution; C- concentration of respective individual component in standard solution, in g/mL; D- dilution factor for sample solution; R_1- Peak ratio of individual component to n-pentanol for standard solution; and S- ethanol content of liquor sample in percent(v/v).
Reference	IS 3752:2005, AOAC 968.09
Approved by	Food Authority based on recommendation of Scientific Panel

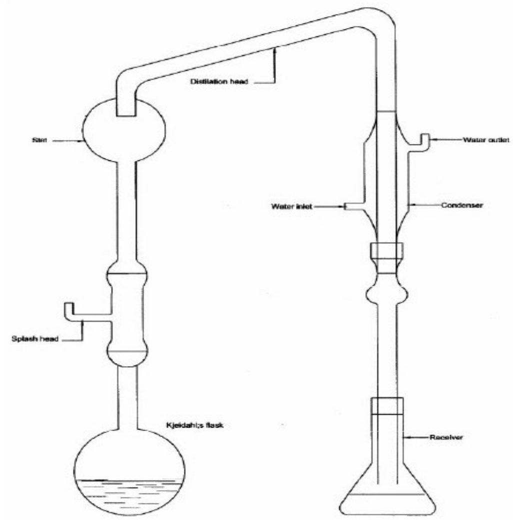
fssai FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of esters**		
Method No.	**6.3**	**Revision No. & Date**	
Introduction/ Caution	Routine Gas Chromatographic Method		
Principle	Sample peak areas in GC are compared with that of standards and esters are determined.		
Apparatus	1. General Glass ware and apparatus (refer page 2). 2. **Gas chromatograph** – Gas chromatograph equipped with flame ionization detector and packed inlet and fixed with a glass column packed with 5 percent Carbowax 20M on carbopak B, 80/120mesh or equivalent packed columns like porapak- Q having the dimensions of 2m in length and 4mm in ID. 3. **Syringe**- 10μL, Hamilton Co. No. 701, or equivalent.		
Chemicals	(see table below)		

S. No.	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-Proponol
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 caporate
16	Ethyl lactate
17	Acetic acid

Preparation of reagents	**Preparation of standard mixture** 1. Transfer accurately known quantity of about 5.0gm of the reagents listed from (3) to (17) in to different 100 ml volumetric flasks and dilute to 100mL with 40 percent (v/) ethanol(methanol-free). 2. Transfer 1.0mL of each of the resulting solutions into a 100ml volumetric flask and dilute to volume with 40 percent (v/v) ethanol (methanol-free). 3. This solution will give approximately 500ppm of each of component listed above.

	Preparation of working standard mixture 4. Transfer 5mL of standard mixture into a 10mL stoppered test tube, add 1ml of internal standard solution (1) and mix well.
Preparation of Test Samples	Transfer 5mL of sample into a 10mL stoppered test tube, add 1mL of n-pentanol internal standard solution and mix well.
Column Chromatography	**Gas chromatograph and operating parameters** 1. Nitrogen or helium may be used as carrier gas at suitable flow rate. 2. The detector and injector port temperatures may be maintained at about 250^0 C. 3. Keep the oven temperature at 45^0C for 4min, raise to 100^0C at the rate of 10^0C/min and finally to 200^0C for 10 min at the rate of 15^0C/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.* 4. Inject 2µL of working standard mixture solution into chromatograph and record the chromatogram. 5. Adjust the operating parameters and attenuation to obtain measurable peaks (at least 25 percent of full-scale deflection). 6. Determine the retention time of methanol and n-pentanol. 7. Inject 2µl sample solution into chromatograph and record the chromatogram (adjust attenuation, if necessary). *Note: - Identify the individual components by injecting respective components standard solutions to the gas chromatograph and record the retention times.*
Calculation	Calculate the individual component in grams per 100 litres of absolute alcohol as follows: Individual component = $\frac{R_2 x C x D x 1000 x 100 x 100}{R_1 x S}$ Where, R_2- peak ratio of respective individual component (with respect to standard) to n-pentanol for sample solution; C- Concentration of respective individual component in standard solution, in g/mL; D- dilution factor for sample solution; R_1- Peak ratio of individual component to n-pentanol for standard solution; and S- ethanol content of liquor sample in percent(v/v).
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**7. Method for Determination of higher alcohols**
Method No.	**7.1** | **Revision No. & Date** |
Introduction/ Caution	Extraction/ Titrimetric Method
Principle	Higher alcohols separated by carbontetrachloride, after satuarion with sodium chloride. Higher alcohols fraction is oxidized using oxidation reagent and formed acid is titrated against alkali and estimated.
Apparatus	1. General Glass ware and apparatus (refer page 2) 2. Separatory funnel, 250mL 3. Volumetric flask, 1L capacity 4. Distillation assembly having Kjeldhal flask, 800mL capacity; With splash head, Liebig condenser, Receiver of capacity 250mL



Chemicals	1. Sulphuric acid GR grade 2. Potassium dichromate 3. Standard NaOH, 0.1N 4. Carbon tetrachloride GR grade, distilled 5. Sodium chloride GR grade 6. Sodium sulphate, AR grade 7. Phenolphthalein indicator
Preparation of reagents	1. Oxidizing mixture - Dissolve Potassium dichromate, 100gm in 500mL distilled water and add sulphuric acid, 100mL and make up to 1L volume with distilled water. 2. Sodium hydroxide solution (0.1N): Sodium hydroxide (4gm) dissolved in 1 L water. 3. Phenolphthalein indicator solution - Dissolve 1.0gm of phenolphthalein in 100mL rectified spirit.
Procedure/ Extraction	1. Transfer the solution obtained from the determination of esters (sec 6.1) into a separatory funnel and add 50mL of distilled water. 2. Saturate it with sodium chloride and extract four times with

	successive portions of 40, 30, 20 and 10mL of carbon tetrachloride. 3. Pool all the extracts and wash 3 times with saturated sodium chloride solution and twice with saturated sodium sulphate solution. 4. Filter the extract and add 50mL of oxidizing mixture. Reflux for 2 hours, cool and wash the reflux with 50mL of distilled water. 5. Transfer it to the distillation assembly using 50mL of water. Distil about 100mL and see that no charring takes place. 6. Titrate the distillate against standard NaOH using phenolphthalein indicator. 7. Run a blank in the same way taking 50mL of distilled water in place of the distillate of the liquor.
Calculation	Higher alcohol expressed Amyl alcohol, in gms. Per 100 liters of abs. alcohol $= \frac{V \times 0.0088 \times 100 \times 1000 \times 2}{V_1 \times V_2}$ Where, V = difference of titer value of Std. alkali used for blank and sample, in mL V_1 = Volume of sample taken for estimation V_2 = alcohol % by volume **Note:** 1 mL of 0.1N NaOH is equivalent to 0.0088gm of Amyl alcohol
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of higher alcohols**		
Method No.	**7.2**	**Revision No. & Date**	
Introduction/ Caution	Spectrophotometric method		
Principle	Higher alcohols react with p–dimethylaminobenzaldehyde in sulphuric acid and forms coloured compounds. Quantity of alcohols is determined by measuring the absorbance at relevant wavelength		
Apparatus	1. General Glass ware and apparatus (refer page 2) 2. Spectrophotometer, double beam 3. Steam bath 4. Test tube, stoppered, 15mL capacity		
Chemicals	1. Alcoholic beverages 2. p-dimethylaminobenzaldehyde 3. Sulphuric acid 4. Iso-butyl alcohol, GR grade 5. Iso-amyl alcohol, GR grade 6. Ethyl alcohol, redistilled, middle 50% fraction.		
Preparation of reagents	1. p-dimethylaminobenzaldehyde solution – Dissolve 1gm in a mixture of 5mL sulphuric acid and 90mL distilled water and transfer to a 100mL volumetric flask and make up to the mark. **Preparation of Synthetic standard of higher alcohols** 2. Weigh 2gm isobutyl alcohol and 8gm iso-amyl alcohol into 1L volumetric flask and dilute to mark with water. 3. Pipette two 10mL portions into 100mL volumetric flasks and dilute to mark, one with water and other with ethyl alcohol. 4. Prepare working standards for products in the range of 1.0 to 6.0gm synthetic higher alcohol per 100L by diluting 1.0 to 6.0mL aliquots of alcohol standards solution to 100mL with alcohol solution. (Solution containing 6mL synthetic standard would give an absorbance of 0.83±0.03 at 530 nm).		
Preparation of Test Samples	Preparation of sample: 1. Transfer 200mL of alcoholic drink into a 500mL distillation flask containing about 25mL of distilled water and a few pieces of pumice stone. 2. Distil the contents in about 35 min and collect the distillate in a 200mL volumetric flask till the volume almost reaches the mark. 3. Bring the distillate to room temperature and make up to volume with distilled water and mix thoroughly. 4. For samples containing 6gm fusel oil per 100L, dilute the distilled sample with distilled water to concentrations of 2.0 to 5.0 g/100L.		
Procedure / Extraction	Determination: 1. Pipette 2 mL of aliquot of sample (or diluted sample), 2 mL of distilled water (for reagent blank) and 2 mL of synthetic standard to each of the test tubes (15mm x 150mm-with stoppers).		

	2. Stopper and place it in ice-bath in a rack. 3. Pipette 1mL p-dimethylaminobenzaldehyde solution into each tube; shake and replace in ice-bath for 3min. 4. With tubes retained in ice- bath, add 10mL sulphuric acid and shake the tubes and replace in ice-bath for 3 min. 5. Transfer the rack containing tubes into steam bath for 3 to 5 min. and bring it to room temperature. 6. Read the % T or Absorbance (OD) of developed colour of samples and series of standards in spectrophotometer at 530/535 nm against reagent blank as reference. 7. Plot higher alcohol g/100 L Concentrations of Standards Vs. %T or OD. 8. From the OD of the sample find out the concentration of Higher alcohol g/100L using the standard curve.
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of higher alcohols**		
Method No.	**7.3**	**Revision No. & Date**	
Introduction/ Caution	Gas chromatography		
Principle	Quantity of alcohols determined using similar procedure as per the esters (pages 18-20) using standard reference materials of alcohols.		
Reference	(IS 3752:2005, AOAC 968.09) (See sec 6.2 pages 18-20)		
Approved by	Food Authority based on recommendation of Scientific Panel		

	Method for Determination of higher alcohols		
Method No.	**7.4**	**Revision No. & Date**	
Introduction/ Caution	Routine Gas Chromatographic Method (See sec 6.3 pages 21-22) Quantity of alcohols determined using similar procedure as per the esters using standard reference materials of alcohols.		
Reference			
Approved by	Food Authority based on recommendation of Scientific Panel		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of higher alcohols**		
Method No.	**7.5**	**Revision No. & Date**	
Introduction/ Caution	Gas Chromatographic method using calibration curves of standards		
Principle	Calibration curves are prepared using GC responses of known concentration of authentic standards. These are used to determine higher alcohols.		
Apparatus	1. General Glass ware and apparatus (refer page 2). 2. Gas chromatograph- Equipped with flame ionization detector. 3. Column- 2% glycerol and 2% 1, 2, 6-hexanetriol. Pack 3m (10ft) × 3mm (1/8in.) od tube. Condition overnight in 80^0C column oven with the flow rate of 10-25ml/min and detector end of column disconnected.		
Chemicals	1. Alcoholic beverages. 2. Absolute alcohol (ethanol); (Use absolute alcohol throughout when alcohol is specified.). 3. n-propyl alcohol. 4. Isobutyl alcohol. 5. Amylalcohol. 6. 3-Pentanol. 7. Ethyl acetate.		
Preparation of reagents	1. Amyl alcohol - Mixture of active-amyl and isoamyl alcohols, ca 22 and 78%, respectively. Concentration composition of reagent (c). Measure areas of 2 peaks by triangulation (height × width at half height), and obtain concentration of each by dividing area of each peak by sum of both peak areas. 2. 3-Pentanol internal standard solution- 40.76mg/mL. Prepare solution containing 10mL reagent in 200mL alcohol-H_2O (1+1) 3. n-Propyl alcohol, isobutyl alcohol, and amyl alcohol standard solutions - Prepare 3 or 4 standard solutions containing varying amounts alcohols as follows: Into tared 100mL volumetric flasks containing alcohol-H_2O (1+1), pipet fusel alcohols and weigh after addition of each component. Proportions of fusel alcohols in each standard solution should vary so that desired concentration range of each is represented in random manner in series of standard solutions. Suggested amounts: 0.25-1.5mL n-propanol, 1.0-2.5 mL isobutyl alcohol, and 2.0-5.0mL amyl alcohol. Dilute each volume with alcohol- H_2O (1+1). 4. n-Propyl alcohol, isobutyl alcohol, and amyl alcohol working standard solution- Dilute 10ml each standard solution and 2.0mL 3-pentanol internal standard solution to 200ml with alcohol- H_2O(1+1) (1:20 dilution). 5. Ethyl acetate standard solutions- Prepare 3 or 4 standard solutions containing 0-0.5 g/l (0-50 g/100L) in water or alcohol- H_2O (1+1). Use for preparing direct standard curve by plotting peak height (mm) against concentration in g/100 L.		

Column Chromatography	Approximate parameters 1. Column, injector and detector temperatures (^{0}C)—80, 100, and 125, respectively; gas flows (ml/min) - He carrier and H 25, air 250-400; attenuation 64×. 2. Optimum operating conditions vary with column and instrument and must be determined by using standard solutions. Adjust parameters for maximum peak sharpness and optimum separation. Analysis is complete in Ca 11 min. Determination 3. Pipet 10mL test portion into convenient vessel (e.g, 1oz French square glass bottle with screw cap), add, by pipet (0.2mL pipet graduated in 0.01mL), 0.1mL 3-pentanol internal standard solution, and mix. 4. Inject 2µL test portion and working standard solutions. 5. Measure peak height of each component in working standard solutions and calculate peak height ratio of each to internal standard. 6. Calculate concentration ratio of each by dividing weight of component by that of internal standard. (Proportion of active-amyl and isoamyl alcohols in mixture must be taken into consideration in calculations of actual weights of each isomer in working standard solutions.) 7. Plot concentration ratios (horizontal axis) against peak height ratios (vertical axis) for each higher alcohol in all working standards to obtain family of curves. 8. For ethyl acetate, plot peak height directly against concentration. 9. Similarly, measure peak height of each component on test portion chromatogram and calculate peak height ratios. 10. Read concentration ratios of all alcohols, using proper standard curve. 11. Multiply concentration ratio of each fusel alcohol in test portion by 40.76 to obtain g/100L. New standard curves need be prepared only when new instruments, parameters, or standards are used.
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**8. Method for Determination of Aldehydes**		
Method No.	**8.1**	**Revision No. & Date**	
Introduction/ Caution	Titrimetric method		
Principle	Aldehydes react with sodium bisulphate and forms adducts. These adducts react with iodine. Excess iodine is titrated and determined. Consumed iodine is correlated with aldehyde content and determined		
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Iodine flask, 250mL capacity 3. Burette, 25/50mL capacity		
Chemicals	1. Sodium bisulphite solution 2. Iodine standard solution 3. Sodium thiosulphate standard 4. Starch indicator		
Preparation of reagents	1. Sodium bisulphite solution (0.05N) – Sodiumbisulphite (2.6gm) dissolved in 1000mL water. 2. Iodine standard solution – 0.05 N. 3. Sodium thiosulphate standard (0.05 N) – Sodium thiosulphate (12.4gm) dissolved in 1000mL water. 4. Starch indicator (1%) – starch (1gm) is dissolved in 100mL water.		
Procedure / Extraction	1. Take 50mL of distillate of liquor (Sec. 2.1) in a 250mL Iodine flask and add 10mL of bisulphite solution. Keep the flask in a dark place for 30 min. with occasional shaking. 2. Add 25mL of standard iodine solution and back titrate excess iodine against standard thiosulphate solution using starch indicator to light green end point. 3. Run a blank taking 50mL of distilled water in the same way. 4. The difference in titer value in milliliters, of sodium thiosulphate solution gives the equivalent aldehyde content.		
Calculation	Aldehydes expressed acetaldehyde, gms. per 100 liters of abs. alcohol $= \dfrac{V \times 0.0011 \times 100 \times 1000 \times 2}{V_1}$ Where, V_1 = alcohol % by volume V = difference in titer of blank and sample, in ml of sodium thiosulphate solution **Note:** 1 mL of 0.05N sodium thiosulphate is equivalent to 0.0011gm of Acetaldehyde.		
Reference			
Approved by	Food Authority based on recommendation of Scientific Panel		

	Method for Determination of Aldehydes		
Method No.	**8.2**	**Revision No. & Date**	
Introduction/ Caution	By Gas chromatography Quantity of aldehydes determined using similar procedure as per the esters using standard reference materials of aldehydes.		
Reference	IS 3752:2005, AOAC 968.09 (See sec 6.2 pages 18-20)		
Approved by	Food Authority based on recommendation of Scientific Panel		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of Aldehydes**		
Method No.	**8.3**	**Revision No. & Date**	
Introduction/ Caution	Routine Gas Chromatographic Method (See sec 6.3 pages 21-22) Quantity of aldehydes determined using similar procedure as per the esters using standard reference materials of alcohols.		
Approved by	Food Authority based on recommendation of Scientific Panel		

FSSAI — FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**9. Method for Determination of Furfural**		
Method No.	**9.1**	**Revision No. & Date**	
Introduction/ Caution	Colorimetric Method		
Principle	Furfural reacts with aniline in presence of hydrochloric acid and develops colour. Developed colours of alcohols with known quantity of furfural and unknown quantity of furfural are compared using nesslers comparator.		
Apparatus	1. Glass ware and apparatus (refer page 2). 2. Nessler tubes with flat bottom tubes of thin high quality glass, 25mm in diameter and 150 mm in length and graduated at 50mL.		
Chemicals	1. Alcoholic beverages 2. Aniline, (distilled and colourless) 3. Hydrochloric acid, sp. gr. 1.125 4. Furfural 5. m-phenylenediamine hydrochloride		
Preparation of reagents	**Furfural free alcohol** 1. Let alcohol containing 5gm of m-phenylenediamine hydrochloride per litre, stand at least for 24h with frequent shaking (previous treatment with potassium hydroxide is not necessary). Reflux for at least 8 h, longer if necessary. 2. Let stand overnight and distill, rejecting the first 100mL and the last 200mL of the distillate. If this gives coloration with aniline hydrochloride, repeat the treatment. **Standard furfural solution** 1. Dissolve 1gm of redistilled, colourless furfural in 100mL of the furfural free alcohol. 2. Prepare standard furfural solution by diluting 1mL of this solution to 100mL with 50% furfural free alcohol. 3. 1 mL of this diluted solution contains 0.1 mg of furfural (strong furfural solution shall retain its strength but the diluted standard solution should be prepared afresh every time).		
Procedure / Extraction	1. Take 5mL of the distillate obtained for ethanol determination, (Sec. 2.1), add 1mL of the colourless aniline and 0.5mL of the hydrochloric acid, and keep for 15 min. Red colour indicates the presence of furfural. Proceed for quantitative estimation if colour develops. 2. Dilute a measured portion of the distillate with 50% furfural free alcohol to 50mL. 3. First add 2mL of the colourless aniline and then 0.5mL of hydrochloric acid. 4. Mix and keep at 15°C for 15 min. 5. Compare the colour developed with standard furfural solution by using a Nessler comparator.		

Calculation	$$\text{Furfural, grams per 100 litres of absolute alcohol} = \frac{W \times 1000 \times 100 \times 100}{V_1 \times V_2}$$ Where, W = is the weight in grams of the furfural present in volume used for matching the experimental solution; V_1 = volume of experimental solution used for estimation; and V_2 = alcohol, % by volume
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of Furfural**		
Method No.	**9.2**	**Revision No. & Date**	
Introduction/ Caution	Determination of Furfural by Gas Chromatography as described under "Determination of Esters". See Sec.6.2 Pg 18-20		
Reference	Procedure of Gas Chromatography (IS 3752:2005, AOAC 968.09)		
Approved by	Food Authority based on recommendation of Scientific Panel		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**10. Method for Determination of Copper / Iron**		
Method No.	**10.1**	**Revision No. & Date**	
Introduction/ Caution	Atomic absorption Spectrophotometric (AAS) Method		
Principle	Liquor (clear) samples/digested samples are aspirated into AAS flame and absorbance are measured for Copper/Iron and compared with absorbance of SRMs.		
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Atomic absorption Spectrophotometer (AAS) – Double beam 3. Hollow Cathode Lamp –Copper 4. Microwave Digester with Quartz tubes for digestion 5. Muffle furnace 6. Fume Hood 7. Steam bath 8. Silica crucible		
Chemicals	1. Alcoholic beverages 2. Acetylene Ultra pure grade 3. Nitrogen – Ultra pure grade 4. Water – triple distilled or Milli-Q /18Ω. 5. Copper SRM and Iron SRM (100 µg/ml) traceable to NIST 6. Alcohol- distilled		
Preparation of reagents	Preparation of Cu / Fe working standard solutions: 1. Take suitable aliquots from Copper/Iron SRM to prepare 0.25, 0.50 and 1.00 µg/mL Cu/Fe solutions and make up to known volume with 1N HNO_3.		
Procedure / Extraction	1. Follow operating instructions of manufacturer for the selection of optimum gas flow, wavelength settings and beam alignment. 2. In case of clear samples direct injection of the liquor sample filtered through 0.45 µm to AAS may be done to determine the quantity of copper present in the sample. 3. In case of samples having high residues, it is not advisable to inject 0.45 µm Millipore-filtered sample, since clogging of the AAS burner head is encountered. Hence wet ashing is preferred. **Preparation of Ash solution:** 4. Wet Ashing - Take 50 to 100mL of wine sample in a glass bowl and evaporate to dryness. 5. Add 5mL of ultra pure nitric acid and transfer to the quartz tube of microwave digester using little distilled water. 6. Pressure Digest the solution in microwave digestion apparatus for 30 min. 7. Cool and make up to 25mL volume. 8. Blank Solution - Prepare a blank by taking 5mL of ultrapure nitric acid and make up to 25mL volume.		

	Determination 9. Aspirate the blank into the AAS flame and set the instrument for zero absorbance. 10. Aspirate the Cu/Fe Std. solutions sequentially for absorbance data acquisition. 11. Now aspirate a) the liquor sample directly or b) nitric acid digested wine sample solution into AAS flame to record the absorbance and in turn note down the displayed concentration of Cu/Fe in µg. 12. Calculate the concentration in the test sample involving the dilutions made.
Calculation	(For directly aspirated liquor sample, dilution part will not appear in the calculation) Reading (in µg) displayed x Dilution Copper / Iron content in = ---wine (in µg/mL or mg/L) Volume of sample
Reference	For Detailed Metal estimation Procedure - Refer Manual of Methods for Analysis of Metals.
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of Copper/Iron**
Method No.	**10.2** **Revision No. & Date**
Introduction/ Caution	By Diethyldithiocarbamate method and Potassium Ferrocyanide method Two methods, namely, diethyldithiocarbamate method and potassium ferrocyanide method are employed. The potassium ferrocyanide method is easier to perform and sufficiently sensitive and accurate for routine type of analysis. The diethyldithiocarbamate method is more sensitive and shall serves as a referee method in case of dispute or where zinc is present.
Principle	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. 2. 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. 3. This method is suitable when the copper content ranges from 0.01 to 0.15mg of copper in the quantity of the material taken. 4. 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. 5. If a quantitative determination is required, the test should be repeated by using proportionately smaller quantities of sample for test.
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Nessler tubes - Flat bottom tubes of thin, colourless glass, about 25mm in diameter and about 150mm in length and graduated at 50ml. The depth measured internally from graduation mark to the bottom shall not vary by more than 2mm in the tubes used for the test.
Chemicals	1. Alcoholic beverages 2. Concentrated Sulphuric acid 3. Concentrated nitric acid 4. Concentrated hydrochloric acid 5. Citric acid, AR grade 6. Ammonium Hydroxide 7. Copper sulphate ($CuSo_4.5H_2O$) 8. Sodium Diethyldithiocarbamate 9. Carbon Tetrachloride, AR grade 10. Acetic acid
Preparation of reagents	1. Dilute sulphuric acid, approximately 10 percent (v/v). 2. Aqua regia, a mixture of one volume of concentrated nitric acid, and three volumes of concentrated hydrochloric acid. 3. Standard copper solution – Dissolve 1.119gm of copper sulphate ($CuSo_4.5H_2O$) in water and dilute to one litre. Dilute 10mL of this solution to

	100mL. One millilitre of the diluted solution contains 0.028545mg of copper. The diluted solution shall always be prepared immediately before use. 4. Sodium Diethyldithiocarbamate- Prepare 0.1 percent by weight solution of sodium diethyldithiocarbamate in water. Sometimes diethyldithiocarbamate available may not be completely soluble in water, in which case the insoluble material may be removed by filtration through an ashless filter paper. The reagent is best prepared just for use, but may stand for one or two weeks in amber coloured bottle without appreciable deterioration. 5. Acetic acid, approximately 5% by weight.
Preparation of Test Samples	1. Transfer 20mL of the material into silica evaporating dish and add 1mL of dilute sulphuric acid. Heat gently in the beginning and then evaporate almost to dryness on a water-bath. 2. Ignite the residue over a smokeless flame to eliminate sulphuric acid. 3. Cool, dissolve the residue in 2mL of water, add three drops of aqua regia and evaporate to dryness on a water bath. 4. Dissolve the residue in water, neutralize, if required, with dilute ammonium hydroxide and make up the volume to 25mL.
Procedure / Extraction	1. To detect copper contamination, if any, in any of the reagents, blank experiment shall be carried out using the same quantities of the reagents. 2. There are two variations of the method- (a) Without extraction, and (b) With extraction (a) Procedure (without extraction) 3. Take in 50mL Nessler tube, 10mL of the test solution prepared as described above. 4. Add 2gm of citric acid and 10ml of dilute ammonium hydroxide. Make up to 50mL with water. 5. Prepare a series of control solutions, each containing in 50mL, 2gm of citric acid and 10mL of dilute ammonium hydroxide together with an increasing amount of copper, namely, 0.1mL, 0.2mL, 0.4mL, 0.6mL, 0.8mL and 1.0mL of standard copper solution. 6. The test solution and controls should be free from any turbidity. 7. Cool all solution to 20^0C, add 2mL of diethyldithiocarbamate solution to each and match the test solution against the control solution. 8. 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. (b) Procedure (with extraction) 9. Extract immediately the copper organometallic compound produced as described in the last paragraph under (a) with four successive portions, 2.5mL each, of carbon tetrachloride and compare the colour of the solution so obtained in a colorimeter with the extracts of control solution similarly prepared. 10. Chloroform may be used but carbon tetrachloride is better as it is almost insoluble in water and forms clearer solution, which separates

	quickly.
Calculation	Calculate copper as follows: Copper (as Cu), in ppm = 0.2845×12.5 V Where V= volume of standard copper solution in the control solution which gives the closest match, in mL.
Reference	AOAC 960.17
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of Copper/Iron**		
Method No.	**10.3**	**Revision No. & Date**	
Introduction/ Caution	By Diethyldithiocarbamate method and Potassium Ferrocyanide method Two methods, namely, diethyldithiocarbamate method and potassium ferrocyanide method are employed. The potassium ferrocyanide method is easier to perform and sufficiently sensitive and accurate for routine type of analysis. The diethyldithiocarbamate method is more sensitive and shall serves as a referee method in case of dispute or where zinc is present.		
Principle	Copper solutions react with potassium Ferrocyanide solutions and forms red-brown solutions of Copper (II) hexacyanoferrate.		
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Nessler tubes - Flat bottom tubes of thin, colourless glass, about 25mm in diameter and about 150mm in length and graduated at 50mL. The depth measured internally from graduation mark to the bottom shall not vary by more than 2mm in the tubes used for the test.		
Chemicals	1. Alcoholic beverages 2. Concentrated Sulphuric acid 3. Concentrated nitric acid 4. Concentrated hydrochloric acid 5. Citric acid, AR grade 6. Ammonium Hydroxide 7. Copper sulphate ($CuSo_4.5H_2O$) 8. Ammonium Chloride, AR grade 9. Acetic acid 10. Potassium Ferrocyanide		
Preparation of reagents	1. Dilute sulphuric acid, approximately 10 percent (v/v). 2. Aqua regia, a mixture of one volume of concentrated nitric acid, and three volumes of concentrated hydrochloric acid. 3. Standard copper solution – Dissolve 1.119gm of copper sulphate ($CuSo_4.5H_2O$) in water and dilute to one litre. Dilute 10mL of this solution to 100mL. One millilitre of the diluted solution contains 0.028545mg of copper. The diluted solution shall always be prepared immediately before use. 4. Acetic acid, approximately 5% by weight. 5. Potassium Ferrocyanide Solution, approximately 4% by weight.		
Preparation of Test Samples	1. Transfer 20mL of the material into silica evaporating dish and add 1mL of dilute sulphuric acid. 2. Heat gently in the beginning and then evaporate almost to dryness on a water-bath. 3. Ignite the residue over a smokeless flame to eliminate sulphuric acid. 4. Cool, dissolve the residue in 2mL of water, add three drops of aqua		

	regia and evaporate to dryness on a water bath. 5. Dissolve the residue in 2mL of water, add three drops of aqua regia and evaporates to dryness on a water bath. 6. Dissolve the residue in 2mL of dilute hydrochloric acid and warm gently till the residue is dissolved. 7. Add 0.5gm of ammonium chloride and dilute to 15mL with water distilled in an all-glass apparatus. 8. Add dilute ammonium hydroxide till alkaline. Boil off excess of ammonia and filter into a clean Nessler tube. 9. Cool and then render the solution acidic with acetic acid (3 to 5 drops are usually sufficient).
Procedure / Extraction	1. Dilute the above solution to 40mL. Add 0.5mL of potassium ferrocyanide solution, stir and make up the volume to 50mL. *Note-If copper is more, a lesser amount, say 10ml of the material may be taken for the test.* 2. Prepare a series of control solutions each containing in 50mL, 0.5gm of ammonium chloride, 3 to 5 drops of acetic acid and 0.5mL of potassium ferrocyanide solution together with an increasing amount of copper, namely, 2mL, 4mL, 6mL, 8mL and 10mL of the standard copper solution. 3. Compare the test solution (1) with control solutions and note the 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.
Calculation	Calculate copper as follows: Copper (as Cu), in ppm = 0.2845×12.5 V Where V= volume of standard copper solution in the control solution which gives the closest match, in ml.
Reference	AOAC 960.17
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Method for Determination of Copper / Iron**		
Method No.	**10.4**	**Revision No. & Date**	
Introduction/ Caution	**By Cuprethol Method**		
Principle	Divalent copper forms a coloured complex with cuprethol. Based on the absorbance of the coloured complex solution copper is determined.		
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Photometer: - spectrophotometer (with blue-green or green filter) set at 445nm and with 40-50 mm cells 3. Copper-free Glassware: - Clean all glassware with 0.1M HNO_3 and rinse thoroughly with Cu-free distilled water		
Chemicals	1. Alcoholic beverages 2. Diethanolamine ($(HOCH_2CH_2)_2NH$) 3. Methanol 4. Carbon disulfide 5. Copper sulphate $CuSO_4.5H_2O$ (free of whitish deposit of lower hydrates) 6. Pure Cu wire or foil 7. HNO_3 8. Anhydrous Sodium Acetate (CH_3COONa) 9. Acetic Acid (CH_3COOH) 10. Copper-free distilled water		
Preparation of reagents	1. Diethanolamine ($(HOCH_2CH_2)_2NH$) solution: - Dissolve 4.0mL diethanolamine in 200mL methanol. 2. Carbon disulfide solution: - Add 1.0 mL CS_2 (Free of precipitate S) to 200mL methanol. 3. Cuprethol solution: - Mix 3 volumes solution (a) and one volume solution (b). Prepare fresh daily. Also mix equal volumes of solution (a) and methanol for blank. 4. Copper standard solutions:- i) Stock solution (conc. 1mg/mL):- Dissolve 3.93gm $CuSO_4.5H_2O$ (free of whitish deposit of lower hydrates) and dilute to 1 L with H_2O. Or dissolve 1.000gm pure Cu wire or foil in 72 mL HNO_3 (1+4) by warming. Boil to expel fumes, cool, and dilute to 1L with H_2O. ii) Working solution (conc.10µg/mL):-Prepare immediately before use by diluting 5ml stock solution with Cu-free distilled H_2O to 500mL in volumetric flask. 5. Buffer solution: - pH 4.4. Dissolve 63.3gm anhydrous Sodium Acetate (CH_3COONa) in ca 800mL H_2O containing 65mL Acetic Acid (CH_3COOH). Dilute to 1 L with H_2O. 6. Copper-free distilled water: - Use distilled water redistilled from all-glass apparatus throughout method.		

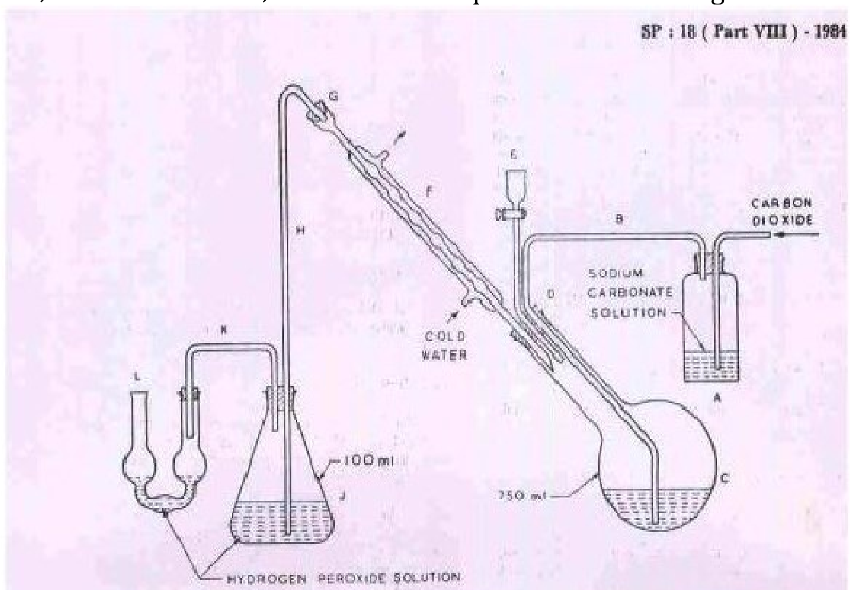
Procedure / Extraction	1. Preparation of standard curve -Into series of glass-stoppered 100ml volumetric flasks add 0.0, 1.0, 2.0, 4.0, 8.0 and 12.0 mL Cu working standard solution containing 0.0, 0.4, 0.8, 1.6, 3.2, and 4.8µg/mL Cu, respectively. 2. Add H_2O to 12 mL in each flask. Dilute to volume with degassed low-Cu beer. 3. Preparation of test portion - Cool bottle or Can of beer/wine and shake thoroughly immediately before opening. 4. Let gas bubbles leave liquid before removing cap or puncturing can. 5. Discard ca 1/3 of beer and degas by swirling. 6. Remove test portion directly from container, mix, and proceed. 7. Use 0.0 Solution to zero instrument, and obtain *A* (absorbance) or scale readings for 0.1, 0.2, 0.4, 0.8, and 1.2 µg/mL added Cu. 8. *A* over this range follows Beer's Law. Calculate average factor, *F*, converting *A* or scale reading to µg/mL Cu. 9. If instrument response is not linear, draw and use smooth curve for calculating µg/mL Cu. Determination 10. Slowly pour 50mL cold beer into 50ml graduate, avoid foaming. Transfer to 125mL flask, add 25mL buffer solution and mix. 11. Measure two 30ml aliquots in 50mL graduate and transfer to separate 50mL flasks. 12. Add 3mL cuprethol solution to one flask and 3mL blank solution to other. Mix each and let stand 10 min. 13. Zero instrument with blank. Determine *A* in same size cell and at same wavelength used in calibration. 14. Calculate µg/mL Cu by multiplying A or scale reading by F, or use curve.
Reference	AOAC 972.12
Approved by	Food Authority based on recommendation of Scientific Panel

	11. Method for Determination of Methyl alcohol		
Method No.	11.1	Revision No. & Date	
Introduction/ Caution	Spectrophotometric method		
Principle	Methanol is oxidized to formaldehyde (methanol) by potassium permanganate (acidified by phosphoric acid). The amount of formaldehyde is determined by the violet color formed by the reaction of chromotropic acid in a sulfuric medium.		
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Separating funnel 3. Spectrophotometer		
Chemicals	1. Alcoholic beverages 2. Potassium permanganate 3. Phosphoricc acid (H_3PO_4) 4. Sodium salt of chromotropic acid (sodium 1,8 - dihydroxynaphthalene - 3,6 disulfonate) 5. Methanol 6. Ethanol 7. Isopropyl alcohol 8. Sulphuric acid (H_2SO_4)		
Preparation of reagents	1. Potassium permanganate solution: 3.0gm $KMnO_4$ and 15.0mL H_3PO_4 shall be dissolved in 100mL water. The solution shall be prepared monthly. 2. Sodium salt of chromotropic acid (sodium 1,8-dihydroxynaphthalene - 3,6 disulfonate) 5 % aqueous solution (w/v). If not clear, the sodium salt chromotropic acid shall be filtered. It shall be prepared weekly. Purification of chromotropic acid 3. If absorbance of blank is greater than 0.05, the reagent shall be purified as follows: 10gm chromotropic acid or its Na salt shall be dissolved in 25mL water (add 2mL H_2SO_4 shall be added to the aqueous solution of the salt to convert it to free acid). 4. Add 50 mL of methanol and heat to just boiling and filter. 5. Add 100 mL isopropyl alcohol to precipitate free chromotropic acid. 6. More isopropyl alcohol may be added to increase yield of purified acid. Methanol Stock solution 7. Dilute 1.0gm methanol (99.99% pure) to 100mL with 40% (v/v) ethanol (methanol free). Dilute to 10mL of this solution to 100mL with 40% ethanol (methanol free). This is 1000ppm solution. Methanol Standard solution: 8. Dilute appropriate volume of methanol (11.1.4) to 100mL vol. flasks with 40% ethanol to get final concentration of 20, 40, 60, 80 and 100 ppm of methanol.		
Procedure / Extraction	1. Take 50mL of sample in a simple still and distil, collecting about		

	40ml of distillate. 2. Dilute 1mL of distillate to 5mL with distilled water and shaken well. 3. Take 1mL of this solution, 1mL of distilled water (for blank) and 1mL of each of the methanol standards in to 50mL stoppered test tubes and keep them in an ice-cold water bath. 4. Add to each test tube, 2mL of $KMnO_4$ reagent and keep aside for 30 min. 5. Decolourize the solution by adding a little sodium bisulphite and add 1mL of chromotropic acid solution. 6. Mix well and add 15ml of sulphuric acid slowly with swirling and place in hot water bath maintaining 80°C for 20 min. Observe the colour development from violet to red. 7. Cool the mixture and measure the absorbance at 575 nm using 1cm cuvette cell.
Calculation	Calculate methanol content in g/100 Litres of absolute alcohol 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 of sample solution C = concentration of methanol std. solution D = dilution factor for sample solution A_1 = absorbance of methanol std. solution
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

	Method for Determination of Methyl alcohol		
Method No.	**11.2**	**Revision No. & Date**	
Introduction/ Caution	**Gas chromatographic method**		
Principle	Methyl alcohol is estimated using GC by the comparison of Peak areas of known quantities of authentic standards of methanol, n-propanol and test sample.		
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Gas Chromatograph, FID Detector, Split injection port, fixed with capillary column (HP Carbowax 20M of 30m x 0.32mm ID x 0.25 μ film thickness or SPB 20 capillary column of 30m x 0.25mm ID x 1.0 μ film thickness). 3. N_2 or He as carrier gas at a flow rate of 1.0mL/min. 4. The detector and injector port temperatures are at 250°C. Oven temperature is at 45^{0}C for 4 min and then raise to100^{0}C / min at the rate of 10^0 C/min and finally at to 200°C for 10min at the rate of 15°C/min. (Optimum operating conditions may vary with type of column used and instrumental characteristics). 5. Syringe - 10µL, Hamilton Co., or equivalent.		
Chemicals	1. Alcoholic beverages 2. Ethanol – Methanol free 3. n-Pentanol 4. Methanol		
Preparation of reagents	1. N-Pentanol Internal standard – 0.05% w/v n-pentanol in 40% ethanol (v/v). 2. Methanol Stock solution: Dilute 1.0gm methanol (99.99% pure) to 100mL with 40% (v/v) ethanol, methanol free. Dilute 10mL of this solution to 100mL with 40% ethanol. 3. Methanol Standard solution: Transfer 5mL of the above solution to a 10mL stoppered test tube and add 1mL of n-pentanol internal std. solution and mix well.		
Preparation of Test Samples	1. Transfer 5mL of sample into a 10mL stoppered test tube and add 1mL of n- pentanol internal standard and mix well.		
Column Chromatography	1. Inject 2 µL of methanol standard solution into GC and record the chromatographic profile. 2. Adjust the operating parameters and attenuation to obtain good resolution of the peaks. 3. Determine the retention time of methanol and n-pentanol. 4. Inject 2 µL sample solution into GC and record the chromatogram.		
Calculation	Methanol, in grams /100L of Absolute alcohol $= \dfrac{R_2 \times C \times D \times 1000 \times 100 \times 100}{R_1 \times S}$ Where,		

	R_2 = peak ratio of methanol to n-pentanol for sample solution C = concentration of methanol in std. solution in g/ml D = dilution factor for sample solution R_1 = peak ratio of methanol to n-pentanol for std. solution S = ethanol content of liquor sample in % (v/v).
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**12. Determination of Total Sulphur Dioxide (for Wines only)**
Method No.	**12.1** **Revision No. & Date**
Introduction/ Caution	Modified Monier Williams Method (Shiphton's Method)
Principle	Sulphur dioxide on treatment with hydrogen peroxide oxidized to sulphuric acid and estimated using sodium hydroxide in presence of indicator Bromophenol blue.
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Round bottom flask – 500mL capacity connected to N_2 or CO_2 inlet source, coiled condenser, receiver and trap as shown in the figure.  FIG. 1 ASSEMBLY OF APPARATUS FOR THE DETERMINATION OF SULPHUR DIOXIDE
Chemicals	1. Alcoholic beverages 2. Hydrogen Peroxide 3. Sodium hydroxide 4. Bromophenol indicator 5. Ethyl alcohol 6. Concentrated Hydrochloric acid – sp gr 1.16 7. Carbon dioxide gas from a cylinder
Preparation of reagents	1. Hydrogen Peroxide solution – Dilute a 30% Hydrogen peroxide solution with distilled water so as to obtain a 3% solution of hydrogen peroxide. 2. Sodium hydroxide – 0.01N. 3. Bromophenol indicator solution – Dissolve 0.1gm of bromophenol blue in 3mL of 0.05N sodium hydroxide solution and 5mL of ethyl alcohol (90%) by warming gently. Make up to 250mL in a volumetric flask with 20% ethyl alcohol.

Procedure / Extraction	1. Transfer 25mL of Hydrogen peroxide solution to Erlenmeyer flask (J) and 5mL to Peligot tube (L), Assemble the apparatus as shown above. 2. Introduce into the flask (C) 300mL water and 20mL of conc.HCl through the dropping funnel (E). 3. Run a steady current of cold water through the condenser (F). 4. To expel air from the system boil the mixture contained in the flask (C) for a short time in a current of Carbon dioxide gas previously passed through the wash bottle (A). 5. Weigh accurately about 25gm of wine sample and transfer with little quantity of water into the flask (C) through the dropping funnel (E). Wash the dropping funnel with a small quantity of water and run the washings into flask (C). 6. Distill by heating the mixture contained in the flask (C) in a slow current of Carbon dioxide gas passed previously through the wash bottle (A) for 1 hour. 7. Just before the end of the distillation stop the flow of water in the condenser (This causes the condenser to become hot and drives off the residual traces of sulphur dioxide retained in the condenser). 8. When the delivery tube (H) just above the Erlenmeyer flask (J) becomes hot to touch disconnect the stopper (G) immediately. 9. Wash the delivery tube (H) and the contents of the Peligot tube (L) with water into the Erlenmeyer flask (J). 10. Cool the contents of the Erlenmeyer flask to room temperature, add a few drops of bromophenol blue indicator and titrate with standard sodium hydroxide solution (Bromophenol blue is unaffected by carbon dioxide and gives a distinct colour change in cold hydrogen peroxide solution). 11. The colour changes from yellow to light blue. Carry out a blank determination using 20mL of concentrated hydrochloric acid diluted with 300mL of water.
Calculation	$\text{Sulphur Dioxide mg / kg} = \dfrac{32000\,(V - v)\,N}{W}$ Where, V = volume in mL of standard sodium hydroxide solution required for the test sample. v = volume of standard sodium hydroxide solution required for the blank determination. N = normality of standard sodium hydroxide solution W = weight in gm of the sample taken for test
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

Logo	Determination of Total Sulphur Dioxide (for Wines only)		
Method No.	**12.2**	**Revision No. & Date**	
Introduction/ Caution	By Rosaniline Colorimetric Method		
Principle	A stable dichlorosulfitomercurate complex, obtained by reaction between SO_2 with potassium /sodium tetrachloromercurate is reacted with pararosaniline and formaldehyde forms pararosaniline methyl sulfonic acid dye. It absorbance measured and sulphur dioxide is estimated.		
Apparatus	Glass ware and apparatus (refer page 2)		
Chemicals	1. Alcoholic beverages 2. p- rosaniline HCl 3. Hydrochloric acid (HCl) 4. Formaldehyde (HCHO) 5. Mercuric chloride ($HgCl_2$) 6. Sodium chloride (NaCl) 7. Sodium bisulphate ($NaHSO_3$) 8. Iodine (I_2) 9. Sodium thiosulphate ($Na_2S_2O_3$) 10. Starch 11. n-Hexyl alcohol		
Preparation of reagents	1. Colour reagent- Weigh 100mg p-rosaniline HCl into 250mL volumetric flask and dissolve in 200mL H_2O. Add 40mL HCl (1+1), mix, and dilute to volume with H_2O. Let stand 15min before use. Store in brown, glass-stoppered bottle in refrigerator. 2. Formaldehyde solution- Dilute 5mL 40% HCHO solution to 1L with H_2O and store in brown, glass-stoppered bottle in refrigerator. 3. Mercury stabilizing solution - Dissolve 27.2gm $HgCl_2$ and 11.7gm NaCl in H_2O and dilute to 1L with H_2O. Calibration 4. Accurately weigh 250mg $NaHSO_3$ into exactly 50mL 0.1M I_2 solution in glass-stoppered flask. Let stand at room temperature for 5 min. Add 1mL HCL, and titrate excess I_2 with 0.1M $Na_2S_2O_3$, using 1% aqueous starch solution as indicator (1mL 0.1M I_2 consumed= 3.203mg SO_2 or 5.20mg $NaHSO_3$). From results of $NaHSO_3$ assay, prepare solution containing 10mg SO_2/mL (ca 8.6-9.0gm $NaHSO_3$/500mL) ***(Solution I)***. 5. Transfer 100mL Hg stabilizing solution to 500mL glass-stoppered volumetric flask. Add 1.00mL Solution I, and dilute to volume with H_2O (1mL=20µg SO_2) ***(Solution II)***. 6. Using 10mL graduate containing 1 drop n-hexyl alcohol as antifoam, transfer 10mL portions of cold, undigested beer (preferably of low SO_2 content) into series of eight 100ml volumetric flasks. 7. To series add 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 8.0mL Solution II (0-160µg SO_2). Dilute to volume with H_2O, and mix. 8. Transfer 25mL aliquots of each solution to separate 50mL		

	volumetric flasks. To each flask, add 5mL color reagent. Mix, and add 5mL HCHO solution. Mix, dilute to volume with H_2O, mix, and hold in 25 °C water bath 30min. 9. Read colour in spectrophotometer at 550nm or in photometer with green filter. 10. Plot absorbance (A) as ordinate against µg SO_2 added to beer as abscissas (colour follows Beer's law over range). 11. Calculate calibration factor F, converting readings to µg SO_2 in 25mL aliquot used, or convert directly to µg/mL SO_2.
Preparation of Test Samples	1. Using pipets, add 2mL Hg stabilizing solution and 5mL 0.05M H_2SO_4 to 100mL volumetric flask. 2. Measure 10mL cold, undegassed beer into 10mL graduate containing 1 drop n-hexyl alcohol, and add to volume flask. 3. Swirl gently, and add 15mL 0.1M NaOH. Swirl, and hold 15s. 4. Add 10mL 0.05M H_2SO_4, then H_2O to volume, and mix thoroughly. Transfer 25mL aliquot to 50mL volumetric flask.
Procedure / Extraction	1. To solution in 50mL volumetric flask, add dilute to volume with H_2O. 2. Mix, and hold in 25ºC bath 30min. 3. Read colour as above, using cells of same size and same instrument settings. 4. Correct for blank as follows: Measure 10mL cold, undegassed beer into 100mL volumetric flask. 5. Add 0.5mL 1% aqueous starch solution, then 0.05M I_2 solution, drop wise until permanent bluish tinge persists. Add 1 drop more, dilute to volume, and mix thoroughly. When blue fades, develop colour in 25mL aliquots as above. (Colour readings for I_2 blanks are usually low and uniform; when test is performed on series of similar beers, blank tests on all may be unnecessary.)
Calculation	SO_2, µg/ml = (A_s-A_b) x F Where, A_s=A of test solution (or photometric reading with green filter equivalent to A) A_b=A of I_2 blank, and F= factor derived from 12.2.2 for converting A to µg SO_2 in aliquot, or directly to µg/ml SO_2.
Reference	AOAC 963.11
Approved by	Food Authority based on recommendation of Scientific Panel

<table>
<tr><td>FSSAI — FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India</td><td colspan="3">13. Method for Determination of Tannins (for Wines only)</td></tr>
<tr><td>Method No.</td><td>13.1</td><td>Revision No. & Date</td><td></td></tr>
<tr><td>Introduction/ Caution</td><td colspan="3">Spectrophotometric Method</td></tr>
<tr><td>Principle</td><td colspan="3">Tannins present in alcoholicbeverages reacts with Folin-Dennis reagent and forms coloured solutions. The absorbances of these colored solutions are measured and tannin quantity is determined.</td></tr>
<tr><td>Apparatus</td><td colspan="3">1. Glass ware and apparatus (refer page 2)
2. Spectrophotometer, Double beam with a working wavelength range of 350-800nm and band width 5nm</td></tr>
<tr><td>Chemicals</td><td colspan="3">1. Alcoholic beverages
2. Sodium tungstate ($Na_2WO_4.2H_2O$)
3. Phosphomolybdic acid
4. Phosphoric acid
5. Anhydrous Sodium carbonate
6. Tannic acid</td></tr>
<tr><td>Preparation of reagents</td><td colspan="3">1. Preparation of Folin-Dennis reagent - Prepare by adding 100gm Sodium tungstate ($Na_2WO_4.2H_2O$), 20gm Phosphomolybdic acid and 50mL phosphoric acid to 750mL water and reflux for 2 hours and dilute to 1 litre.
2. Preparation of Sodium carbonate solution - Prepare by adding 35gm anhydrous Sodium carbonate to 100mL water at about 80^{o}C. Allow to cool overnight and seed with few crystals of sodium carbonate. Filter.
3. Preparation of standard Tannic acid solution - Prepare fresh daily, by dissolving 100mg Tannic acid in 1000mL water.
(1 mL = 0.1mg of tannic acid).</td></tr>
<tr><td>Procedure/Extraction</td><td colspan="3">Preparation of standard curve
1. Pipette 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of standard tannic acid solution into 100mL volumetric flasks containing 75mL water.
2. Add 5mL Folin-Dennis reagent and 10mL sodium carbonate solution. Make up to volume.
3. Mix well and after 30 min. determine absorbance of each standard using reagent blank.
4. Plot absorbance against mg of tannic acid and use the graph for the determination of concentration of tannin in wine.
Determination
5. Pipette 1mL of wine into a 100mL volumetric flask containing about 80 mL water.
6. Add 5mL Folin-Dennis reagent and 10mL sodium carbonate solution. Make up to volume.
7. Mix well and after 30 minutes, against reagent blank read the absorbance.
8. If the absorbance is beyond 0.8, dilute the solution 1:4 times and read.</td></tr>
</table>

Calculation	Obtain the mg of tannic acid using the standard curve and calculate to express the value in g/L of wine.
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**14. Method for Determination of extracts in wine**
Method No.	**14.1** | **Revision No. & Date** |
Introduction/ Caution	**Evaporation Method**
Principle	Extracts are estimated by evaporating the known quantity of the sample of wine on a steam bath
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Pipette, 50 ml 3. Evaporating dishes, aluminium , flat bottom with lids, 75ml capacity 4. Oven- calibrated to maintain temperature of 100 ± 2°C 5. Steam bath 6. Desiccators 7. Electronic balance, 0.1 mg sensitivity
Chemicals	1. Alcoholic beverages
Procedure / Extraction	1. Weigh, dried and cooled aluminium dish (W_1). 2. Mix the wine sample well and draw 50mL sample (dry wines) or 25mL sample (sweet wines) into the aluminium dish and evaporate on steam bath to almost dryness. 3. Transfer the dish to an air oven maintained at 100°C and dry for 4-5 hours. 4. Remove the dish and cool in a desiccator and weigh to constant weight (W_2). 5. Calculate the extract in g/L of wine.
Calculation	$\text{Extract, g/L} = \dfrac{(W_2 - W_1) \times 1000}{\text{Volume of sample}}$
Reference	
Approved by	Food Authority based on recommendation of Scientific Panel

	15. Method for Determination of Sorbic acid		
Method No.	**15.0**	**Revision No. & Date**	
Introduction/ Caution	Spectrphotometric method		
Principle	Sorbic acid (2,4-hexadienoic acid) shows UV absorbance at 260 nm due to its inherent conjugation system present in the molecule. This absorbance is used for its quantification.		
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Cash Electric still 3. UV Spectrophotometer		
Chemicals	1. Alcoholic beverages 2. Hydrochloric acid 3. Potassium sorbate		
Preparation of reagents	1. Hydrochloric acid.—0.1M. Dilute 8.2 mL HCl to 1 L with H_2O. 2. Sorbic acid standard solution.—1.0 mg/mL. Accurately weigh 1.340 g potassium sorbate (equiv a lent to 1.000 gm sorbic acid) in 1 L volumetric flask, and dissolve and dilute to volume with H_2O. Solution is stable several days when refrigerated.		
Procedure / Extraction	Preparation of Standard Curve 1. Pipet 0, 10, 20, 30, and 40 mL sorbic acid standard solution into separate 100mL volumetric flasks, and dilute to volume with H_2O. 2. Pipet 2mL of each solution into separate 200mL volumetric flasks, add 0.5mL 0.1M HCl, and dilute to volume with H_2O. 3. Read A at 260 nm in 1 cm cell and plot A against concentration. Determination. 4. Pipet 2mL wine into Cash still. 5. Rinse in with 2–3mL H_2O. 6. Steam distill into 200mL volumetric flask containing 0.5mL 0.1M HCl. 7. Collect ca 190mL distillate; dilute to volume with H_2O. 8. Read A at 260 nm in 1 cm cell. Determine concentration from standard curve.		
Reference	AOAC 974.08		
Approved by	Food Authority based on recommendation of Scientific Panel		

	16. Method for Determination of Reducing Sugar		
Method No.	**16.0**	**Revision No. & Date**	
Introduction/ Caution	Lane and Eynon (Fehling) Method		
Principle	Known quantity of Fehling (Soxhlet) solution titrated with dextrose solution and used quantity is determined. Known quantity of Fehling solution is taken and known quantity of clarified wine is added and titrated with dextrose solution and used quantity is determined. The difference in the quantities of dextrose used will provide the reducing sugar present in wine.		
Apparatus	Glass ware and apparatus (refer page 2)		
Chemicals	1. Alcoholic beverages. 2. Copper sulphate. 3. Sulphuric acid (conc. H_2SO_4). 4. Rochelle salt (Potassium sodium tartarate). 5. Sodium hydroxide. 6. Lead acetate. 7. Glacial acetic acid. 8. Disodium hydrogen phosphate (Na_2HPO_4). 9. Methylene blue. 10. Anhydrous dextrose. 11. Benzoic acid. 12. Sodium Hydroxide.		
Preparation of reagents	Soxhlet solution 1. Solution A - Dissolve 34.639gm of copper sulphate in water, add 0.5mL of conc. H_2SO_4 and dilute to 500mL. Filter the solution. 2. Solution B - Dissolve 173gm of Rochelle salt (Potassium sodium tartarate) and 50gm of sodium hydroxide dilute to 500mL and allow the solution to stand for 2 days. Filter the solution. 3. Mix equal amounts of solution A and solution B. 4. Lead acetate solution (Saturated and neutral). 5. Methylene blue solution - 0.05gm of Methylene blue is dissolved in 100mL water. **Standard invert sugar solution** 6. Stock solution of dextrose – Anhydrous dextrose (10gm) dissolved in water in a 1 litre graduated flask. Benzoic acid (2.5gm) is added and dissolved while shaking. Make up the volume to the mark with water. This solution is prepared daily. 7. Standard dextrose solution – Dilute known amount of dextrose stock solution (6) to such a concentration that more than 15mL but less than 50mL of it will be required to reduce all the copper in the Fehling solution taken for titration. Note the concentration of anhydrous dextrose in the solution as mg per 100mL. Prepare this solution everyday. 8. Sodium Hydroxide – 1 normal solution.		

	Preparation of control 9. Pipette 25mL of Soxhlet reagent into a 250mL flask. Add 10mL of 0.5% standard invert sugar solution, bring it to boil in 3min and keep it boiling for 3min (use glass beads to prevent bumping). Add 5 drops of methylene blue indicator and titrate the solution while still hot with standard 0.5% invert sugar till faint blue and then add dropwise until the solution is reddish in colour.
Preparation of Test Samples	De-alcoholization and Decolourization of Wine Sample 1. Take 100mL of wine sample in a porcelain dish. 2. Exactly neutralize with sodium hydroxide calculating the acidity and evaporate to 50mL. 3. To this add 5mL of lead acetate solution, enough activated charcoal and 2 drops of glacial acetic acid. 4. Make the volume to 100mL with distilled water. Filter this mixture into 2gm of disodium hydrogen phosphate in a beaker.
Procedure / Extraction	1. Pipette 20mL of the clarified wine into an Erlen-meyer flask containing 25mL of Soxhlet reagent. 2. Bring it to boil and titrate with 0.5 percent invert sugar, with methylene blue indicator, to a brick red end point. Calculate the reducing sugar from the standard tables.
Reference	IS 7585(1995)
Approved by	Food Authority based on recommendation of Scientific Panel

17. Method for Determination of Total sugar

Method No.	**17.0**	**Revision No. & Date**	
Introduction/ Caution	The presence of added sucrose can be detected by determining sugars before and after inversion by copper- reduction methods.		
Principle	Fehling solution is standardized using standard dextrose solution. First reducing sugars are estimated in the alcoholic beverage. Later, Alcoholic beverage is inverted and total sugars are estimated.		
Apparatus	1. Glass ware and apparatus (refer page 2) 2. Amber coloured bottles		
Chemicals	1. Alcoholi beverages 2. Copper sulphate ($CuSO_4.5H_2O$) 3. Rochelle salt (potassium sodium tartrate) ($K Na C_4H_4O_6. 4H_2O$) 4. Hydrochloric acid 5. Sodium hydroxide 6. Lead acetate 7. Potassium or sodium oxalate 8. Phenolphthalein indicator		
Preparation of reagents	1. Fehling A: Dissolve 69.28gm copper sulphate ($CuSO_4.5H_2O$) in distilled water. Dilute to 1000mL. Filter and store in amber coloured bottle. 2. Fehling B: Dissolve 346gm Rochelle salt (potassium sodium tartrate) ($K Na C_4H_4O_6. 4H_2O$) and 100gm NaOH in distilled water. Dilute to 1000mL. Filter and store in amber coloured bottle. 3. Saturated neutral Lead acetate solution.		
Preparation of Test Samples	1. Transfer test sample representing about 2- 2.5gm sugar to 200mL volumetric flask, dilute to about 100mL. 2. Add excess of saturated neutral Lead acetate solution (about 2mL is usually enough). 3. Mix, dilute to volume and filter, discarding the first few ml filterate. 4. Add dry Potassium or Sodium Oxalate to precipitate excess lead used in clarification, mix and filter, discarding the first few mL filterate. **Note:** Use of Potassium Ferrocyanide and Zinc acetate is preferable instead of Lead acetate and Sodium oxalate, due to safety issues.		
Procedure / Extraction	Standardization of Fehling's solution 1. Prepare standard dextrose solution into a 100mL volumetric flask. Find the titre (volume of dextrose solution required to reduce all the copper in 10mL of Fehling solution) corresponding to the standard dextrose solution (Refer table below). 2. Pipette 10mL of Fehling's solution into a 300mL of conical flask and run in from the burette almost the whole of the standard dextrose solution required to effect reduction of all the copper, so that more than one mL will be required later to complete the titration. 3. Heat the flask containing mixture over wire gauze. Gently boil the contents of the flask for 2 minutes.		

	4. At the end of two minutes of boiling add without interrupting boiling, one mL of methylene blue indicator solution. 5. While the contents of the flask begins to boil, begin to add standard dextrose solution (one or two drops at a time) from the burette till blue color of indicator disappears. 6. The titration should be completed within one minute so that the contents of the flask boil together for 3 minutes without interpretation. 7. Note the titre (that is total volume in mL. of std. dextrose solution used for the reduction of all the copper in 10mL of Fehling's solution. 8. Multiply the titre (obtained by direct titration) by the number of mg of anhydrous dextrose in one millilitre of standard dextrose solution to obtain the dextrose factor. 9. Compare this factor with the dextrose factor and determine correction.

Dextrose factors for 10 mL of Fehling's Solution		
Titre (ml)	Dextrose factor	Dextrose content per 100 ml of solution (mg)
15	49.1	327
16	49.2	307
17	49.3	289
18	49.3	274
19	49.4	260
20	49.5	247.4
21	49.5	235.8
22	49.6	225.5
23	49.7	216.1
24	49.8	207.4
25	49.8	199.3
26	49.9	191.8
27	49.9	184.9
28	50.0	178.5
29	50.0	172.5
30	50.1	167.0
31	50.2	161.8
32	50.2	156.9
33	50.3	152.4
34	50.3	148.0
35	50.4	148.9
36	50.4	140.0
37	50.5	136.4
38	50.5	132.9
39	50.6	129.6
40	50.6	126.5
41	50.7	123.6
42	50.7	120.8
43	50.8	118.1
44	50.8	115.5
45	50.9	113.0
46	50.9	110.6
47	51.0	108.4
48	51.0	106.2
49	51.0	104.1
50	51.1	102.2
Miligrams of anhydrous dextrose corresponding to 10 mL of Fehlings solution		

1. Take 25mL filterate or aliquot containing (if possible) 50 – 200 mg reducing sugars and titrate with mixed Fehling A and B solution using Lane and Eynon Volumetric method.

	2. For inversion at room temperature, transfer 50mL aliquot clarified and deleaded solution to a 100mL volumetric flask, add 10mL HCl (1+ 1) and let stand at room temperature for 24 hours. (For immediate inversion, the sample with HCl can be heated at 70^{0}C for 1 hr). 3. Neutralise exactly with conc. NaOH solution using phenolphthalein indicator and dilute to 100mL. Titrate against mixed Fehling A and B solution (25mL of Fehling's Solution can be considered for the purpose) and determine total sugar as invert sugar (Calculate added sugar by deducting reducing sugars from total sugars).
Calculation	Reducing and total reducing sugar can be calculated as, $\text{Reducing sugar (\%)} = \frac{\text{mg of invert sugar x vol. made up x 100}}{\text{TR x Wt. of sample x 1000}}$ $\text{Total reducing sugar (\%)} = \frac{\text{mg of invert sugar x final vol. made up x original volume x 100}}{\text{TR x Wt. of sample x aliquot taken for inversion x1000}}$ Total sugar (as sucrose) (%) = (Total reducing sugar – Reducing sugar) x 0.95 + Reducing sugar Added sugar = Total sugars – Reducing sugars
Reference	1. Table 2: IS 6287:1985, Methods for sampling and analysis for sugar confectionery, Pg.11. 2. AOAC 17th edn, 2000 Official Method 925.35 Sucrose in Fruits and Fruit Products read with AOAC Official method 923.09 Lane and Eynon general volumetric method. 3. AOAC 984.17: 'Method for the determination of Sugars in foods', *Jr. Agri. and Food Chemistry,* 19(3):551-54, (1971) (Modified) Brobst, K.M. 4. Gas-Liquid Chromatography of Trimethylsilyl Derivatives, Methods in Carbohydrate Chemistry, 6:3-8, Academic Press, New York, NY, (1972) (Modified)
Approved by	Food Authority based on recommendation of Scientific Panel

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**18. Method for Determination of carbonation (GV)**		
Method No.	**18.0**	**Revision No. & Date**	
Introduction/ Caution	In case of carbonated RTD low alcoholic beverages, they shall be carbonated with carbon dioxide conforming to Grade 2 of IS 307 to a pressure in accordance with their character. However, the carbonated RTD low alcoholic beverages shall have a minimum of one volume of carbon dioxide. The gas volume is the amount of carbon dioxide the water will absorb at the normal atmospheric pressure at 15,56T,		
Principle	Amount of carbonation is determined using the pressure guage.		
Apparatus	1. Glass ware and apparatus (refer page 2) 2. The apparatus consists of-a pressure gauge having a hollow spike with holes in its side. The bottle is inserted from the side into the slot provided in the neck of the carbon dioxide tester and is secured in place by tightening with a threaded system, The pressure gauge is inserted until the needlepoint touches the crown cork. There is a sniff valve on the gauge stem, which is kept closed until the needlepoint of the pressure gauge is forced through the crown cork. The reading is noted on the gauge.		
Chemicals	Alcoholic beverages		
Procedure / Extraction	1. Clamp the bottle in the frame of the gas volume tester. 2. Pierce the crown cork but do not shake the bottle. Sniff off the top gas quickly until the gauge reading drops to zero. 3. Make certain to close the valve the instant the needle touches zero in the pressure gauge, Shake the bottle vigorously until the gauge gives a reading that additional shaking does not change. 4. Record the pressure. 5. Note the temperature and record it. 6. Obtain -the volume of gas from Table 2 of IS 2346.		
Reference	IS:15588 (2005)		
Approved by	Food Authority based on recommendation of Scientific Panel		

19.0 **<u>References</u>-**

1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test.

2. IS Standard – IS 7585:1995, Wines, Methods of Analysis.

3. Amerine, M.A., Ough, C.S. Methods of analysis of Musts and Wines. New York: John Wiley & Sons; 1980: 83–85, 88–89.

4. AOAC Official Methods of Analysis, 18th Edn. (2005), Ch.26, Method, 967.08, Copper in distilled liquors by Atomic Absorption Spectrophotometry.

5. I.S.I.Hand book of Food Analysis (Part VIII) – 1984 page 12, Determination of Sulphur dioxide. + Additional references for Secs. 15, 16, 17 and 18 to be included(see contents)

6. Determination of sorbic acid AOAC, 974.08; JAOAC 57, 951(1974); 58, 133(1975).

ANNEXURE-I

DETERMINATION OF ALCOHOL CONTENT % BY VOL. OF BEVERAGES USING SPECIFIC GRAVITY Vs. ALCOHOL% TABLE

Sp.gr 20/20^{0} C	% by vol	Sp.gr 20/20^{0} C	% by vol
0.99	7.15	0.985	11.26
0.9899	7.23	0.9849	11.34
0.9898	7.31	0.9848	11.43
0.9897	7.39	0.9847	11.51
0.9896	7.47	0.9848	11.59
0.9895	7.55	0.9845	11.68
0.9894	7.63	0.9844	11.76
0.9893	7.71	0.9843	11.85
0.9892	7.79	0.9842	11.93
0.9891	7.87	0.9841	12.02
0.989	7.95	0.984	12.1
0.9889	8.03	0.9839	12.19
0.9888	8.11	0.9838	12.28
0.9887	8.19	0.9837	12.36
0.9886	8.27	0.9836	12.45
0.9885	8.35	0.9835	12.53
0.9884	8.44	0.9834	12.62
0.9883	8.52	0.9833	12.71
0.9882	8.6	0.9832	12.8
0.9881	8.68	0.9831	12.88
0.988	8.76	0.983	12.97
0.9879	8.84	0.9829	1306
0.9878	8.93	0.9828	13.14
0.9877	9.01	0.9827	13.23
0.9876	9.09	0.9826	13.32
0.9875	9.17	0.9825	13.41
0.9874	9.26	0.9824	13.49
0.9873	9.34	0.9823	13.58
0.9872	9.42	0.9822	13.67
0.9871	9.51	0.9821	13.76
0.987	9.59	0.982	13.85
0.9869	9.67	0.9819	13.94
0.9868	9.75	0.9818	14.02
0.9867	9.84	0.9817	14.11
0.9866	9.92	0.9816	14.2
0.9865	10	0.9815	14.29
0.9864	10.09	0.9814	14.38
0.9863	10.17	0.9813	14.47

Sp.gr $20/20^{0}$ C	% by vol	Sp.gr $20/20^{0}$ C	% by vol
0.9862	10.25	0.9812	14.56
0.9861	10.34	0.9811	14.65
0.986	10.42	0.981	14.74
0.9859	10.5	0.9809	14.83
0.9858	10.59	0.9808	14.92
0.9857	10.67	0.9807	15.01
0.9856	10.75	0.9806	15.1
0.9855	10.84	0.9805	15.19
0.9854	10.92	0.9804	15.28
0.9853	11	0.9803	15.37
0.9852	11.09	0.9802	15.46
0.9851	11.17	0.9801	15.54
0.98	15.64	0.975	20.3
0.9799	15.73	0.9749	20.4
0.9798	15.82	0.9748	20.49
0.9797	15.91	0.9747	20.59
0.9796	16	0.9746	20.68
0.9795	16.09	0.9745	20.77
0.9794	16.18	0.9744	20.87
0.9793	16.27	0.9743	20.96
0.9792	16.36	0.9742	21.05
0.9791	16.45	0.9741	21.15
0.979	16.54	0.974	21.24
0.9789	16.64	0.9739	21.33
0.9788	16.73	0.9738	21.42
0.9787	16.82	0.9737	21.52
0.9786	16.91	0.9736	21.61
0.9785	17	0.9735	21.7
0.9784	17.1	0.9734	21.79
0.9783	17.19	0.9733	21.89
0.9782	17.28	0.9732	21.98
0.9781	17.38	0.9731	22.07
0.978	17.47	0.973	22.16
0.9779	17.56	0.9729	22.25
0.9778	17.66	0.9728	22.34
0.9777	17.75	0.9727	22.43
0.9776	17.84	0.9726	22.52

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.9775	17.94	0.9725	22.62
0.9774	18.03	0.9724	22.71
0.9773	18.12	0.9723	22.8
0.9772	18.22	0.9722	22.89
0.9771	18.31	0.9721	22.98
0.977	18.41	0.972	23.07
0.9769	18.5	0.9719	23.16
0.9768	18.6	0.9718	23.25
0.9767	18.69	0.9717	23.34
0.9766	18.79	0.9716	23.43
0.9765	18.88	0.9715	23.52
0.9764	18.98	0.9714	23.61
0.9763	19.07	0.9713	23.7
0.9762	19.17	0.9712	23.79
0.9761	19.26	0.9711	23.88
0.976	19.36	0.971	23.97
0.9759	19.45	0.9709	24.06
0.9758	19.55	0.9708	24.15
0.9757	19.64	0.9707	24.24
0.9756	19.74	0.9706	24.33
0.9755	19.83	0.9705	24.42
0.9754	19.93	0.9704	24.51
0.9753	20.02	0.9703	24.59
0.9752	20.12	0.9702	24.68
0.9751	20.21	0.9701	24.77
0.97	24.86	0.965	29.14
0.9699	24.95	0.9649	29.22
0.9698	25.04	0.9648	29.31
0.9697	25.12	0.9647	29.39
0.9696	25.21	0.9646	29.47
0.9695	25.3	0.9645	29.55
0.9694	25.39	0.9644	29.64
0.9693	25.48	0.9643	29.72
0.9692	25.56	0.9642	29.8
0.9691	25.65	0.9641	29.88

Sp.gr 20/20 °C	% by vol	Sp.gr 20/20 °C	% by vol
0.969	25.74	0.964	29.96
0.9689	25.83	0.9639	30.04
0.9688	25.91	0.9638	30.12
0.9687	26	0.9637	30.20
0.9686	26.09	0.9636	30.29
0.9685	26.17	0.9635	30.37
0.9684	26.26	0.9634	30.45
0.9683	26.35	0.9633	30.53
0.9682	26.43	0.9632	30.61
0.9681	26.52	0.9631	30.69
0.968	26.61	0.963	30.77
0.9679	26.69	0.9629	30.85
0.9678	26.78	0.9628	30.92
0.9677	26.86	0.9627	31
0.9676	26.95	0.9626	31.08
0.9675	27.04	0.9625	31.16
0.9674	27.12	0.9624	31.24
0.9673	27.21	0.9623	31.32
0.9672	27.29	0.9622	31.4
0.9671	27.38	0.9621	31.47
0.967	27.46	0.962	31.55
0.9669	27.55	0.9619	31.63
0.9668	27.63	0.9618	31.71
0.9667	27.72	0.9617	31.78
0.9666	27.8	0.9616	31.86
0.9665	27.89	0.9615	31.94
0.9664	27.97	0.9614	32.01
0.9663	28.05	0.9613	32.09
0.9662	28.14	0.9612	32.17
0.9661	28.22	0.9611	32.24
0.966	28.31	0.961	32.32
0.9659	28.39	0.9609	32.39
0.9658	28.47	0.9608	32.47
0.9657	28.56	0.9607	32.54
0.9656	28.64	0.9606	32.62

Sp.gr 20/20 ⁰C	% by vol	Sp.gr 20/20 ⁰C	% by vol
0.9655	28.73	0.9605	32.69
0.9654	28.81	0.9604	32.77
0.9653	28.89	0.9603	32.84
0.9652	28.98	0.9602	32.92
0.9651	29.06	0.9601	32.99
0.96	33.07	0.955	36.6
0.9599	33.14	0.9549	36.66
0.9598	33.22	0.9548	36.73
0.9597	33.29	0.9547	36.8
0.9596	33.36	0.9546	36.87
0.9595	33.44	0.9545	36.93
0.9594	33.51	0.9544	37
0.9593	33.59	0.9543	37.07
0.9592	33.66	0.9542	37.13
0.9591	33.73	0.9541	37.2
0.959	33.8	0.954	37.27
0.9589	33.88	0.9539	37.33
0.9588	33.95	0.9538	37.4
0.9587	34.02	0.9537	37.46
0.9586	34.09	0.9536	37.53
0.9585	34.16	0.9535	37.6
0.9584	34.24	0.9534	37.66
0.9583	34.31	0.9533	37.73
0.9582	34.38	0.9532	37.79
0.9581	34.45	0.9531	37.86
0.958	34.52	0.953	37.92
0.9579	34.59	0.9529	37.99
0.9578	34.66	0.9528	38.05
0.9577	34.73	0.9527	38.12
0.9576	34.8	0.9526	38.18
0.9575	34.88	0.9525	38.25
0.9574	34.95	0.9524	38.31
0.9573	35.02	0.9523	38.38
0.9572	35.09	0.9522	38.44
0.9571	35.16	0.9521	38.51

Sp.gr 20/20 °C	% by vol	Sp.gr 20/20 °C	% by vol
0.957	35.23	0.952	38.57
0.9569	35.3	0.9519	38.63
0.9568	35.37	0.9518	38.7
0.9567	35.43	0.9517	38.76
0.9566	35.5	0.9516	38.83
0.9565	35.57	0.9515	38.89
0.9564	35.64	0.9514	38.95
0.9563	35.71	0.9513	39.02
0.9562	35.78	0.9512	39.08
0.9561	35.85	0.9511	39.14
0.956	35.92	0.951	39.21
0.9559	35.99	0.9509	39.27
0.9558	36.05	0.9508	39.33
0.9557	36.12	0.9507	39.4
0.9556	36.19	0.9506	39.46
0.9555	36.26	0.9505	39.52
0.9554	36.33	0.9504	39.58
0.9553	36.39	0.9503	39.65
0.9552	36.46	0.9502	39.71
0.9551	36.53	0.9501	39.77
0.95	39.83	0.945	42.85
0.9499	39.9	0.9449	42.91
0.9498	39.96	0.9448	42.97
0.9497	40.02	0.9447	43.03
0.9496	40.08	0.9446	43.09
0.9495	40.15	0.9445	43.15
0.9494	40.21	0.9444	43.2
0.9493	40.27	0.9443	43.26
0.9492	40.33	0.9442	43.32
0.9491	40.39	0.9441	43.38
0.949	40.46	0.944	43.43
0.9489	40.52	0.9439	43.49
0.9488	40.58	0.9438	43.55
0.9487	40.64	0.9437	43.61
0.9486	40.70	0.9436	43.66

Sp.gr 20/20 °C	% by vol	Sp.gr 20/20 °C	% by vol
0.9485	40.76	0.9435	43.72
0.9484	40.82	0.9434	43.78
0.9483	40.88	0.9433	43.84
0.9482	40.95	0.9432	43.89
0.9481	41.01	0.9431	43.95
0.948	41.07	0.943	44.01
0.9479	41.13	0.9429	44.06
0.9478	41.19	0.9428	44.12
0.9477	41.25	0.9427	44.18
0.9476	41.31	0.9426	44.23
0.9475	41.37	0.9425	44.29
0.9474	41.43	0.9424	44.35
0.9473	41.49	0.9423	44.4
0.9472	41.55	0.9422	44.46
0.9471	41.61	0.9421	44.52
0.947	41.67	0.942	44.57
0.9469	41.73	0.9419	44.63
0.9468	41.79	0.9418	44.69
0.9467	41.85	0.9417	44.74
0.9466	41.91	0.9416	44.8
0.9465	41.97	0.9415	44.86
0.9464	42.03	0.9414	44.91
0.9463	42.09	0.9413	44.97
0.9462	42.15	0.9412	45.02
0.9461	42.21	0.9411	45.08
0.946	42.27	0.941	45.13
0.9459	42.32	0.9409	45.19
0.9458	42.38	0.9408	45.24
0.9457	42.44	0.9407	45.3
0.9456	42.5	0.9406	45.36
0.9455	42.56	0.9405	45.41
0.9454	42.62	0.9404	45.47
0.9453	42.68	0.9403	45.52
0.9452	42.74	0.9402	45.58
0.9451	42.8	0.9401	45.63

Sp.gr **20/20 ^{0}C**	**% by vol**	**Sp.gr** **20/20 ^{0}C**	**% by vol**
0.94	45.69	0.935	48.36
0.9399	45.74	0.9349	48.41
0.9398	45.8	0.9348	48.47
0.9397	45.85	0.9347	48.52
0.9396	45.9	0.9346	48.57
0.9395	45.96	0.9345	48.62
0.9394	46.01	0.9344	48.67
0.9393	46.07	0.9343	48.73
0.9392	46.12	0.9342	48.78
0.9391	46.18	0.9341	48.83
0.939	46.23	0.934	48.88
0.9389	46.28	0.9339	48·93
0.9388	46.34	0.9338	48.99
0.9387	46.39	0.9337	49.04
0.9386	46.45	0.9336	49.09
0.9385	46.5	0.9335	49.14
0.9384	46.55	0.9334	49.19
0.9383	46.61	0.9333	49.24
0.9382	46.66	0.9332	49.3
0.9381	46.72	0.9331	49.35
0.938	46.77	0.933	49.4
0.9379	46.82	0.9329	49.45
0.9378	46.88	0.9328	49.5
0.9377	46.93	0.9327	49.55
0.9376	46.98	0.9326	49.6
0.9375	47.04	0.9325	49.65
0.9374	47.09	0.9324	49.71
0.9373	47.14	0.9323	49.76
0.9372	47.2	0.9322	49.81
0.9371	47.25	0.9321	49.86
0.937	47.3	0.932	49.91
0.9369	47.36	0.9319	49.96
0.9368	47.41	0.9318	50.01
0.9367	47.46	0.9317	50.06
0.9366	47.52	0.9316	50.11

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.9365	47.57	0.9315	50.16
0.9364	47.62	0.9314	50.21
0.9363	47.68	0.9313	50.26
0.9362	47.73	0.9312	50.31
0.9361	47.78	0.9311	50.36
0.936	47.84	0.931	50.41
0.9359	47.89	0.9309	50.46
0.9358	47.94	0.9308	50.51
0.9357	47.99	0.9307	50.56
0.9356	48.05	0.9306	50.62
0.9355	48.1	0.9305	50.67
0.9354	48.15	0.9304	50.72
0.9353	48.2	0.9303	50.77
0.9352	48.26	0.9302	50.82
0.9351	48.31	0.9301	50.87
0.93	50.92	0.925	53.38
0.9299	50.97	0.9249	53.43
0.9298	51.02	0.9248	53.48
0.9297	51.07	0.9247	53.52
0.9296	51.12	0.9246	53.57
0.9295	51.16	0.9245	53.62
0.9294	51.21	0.9244	53.67
0.9293	51.26	0.9243	53.72
0.9292	51.31	0.9242	53.77
0.9291	51.36	0.9241	53.82
0.929	51.41	0.924	53.86
0.9289	51.46	0.9239	53.91
0.9288	51.51	0.9238	53.96
0.9287	51.56	0.9237	54.01
0.9286	51.61	0.9236	54.06
0.9285	51.66	0.9235	54.1
0.9284	51.71	0.9234	54.15
0.9283	51.76	0.9233	54.2
0.9282	51.81	0.9232	54.25
0.9281	51.86	0.9231	54.3

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.928	51.91	0.923	54.35
0.9279	51.96	0.9229	54.39
0.9278	52.01	0.9228	54.44
0.9277	52.06	0.9227	54.49
0.9276	52.11	0.9226	54.54
0.9275	52.16	0.9225	54.59
0.9274	52.21	0.9224	54.63
0.9273	52.26	0.9223	54.68
0.9272	52.31	0.9222	54.73
0.9271	52.35	0.9221	54.78
0.927	52.4	0.922	54.82
0.9269	52.45	0.9219	54.87
0.9268	52.5	0.9218	54.92
0.9267	52.55	0.9217	54.97
0.9266	52.6	0.9216	55.01
0.9265	52.65	0.9215	55.06
0.9264	52.7	0.9214	55.11
0.9263	52.75	0.9213	55.16
0.9262	52.8	0.9212	55.2
0.9261	52.84	0.9211	55.25
0.926	52.89	0.921	55.3
0.9259	52.94	0.9209	55.35
0.9258	52.99	0.9208	55.39
0.9257	53.04	0.9207	55.44
0.9256	53.09	0.9206	55.49
0.9255	53.14	0.9205	55.54
0.9254	53.19	0.9204	55.58
0.9253	53.23	0.9203	55.63
0.9252	53.28	0.9202	55.68
0.9251	53.33	0.9201	55.72
0.92	55.77	0.915	58.1
0.9199	55.82	0.9149	58.14
0.9198	55.87	0.9148	58.19
0.9197	55.91	0.9147	5823

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.9196	55.96	0.9146	58.28
0.9195	56.01	0.9145	58.32
0.9194	56.05	0.9144	58.37
0.9193	56.1	0.9143	58.41
0.9192	56.15	0.9142	58.46
0.9191	56.19	0.9141	58.5
0.919	56.24	0.914	58.55
0.9189	56.29	0.9139	58.59
0.9188	56.33	0.9138	58.64
0.9187	56.38	0.9137	58.68
0.9186	56.43	0.9136	58.73
0.9185	56.47	0.9135	58.77
0.9184	56.52	0.9134	58.82
0.9183	56.57	0.9133	58.86
0.9182	56.61	0.9132	58.91
0.9181	56.66	0.9131	58.95
0.918	56.71	0.913	59
0.9179	56.75	0.9129	59.04
0.9178	56.8	0.9128	59.09
0.9177	56.85	0.9127	59.13
0.9176	56.9	0.9126	59.18
0.9175	56.94	0.9125	59.22
0.9174	56.99	0.9124	59.27
0.9173	57.04	0.9123	59.31
0.9172	57.08	0.9122	59.36
0.9171	57.13	0.9121	59.4
0.917	57.17	0.912	59.45
0.9169	57.22	0.9119	59.49
0.9168	57.27	0.9118	59.54
0.9167	57.31	0.9117	59.58
0.9166	57.36	0.9116	59.63
0.9165	57.41	0.9115	59.67
0.9164	57.46	0.9114	59.72
0.9163	57.5	0.9113	59.76

Sp.gr 20/20 ⁰C	% by vol	Sp.gr 20/20 ⁰C	% by vol
0.9162	57.55	0.9112	59.8
0.9161	57.59	0.9111	59.85
0.916	57.64	0.911	59.89
0.9159	57.69	0.9109	59.94
0.9158	57.73	0.9108	59.98
0.9157	57.78	0.9107	60.03
0.9156	57.82	0.9106	60.07
0.9155	57.87	0.9105	60.12
0.9154	57.91	0.9104	60.16
0.9153	57.96	0.9103	60.21
0.9152	58	0.9102	60.25
0.9151	58.05	0.9101	60.3
0.91	60.34	0.905	62.53
0.9099	60.38	0.9049	62.58
0.9098	60.43	0.9048	62.62
0.9097	60.47	0.9047	62.66
0.9096	60.52	0.9046	62.71
0.9095	60.56	0.9045	62.75
0.9094	60.61	0.9044	62.8
0.9093	60.65	0.9043	62.84
0.9092	60.69	0.9042	62.88
0.9091	60.74	0.9041	62.93
0.909	60.78	0.904	62.97
0.9089	60.83	0.9039	63.01
0.9088	60.87	0.9038	63.06
0.9087	60.92	0.9037	63.10
0.9086	60.96	0.9036	63.14
0.9085	61	0.9035	63.19
0.9084	61.05	0.9034	63.23
0.9083	61.09	0.9033	63.27
0.9082	61.14	0.9032	63.31
0.9081	61.18	0.9031	63.36
0.908	61.22	0.903	63.4
0.9079	61.27	0.9029	63.44
0.9078	61.31	0.9028	63.49

Sp.gr 20/20 °C	% by vol	Sp.gr 20/20 °C	% by vol
0.9077	61.36	0.9027	63.53
0.9076	61.4	0.9026	63.57
0.9075	61.44	0.9025	63.62
0.9074	61.49	0.9024	63.66
0.9073	61.53	0.9023	63.7
0.9072	61.58	0.9022	63.75
0.9071	61.62	0.9021	63.79
0.907	61.66	0.902	63.83
0.9069	61.71	0.9019	63.88
0.9068	61.75	0.9018	63.92
0.9067	61.79	0.9017	63.96
0.9066	61.84	0.9016	64
0.9065	61.88	0.9015	64.05
0.9064	61.93	0.9014	64.09
0.9063	61.97	0.9013	64.13
0.9062	62.01	0.9012	64.18
0.9061	62.06	0.9011	64.22
0.906	62.1	0.901	64.26
0.9059	62.14	0.9009	64.3
0.9058	62.19	0.9008	64.35
0.9057	62.23	0.9007	64.39
0.9056	62.27	0.9006	64.43
0.9055	62.32	0.9005	64.47
0.9054	62.36	0.9004	64.52
0.9053	62.4	0.9003	64.56
0.9052	62.45	0.9002	64.6
0.9051	62.49	0.9001	64.65
0.9	64.69	8950	66.79
0.8999	64.73	0.8949	66.83
0.8998	64.77	0.8948	66.87
0.8997	64.82	0.8947	66.92
0.8996	64.86	0.8946	66.96
0.8995	64.9	0.8945	67
0.8994	64.94	0.8944	67.04
0.8993	64.99	0.8943	67.08

Sp.gr 20/20 °C	% by vol	Sp.gr 20/20 °C	% by vol
0.8992	65.03	0.8942	67.12
0.8991	65.07	0.8941	67.16
0.899	65.11	0.894	67.21
0.8989	65.16	0.8939	67.25
0.8988	65.2	0.8938	67.29
0.8987	65.24	0.8937	67.33
0.8986	65.28	0.8936	67.37
0.8985	65.32	0.8935	67.41
0.8984	65.37	0.8934	67.45
0.8983	65.41	0.8933	67.49
0.8982	65.45	0.8932	67.54
0.8981	65.49	0.8931	67.58
0.898	65.54	0.893	67.62
0.8979	65.58	0.8929	67.66
0.8978	65.62	0.8928	67.7
0.8977	65.66	0.8927	67.74
0.8976	65.7	0.8926	67.78
0.8975	65.75	0.8925	67.82
0.8974	65.79	0.8924	67.87
0.8973	65.83	0.8923	67.91
0.8972	65.87	0.8922	67.95
0.8971	65.91	0.8921	67.99
0.897	65.96	0.892	68.43
0.8969	66	0.8919	68.07
0.8968	66.04	0.8918	68.11
0.8967	66.08	0.8917	68.15
0.8966	66.12	0.8916	68.19
0.8965	66.17	0.8915	68.24
0.8964	66.21	0.8914	68.28
0.8963	66.25	0.8913	68.32
0.8962	66.29	0.8912	68.36
0.8961	66.33	0.8911	68.4
0.896	66.37	0.891	68.44
0.8959	66.42	0.8909	68.48
0.8958	66.46	0.8908	68.52

Sp.gr 20/20 °C	**% by vol**	**Sp.gr** 20/20 °C	**% by vol**
0.8957	66.5	0.8907	68.56
0.8956	66.54	0.8906	68.6
0.8955	66.58	0.8905	68.65
0.8954	66.62	0.8904	68.69
0.8953	66.67	0.8903	68.73
0.8952	66.71	0.8902	68.77
0.8951	66.75	0.8901	68.81
0.89	68.85	0.885	70.86
0.8899	68.89	0.8849	70.9
0.8898	68.93	0.8848	70.94
0.8897	68.97	0.8847	70.98
0.8896	69.01	0.8846	71.02
0.8895	69.05	0.8845	71.06
0.8894	69.09	0.8844	71.1
0.8893	69.13	0.8843	71.14
0.8892	69.17	0.8842	71.18
0.8891	69.22	0.8841	71.22
0.889	69.26	0.884	71.26
0.8889	69.34	0.8838	71.34
0.8887	69.38	0.8837	71.38
0.8886	69.42	0.8836	71.42
0.8885	69.46	0.8835	71.46
0.8884	69.5	0.8834	71.5
0.8883	69.54	0.8833	71.54
0.8882	69.58	0.8832	71.58
0.8881	69.62	0.8831	71.61
0.888	69.66	0.883	71.65
0.8879	69.7	0.8829	71.69
0.8878	69.74	0.8828	71.73
0.8877	69.78	0.8827	71.77
0.8876	69.82	0.8826	71.81
0.8875	69.86	0.8825	71.85
0.8874	69.9	0.8824	71.89
0.8873	69.94	0.8823	71.93
0.8872	69.98	0.8822	71.97

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.8871	70.02	0.8821	72.01
0.887	70.06	0.882	72.05
0.8869	70.1	0.8819	72.09
0.8868	70.14	0.8818	72.12
0.8867	70.18	0.8817	72.16
0.8866	70.22	0.8816	72.2
0.8865	70.26	0.8815	72.24
0.8864	70.3	0.8814	72.28
0.8863	70.34	0.8813	72.32
0.8862	70.38	0.8812	72.36
0.8861	70.42	0.8811	72.4
0.886	70.46	0.881	72.44
0.8859	70.5	0.8809	72.48
0.8858	70.54	0.8808	72.52
0.8857	70.58	0.8807	72.56
0.8856	70.62	0.8806	72.59
0.8855	70.66	0.8805	72.63
0.8854	70.7	0.8804	72.67
0.8853	70.74	0.8803	72.71
0.8852	70.78	0.8802	72.75
0.8851	70.82	0.8801	72.79
0.88	72.83	0.875	74.76
0.8799	72.87	0.8749	74.8
0.8798	72.91	0.8748	74.83
0.8797	72.95	0.8747	74.87
0.8796	72.99	0.8746	74.91
0.8795	73.02	0.8745	74.95
0.8794	73.06	0.8744	74.99
0.8793	73.1	0.8743	75.03
0.8792	73.14	0.8742	75.06
0.8791	73.18	0.8741	75.1
0.879	73.22	0.874	75.14
0.8789	73.26	0.8739	75.18
0.8788	73.3	0.8738	75.22
0.8787	73.33	0.8737	75.25

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.8786	73.37	0.8736	75.29
0.8785	73.41	0.8735	75.33
0.8784	73.45	0.8734	75.37
0.8783	73.49	0.8733	75.41
0.8782	73.53	0.8732	75.44
0.8781	73.57	0.8731	75.48
0.878	73.61	0.873	75.52
0.8779	73.64	0.8729	75.56
0.8778	73.68	0.8728	75.6
0.8777	73.72	0.8727	75.63
0.8776	73.76	0.8726	75.67
0.8775	73.8	0.8725	75.71
0.8774	73.84	0.8724	75.75
0.8773	73.87	0.8723	75.78
0.8772	73.91	0.8722	75.82
0.8771	73.95	0.8721	75.86
0.877	73.99	0.872	75.9
0.8769	74.03	0.8719	75.93
0.8768	74.07	0.8718	75.97
0.8767	74.11	0.8717	76.01
0.8766	74.14	0.8716	76.05
0.8765	74.18	0.8715	76.09
0.8764	74.22	0.8714	76.12
0.8763	74.26	0.8713	76.16
0.8762	74.3	0.8712	76.2
0.8761	74.34	0.8711	76.24
0.876	74.37	0.871	76.27
0.8759	74.41	0.8709	76.31
0.8758	74.45	0.8708	76.35
0.8757	74.49	0.8707	76.39
0.8756	74.53	0.8706	76.42
0.8755	74.57	0.8705	76.46
0.8754	74.6	0.8704	76.5
0.8753	74.64	0.8703	76.54

Sp.gr 20/20 °C	**%** **by vol**	**Sp.gr** 20/20 °C	**%** **by vol**
0.8752	74.68	0.8702	76.57
0.8751	74.72	0.8701	76.61
0.87	76.65	0.865	78.49
0.8699	76.68	0.8649	78.52
0.8698	76.72	0.8648	78.56
0.8697	76.76	0.8647	78.6
0.8696	76.8	0.8646	78.63
0.8695	76.83	0.8645	78.67
0.8694	76.87	0.8644	78.71
0.8693	76.91	0.8643	78.74
0.8692	76.94	0.8642	78.78
0.8691	76.98	0.8641	78.82
0.869	77.02	0.864	78.85
0.8689	77.06	0.8639	78.89
0.8688	77.09	0.8638	78.93
0.8687	77.13	0.8637	78.96
0.8686	77.17	0.8636	79
0.8685	77.2	0.8635	79.03
0.8684	77.24	0.8634	79.07
0.8683	77.28	0.8633	79.11
0.8682	77.32	0.8632	79.14
0.8681	77.35	0.8631	79.18
0.868	77.39	0.863	79.22
0.8679	77.43	0.8629	79.25
0.8678	77.46	0.8628	79.29
0.8677	77.5	0.8627	79.32
0.8676	77.54	0.8626	79.36
0.8675	77.57	0.8625	79.4
0.8674	77.61	0.8624	79.43
0.8673	77.65	0.8623	79.47
0.8672	77.68	0.8622	79.5
0.8671	77.72	0.8621	79.54
0.867	77.76	0.862	79.58
0.8669	77.79	0.8619	79.61
0.8668	77.83	0.8618	79.65

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.8667	77.87	0.8617	79.68
0.8666	77.9	0.8616	79.72
0.8665	77.94	0.8615	79.76
0.8664	77.98	0.8614	79.79
0.8663	78.01	0.8613	79.83
0.8662	78.45	0.8612	79.86
0.8661	78.09	0.8611	79.9
0.8643	78.12	0.861	79.94
0.8659	78.16	0.8609	79.97
0.8658	78.2	0.8608	80.01
0.84357	78.23	0.8607	80.04
0.8656	78.27	0.8606	80.08
0.8655	78.31	0.8605	80.12
0.8654	78.34	0.8604	80.15
0.8653	78.38	0.8603	80.19
0.8652	78.42	0.8602	80.22
0.8651	78.45	0.8601	80.26
0.86	80.29	8550	82.06
0.8599	80.33	0.8549	82.09
0.8598	80.36	0.8548	82.13
0.8597	80.4	0.8547	82.16
0.8596	80.44	0.8546	82.2
0.8595	80.47	0.8545	82.23
0.8594	80.51	8544	82.27
0.8593	80.54	0.8543	82.3
0.8592	80.58	0.8542	82.34
0.8591	80.61	0.8541	82.37
0.859	80.65	0.854	82.41
0.8589	80.68	0.8539	82.44
0.8588	80.72	8538	82.48
0.8587	80.76	0.8537	82.51
0.8586	80.79	0.8536	82.54
0.8585	80.83	0.8535	82.58
0.8584	80.86	0.8534	82.61
0.8583	80.9	0.8533	82.65

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.8582	80.93	0.8532	82.68
0.8581	80.97	0.8531	82.72
0.858	81	0.853	82.75
0.8579	81.04	0.8529	82.79
0.8578	81.07	0.8528	82.82
0.8577	81.11	0.8527	82.86
0.8576	81.14	0.8526	82.89
0.8575	81.18	0.8525	82.92
0.8574	81.21	0.8524	82.96
0.8573	81.25	0.8523	82.99
0.8572	81.28	0.8522	83.03
0.8571	81.32	0.8521	83.06
0.857	81.35	0.852	83.1
0.8569	81.39	0.8519	83.13
0.8568	81.43	0.8518	83.17
0.8567	81.46	0.8517	83.2
0.8566	81.5	0.8516	83.23
0.8565	81.53	0.8515	83.27
0.8564	81.57	0.8514	83.3
0.8563	81.6	0.8513	83.34
0.8562	81.64	0.8512	83.37
0.8561	81.67	0.8511	83.41
0.856	81.71	0 .8510	83.44
0.8559	81.74	0.8509	83.47
0.8558	81.78	0.8508	83.51
0.8557	81.81	0.8507	83.54
0.8556	81.85	0.8506	83.58
0.8555	81.88	0.8505	83.61
0.8554	81.92	0.8504	83.65
0.8553	81.95	0.8503	83.68
0.8552	81.99	0.8502	83.71
0.8551	82.02	0.8501	83.75
0.85	83.78	0.845	85.46
0.8499	83.82	0.8449	85.49
0.8498	83.85	0.8448	85.53

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.8497	83.88	0.8447	85.56
0.8496	83.92	0.8446	85.59
0.8495	83.95	0.8445	85.63
0.8494	83.99	0.8444	85.66
0.8493	84.02	0.8443	85.69
0.8492	84.05	0.8442	85.73
0.8491	84.09	0.8441	85.76
0.849	84.12	0.8440	85.79
0.8489	84.15	0.8439	85.82
0.8488	84.19	0.8438	85.86
0.8487	84.22	0.8437	85.89
0.8486	84.26	0.8436	85.92
0.8485	84.29	0.8435	85.95
0.8484	84.32	0.8434	85.99
0.8483	84.36	0.8433	86.02
0.8482	84.39	0.8432	86.05
0.8481	84.42	0.8431	86.08
0.848	84.46	0.843	86.12
0.8479	84.49	0.8429	86.15
0.8478	84.53	0.8428	86.18
0.8477	84.56	0.8427	86.22
0.8476	84.59	0.8426	86.25
0.8475	84.63	0.8425	86.28
0.8474	84.66	0.8424	86.31
0.8473	84.69	0.8423	86.35
0.8472	84.73	0.8422	86.38
0.8471	84.76	0.8421	86.41
0.847	84.79	0.842	86.44
0.8469	84.83	0.8419	86.48
0.8468	84.86	0.8418	86.51
0.8467	84.90	0.8417	86.54
0.8466	84.93	0.8416	86.57
0.8465	84.96	0.8415	86.61
0.8464	85.00	0.8414	86.64
0.8463	85.03	0.8413	86.67

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.8462	85.06	0.8412	86.7
0.8461	85.10	0.8411	86.74
0.846	85.13	0.841	86.77
0.8459	85.16	0.8409	86.8
0.8458	85.2	0.8408	86.83
0.8457	85.23	0.8407	86.87
0.8456	85.26	0.8406	86.9
0.8455	8530	0.8405	86.93
0.8454	85.33	0.8404	86.96
0.8453	85.36	0.8403	87
0.8452	85.40	8402	87.03
0.8451	85.43	0.8401	87.06
0.84	87.09	0.835	88.68
0.8399	87.13	0.8349	88.72
0.8398	87.16	0.8348	88.75
0.8397	87.19	0.8347	88.78
0.8396	87.22	0.8346	88.81
0.8395	87.26	0.8345	88.84
0.8394	87.29	0.8344	88.87
0.8393	87.32	0.8343	88.9
0.8392	87.35	0.8342	88.93
0.8391	87.38	0.8341	88.96
0.839	87.42	0.834	89
0.8389	87.45	0.8339	89.03
0.8388	87.48	0.8338	89.06
0.8387	87.51	0.8337	89.09
0.8386	87.55	0.8336	89.12
0.8385	87.58	0.8335	89.15
0.8384	87.61	0.8334	89.18
0.8383	87.64	0.8333	89.21
0.8382	87.67	0.8332	89.24
0.8381	87.71	0.8331	89.27
0.838	87.74	0.833	89.3
0.8379	87.77	0.8329	89.33
0.8378	87.8	0.8328	89.37

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.8377	87.83	0.8327	89.4
0.8376	87.86	0.8326	89.43
0.8375	87.90	0.8325	89.46
0.8374	87.93	0.8324	89.49
0.8373	87.96	0.8323	89.52
0.8372	87.99	0.8322	89.55
0.8371	88.02	0.8321	89.58
0.837	88.06	0.832	89.61
0.8369	88.09	0.8319	89.64
0.8368	88.12	0.8318	89.67
0.8367	88.15	0.8317	89.7
0.8366	88.18	0.8316	89.73
0.8365	88.21	0.8315	89.76
0.8364	88.24	0.8314	89.79
0.8363	88.28	0.8313	89.82
0.8362	88.31	0.8312	89.85
0.8361	88.34	0.8311	89.88
0.836	88.37	0.831	89.91
0.8359	88.4	0.8309	89.94
0.8358	88.43	0.8308	89.97
0.8357	88.47	0.8307	90
0.8356	88.5	0.8306	90.04
0.8355	88.53	0.8305	90.07
0.8354	88.56	0.8304	90.1
0.8353	88.59	0.8303	90.13
0.8352	88.62	0.8302	90.16
0.8351	88.65	0.8301	90.19
0.83	90.22	0.825	91.69
0.8299	90.25	0.8249	91.72

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