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Food Safety and Standards Authority of India

(A statutory Authority established under the Food Safety and Standards Act, 2006)

(Quality Assurance Division)

FDA Bhawan, Kotla Road, New Delhi – 110002

Dated, the 20 July, 2021

ORDER

Subject: **Revised FSSAI Manual of Methods of Analysis of Foods – Alcoholic Beverages – reg.**

Revised FSSAI "Manual of Methods of Analysis of Foods – Alcoholic Beverages" which has been approved by the Food Authority in its 35th meeting held on 24.06.2021 is enclosed herewith.

2. This manual shall be used by the laboratories with immediate effect. It supersedes the earlier manual on Alcoholic Beverages issued vide Office Order No. 1-90/FSSAI/SP (MS&A)/2009 dated 03.07.2019.

3. Since the process of updation of test methods is dynamic, any changes happening from time to time will be notified separately. Queries/concerns, if any, may be forwarded to *email: sp-sampling@fssai.gov.in, dinesh.k@fssai.gov.in*

Encl: as above

(Harinder Singh Oberoi)
Advisor (QA)

To:

1. All FSSAI Notified Laboratories
2. All State Food Testing Laboratories

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA

Inspiring Trust, Assuring Safe & Nutritious Food

Ministry of Health and Family Welfare, Government of India

MANUAL OF METHODS OF ANALYSIS OF FOODS

ALCOHOLIC BEVERAGES

PREFACE

Food safety requires an assurance that food will not cause any harm to the consumer, when it is prepared and/or consumed according to its intended use. There is a significant challenge in ensuring food safety to protect public health. Safeguarding food safety in today's complex world is a formidable task and is possible only with an intensive effort of all the stakeholders including regulatory authorities, industry and consumers.

The FSSAI Manual of Methods for Analysis of Alcoholic Beverages is principally intended to provide unified, up-to-date testing methods for regulatory compliance. The manual brings together testing methodologies approved by FSSAI for use in surveillance and implementing the regulatory program. The objective here is to adopt "One Parameter - One Method" approach. These methods are dynamic and will be constantly updated, commensurate with the latest technological advancements in food analysis. The FSSAI notified laboratories shall use these testing methods only for analyzing samples under the Food Safety and Standards Act, 2006 and Food Safety and Standards Regulations, 2011.

Any suggestions/feedback from the stakeholders, which will contribute towards updating the manuals from time to time are welcome.

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ACKNOWLEDGEMENT

My deepest sense of gratitude and indebtedness to all the Members of the Panel on "Methods of Sampling and Analysis" especially Dr. Jagan Mohan Rao whose help, knowledge and insight has led to the successful revision of this manual.

Sincere thanks to the Panel, Chairman for their valuable guidance and encouragement and the Secretariat of this panel who have extended their support during this revision process.

Deepest appreciation to the Chairperson, FSSAI and CEO, FSSAI for their cooperation, support and constant encouragement without which the work would not have seen the light of day.

July 2021

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FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA

Inspiring Trust, Assuring Safe & Nutritious Food

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Note: The test methods given in the manual are standardized / validated/ taken from national or international methods or recognized specifications, however it would be the responsibility of the respective testing laboratory to verify the performance of these methods onsite and ensure that it gives proper results before putting these methods in to use.

MANUAL FOR ANALYSIS OF ALCOHOLIC BEVERAGES

1.0 Alcoholic Beverages and Types

Alcoholic beverages comprise a large group of beverages that contain varying amounts of alcohol (ethanol).These are produced by fermentation of grains, fruits, or other sources of sugar. The consumption of alcohol plays an important social role in many cultures. Most countries have laws regulating the production, sale, and consumption of alcoholic beverages. Following types alcoholic beverages are produced industrially and consumed.

- Rum: Rum is a liquor made by fermenting then distilling sugarcane molasses or sugarcane juice, has a typical alcohol concentration of 40% ABV. The distillate, a clear liquid, is usually aged in oak barrels.

- Gin: Gin is a distilled alcoholic drink (have anywhere from 35% to 55% ABV) that derives its predominant flavour from juniper berries (*Juniperus communis*). Gin originated as a medicinal liquor made by monks and alchemists across Europe, particularly in southern France, Flanders and the Netherlands, to provide aqua vita from distillates of grapes and grains.

- Whisky: Whisky is a type of distilled alcoholic beverage (ABV of whiskey ranges from 40% to 50%) made from fermented grain mash or by distilling beer. Various grains are used for different varieties, including barley, corn, rye, and wheat. Whisky is typically aged in wooden casks, generally made of charred white oak

- Brandy: Brandy is a liquor produced by distilling wine. Brandy generally contains 35–60% alcohol by volume and is typically consumed as an after-dinner digestif. Some brandies are aged in wooden casks. Varieties of wine brandy can be found. The most renowned are Cognac and Armagnac.

- Beer: Beer (have ~5% ABV) is brewed from cereal grains—most commonly from malted barley, wheat, maize, and rice.

- Vodka: Vodka (have ~40% ABV) is a clear distilled alcoholic beverage. In general, it is made by distilling the liquid from cereal grains (e.g., wheat) and vegetables (e.g., Potatoes) containing starch, that are fermented with yeast. There are different varieties originating in Poland, Russia and Sweden. It is composed primarily of water and ethanol, but sometimes with traces of flavorings (essences of herbs, fruits, grasses, and spices). Some modern brands are using fruits, honey or maple sap as the base.

- Wine: Wine is an alcoholic drink typically made from fermented grape juice. Yeast consumes the sugar in the grapes and converts it to ethanol, carbon dioxide, and heat. Different varieties of grapes and strains of yeasts produce different styles of wine. The range of ABV for unfortified wine is about 5.5% to 16%, with an average of 11.6%.

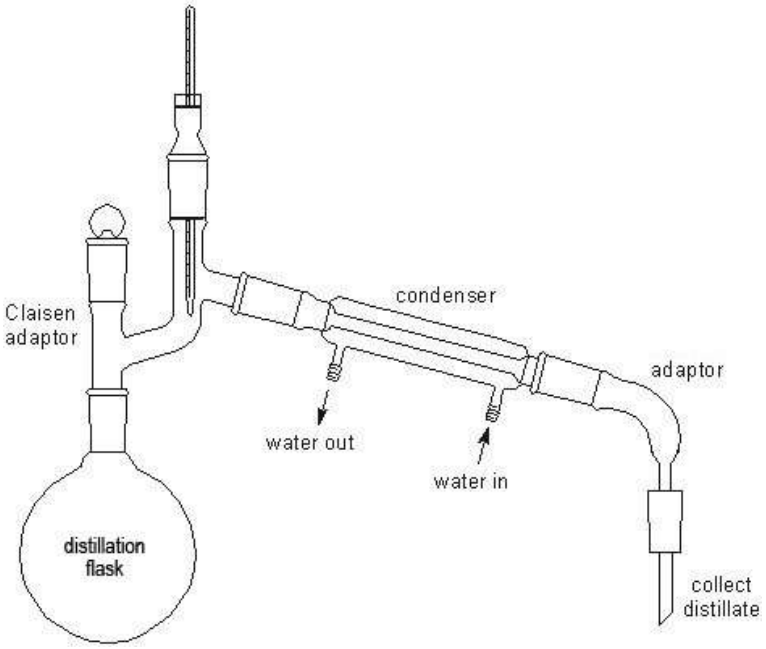
- Rice Wine: It is an alcoholic beverage fermented and distilled from rice and typically has an alcohol content of 18-25% ABV. Rice wine is made by the fermentation of rice starch that has been converted to sugars. Microbes are the source of the enzymes that convert the starches to sugar. It is traditionally consumed in East Asia, Southeast Asia and Northeast India at formal dinners and banquets and in cooking.

- Toddy: Toddy (have 4-6% ABV), known by several local names, is an alcoholic beverage created from the sap of various species of palm tree such as the palmyra, date palms, and coconut palms

- Fenny (Cashew & Coconut etc.): Fenny (have 42-43 ABV), is a spirit produced in Goa, India. The two most popular types of feni are cashew fenny and toddy coconut palm fenny, depending on the original ingredient; however, many other varieties are sold.

2.0 General Glassware and Apparatus

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)

	Determination of Ethyl Alcohol Content - Pycnometer Method or Hydrometer Method		
Method No.	FSSAI 13.001:2021	**Revision No. & Date**	0.0
Scope	Pycnometer Method or Hydrometer Method (after distillation)-Specific gravity of the alcoholic beverages can be determined. The method is applicable to all alcoholic beverages.		
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 and Annexure - II).		
Apparatus /Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Distillation Unit: Distillation flask of 500 mL 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.  (Figure is adopted from FSSAI Manual of Methods of Analysis of Foods: Alcoholic beverages, 2019, Page 5). 3. Pycnometer: 50 mL capacity/ SG Hydrometer, Short range (0.96 – 1.00). 4. Thermometer: 0-100 °C 5. Volumetric flask: 200 mL capacity		
Materials and reagents	Alcoholic beverages		
Method of Analysis	1. Transfer exactly 200 mL of alcoholic drink into a 500 mL distillation flask containing about 25 mL of distilled water and a few pieces of pumice stone. 2. Distil the contents in about 35 min and collect the distillate in a 200 mL 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.		

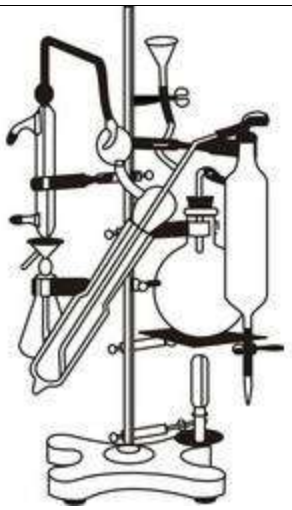
	6. Wipe the Liquid that spills out using water absorbing filter paper and weigh at 20 °C(W1). 7. Next remove the liquor sample distillate and wash it with distilled water. 8. Fill the pycnometer with distilled water in the same manner as described above and at 20 °C take the weight (W2).
Calculation with units of expression	Specific gravity= $\frac{W1-W2}{W2-W}$ W: Weight of Empty Pycnometer W1: Weight of Empty Pycnometer with liquor sample W2: Weight of Empty Pycnometer with water Find out the corresponding alcohol percent by volume from the table showing Specific Gravity Vs Alcohol percent (Refer Annexure I). Alternatively, use a SG hydrometer to find out the specific gravity (SG) and use the following equation to convert SG to % Alcohol. % Alcohol (v/v) = 8610.6 – (16584× SG) + (7973.3 × SG 2) (One can use computer program to automate this process).
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Ethyl Alcohol Content - Distillation Method (for products containing high volatile acids)**		
Method No.	FSSAI 13.002:2021	**Revision No. & Date**	0.0
Scope	Distillation method is used for alcoholic beverages products containing high volatile acids.		
Caution	1. Petroleum ether: Harmful when inhaled in high concentrations or ingested. Petroleum ether may cause dizziness and drowsiness if inhaled, and high concentrations may result in central nervous system depression, and loss of consciousness. 2. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage.		
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 /Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Volumetric flask, 200 mL capacity. 3. Separatory funnels, 500 mL capacity. 4. Distillation unit with assembly		
Materials and Reagents	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 (4g) dissolved in 1 L water. 2. Phenolphthalein indicator solution - Dissolve 1.0 g of phenolphthalein in 100 mL rectified spirit.		
Method of Analysis	1. Measure 200 mL of liquor sample in a volumetric flask. 2. Transfer to aseparatory funnel and wash the volumetric flask with about 100 mL 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 20-30 mL 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 with units of expression	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	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Gas Chromatography-FID Method of Alcohol Estimation using Chromosorb Support Columns**		
Method No	FSSAI 13.003:2021	**Revision No. & Date**	0.0
Scope	Gas Chromatography-Flame Ionization Detection Method of alcohol estimation using chromosorb support columns and is applicable to all alcoholic beverages		
Caution	Propanol: Exposure to propyl alcohol might irritate eyes, nose, and throat. Exposure to high concentrations can cause headache, drowsiness, dizziness, confusion, nausea and vomiting. Propyl alcohol may cause liver damage. Propyl alcohol is a flammable liquid and a dangerous fire hazard.		
Principle	n-Propanol internal standard is added to sample and ethanol is determined by GC - flame ionization detection.		
Apparatus / Instruments	1. General Glass ware and apparatus (Refer 2.0 at page no. 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 °C, column temperature 185 °C isothermal (adjust temperature so ethanol elutes in 1min, n-propanol in 1.6 min); detector temperature 250 °C; chart speed and attenuation as required based on instrument used. *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.*		
Materials and reagents	1. Alcoholic beverages. 2. n-Propanol. 3. Ethanol.		
Preparation of reagents	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 or hydrometer. Alternatively, prepare standard solutions by quantitative dilution of concentrated ethanol solution analyzed by one of above techniques. Keep solutions refrigerated.		
Method of analysis	1. Pipet 5.0 mL ethanol standard solutions into separate glass-stoppered flasks. 2. Add 5.0 mL internal standard solution to each and mix well. 3. De-carbonate beer by filtering through S&S 560 or equivalent paper. Pipet 5.0 mL into glass-stoppered flask. Add 5.0 mL aqueous n-propanol internal standard solution. Mix thoroughly by swirling. 4. Inject 0.2 µL 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. Repeat analysis of 5% ethanol standard solution each day. 5. Inject 0.2 µL of beverage (prepared beer solution) onto GC column, and determine ratio of ethanol to n-propanol peaks.		
Calculation with units of expression	$\text{Ethanol, \% (v/v)} = \text{(peak area ethanol / peak area n} - \text{propanol)}$		
Reference	AOAC 984.14, 1988, Gas chromatographic method		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Ethyl Alcohol Content - Dichromate Oxidation Method**		
Method No.	FSSAI 13.004:2021	**Revision No. & Date**	0.0
Scope	Dichromate oxidation method is used to determine the alcohol content in alcoholic beverages.		
Caution	1. Sulphuric acid: Concentrated sulfuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If sulfuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 2. Potassium dichromate: Corrosive. Causes severe burns to every area of contact. Harmful if swallowed or inhaled. Affects the respiratory system, liver, kidneys, eyes, skin and blood. 3. Ferrous Ammonium Sulfate: Ferrous Ammonium Sulfate can affect, when breathed in. Contact can irritate the skin and eyes. Breathing Ferrous Ammonium Sulfate can irritate the nose and throat causing coughing and wheezing. High exposure may cause nausea, stomach pain, diarrhea, vomiting and drowsiness. 4. 1,10-Phenanthroline: 1,10-Phenanthroline is absorbed through the skin. Symptoms/effects after inhalation: Slight irritation. Symptoms/effects after skin contact: Slight irritation.		
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 / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 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.		

	 (Figure is adopted from FSSAI Manual of Methods of Analysis of Foods: Alcoholic beverages, 2019, Page 10).
Materials and Reagents	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 325 mL H_2SO_4 to ca 400 mL H_2O in 1 L volumetric flask. Mix and cool to 80- 90 °C. Add 33.768 g $K_2Cr_2O_7$ (primary standard). Dissolve, cool, and dilute to volume with H_2O at 20 °C. 2. Ferrous ammonium sulfate solution - Dissolve 135.5 g $FeSO_4$ $(NH_4)_2SO_4 \cdot 6H_2O$ 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 0.695 g $FeS0_4.7H_2O$ in ca 50 mL H_2O, add 1.485 g o-phenanthroline$\cdot H_2O$, and dilute to 100 mL with H_2O.
Method of Analysis	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 25 mL $K_2Cr_2O_7$ solution in 50 mL 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 slightly over mark, and wipe excess wine from exterior. Hold pipet vertical with tip touching inside neck of test bottle, drain to mark. Drain pipette 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 40 mL, 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 1 mL test portion by filling 1 mL 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 pipette tip rests just above H_2O. Let pipette drain 15 s 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 25 mL $K_2Cr_2O_7$ solution in 50 mL 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 40 mL. 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 500 mL 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 25 mL $K_2Cr_2O_7$ (V' mL). Discard $FeSO_4(NH_4)_2SO_4$ solution that has been standing in buret >30 min.
Calculation with units of expression	Calculate % alcohol by volume $= 25.00 - (25 \times V/V')$ V –Volume of $FeSO_4(NH_4)_2SO_4$ solution used for reaction. V' –Volume of $FeSO_4(NH_4)_2SO_4$ solution used for blank.
Reference	AOAC 969.12-1988, alcohol in wines by dichromate oxidation
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Gas Chromatography-FID Method of Ethyl Alcohol Estimation using Carbowax (on carbopack support) Column**		
Method No.	FSSAI 13.005:2021	**Revision No. & Date**	0.0
Scope	Gas Chromatography/FID method using carbowax (on carbopack support) column for determination of alcoholic content in alcoholic beverages.		
Caution	2-Propanol: Non-toxic in contact with skin (LD50 skin> 5000 mg/kg). May cause drowsiness or dizziness. Causes serious eye irritation. Symptoms/effects after inhalation: exposure to high concentrations: coughing.		
Principle	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.		
Apparatus/ Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 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.		
Materials and Reagents	1. Alcoholic beverages 2. 2-propanol 3. Ethanol		
Preparation of reagents	1. Internal standard solution - 0.2% (v/v) 2-propanol in H_2O. 2. Alcohol standard solution - Prepare Alcohol-H_2O 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).		
Method of Analysis	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). 5. 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 count for internal standard peak. 6. Gas chromatograph specifications:		

Carrier gas	N_2
Flow rate, mL/min	15
Oven temperature	105 °C
Injector temperature	175 °C
Detector temperature	175 °C

Calculation with units of expression	Alcohol % = (RR × % alcohol in standard) ÷ RR′ RR-Response ratio with known quantities. RR'- Response ratio with test sample
Reference	AOAC 983.13-1988, Alcohol in wines. Gas chromatographic method
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Residue on Evaporation**		
Method No.	FSSAI 13.006:2021	**Revision No. & Date**	0.0
Scope	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 / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Hot Air oven. 3. Water bath. 4. Desiccator. 5. Glass bowl, 250 mL capacity. 6. Volumetric flask, 200 mL.		
Method of Analysis	1. Transfer 200 mL 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 °C for 2 h. 3. Cool in a desiccator and weigh the dish (W1). 4. Repeat till constant weight is obtained. 5. Calculate the % residual solids.		
Calculation with units of expression	$\text{Residue on evaporation \%}\left(\frac{w}{v}\right) = \frac{W1 - W}{V} \times 100$ Where, W1 = weight of glass bowl with dry residue, in g W = weight of empty glass bowl, in g V = volume of liquor taken for the estimation, in mL		
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Total Acids (as Tartaric Acid) - Method I (for colourless liquors)		
Method No.	FSSAI 13.007:2021	**Revision No. & Date**	0.0
Scope	Method I – This method is used to determine total acidity of colorless alcoholic beverages only.		
Caution	Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage.		
Principle	Total acids present in alcoholic beverages are estimated using acid –base titration using phenolphthalein as indicator.		
Apparatus /Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2).		
Materials and Reagents	1. Sodium hydroxide. 2. Phenolphthalein indicator. 3. Rectified spirit.		
Preparation of reagents	1. Sodium hydroxide solution (0.05N): Sodium hydroxide (2 g) dissolved in 1 L water. 2. Phenolphthalein indicator solution - Dissolve 1.0 g of phenolphthalein in 100 mL rectified spirit.		
Method of Analysis	1. Take 50 mL of liquor sample and add about 200 mL neutral distilled water. 2. Titrate against standard sodium hydroxide using Phenolphthalein indicator.		
Calculation with units of expression	Total acids as tartaric acid, g per 100 liters absolute alcohol $= (V \times 0.00375 \times 100 \times 1000 \times 2)/V_1$ Where, V_1 = alcohol % by volume V = volume of std. NaOH used for titration, in mL		
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Total Acids (as Tartaric Acid) - Method II (for coloured liquors such as Wine, Toddy)		
Method No.	FSSAI 13.008:2021	**Revision No. & Date**	0.0
Scope	Method II – This method is used to determine total acidity of coloured alcoholic beverages such as Wine, Toddy.		
Caution	Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage.		
Principle	Total acids present in alcoholic beverages are estimated using acid –base titration using pH meter.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. pH Meter. 3. Magnetic stirrer. 4. Beaker 250 mL capacity		
Materials and Reagents	1. Alcoholic beverages. 2. Sodium Hydroxide. 3. Buffer solutions of pH 4.0, 7.0 and 9.2		
Preparation of reagents	1. Sodium hydroxide solution (0.05N): Sodium hydroxide (2 g) dissolved in 1 L water.		
Method of analysis	1. Calibrate and standardize the pH meter using the buffer solutions of pH 4.0, 7.0 and 9.2. 2. Take approximately 100 mL 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 50 mL 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 with units of expression	Total acidity as tartaric acid(g per liter of wine or toddy) $= (V \times 0.00375 \times 1000) \div V_1$ Where, V_1 = 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 g of tartaric acid.		
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Volatile Acids (as Acetic Acid)		
Method No.	FSSAI 13.009:2021	**Revision No. & Date**	0.0
Scope	Volatile acids present in alcoholic beverages are estimated using this method. The method is applicable to all alcoholic beverages		
Caution	Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage.		
Principle	Alcoholic beverages are distilled and the volatile acids present, in the distillate are estimated.		
Apparatus/Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2).		
Materials and Reagents	1. Sodium Hydroxide. 2. Phenolphthalein indicator. 3. Rectified spirit.		
Preparation of reagents	1. Sodium hydroxide solution (0.05N): Sodium hydroxide (2 g) dissolved in 1 L water. 2. Phenolphthalein indicator solution - Dissolve 1.0 g of phenolphthalein in 100 mL rectified spirit.		
Method of Analysis	1. Take 50 mL distillate collected during the determination of ethyl alcohol for volatile acidity determination (FSSAI 13.001:2021). 2. Titrate against standard NaOH using phenolphthalein indicator		
Calculation with units of expression	1. For liquors: $\text{Volatile acidity as acetic acid(g per 100 liters of absolute alcohol)} = (V \times 0.003 \times 100 \times 1000 \times 2) \div V_1$ Where, V = volume of standard NaOH used for titration, in mL V_1 = alcohol % by volume 2. For wines: $\text{Volatile acidity as acetic acid(g per liter of wine)} = (V \times 0.003 \times 1000) \div V_1$ Where, V_1 = Volume of wine taken for estimation V = volume of standard NaOH used for titration, in mL **Note:** 1 mL of 0.05N NaOH is equivalent to 0.003 g of acetic acid.		
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Total Esters**		
Method No.	FSSAI 13.010:2021	**Revision No. & Date**	0.0
Scope	Total esters present in the alcoholic beverages are determined. The method is applicable to all alcoholic beverages		
Caution	1. Sulphuric acid: Concentrated sulfuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If sulfuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 2. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage.		
Principle	Esters present in the neutralised alcoholic beverages are hydrolysed and estimated.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2)		
Materials and Reagents	1. Alcoholic beverages 2. Sodium Hydroxide 3. Sulphuric acid		
Preparation of reagents	1. Sodium hydroxide solution (0.1N): Sodium hydroxide (4 g) dissolved in 1 L water. 2. Standard Sulphuric acid, 0.1N: Sulphuric acid (4.9 g) dissolved in 1 L water.		
Method of Analysis	1. To the neutralized distillate from the volatile acidity determination (FSSAI 13.009:2021), add 10 mL of std. NaOH and reflux on a steam bath for 1 h. 2. Cool and back titrate the unspent alkali against standard sulphuric acid. 3. Carry out a blank titration simultaneously taking 50 mL 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 with units of expression	Esters expressed as ethyl acetate(g per 100 liters of abs. alcohol) $= (V \times 0.0088 \times 100 \times 1000 \times 2) \div V_1$ Where, V = difference of titer value of standard H_2SO_4used for blank and sample, in mL V_1 = alcohol % by volume. **Note:** 1 mL of 0.1N NaOH is equivalent to 0.0088 g of Ethyl acetate.		
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Esters - Gas Chromatographic Method using Capillary Column**		
Method No.	FSSAI 13.011:2021	**Revision No. & Date**	0.0
Scope	This method is used to determine esters using Gas chromatography equipped with capillary column. The method is applicable to all alcoholic beverages		
Caution	1. Methanol: Methanol is highly flammable and toxic. Direct ingestion of more than 10 mL can cause permanent blindness by destruction of the optic nerve, poisoning of the central nervous system, coma and possibly death. These hazards are also true if methanol vapors are inhaled. It is best to avoid direct exposure. 2. Isobutyraldehyde: Breathing Isobutyraldehyde can irritate the lungs causing coughing and/or shortness of breath. Exposure to Isobutyraldehyde can cause headache, nausea and vomiting. High levels can cause to feel dizzy, lightheaded and to pass out. Isobutyraldehyde is a flammable liquid and a fire hazard. 3. Methyl acetate: Methyl Acetate can affect you when breathed in and by passing through your skin. Contact can irritate and burn the eyes with possible permanent damage. Methyl Acetate can irritate the skin and cause itching, redness, rash, drying and cracking. Methyl Acetate is a flammable liquid and a fire hazard. 4. n-Propyl acetate: Causes eye, skin, and respiratory tract irritation. Breathing vapors may cause drowsiness and dizziness. 5. t-Amyl alcohol: Ingestion Harmful if swallowed. Skin Harmful if absorbed through skin. Causes skin irritation. Eyes Causes eye irritation. 6. n-Butyl acetate: Breathing vapors may cause drowsiness and dizziness. Causes eye and respiratory tract irritation. Repeated exposure may cause skin dryness or cracking. Target Organs: Central nervous system, respiratory system, eyes, skin. 7. Ethyl propionate: Ethyl propionate can affect when breathed in and may be absorbed through the skin. Contact can irritate and burn the skin and eyes. Breathing Ethyl Propionate can irritate the nose and throat causing coughing and wheezing. High exposure to Ethyl Propionate can cause drowsiness and sleepiness. 8. n-Propanol: Exposure to Propyl alcohol can irritate the eyes, nose, and throat. Exposure to high concentrations can cause headache, drowsiness, dizziness, confusion, nausea and vomiting. Propyl alcohol may cause liver damage. Propyl alcohol is a flammable liquid and a dangerous fire hazard. 9. Isoutanol: Inhalation of high concentrations of vapors may cause irritation of the respiratory tract with sore throat, coughing, shortness of breath, headaches, nausea, dizziness, dullness, narcosis and unconsciousness. 10. Iso-amyl acetate: Exposure to high concentrations of Isoamyl acetate can cause headache, drowsiness, dizziness, lightheadedness, fatigue, and may cause you to pass out. Prolonged or repeated contact can cause drying and cracking of the skin. Isoamyl acetate is a flammable liquid and a fire hazard. 11. Phenyl acetate: Harmful if swallowed, Exposure: skin – redness; eyes-redness, pain. 12. Caprylic acid: Caprylic acid can lower blood pressure. In theory, caprylic acid might cause blood pressure to go too low if used by people prone to low		

	blood pressure; Caprylic acid is broken down by the liver. There is some concern that people with liver disease might not be able to break down caprylic acid. This might cause blood levels of caprylic acid to increase. 13. n-Butanol: Flammable Liquid, Oral and dermal Toxicity, Acute Toxicity on Inhalation, Skin Corrosion/Irritation, Eye Damage Category, Acute Vertebrate Hazard. 14. Iso-amyl alcohol: Iso-amyl Alcohol can cause nausea, vomiting and diarrhea. Exposure can cause headache, dizziness, lightheadedness, and passing out. cracking of the skin. 15. Ethyl caprylate: Causes eye, skin, and respiratory tract irritation. Combustible liquid and vapor. Target Organs: Respiratory system, eyes, skin. Potential Health Effects. 16. Furfural: Toxic if swallowed; Harmful in contact with skin; Causes skin irritation; Causes serious eye irritation; Toxic if inhaled; May cause respiratory irritation; Respiratory tract irritation; Suspected of causing cancer. 17. Ethyl laurate: May irritate eyes, skin, and respiratory tract Alfa Aesar. 18. Phenethyl alcohol: Harmful if absorbed through the skin. Causes eye, skin, and respiratory tract irritation. May be harmful if swallowed. 19. Isovaleric acid: Harmful if swallowed. Toxic in contact with skin. Causes burns. 20. Ethyl caproate: Difficulty in breathing. Symptoms of overexposure may be headache, dizziness, tiredness, nausea and vomiting. 21. Phenethyl acetate: Serious eye damage/eye irritation. 22. Ethyl lactate: Ethyl Lactate can affect you when breathed in and may be absorbed through the skin. Prolonged contact can irritate the skin and eyes. Breathing Ethyl Lactate may cause dizziness, lightheadedness, and passing out. 23. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 24. Isobutyric acid: Isobutyric Acid can affect you when breathed in and may be absorbed through the skin. Contact can irritate and burn the skin and eyes. Breathing Isobutyric Acid can irritate the nose, throat and lungs causing coughing, wheezing and/or shortness of breath. 25. Pelargonic acid: Causes skin irritation. Causes serious eye irritation. 26. Capric acid: Causes skin irritation. May be harmful if absorbed through the skin. Ingestion: May cause gastrointestinal irritation with nausea, vomiting and diarrhea. Inhalation: May cause respiratory tract irritation.
Principle	Sample peak areas in GC are compared with that of standards and esters are determined.
Apparatus/ Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 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 25 m length, 0.32 mm ID and 0.30 µm film thickness. 3. **Syringe –** 10 µL; Hamilton Co. No. 701, or equivalent.

Materials and Reagents

S. No.	Reagents
1	Internal standard: 0.5% (v/v) n-Pentanol in 40% (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
28	Phenethyl acetate
29	Ethyl lactate
30	Acetic acid
31	Isobutyric acid
32	Ethyl myristate
33	Pelargonic acid
34	Capric acid
35	Diacetyl

Preparation of reagents

Preparation of standard mixture

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

Preparation of working standard mixture

	4. Transfer 5 mL of standard mixture into a 10 mL stoppered test tube. Add 1 mL of internal standard solution (1) and mix well.
Sample Preparation	Transfer 5 mL of sample into a 10 mL stoppered test tube, add 1 mL of n-pentanol internal standard solution and mix well.
Method of Analysis	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 1.7 mL/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 4 min, raise to 100 ^{0}C at the rate of 10 ^{0}C/min and finally to 200 ^{0}C for 10 min 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 2 µL 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% of full-scale deflection). 6. Determine the retention time of methanol and n-pentanol. 7. Inject 2 µL sample solution into chromatograph and record the chromatogram (adjust attenuation, if necessary). *Note: -Identify the individual components by injecting respective component standard solutions into the gas chromatograph and record the retention times.*
Calculation with units of expression	Calculate the individual component in gram per 100 litres of absolute alcohol as follows: $\text{Individual component} = (R_2 \times C \times D \times 1000 \times 100 \times 100) \div (R_1 \times S)$ 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; S- Ethanol content of liquor sample in percent(v/v).
Reference	1. IS 3752:2005 2. AOAC 968.09-1969, alcohols (higher) and ethyl acetate in distill
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Esters - Gas Chromatographic Method using Packed Column**		
Method No.	FSSAI 13.012:2021	**Revision No. & Date**	0.0
Scope	This method is used to determine esters using Gas chromatography equipped with packed column. The method is applicable to all alcoholic beverages.		
Caution	See FSSAI 13.011:2021		
Principle	Sample peak areas in GC are compared with that of standards and esters are determined.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2.**Gas chromatograph** – Gas chromatograph equipped with flame ionization detector and packed inlet and fixed with a glass column packed with 5% Carbowax 20M on carbopak B, 80/120 mesh or equivalent packed columns like porapak- Q having the dimensions of 2 m in length and 4 mm in ID. 3. **Syringe**- 10 µL, Hamilton Co. No. 701, or equivalent.		

Materials and Reagents

S. No.	Reagents
1	Internal standard:0.5% (v/v) n-Pentanol in 40% (v/v) Ethanol (methanol-free)
2	Ethanol (Methanol-free)
3	Methanol
4	Acetaldehyde
5	Ethyl acetate
6	n-Propanol
7	Iso-butanol
8	Iso-amyl acetate
9	Iso-amyl alcohol
10	Ethyl caprylate
11	Furfural
12	Ethyl caprate
13	Ethyl laurate
14	Phenethyl alcohol
15	Ethyl caporate
16	Ethyl lactate
17	Acetic acid

Preparation of reagents

Preparation of standard mixture

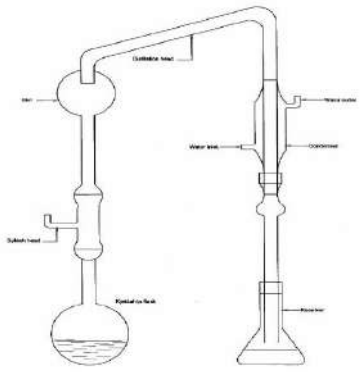
1. Transfer accurately known quantity of about 5.0 g of the reagents listed from (3) to (17) in to different 100 mL volumetric flasks and dilute to 100 mL with 40 percent (v/) ethanol (methanol-free).
2. Transfer 1.0 mL of each of the resulting solutions into a 100 mL volumetric flask and dilute to volume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

Preparation of working standard mixture

Transfer 5 mL of standard mixture into a 10 mL stoppered test tube, add 1 mL of internal standard solution (1) and mix well.

Sample Preparation	Transfer 5 mL of sample into a 10 mL stoppered test tube, add 1 mL of n-pentanol internal standard solution and mix well.

Method of Analysis	Gas chromatograph and operating parameters Nitrogen or helium may be used as carrier gas at suitable flow rate. The detector and injector port temperatures may be maintained at about 250 ^{0}C. 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 10 min 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.* Inject 2 µL of working standard mixture solution into chromatograph and record the chromatogram. Adjust the operating parameters and attenuation to obtain measurable peaks (at least 25% of full-scale deflection). Determine the retention time of methanol and n-pentanol. Inject 2 µL 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 with units of expression	Calculate the individual component in grams per 100 litres of absolute alcohol as follows: $\text{Individual component} = (R_2 \times C \times D \times 1000 \times 100 \times 100) \div (R_1 \times S)$ 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	1. IS 3752:2005 2.AOAC 968.09
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Higher Alcohols - Titrimetric Method**		
Method No.	FSSAI 13.013:2021	**Revision No. & Date**	0.0
Scope	Titrimetric method for determination of higher alcohols present in alcoholic beverages.		
Caution	1. Sulphuric acid: Concentrated sulfuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If sulfuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 2. Potassium dichromate: Corrosive. Causes severe burns to every area of contact. Harmful if swallowed or inhaled. Affects the respiratory system, liver, kidneys, eyes, skin and blood. 3. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage.		
Principle	Higher alcohols separated by carbon tetrachloride, after saturation with sodium chloride. Higher alcohols fraction is oxidized using oxidation reagent and formed acid is titrated against alkali and estimated.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Separating funnel, 250 mL. 3. Volumetric flask, 1 L capacity. 4. Distillation assembly having Kjeldhal flask, 800 mL capacity; With splash head, Liebig condenser, Receiver of capacity 250 mL.  (Figure is adopted from FSSAI Manual of Methods of Analysis of Foods: Alcoholic beverages, 2019, Page 24)		
Materials and Reagents	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, 100 g in 500 mL distilled water and add sulphuric acid, 100 mL and make up to 1 L volume with distilled water. 2. Sodium hydroxide solution (0.1N): Sodium hydroxide (4 g) dissolved in 1 L water. 3. Phenolphthalein indicator solution - Dissolve 1.0 g of phenolphthalein in 100 mL rectified spirit.
Method of Analysis	1. Transfer the solution, obtained from the determination of esters (FSSAI 13.010:2021) into a separatory funnel and add 50 mL of distilled water. 2. Saturate it with sodium chloride and extract four times with successive portions of 40, 30, 20 and 10 mL 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 50 mL of oxidizing mixture. Reflux for 2 h, cool and wash the reflux with 50 mL of distilled water. 5. Transfer it to the distillation assembly using 50 mL of water. Distil about 100 mL 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 50 mL of distilled water in place of the distillate of the liquor.
Calculation with units of expression	Higher alcohol expressed Amyl alcohol, in grams. Per 100 liters of abs. alcohol $= (V \times 0.0088 \times 100 \times 1000 \times 2) \div (V_1 \times V_2)$ 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 0.0088 g of Amyl alcohol
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Higher Alcohols - Spectrophotometric Method		
Method No.	FSSAI 13.014:2021	**Revision No. & Date**	0.0
Scope	Spectrophotometric method for determination of higher alcohols present in alcoholic beverages.		
Caution	1. p-Dimethylaminobenzaldehyde: Inhalation may be harmful if inhaled. May cause respiratory tract irritation. Ingestion may be harmful if swallowed. Skin May be harmful if absorbed through skin. 2. Sulphuric acid: Concentrated sulfuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If sulfuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 3. Isobutylalcohol: Breathing Isobutyl Alcohol can irritate the nose, mouth and throat causing coughing and wheezing. Exposure to Isobutyl Alcohol can cause headache, dizziness, drowsiness, confusion and loss of coordination. Isobutyl Alcohol may affect the liver. Isobutyl Alcohol is a flammable liquid and a dangerous fire hazard. 4. Isoamyl alcohol: Isoamyl Alcohol can cause nausea, vomiting and diarrhea. Exposure can cause headache, dizziness, lightheadedness, and passing out. cracking of the skin.		
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 / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Spectrophotometer, double beam. 3. Steam bath. 4. Test tube, stoppered, 15 mL capacity		
Materials and Reagents	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 1 g in a mixture of 5 mL sulphuric acid and 90 mL distilled water and transfer to a 100 mL volumetric flask and make up to the mark. Preparation of Synthetic standard of higher alcohols 2. Weigh 2 g isobutyl alcohol and 8 g iso-amyl alcohol into 1 L volumetric flask and dilute to mark with water. 3. Pipette two 10 mL portions into 100 mL 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 6.0 g synthetic		

	higher alcohol per 100 L by diluting 1.0 to 6.0 mL aliquots of alcohol standards solution to 100 mL with alcohol solution. (Solution containing 6 mL synthetic standard would give an absorbance of 0.83±0.03 at 530 nm).
Sample Preparation	1. Transfer 200 mL of alcoholic drink into a 500 mL distillation flask containing about 25 mL of distilled water and a few pieces of pumice stone. 2. Distil the contents in about 35 min and collect the distillate in a 200 mL 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 6 g fuel oil per 100 L, dilute the distilled sample with distilled water to concentrations of 2.0 to 5.0 g/100L.
Method of analysis	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 1 mL p-dimethylaminobenzaldehyde solution into each tube; shake and replace in ice-bath for 3 min. 4. With tubes retained in ice- bath, add 10 mL 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.
Calculation with units of expression	From the OD of the sample find out the concentration of Higher alcohol g/100L using the standard curve.
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Higher Alcohols - Gas Chromatographic Method using Capillary Column**		
Method No.	FSSAI 13.015:2021	**Revision No. & Date**	0.0
Scope	Gas chromatographic method using capillary column for determination of higher alcohols present in alcoholic beverages.		
Principle	Quantity of alcohols determined using similar procedure as per the esters (See FSSAI 13.011:2021) using standard reference materials of alcohols.		
Reference	1. IS 3752:2005 2. AOAC 968.09		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Higher Alcohols - Gas Chromatographic Method using Packed Column		
Method No.	FSSAI 13.016:2021	**Revision No. & Date**	0.0
Scope	Gas Chromatographic Method using packed column(See FSSAI 13.012:2021) Quantity of alcohols determined using similar procedure as per the esters using standard reference materials of alcohols.		
Reference	1. IS 3752:2005 2. AOAC 968.09		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Higher Alcohols - Gas Chromatographic Method using Calibration Curves of Standards		
Method No.	FSSAI 13.017:2021	**Revision No. & Date**	0.0
Scope	Gas Chromatographic method for determination of higher alcohols present in alcoholic beverages using calibration curves of standards.		
Caution	1. Isobutyl alcohol: Breathing Isobutyl Alcohol can irritate the nose, mouth and throat causing coughing and wheezing. Exposure to Isobutyl Alcohol can cause headache, dizziness, drowsiness, confusion and loss of coordination. Isobutyl Alcohol may affect the liver. Isobutyl Alcohol is a flammable liquid and a dangerous fire hazard. 2. Isoamyl alcohol: Isoamyl alcohol can cause nausea, vomiting and diarrhea. Exposure can cause headache, dizziness, lightheadedness, and passing out. Cracking of the skin. 3. Propanol: Exposure to propyl alcohol can irritate the eyes, nose, and throat. Exposure to high concentrations can cause headache, drowsiness, dizziness, confusion, nausea and vomiting. Propyl alcohol may cause liver damage. Propyl alcohol is a flammable liquid and a dangerous fire hazard. 4. 3-Pentanol: Flammable liquid and vapour. Harmful if swallowed. 5. Ethyl acetate: Ethyl acetate is highly flammable, as well as toxic when ingestion or inhaled, and this chemical can be seriously damaging to internal organs in the case of repeated or prolonged exposure. Ethyl acetate can also cause irritation when it comes into contact with the eyes or skin.		
Principle	Calibration curves are prepared using GC responses of known concentration of authentic standards. These are used to determine higher alcohols.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 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 ^{0}C column oven with the flow rate of 10-25 mL/min and detector end of column disconnected.		
Materials and Reagents	1. Alcoholic beverages 2. Absolute alcohol (ethanol); (Use absolute alcohol throughout when alcohol is specified) 3. n-Propyl alcohol 4. Isobutyl alcohol 5. Amyl alcohol 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. 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- 40.76 mg/mL. Prepare solution containing 10 mL reagent in 200 mL 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 100 mL volumetric flasks containing alcohol- H_2O (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: 0.25-1.5 mL n-propanol, 1.0-2.5 mL isobutyl alcohol, and 2.0-5.0 mL amyl alcohol. Dilute each volume with alcohol- H_2O (1+1). 4. n-Propyl alcohol, isobutyl alcohol, and amyl alcohol working standard solution- Dilute 10 mL each standard solution and 2.0 mL 3-pentanol internal standard solution to 200 mL 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.
Method of Analysis	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 10 mL test portion into convenient vessel (e.g, 1oz French square glass bottle with screw cap), add, by pipet (0.2 mL pipet graduated in 0.01 mL), 0.1 mL 3-pentanol internal standard solution, and mix. 4. Inject 2 µL 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.
Calculation with units of expression	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	1. IS 3752:2005 2. AOAC 968.09
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Aldehydes - Titrimetric Method		
Method No.	FSSAI 13.018:2021	**Revision No. & Date**	0.0
Scope	Titrimetric method for determination of aldehydes present in alcoholic beverages.		
Caution	1. Sodium bisulphate: Harmful if swallowed. Contact with acids liberates toxic gas. 2. Sodium thiosulphate: Sodium thiosulphate is moderately toxic when ingested. Remove contaminated clothing and wash the affected area on the skin with soap or mild detergent and large amounts of water until all evidence of the chemical has been removed (approximately 15 min). Wash contaminated clothing before reuse.		
Principle	Aldehydes react with sodium bisulphite and forms adducts. These adducts react with iodine. Excess iodine is titrated and determined. Consumed iodine is correlated with aldehyde content and determined		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Iodine flask, 250 mL capacity. 3. Burette, 25/50 mL capacity.		
Materials and Reagents	1. Sodium bisulphite solution. 2. Iodine standard solution. 3. Sodium thiosulphate standard. 4. Starch indicator.		
Preparation of reagents	1. Sodium bisulphite solution (0.05N) – Sodium bisulphite (2.6 g) dissolved in 1000 mL water. 2. Iodine standard solution – 0.05N. 3. Sodium thiosulphate standard (0.05N) – Sodium thiosulphate (12.4 g) dissolved in 1000 mL water. 4. Starch indicator (1%) – starch (1 g) is dissolved in 100 mL water.		
Method of Analysis	1. Take 50 mL of distillate of liquor (FSSAI 13.001:2021) in a 250 mL Iodine flask and add 10 mL of bisulphite solution. Keep the flask in a dark place for 30 min. with occasional shaking. 2. Add 25 mL 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 50 mL 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 with units of expression	Aldehydes expressed acetaldehyde (g per 100 liters of absolute alcohol) $= (V \times 0.0011 \times 100 \times 1000 \times 2) \div V_1$ 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 0.0011 g of Acetaldehyde.		
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Aldehydes – Gas Chromatographic Method using Capillary Column		
Method No.	FSSAI 13.019:2021	**Revision No. & Date**	0.0
Scope	Gas chromatographic method using capillary column (See FSSAI 13.011:2021) Quantity of aldehydes determined using similar procedure as per the esters using standard reference materials of aldehydes.		
Reference	1. IS 3752:2005 2. AOAC 968.09		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Aldehydes - Gas Chromatographic Method using Packed Column		
Method No.	FSSAI 13.020:2021	**Revision No. & Date**	0.0
Scope	Gas Chromatographic Method using packed column (See FSSAI 13.012:2021) Quantity of aldehydes determined using similar procedure as per the esters using standard reference materials of alcohols.		
Reference	1. IS 3752:2005 2. AOAC 968.09		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Furfural - Colorimetric Method**		
Method No.	FSSAI 13.021:2021	**Revision No. & Date**	0.0
Scope	Colorimetric Method for determination of furfural present in alcoholic beverages.		
Caution	1. Hydrochloric acid: Hydrochloric acid is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 2. Aniline: Aniline vapor is heavier than air and may accumulate in low-lying areas. The vapor is combustible. Aniline has a characteristic aromatic or fishy odor which provides adequate warning of acute exposure. Aniline is rapidly absorbed after inhalation and ingestion. 3. Furfural: Toxic if swallowed; Harmful in contact with skin; Causes skin irritation; Causes serious eye irritation; Toxic if inhaled; May cause respiratory irritation; Respiratory tract irritation; Suspected of causing cancer. 4. m-Phenylenediamine hydrochloride: Causes serious eye irritation		
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 Nessler comparator.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Nessler tubes with flat bottom tubes of thin high quality glass, 25 mm in diameter and 150 mm in length and graduated at 50mL.		
Materials and Reagents	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 5 g of m-phenylenediamine hydrochloride per litre, stand at least for 24 h 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 100 mL and the last 200 mL of the distillate. If this gives coloration with aniline hydrochloride, repeat the treatment. Standard furfural solution 3. Dissolve 1 g of redistilled, colourless furfural in 100 mL of the furfural free alcohol. 4. Prepare standard furfural solution by diluting 1 mL of this solution to 100 mL with 50% furfural free alcohol. 5. One 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).		
Method of Analysis	1. Take 5 mL of the distillate obtained for ethanol determination, (FSSAI 13.001:2021), add 1 mL of the colourless aniline and 0.5 mL 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 50 mL. 3. First add 2 mL of the colourless aniline and then 0.5 mL 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 with units of expression	Furfural (g per 100 liters of absolute alcohol) $= (W \times 1000 \times 100 \times 100) \div (V_1 \times V_2)$ 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	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Furfural - Gas Chromatographic Method		
Method No.	FSSAI 13.022:2021	**Revision No. & Date**	0.0
Scope	Determination of Furfural by Gas Chromatography as described under "Determination of Esters"(See FSSAI 13.011:2021)		
Reference	1. IS 3752:2005 2. AOAC 968.09		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Copper / Iron - Atomic Absorption Spectrophotometric (AAS) Method		
Method No.	FSSAI 13.023:2021	**Revision No. & Date**	0.0
Scope	Atomic absorption Spectrophotometric (AAS) method for determination of Copper / Iron present in alcoholic beverages.		
Caution	1. Acetylene: Acetylene combines with air or oxygen to form an explosive mixture that can be ignited by a spark or the like, and can cause a serious Explosion. 2. Nitric acid: May be fatal if inhaled. Causes severe eye and skin burns. Causes severe respiratory and digestive tract burns. Strong oxidizer. Contact with other material may cause a fire. Acute pulmonary edema or chronic obstructive lung disease may occur from inhalation of the vapors of nitric acid. Corrosive to metal. Target Organs: Lungs, eyes, skin, mucous membranes.		
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 / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 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.		
Materials and Reagents	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.		
Method of Analysis	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 100 mL of wine sample in a glass bowl and evaporate to dryness. 5. Add 5 mL 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 25 mL volume. 8. Blank Solution - Prepare a blank by taking 5 mL of ultrapure nitric acid and make up to 25 mL 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 with units of expression	Copper / Iron content in wine (in µg/mL or mg/L) $= \frac{[\text{Reading (in µg)displayed} \times \text{Dilution}]}{\text{Volume of sample}}$ Note: For directly aspirated liquor sample, dilution part will not appear in the calculation
Reference	1. A.O.A.C 17thedn, 2000 Official Method 999.11 Determination of Lead, Cadmium, Copper, Iron and Zinc in Foods Atomic Absorption Spectrophotometry after dry ashing. 2. For Detailed Metal Estimation Procedure - Refer Manual of Methods for Analysis of Metals, FSSAI.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Copper using Diethyldithiocarbamate		
Method No.	FSSAI 13.024:2021	**Revision No. & Date**	0.0
Scope	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 serve as a referee method in case of dispute or where zinc is present.		
Caution	1. Sulphuric acid: Concentrated sulfuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If sulfuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 2. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Ammonia solution: Contact with concentrated ammonia solutions may cause corrosive injury including skin burns, permanent eye damage or blindness. The full extent of eye injury may not be apparent for up to a week after the exposure. Contact with liquefied ammonia can also cause frostbite injury. 4. Nitric acid: May be fatal if inhaled. Causes severe eye and skin burns. Causes severe respiratory and digestive tract burns. Strong oxidizer. Contact with other material may cause a fire. Acute pulmonary edema or chronic obstructive lung disease may occur from inhalation of the vapors of nitric acid. Corrosive to metal. Target Organs: Lungs, eyes, skin, mucous membranes. 5. Citric acid: Ingestion May irritate and cause stomach pain, vomiting and diarrhoea. Skin contact Skin irritation is not anticipated when used normally. Eye contact Causes serious eye irritation. Particles in the eyes may cause irritation and smarting. 6. Copper sulphate: Copper sulfate can cause severe eye irritation. Eating large amounts of copper sulfate can lead to nausea, vomiting, and damage to body tissues, blood cells, the liver, and kidneys. 7. Sodium diethyldithiocarbamate: Harmful if swallowed or inhaled. Cause irritation to skin, eyes, and respiratory tract. 8. Carbon tetrachloride: Carbon tetrachloride can cause nausea, vomiting, diarrhea and abdominal pain. Carbon tetrachloride can damage the liver and kidneys. 9. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation.		

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 0.15 mg 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 / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Nessler tubes - Flat bottom tubes of thin, colourless glass, about 25 mm in diameter and about 150 mm in length and graduated at 50 mL. The depth measured internally from graduation mark to the bottom shall not vary by more than 2 mm in the tubes used for the test.
Materials and Reagents	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% (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 1.119 g of copper sulphate ($CuSo_4.5H_2O$) in water and dilute to one litre. Dilute 10 mL of this solution to 100 mL. One millilitre of the diluted solution contains 0.028545 mg of copper. The diluted solution shall always be prepared immediately before use. 4. Sodium diethyldithiocarbamate- Prepare 0.1% 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.
Sample Preparation	1. Transfer 20 mL of the material into silica evaporating dish and add 1 mL of dilute sulphuric acid. Heat gently in the beginning and then evaporate almost to dryness on a water-bath. 2. Ignite the residue over a smokeless flame to eliminate sulphuric acid. 3. Cool, dissolve the residue in 2 mL 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 25 mL.
Method of Analysis	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 50 mL Nessler tube, 10 mL of the test solution prepared as described above. 4. Add 2 g of citric acid and 10 mL of dilute ammonium hydroxide. Make up to 50 mL with water. 5. Prepare a series of control solutions, each containing in 50 mL, 2 g of citric acid and 10 mL of dilute ammonium hydroxide together with an increasing amount of copper, namely, 0.1 mL, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL and 1.0 mL of standard copper solution. 6. The test solution and controls should be free from any turbidity. 7. Cool all solution to 20 °C, add 2 mL 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, 2.5 mL each, of carbon tetrachloride and compare the colour of the solution so obtained in a colorimeter with the extracts of control solution similarly prepared. 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 with units of expression	Calculate copper as follows: Copper (as Cu), in ppm $= 0.2845 \times 12.5$ V Where V= volume of standard copper solution in the control solution which gives the closest match, in mL.
Reference	1. AOAC 960.17, Copper in Beer, Direct, Non ashing Method 2. A.O.A.C 15th edn, Official Method 960.40 Copper in Food
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Copper using Potassium Ferrocyanide**		
Method No.	FSSAI 13.025:2021	**Revision No. & Date**	0.0
Scope	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.		
Caution	1. Sulphuric acid: Concentrated sulfuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If sulfuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 2. Hydrochloric acid: It is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Ammonia solution: Contact with concentrated ammonia solutions may cause corrosive injury including skin burns, permanent eye damage or blindness. The full extent of eye injury may not be apparent for up to a week after the exposure. Contact with liquefied ammonia can also cause frostbite injury. 4. Nitric acid: May be fatal if inhaled. Causes severe eye and skin burns. Causes severe respiratory and digestive tract burns. Strong oxidizer. Contact with other material may cause a fire. Acute pulmonary edema or chronic obstructive lung disease may occur from inhalation of the vapors of nitric acid. Corrosive to metal. Target Organs: Lungs, eyes, skin, mucous membranes. 5. Citric acid: Ingestion May irritate and cause stomach pain, vomiting and diarrhea. Skin contact Skin irritation is not anticipated when used normally. Eye contact Causes serious eye irritation. Particles in the eyes may cause irritation and smarting. 6. Copper sulphate: Copper sulfate can cause severe eye irritation. Eating large amounts of copper sulfate can lead to nausea, vomiting, and damage to body tissues, blood cells, the liver, and kidneys. 7. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 8. Potassium ferrocyanide: Potassium ferrocyanide can be absorbed into the body by inhalation or ingestion of the powder. It is a skin and eye irritant. Inhalation will cause sore throat and coughing. 9. Ammonium chloride: Exposure to Ammonium chloride is moderately hazardous, causing irritation, shortness of breath, cough, nausea, and		

	headache.
Principle	Copper solutions react with Potassium ferrocyanide solutions and forms red-brown solutions of Copper (II) hexacyanoferrate.
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Nessler tubes - Flat bottom tubes of thin, colourless glass, about 25 mm in diameter and about 150 mm in length and graduated at 50 mL. The depth measured internally from graduation mark to the bottom shall not vary by more than 2 mm in the tubes used for the test.
Materials and Reagents	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% (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 1.119 g of copper sulphate ($CuSO_4.5H_2O$) in water and dilute to one litre. Dilute 10 mL of this solution to 100 mL. One millilitre of the diluted solution contains 0.028545 mg 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.
Sample Preparation	1. Transfer 20 mL of the material into silica evaporating dish and add 1 mL 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 2 mL of water, add three drops of aqua regia and evaporate to dryness on a water bath. 5. Dissolve the residue in 2 mL of water, add three drops of aqua regia and evaporates to dryness on a water bath. 6. Dissolve the residue in 2 mL of dilute hydrochloric acid and warm gently till the residue is dissolved. 7. Add 0.5 g of ammonium chloride and dilute to 15 mL 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).
Method of Analysis	1. Dilute the above solution to 40 mL. Add 0.5 mL of potassium ferrocyanide solution, stir and make up the volume to 50 mL. *Note-If copper is more, a lesser amount, say 10 mL of the material may be taken for the test.* 2. Prepare a series of control solutions each containing in 50 mL, 0.5 g of

	ammonium chloride, 3 to 5 drops of acetic acid and 0.5 mL of potassium ferrocyanide solution together with an increasing amount of copper, namely, 2 mL, 4 mL, 6 mL, 8 mL and 10 mL of the standard copper solution. 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 with units of expression	Calculate copper as follows: Copper (as Cu), in ppm $= 0.2845 \times 12.5V$ Where V= volume of standard copper solution in the control solution which gives the closest match, in mL.
Reference	1. AOAC 960.17, Copper in Beer, Direct, Non ashing Method 2. A.O.A.C 960.40, 1965, 15th edn, Official Method, Copper in Food
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Copper - Cuperthol Method**		
Method No.	FSSAI 13.026:2021	**Revision No. & Date**	0.0
Scope	Estimation of copper present in alcoholic beverages by Cuperthol Method		
Caution	1. Nitric acid: May be fatal if inhaled. Causes severe eye and skin burns. Causes severe respiratory and digestive tract burns. Strong oxidizer. Contact with other material may cause a fire. Acute pulmonary edema or chronic obstructive lung disease may occur from inhalation of the vapors of nitric acid. Corrosive to metal. Target Organs: Lungs, eyes, skin, mucous membranes. 2. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 3. Diethanolamine: Causes serious eye damage. Causes skin irritation. Suspected of causing cancer. May cause respiratory irritation. May cause damage to organs through prolonged or repeated exposure. 4. Methanol: Methanol is highly flammable and toxic. Direct ingestion of more than 10 mL can cause permanent blindness by destruction of the optic nerve, poisoning of the central nervous system, coma and possibly death. These hazards are also true if methanol vapors are inhaled. 5. Carbon disulfide: Highly flammable liquid and vapour. Harmful if swallowed. Causes skin irritation. Causes serious eye irritation. 6. Sodium acetate: May cause irritation to skin, eyes, and respiratory tract		
Principle	Divalent copper forms a coloured complex with Cuperthol. Based on the absorbance of the coloured complex solution copper is determined.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Photometer - Spectrophotometer (with blue-green or green filter) set at 445 nm 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.		
Materials and Reagents	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. Nitric acid. 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 4.0 mL diethanolamine in 200 mL methanol. 2. Carbon disulfide solution: - Add 1.0 mL CS_2 (Free of precipitate S) to 200 mL 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.		

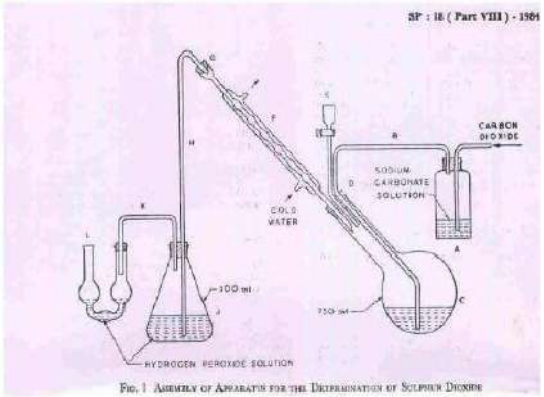
	Copper standard solutions: - i) Stock solution (conc. 1mg/mL): - Dissolve 3.93 g $CuSO_4.5H_2O$ (free of whitish deposit of lower hydrates) and dilute to 1 L with H_2O or dissolve 1.000 g pure Cu wire or foil in 72 mL HNO_3 (1+4) by warming. Boil to expel fumes, cool, and dilute to 1 L with H_2O. ii) Working solution (conc.10 µg/mL): -Prepare immediately before use by diluting 5 mL stock solution with Cu-free distilled H_2O to 500 mL in volumetric flask. 4. Buffer solution: - pH 4.4. Dissolve 63.3 g anhydrous sodium acetate (CH_3COONa) in ca 800 mL H_2O containing 65 mL acetic acid (CH_3COOH). Dilute to 1 L with H_2O. 5. 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 100 mL 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 50 mL cold beer into 50 mL graduate, avoid foaming. Transfer to 125 mL flask, add 25 mL buffer solution and mix. 11. Measure two 30 mL aliquots in 50 mL graduate and transfer to separate 50 mL flasks. 12. Add 3 mL cuprethol solution to one flask and 3 mL 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.
Calculation with units of expression	Calculate µg/mL Cu by multiplying *A* or scale reading by *F*, or use curve.
Reference	AOAC 972.12-1973, copper in beer. Cuprethol method
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Methyl Alcohol - Spectrophotometric Method		
Method No.	FSSAI 13.027:2021	**Revision No. & Date**	0.0
Scope	This spectrophotometric method determines the methyl alcohol present in alcoholic beverages.		
Caution	1. Sulphuric acid: Concentrated sulfuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If sulfuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 2. Methanol: Methanol is highly flammable and toxic. Direct ingestion of more than 10mL can cause permanent blindness by destruction of the optic nerve, poisoning of the central nervous system, coma and possibly death. These hazards are also true if methanol vapors are inhaled. 3. Phosphoric acid: Repeated or prolonged exposure to phosphoric acid mist can lead to chronic eye irritation, severe skin irritation, or prolonged respiratory tract issues. 4. Potassium permanganate: Potassium Permanganate can affect you when breathed in. Contact can severely irritate and burn the skin and eyes with possible **eye** damage. Breathing Potassium Permanganate can irritate the nose and throat. Breathing Potassium Permanganate can irritate the lungs causing coughing and/or shortness of breath. 5. Sodium salt of chromotropic acid: Causes skin irritation. Causes serious eye irritation. 6. Isopropyl alcohol: Swallowing or inhaling isopropyl alcohol can cause headache, dizziness, vomiting, nausea, and unconsciousness. Long-term effects of working with this substance are not well-known at this time, so care and caution should be taken when handling isopropyl alcohol and isopropyl products as a preventative measure.		
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 / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Separating funnel. 3. Spectrophotometer		
Materials and Reagents	1. Alcoholic beverages 2. Potassium permanganate 3. Phosphoric 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: 3.0 g $KMnO_4$ and 15.0 mL H_3PO_4 shall be dissolved in 100 mL 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: 10 g chromotropic acid or its Na salt shall be dissolved in 25 mL water (add 2 mL 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 1.0 g methanol (99.99% pure) to 100 mL with 40% (v/v) ethanol (methanol free). Dilute to 10 mL of this solution to 100 mL with 40% ethanol (methanol free). This is 1000 ppm solution. Methanol Standard solution: 8. Dilute appropriate volume of methanol (11.1.4) to 100 mL vol. flasks with 40% ethanol to get final concentration of 20, 40, 60, 80 and 100 ppm of methanol.
Method of Analysis	1. Take 50 mL of sample in a simple still and distil, collecting about 40 mL of distillate. 2. Dilute 1 mL of distillate to 5mL with distilled water and shaken well. 3. Take 1 mL of this solution, 1 mL of distilled water (for blank) and 1 mL of each of the methanol standards in to 50 mL stoppered test tubes and keep them in an ice-cold water bath. 4. Add to each test tube, 2 mL of $KMnO_4$ reagent and keep aside for 30 min. 5. Decolourize the solution by adding a little sodium bisulphite and add 1 mL of chromotropic acid solution. 6. Mix well and add 15 mL 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 with units of expression	Calculate methanol content in g/100 litres of absolute alcohol as follows: $\text{Methanol} = (A_2 \times C \times D \times 1000 \times 100 \times 100)/(A_1 \times S)$ 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 S = ethanol content (%) of liquor sample (v/v)
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Methyl Alcohol - Gas Chromatographic Method		
Method No.	FSSAI 13.028:2021	**Revision No. & Date**	0.0
Scope	This Gas chromatographic method determines the methyl alcohol present in alcoholic beverages.		
Caution	1. Methanol: Methanol is highly flammable and toxic. Direct ingestion of more than 10 mL can cause permanent blindness by destruction of the optic nerve, poisoning of the central nervous system, coma and possibly death. These hazards are also true if methanol vapors are inhaled. 2. n-Pentanol: The substance is irritating to the eyes, skin and respiratory tract. If swallowed the substance may cause vomiting and could result in aspiration pneumonitis. The substance may cause effects on the central nervous system.		
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 / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 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 µm film thickness or SPB 20 capillary column of 30m x 0.25mm ID x 1.0 µm 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 °C for 4 min and then raise to 100 °C/min at the rate of 10 °C/min and finally at to 200 °C for 10 min at the rate of 15 °C /min. (Optimum operating conditions may vary with type of column used and instrumental characteristics). 5. Syringe – 10 µL, Hamilton Co., or equivalent.		
Materials and Reagents	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 1.0 g methanol (99.99% pure) to 100 mL with 40% (v/v) ethanol, methanol free. Dilute 10 mL of this solution to 100 mL with 40% ethanol. 3. Methanol Standard solution: Transfer 5 mL of the above solution to a 10 mL stoppered test tube and add 1 mL of n-pentanol internal std. solution and mix well.		
Preparation of Test Samples	Transfer 5 mL of sample into a 10 mL stoppered test tube and add 1 mL of n-pentanol internal standard and mix well.		
Method of Analysis	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 with units of expression	Methanol (in grams /100L of Absolute alcohol) $= (R_2 \times C \times D \times 1000 \times 100 \times 100) \div (R_1 \times S)$ 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	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Total Sulphur Dioxide (for Wines only) - Modified Monier Williams Method (Shiphton's Method)**		
Method No.	FSSAI 13.029:2021	**Revision No. & Date**	0.0
Scope	Modified Monier Williams Method (Shiphton's Method) - This method is useful to determine total sulphur dioxide present in wines.		
Caution	1. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage. 2. Hydrochloric acid: Hydrochloric acid is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Hydrogen peroxide: Hydrogen peroxide is a strong oxidizer (moderate oxidizer in lower concentrations), and can be corrosive to the eyes, skin, and respiratory system. This chemical can cause burns to the skin and tissue damage to the eyes. Take special caution to avoid contact with hydrogen peroxide. 4. Carbon dioxide: Carbon dioxide gas can cause injury or death. A high carbon dioxide gas concentration can cause suffocation. This sign should be posted outside each entrance to a carbon dioxide storage room.		
Principle	Sulphur dioxide on treatment with hydrogen peroxide oxidized to sulphuric acid and estimated using sodium hydroxide in presence of indicator bromophenol blue.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Round bottom flask – 500 mL 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 Figure is adopted from FSSAI Manual of Methods of Analysis of Foods: Alcoholic beverages, 2019, Page 51.		
Materials and Reagents	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 0.1 g of bromophenol blue in 3 mL of 0.05N sodium hydroxide solution and 5 mL of ethyl alcohol (90%) by warming gently. Make up to 250 mL in a volumetric flask with 20% ethyl alcohol.
Method of Analysis	1. Transfer 25 mL of Hydrogen peroxide solution to Erlenmeyer flask (J) and 5 mL to Peligot tube (L), Assemble the apparatus as shown above. 2. Introduce into the flask (C) 300 mL water and 20 mL 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 25 g 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 h. 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 20 mL of concentrated hydrochloric acid diluted with 300 mL of water.
Calculation with units of expression	$\text{Sulphur Dioxide}(\frac{\text{mg}}{\text{kg}}) = [32000(\text{V} - \text{v})\ \text{N}] \div \text{W}$ 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 g of the sample taken for test
Reference	I.S.I. Hand book of Food Analysis (Part VIII) – 1984 page 12, Determination of Sulphur dioxide
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Total Sulphur Dioxide (for Wines only) - Rosaniline Colorimetric Method		
Method No.	FSSAI 13.030:2021	**Revision No. & Date**	0.0
Scope	Rosaniline Colorimetric Method - This method is useful to determine total sulphur dioxide present in wines.		
Caution	1. p- Rosaniline hydrochloride: When heated to decomposition this compound emits very toxic fumes of hydrogen chloride and nitrogen oxides. 2. Hydrochloric acid: Hydrochloric acid is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Formaldehyde has been shown to cause cancer in laboratory animals and may cause cancer in humans. It also may cause birth defects. It is highly toxic if swallowed, inhaled, or absorbed through skin or mucous membranes. Formaldehyde is corrosive, and the eyes are especially vulnerable. An air concentration of two parts per million (2 ppm) is quickly irritating to the eyes, and 20 ppm can cause permanent clouding of the cornea after only one exposure. Formaldehyde is also a sensitizing agent. Subsequent exposures can produce symptoms more quickly and at lower concentrations. Symptoms of exposure may include coughing, eye or skin irritation, allergic reactions, vomiting, and diarrhea. 4. Mercuric chloride: Ingestion of metallic chloride-Metallic taste. Sore throat. Burning sensation. Nausea. Abdominal pain. Vomiting. Diarrhoea. Shock or collapse. 5. Sodium thiosulphate: Inhalation: Sore throat, shortness of breath coughing, and congestion. Eye Contact: Irritation to eyes and mucous. Skin Contact: Irritation, itching, dermatitis Ingestion: Irritation to mucous membranes. 6. n-Hexyl alcohol: May cause toxic effects if inhaled or absorbed through skin. Inhalation or contact with material may irritate or burn skin and eyes. Fire will produce irritating, corrosive and/or toxic gases. Vapors may cause dizziness or suffocation		
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 / Instruments	General Glassware and apparatus (Refer 2.0 at page no. 2).		
Materials and Reagents	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 100 mg p- rosaniline HCl into 250 mL volumetric flask and dissolve in 200 mL H_2O. Add 40 mL HCl (1+1), mix, and dilute to volume with H_2O. Let stand 15 min before use. Store in brown, glass-stoppered bottle in refrigerator. 2. Formaldehyde solution- Dilute 5 mL 40% HCHO solution to 1 L with H_2O and store in brown, glass-stoppered bottle in refrigerator. 3. Mercury stabilizing solution - Dissolve 27.2 g $HgCl_2$ and 11.7 g NaCl in H_2O and dilute to 1 L with H_2O. Calibration 4. Accurately weigh 250 mg $NaHSO_3$ into exactly 50 mL 0.1M I_2 solution in glass-stoppered flask. Let stand at room temperature for 5 min. Add 1 mL HCL, and titrate excess I_2 with 0.1M $Na_2S_2O_3$, using 1% aqueous starch solution as indicator (1 mL 0.1M I_2 consumed= 3.203 mg SO_2 or 5.20 mg $NaHSO_3$). From results of $NaHSO_3$ assay, prepare solution containing 10 mg SO_2/mL (ca 8.6-9.0 g $NaHSO_3$/500mL) ***(Solution I)***. 5. Transfer 100 mL Hg stabilizing solution to 500 mL glass-stoppered volumetric flask. Add 1.00 mL Solution I, and dilute to volume with H_2O (1mL=20µg SO_2) ***(Solution II)***. 6. Using 10 mL graduate containing 1 drop n-hexyl alcohol as antifoam, transfer 10 mL 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 8.0 mL Solution II (0-160 µg SO_2). Dilute to volume with H_2O, and mix. 8. Transfer 25 mL aliquots of each solution to separate 50 mL volumetric flasks. To each flask, add 5 mL color reagent. Mix, and add 5 mL HCHO solution. Mix, dilute to volume with H_2O, mix, and hold in 25 °C water bath 30 min. 9. Read colour in spectrophotometer at 550 nm 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 25 mL aliquot used, or convert directly to µg/mL SO_2.
Sample Preparation	1. Using pipets, add 2 mL Hg stabilizing solution and 5 mL 0.05M H_2SO_4 to 100 mL volumetric flask. 2. Measure 10 mL cold, undegassed beer into 10 mL graduate containing 1 drop n-hexyl alcohol, and add to volume flask. 3. Swirl gently, and add 15 mL 0.1M NaOH. Swirl, and hold 15 s. 4. Add 10 mL 0.05M H_2SO_4, then H_2O to volume, and mix thoroughly. Transfer 25 mL aliquot to 50 mL volumetric flask.
Method of Analysis	1. To solution in 50 mL volumetric flask, add dilute to volume with H_2O. 2. Mix, and hold in 25 ºC bath 30 min. 3. Read colour as above, using cells of same size and same instrument settings. 4. Correct for blank as follows: Measure 10 mL cold, undegassed beer into 100 mL volumetric flask. 5. Add 0.5 mL 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 25 mL 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 with units of expression	$\text{Sulphur dioxide } \left(\frac{\mu g}{mL}\right) = (A_s - A_b) \times F$ Where, A_s=A of test solution (or photometric reading with green filter equivalent to *A*) A_b=A of I_2 blank, F= factor derived from point no. 11 (Preparation of reagents) for converting *A* to µg SO_2 in aliquot, or directly to µg/mL SO_2.
Reference	AOAC 963.11-1964, Sulfur dioxide in beer. Colorimetric method
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Tannins (for Wines only)**		
Method No.	FSSAI 13.031:2021	**Revision No. & Date**	0.0
Scope	Spectrophotometric Method - This method is useful for the determination of tannins present in alcoholic beverages.		
Caution	1. Sodium tungstate: Acute oral toxicity. 2. Phosphomolybdic acid: Contact with skin causes irritation and possible burns, especially if the skin is wet or moist. May be harmful if absorbed through the skin. Ingestion: May cause severe gastrointestinal tract irritation with nausea, vomiting and possible burns. Inhalation: May cause burns to the respiratory tract. 3. Sodium Carbonate: Eye contact can cause permanent corneal injury and possible burns. Avoid ingestion or inhalation of dust. Due to these potential hazards, sodium carbonate should be handled with care. 4. Phosphoric acid: Repeated or prolonged exposure to phosphoric acid mist can lead to chronic eye irritation, severe skin irritation, or prolonged respiratory tract issues. 5. Tannic acid: Very large amounts of tannic acid can cause stomach irritation, nausea, and vomiting. When applied to the skin: Tannic acid is possibly unsafe when applied to skin that is tender or damaged.		
Principle	Tannins present in alcoholic beverages reacts with Folin - Dennis reagent and forms coloured solutions. The absorbance of these colored solutions are measured and tannin quantity is determined.		
Apparatus /Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Spectrophotometer, Double beam with a working wavelength range of 350-800 nm and band width 5 nm.		
Materials and Reagents	1. Alcoholic beverages 2. Sodium tungstate ($Na_2WO_4.2H_2O$) 3. Phosphomolybdic acid 4. Phosphoric acid 5. Anhydrous Sodium carbonate 6. Tannic acid		
Preparation of reagents	1. Preparation of Folin - Dennis reagent – Prepare by adding 100 g Sodium tungstate ($Na_2WO_4.2H_2O$), 20 g Phosphomolybdic acid and 50 mL phosphoric acid to 750 mL water and reflux for 2 h and dilute to 1 L. 2. Preparation of Sodium carbonate solution–Prepare by adding 35 g anhydrous Sodium carbonate to 100 mL water at about 80 °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 100 mg Tannic acid in 1000 mL water. (1 mL = 0.1 mg of tannic acid).		
Method of Analysis	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 100 mL volumetric flasks containing 75 mL water. 2. Add 5 mL Folin - Dennis reagent and 10 mL 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 1 mL of wine into a 100 mL volumetric flask containing about 80 mL water. 6. Add 5 mL Folin-Dennis reagent and 10 mL sodium carbonate solution. Make up to volume. 7. Mix well and after 30 min, against reagent blank read the absorbance. 8. If the absorbance is beyond 0.8, dilute the solution 1:4 times and read.
Calculation with units of expression	Obtain the mg of tannic acid using the standard curve and calculate to express the value in g/L of wine.
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Extracts in Wines		
Method No.	FSSAI 13.032:2021	**Revision No. & Date**	0.0
Scope	Evaporation Method – This method is useful to determine the extracts present in alcoholic beverages.		
Principle	Extracts are estimated by evaporating the known quantity of the sample of wine on a steam bath		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Pipette, 50 mL. 3. Evaporating dishes, aluminum, flat bottom with lids, 75 mL capacity. 4. Oven- calibrated to maintain temperature of 100 ± 2 °C. 5. Steam bath. 6. Desiccators. 7. Electronic balance, 0.1 mg sensitivity		
Materials and Reagents	Alcoholic beverages		
Method of Analysis	1. Weigh, dried and cooled aluminum dish (W_1). 2. Mix the wine sample well and draw 50 mL sample (dry wines) or 25 mL sample (sweet wines) into the aluminum 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 h. 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 with units of expression	$\text{Extract } \left(\frac{\text{g}}{\text{L}}\right) = \frac{[(W_2 - W_1) \times 1000]}{\text{Volume of sample}}$ W_1 – Weight of empty aluminum dish W_2 - Weight of aluminum dish with extract residue.		
Reference	1. IS Standard – IS 3752:2005, Alcoholic Drinks, Methods of Test 2. IS Standard – IS 7585:1995, Wines, Methods of Analysis		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Sorbic Acid**		
Method No.	FSSAI 13.033:2021	**Revision No. & Date**	0.0
Scope	Spectrophotometric method – This method is useful to determine sorbic acid present in alcoholic beverages.		
Caution	Hydrochloric acid: Hydrochloric acid is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases.		
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 / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Cash Electric still. 3. UV Spectrophotometer		
Materials and Reagents	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 (equivalent to 1.000 g sorbic acid) in 1 L volumetric flask, and dissolve and dilute to volume with H_2O. Solution is stable several days when refrigerated.		
Method of analysis	Preparation of Standard Curve 1. Pipet 0, 10, 20, 30, and 40 mL sorbic acid standard solution into separate 100 mL volumetric flasks, and dilute to volume with H_2O. 2. Pipet 2 mL of each solution into different 200 mL volumetric flasks and, add 0.5 mL 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 2 mL wine into Cash still. 5. Rinse in with 2–3 mL H_2O. 6. Steam-distill into 200 mL volumetric flask containing 0.5 mL 0.1M HCl. 7. Collect ca 190 mL distillate; dilute to volume with H_2O. 8. Read A at 260 nm in 1 cm cell.		
Calculation with units of expression	Determine concentration from standard plot/ curve.		
Reference	1. Determination of sorbic acid AOAC, 974.08 2. Determination of sorbic acid in wine; Arthur Caputi, Masao Ueda, Bruno Trombella; Journal of Association of Official Analytical Chemists, Volume 57, Issue 4, 1 July 1974, Pages 951–953 3. Collaborative Study of the Determination of sorbic acid in wine; Arthur Caputi, Jr, Karen Slinkard; Journal of Association of Official Analytical Chemists**,** Volume 58, Issue 1, 1 January 1975, Pages 133–135, https://doi.org/10.1093/jaoac/58.1.133		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

	Determination of Reducing Sugar - Lane and Eynon (Fehling) Method		
Method No.	FSSAI 13.034:2021	**Revision No. & Date**	0.0
Scope	Lane and Eynon (Fehling) Method –This method is useful to determine reducing sugars present in alcoholic beverages.		
Caution	1. Copper sulphate: Copper sulfate can cause severe eye irritation. Eating large amounts of copper sulfate can lead to nausea, vomiting, and damage to body tissues, blood cells, the liver, and kidneys. 2. Sulphuric acid: Concentrated sulfuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If sulfuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 3. Potassium sodium tartrate: May cause irritation to skin, eyes, and respiratory tract. Inhalation: may cause irritation to the respiratory tract. 4. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage. 5. Lead acetate: may be fatal if swallowed, inhaled or absorbed through skin. Suspect cancer hazard. May cause cancer. Risk of cancer depends on level and duration of exposure. Causes irritation to skin, eyes and respiratory tract. Neurotoxin. Affects the gum tissue, central nervous system, kidneys, blood and reproductive system. 6. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 7. Disodium hydrogen phosphate: Causes mild skin irritation; Causes eye irritation. 8. Benzoic acid: Immediately or shortly after exposure to benzoic acid, the following health effects can occur: Eye damage. Irritation of the skin, resulting in a rash, redness, and/or a burning feeling. Irritation to the nose, throat and lungs if inhaled, which may cause coughing, wheezing and/or shortness of breath.		
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 / Instruments	General Glassware and apparatus (Refer 2.0 at page no. 2).		
Materials and Reagents	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
Preparation of reagents	Soxhlet solution 1. Solution A - Dissolve 34.639 g of copper sulphate in water, add 0.5 mL of conc. H_2SO_4 and dilute to 500 mL. Filter the solution. 2. Solution B - Dissolve 173 g of Rochelle salt (Potassium sodium tartarate) and 50 g of sodium hydroxide dilute to 500 mL 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 - 0.05 g of Methylene blue is dissolved in 100 mL water. Standard invert sugar solution 6. Stock solution of dextrose – Anhydrous dextrose (10 g) dissolved in water in a 1 L graduated flask. Benzoic acid (2.5 g) 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 15 mL but less than 50 mL 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 100 mL. Prepare this solution every day. 8. Sodium Hydroxide – 1 normal solution. Preparation of control 9. Pipette 25 mL of Soxhlet reagent into a 250 mL flask. Add 10 mL of 0.5% standard invert sugar solution, bring it to boil in 3 min and keep it boiling for 3 min (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.
Sample Preparation	De-alcoholization and Decolourization of Wine Sample 1. Take 100 mL of wine sample in a porcelain dish. 2. Exactly neutralize with sodium hydroxide calculating the acidity and evaporate to 50 mL. 3. To this add 5 mL of lead acetate solution, enough activated charcoal and 2 drops of glacial acetic acid. 4. Make the volume to 100 mL with distilled water. Filter this mixture into 2 g of disodium hydrogen phosphate in a beaker.
Method of Analysis	1. Pipette 20 mL of the clarified wine into an Erlen-meyer flask containing 25 mL of Soxhlet reagent. 2. Bring it to boil and titrate with 0.5% invert sugar, with methylene blue indicator, to a brick red end point.
Calculation with units of expression	Calculate the reducing sugar from the standard tables.

Reference	IS Standard – IS 7585:1995, Wines, Methods of Analysis
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Reducing Sugar – Dinitrosalicylic Acid Method**		
Method No.	FSSAI 13.035:2021	**Revision No. & Date**	0.0
Scope	Reducing sugars (contain free carbonyl group) have the property to reduce many of the reagents. Dinitrosalicylic acid (DNS) is one such reagent. This method is useful to determine reducing sugars present in alcoholic beverages using dinitrosalicylic acid.		
Caution	1. Potassium sodium tartarate: May cause irritation to skin, eyes, and respiratory tract. Inhalation: may cause irritation to the respiratory tract. 2. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage. 3. 3,5-Dinitrosalicylic acid: Causes eye burns. Harmful if absorbed through the skin. Causes skin burns. Harmful if swallowed. Causes gastrointestinal tract burns. Harmful if inhaled. Causes chemical burns to the respiratory tract. Chronic exposure may cause effects similar to those of acute exposure. 4. Phenol: Phenol can pose a severe health hazard and should be handled with extreme caution. Phenol is highly corrosive to the skin and readily absorbed through it, whereupon it can affect the central nervous system and cause damage to the liver and kidneys. It is also a mutagen, and there is some evidence that phenol may be a reproductive hazard. When heated, phenol will produce flammable vapors that are highly toxic (at just a few parts per million) and explosive (at concentrations of 3% to 10% in air). 5. Sodium sulphite: Dust or mist may cause skin irritation from prolonged contact. Solutions will cause skin irritation. Inhalation of dust may cause coughing and sneezing. Ingestion may result in irritation of the mouth and gastrointestinal tract		
Principle	When alkaline solution of 3,5-dinitrosalicylic acid reacts with reducing sugars (e.g. Glucose, lactose.), it is converted into 3-amino-5-nitrosalicylic acid with orange color.		
Apparatus	1. General Glassware and apparatus (Refer 2.0 at page no. 2) 2. Spectrophotometer UV-Visible (variable wavelength) 3. Amber color bottle		
Materials and reagents	1. Alcoholic beverages 2. Sodium potassium tartrate 3. 3,5-Dinitrosalicylic acid 4. Sodium hydroxide 5. Phenol – Crystalline 6. Sodium sulphite 7. Glucose (Standard)		
Preparation of reagents	1. Sodium hydroxide (1%): Dissolve sodium hydroxide(1 g) in distilled water (100 mL) 2. Dinitrosalicyclic acid reagent (DNS Reagent): Dissolve by stirring 1 g dinitrolsalicyclic acid, 200 mg crystalline phenol and 50 mg sodium sulphite in 100 mL 1% NaOH. Store at 4 ˚C in amber bottle. Since, the reagent deteriorates due to sodium sulphite, if long storage is required, sodium		

	sulphite may be added at the time of use. 3. Rochelle salt solution (Potassium sodium tartrate - 40%): Dissolve potassium sodium tartrate (40 g) in distilled water (100 mL).
Sample Preparation	1. Stock standard Glucose solution: Glucose solutions of different concentrations are obtained by dilutions from a stock solution of 2 g/L. 2. Working standard Glucose solution: Stock standard Glucose solution (10 mL) is diluted to 100 mL.
Method of Analysis	1. Take 100 mL of alcoholic beverage and remove alcohol completely by distillation on water bath at 80 ˚C. Note down the weight (A mg) of the residue 2. Weigh 100 mg of the sample (residue) and extract the sugars with hot 80% ethanol twice (5 mL each time) 3. Collect the supernatant and evaporate it by keeping it on a water bath at 80 ˚C 4. Add 10 mL water and dissolve the sugars 5. Pipette out 0.5 to 3 mL of the extract in test tubes and equalize the volume to 3 mL with water in all the tubes. 6. Add 3 mL of DNS reagent. 7. Heat the contents in a boiling water bath for 5 min. 8. When the contents of the tubes are still warm, add 1mL of 40% Rochelle salt solution. 9. Cool to room temperature make up to 7 mL with distilled water. 10. Read the intensity of dark red colour at 510 nm. 11. Run a series of standards using glucose (0 to 500 µg) and plot a calibration graph. 12. Calculate the reduced sugars (B µg) using calibration curve present in C mL of residue solution .
Calculation with units of expression	1. Reducing sugars present in 10 mL of residue solution (reducing sugars present in 100 mg of the residue sample) = $10 \times B/C$ 2. Reducing sugars present in100 mL of alcoholic beverage (total residue i.e., A mg)= $\frac{A \times 10 \times B}{100 \times C}$(in micro grams) A – Weight of residue from 100 mL of beverage. B –Weight of reduced sugars from C mL of residue solution.
Reference	Miller, G. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry 31, pp. 426-428
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Individual Sugars - HPLC**		
Method No.	FSSAI 13.036:2021	**Revision No. & Date**	0.0
Scope	Determination of individual sugars in alcoholic beverages using High Performance Liquid Chromatography with refractive index detector.		
Principle	Retention times of individual sugars in HPLC are different. All sugars show refractive index.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. HPLC with RI Detector and temperature oven. 3. Hi-Plex H column (7.7 × 300 mm).		
Materials and Reagents	1. Alcoholic beverages 2. Ethanol 3. Glucose (Extra Pure) 4. Fructose (analytical reagent) 5. Sucrose (analytical reagent) 6. Distilled deionized water		
Preparation of reagents	1. Stock Standard sugar solutions: Prepare 5% stock standard sugar solutions. 2. Working standard sugar solution: Dilute the stock standard sugar solution to working standard sugar solutions (1%).		
Samples Preparation	1. Extract sugars from Alcoholic beverages using methanol (80%) in ethanol for 90 min.		
Method of Analysis	1. Dilute working standard sugar solutions to Standard solutions of glucose, fructose, and sucrose from 0.03% (w/w) to 0.2% (w/w) for preparation of calibration curves. 2. Inject these solutions to HPLC under the following conditions. 3. Note the retention time of each standard sugar. 4. Prepare calibration curves using the concentration of sugars (x-axis) vs detector response (y-axis). Note: The limit of detection (%, w/w) and recovery (%) of the individual sugars by the HPLC-RI method were fructose 0.001, 89.4–106; glucose 0.002, 92.4–109; and sucrose 0.002, 94.2–95.1. 5. Inject test samples of sugar solutions to HPLC as per the conditions used for the preparation of calibration curves. 6. Note detector responses for each peak. 7. Make triplicate injections and calculate average detector response for each peak. HPLC conditions 1. Mobile phase: Distilled deionized water 2. Mobile phase flow rate: 0.5 mL /min. 3. Sample injection volumes: 10 µL. 4. Column temperature : 35 °C		
Calculation with units of expression	Calculate quantity of each sugar using detector response (average of triplicate) of the respective peak and calibration curve.		

Reference	Improvement in Analytical Methods for Determination of Sugars in Fermented Alcoholic Beverages; Ayalew Debebe, Shibru Temesgen, Mesfin Redi-Abshiro, Bhagwan Singh Chandravanshi ,and Estifanos Ele; Journal of Analytical Methods in Chemistry; Volume 2018, Article ID 4010298, 10 pages; https://doi.org/10.1155/2018/4010298
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Total Sugar – Fehling Solution Method**		
Method No.	FSSAI 13.037:2021	**Revision No. & Date**	0.0
Scope	The presence of added sucrose can be detected by determining sugars before and after inversion by copper- reduction methods.		
Caution	1. Copper sulphate: Copper sulfate can cause severe eye irritation. Eating large amounts of copper sulfate can lead to nausea, vomiting, and damage to body tissues, blood cells, the liver, and kidneys. 2. Hydrochloric acid: Hydrochloric acid is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 3. Potassium sodium tartrate: May cause irritation to skin, eyes, and respiratory tract. Inhalation: may cause irritation to the respiratory tract. 4. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage. 5. Lead acetate: may be fatal if swallowed, inhaled or absorbed through skin. Suspect cancer hazard. May cause cancer. Risk of cancer depends on level and duration of exposure. Causes irritation to skin, eyes and respiratory tract. Neurotoxin. Affects the gum tissue, central nervous system, kidneys, blood and reproductive system. 6. Sodium oxalate: Like several other oxalates, sodium oxalate is toxic to humans. It can cause burning pain in the mouth, throat and stomach, bloody vomiting, headache, muscle cramps, cramps and convulsions, drop in blood pressure, heart failure, shock, coma, and possible death. 7. Potassium oxalate: Harmful if swallowed. Causes eye, skin, and respiratory tract irritation.		
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 / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Amber coloured bottles.		
Materials and Reagents	1. Alcoholic beverages 2. Copper sulphate ($CuSO_4.5H_2O$) 3. Rochelle salt (potassium sodium tartrate) ($KNaC_4H_4O_6.4H_2O$). 4. Hydrochloric acid 5. Sodium hydroxide 6. Lead acetate 7. Potassium or sodium oxalate 8. Phenolphthalein indicator		
Preparation of reagents	1. Fehling A: Dissolve 69.28 g copper sulphate ($CuSO_4.5H_2O$) in distilled water. Dilute to 1000 mL. Filter and store in amber coloured bottle. 2. Fehling B: Dissolve 346 g Rochelle salt (potassium sodium tartrate) (K Na $C_4H_4O_6.4H_2O$) and 100 g NaOH in distilled water. Dilute to 1000 mL. Filter and store in amber coloured bottle.		

<table>
<tr><td></td><td>3. Saturated neutral Lead acetate solution.</td></tr>
<tr><td>Sample Preparation</td><td>1. Transfer test sample representing about 2-2.5 g sugar to 200 mL volumetric flask, dilute to about 100 mL.
2. Add excess of saturated neutral Lead acetate solution (about 2 mL is usually enough).
3. Mix, dilute to volume and filter, discarding the first few mL filtrate.
4. Add dry Potassium or Sodium Oxalate to precipitate excess lead used in clarification, mix and filter, discarding the first few mL filtrate.
Note: Use of Potassium Ferrocyanide and Zinc acetate is preferable instead of Lead acetate and Sodium oxalate, due to safety issues.</td></tr>
<tr><td>Method of Analysis</td><td>Standardization of Fehling’s solution
1. Prepare standard dextrose solution into a 100 mL volumetric flask. Find the titre (volume of dextrose solution required to reduce all the copper in 10 mL of Fehling solution) corresponding to the standard dextrose solution (Refer table below).
2. Pipet 10 mL of Fehling’s solution into a 300 mL 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 min.
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 begin 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 min 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 10 mL 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.</td></tr>
</table>

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
Milligrams of anhydrous dextrose corresponding to 10 mL of Fehlings solution		

a) Take 25 mL filtrate 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.
b) For inversion at room temperature, transfer 50 mL aliquot clarified and de-leaded solution to a 100 mL volumetric flask, add 10 mL HCl (1+ 1) and let stand at room temperature for 24 h. (For immediate inversion, the sample with HCl can be heated at 70 ˚C for 1 h).
c) Neutralise exactly with conc. NaOH solution using phenolphthalein indicator and dilute to 100 mL. Titrate against mixed Fehling A and B solution (25 mL 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 with units of expression

Reducing and total reducing sugar can be calculated as below:

Reducing sugar (%)

$$= \frac{(\text{mg of invert sugar} \times \text{volume made up} \times 100)}{\text{TR} \times \text{Weight of sample} \times 1000}$$

Total reducing sugar (%)

	$= \frac{\text{mg of invert sugar} \times \text{final volume made up} \times \text{original volume} \times 100}{\text{TR} \times \text{Weight of sample} \times \text{aliquot taken for inversion} \times 1000}$ Total sugar (as sucrose) (%) $= (\text{Total reducing sugar} - \text{Reducing sugar}) \times 0.95 + \text{Reducing sugar}$ 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	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Total Sugar –Anthrone Method**		
Method No.	FSSAI 13.038:2021	**Revision No. & Date**	0.0
Scope	Anthrone method – Total sugars in alcoholic beverages are determined using anthrone method.		
Caution	1. Hydrochloric acid: Hydrochloric acid is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases. 2. Sodium carbonate: Eye contact can cause permanent corneal injury and possible burns. Avoid ingestion or inhalation of dust. Due to these potential hazards, sodium carbonate should be handled with care. 3. Sulphuric acid: Concentrated sulphuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If sulphuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 4. Anthrone: Causes skin irritation. Causes serious eye irritation. May cause respiratory irritation. 5. Toluene: Toluene is a highly flammable liquid and it can cause mild damage to the skin and the eyes. However, the most-common hazard associated with this chemical is inhalation. Products containing toluene can produce dangerous fumes which can cause nausea, headaches, unconsciousness, and even death if inhaled.		
Principle	Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green colored product with an absorption maximum at 630 nm.		
Apparatus/ Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Spectrophotometer UV-Visible (variable wavelength).		
Materials and Reagents	1. Alcoholic beverages 2. Hydrochloric acid (36%) 3. Sodium carbonate 4. Anthrone 5. Sulphuric acid 6. Standard Glucose 7. Toluene		
Preparation of reagents	1. Hydrochloric acid (2.5 N): Dilute Hydrochloric acid (21.5 mL) to 100 mL. 2. Anthrone reagent: Dissolve 200 mg anthrone in 100 mL of ice cold 95% Sulphuric acid. Prepare fresh before use. 3. Stock Standard Glucose solution: Dissolve 100 mg of standard glucose in 100 mL water. 4. Working standard Glucose solution: 10 mL of stock Standard Glucose		

	solution diluted to 100 mL with distilled water. Store refrigerated after adding a few drops of toluene.
Sample Preparation	1. Take 100 mL of alcoholic beverage and remove alcohol completely by distillation on water bath at 80 °C. Note down the weight (A mg) of the residue 2. Weigh 100 mg of the residue into a boiling tube. 3. Hydrolyze by keeping it in a boiling water bath for three hours with 5 mL of 2.5 N HCI and cool to room temperature. 4. Neutralize it with solid sodium carbonate until the effervescence ceases. 5. Make up the volume to 100 mL and centrifuge. 6. Collect the supernatant and take 0.5 and 1 mL for analysis as test sample.
Method of analysis	1. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard glucose solution. '0' serves as blank. 2. Make up the volume to 1 mL in all the tubes including the sample tubes by adding distilled water. 3. Then add 4 mL of anthrone reagent. 4. Heat for eight minutes in a boiling water bath. 5. Cool rapidly to room temperature and make upto 5 mL with distilled water 6. Read the green to dark green colour at 630 nm. 7. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. 8. From the graph calculate the amount of carbohydrate present in the sample tube.
Calculation with units of expression	Amount of carbohydrate present in 100 mg of the sample residue (B) $= (\text{mg of glucose} \div \text{Volume of test sample}) \times 100$ Amount of carbohydrate present in 100 mL of the alcoholic beverage $= \frac{B \times A}{100}$
Reference	Hedge, J E and Hofreiter, B T (1962) In: Carbohydrate Chemistry 17 (Eds Whistler R L and Be Miller, J N) Academic Press New York
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Carbonation (GV)		
Method No.	FSSAI 13.039:2021	**Revision No. & Date**	0.0
Scope	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 15,56 T.		
Principle	Amount of carbonation is determined using the pressure gauge.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 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 needle point 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.		
Materials and Reagents	Alcoholic beverages		
Method of Analysis	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.		
Calculation with units of expression	Obtain the volume of gas from Table 2 of IS 2346.		
Reference	1. IS: 15588 (2005), Alcoholic drinks - Low alcoholic beverages. 2. IS: 2346 Carbonated beverages specification		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of pH**		
Method No.	FSSAI 13.040:2021	**Revision No. & Date**	0.0
Scope	The pH is closely related to the concentration of hydrogen ions (H+) present in alcoholic beverages (the pH characteristics of alcoholic beverages depend on various parameters, such as the quality of the reducing water, the duration of maturation in casks, the nature of the aromatic raw materials, and of any additives). Due to the presence of ethyl alcohol in alcoholic beverages, the pH should be measured according to specific procedures.		
Caution	1. Potassium tartrate monobasic: Accidental ingestion of the material may be damaging to the health of the individual. Excessive amounts or overuse may bring irritant and / or harmful effects. Potassium causes a slow, weak pulse, irregularities in heart rhythm, heart block and an eventual fall in blood pressure. The acid itself have all produced serious poisonings or fatalities in man. Gastrointestinal symptoms are marked and include violent vomiting, diarrhea, abdominal pain and thirst followed by cardiovascular collapse and/or kidney failure. This material can cause eye irritation and damage in some persons. This material can cause inflammation of the skin on contact in some persons and produce health damage following entry through wounds, lesions or abrasions. Examine the skin prior to the use of the material and ensure that any external damage is suitably protected. If inhaled, the material can cause respiratory irritation in some persons. The body's response to such irritation can cause further lung damage. 2. Thymol: Ingestion may cause burning pain in the oesophagus, nausea, abdominal pain, vomiting, dizziness, convulsions, coma, cyanosis, central hyperactivity (e.g., talkativeness), cardiac and respiratory arrest. 3. Potassium hydrogen phthalate: May cause eye, skin, and respiratory tract irritation. 4. Decahydrate Borax: Borax can be irritating when exposure occurs through skin or eye contact, inhalation or ingestion. Poison reports suggest misuse of borax-based pesticides can result in acute toxicity, with symptoms including vomiting, eye irritation, nausea, skin rash, oral irritation and respiratory effects.		
Principle	Principle applied to alcoholic beverages - The measurement of pH in organic media 1. The traditional pH range extending from 0 to 14 is determined by the dissociation of water. If the water content of a solution is gradually reduced or the water is replaced by another solvent, it is the dissociation equilibrium, i.e. the latter's ionic product which is taken into account instead of that of the water. This results in totally different concentration ranges for the "free" H+ ions (i.e. which are not chemically bound). In non-aqueous media, it is not possible to carry out absolute measurements of pH. Only relative measurements can be made. In addition, partially aqueous media are often low-ion. 2. However, from a water content of at least 5%, the classic definition of pH can be used, i.e. expressed in terms of absolute values and not just relative values. Under these operating conditions, at the interface between the electrolyte and solution to be measured a phase separation is often formed which makes the		

	signal unstable. There is also a risk of precipitation at the membrane level. The same problem is also encountered when using concentrated solutions of KCl as the reference electrolyte. 3. Specific measurement conditions - To avoid the problems described above, the basic requirement is that the electrolyte solution to measure and form a homogeneous solution without phase separation or precipitation. This condition can be met using lithium chloride (LiCl) in an ethanol medium. A second condition is the use of an electrode with cylindrical membrane and a ground-in diaphragm, to ensure optimum contact between the reference electrolyte and the solution to be measured.
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. pH meter - pH meter calibrated in pH units, enabling measurements to a minimum accuracy of: ± 0.01 pH i.e. ± 1 mV. The instrument is preferably to be equipped with an electronic device for the automatic compensation of the temperature to a minimum accuracy of ± 0.5 °C. The pH meter should be used in a place sheltered from pollutants, acid or alkaline vapours in particular, hydrogen sulphide (H_2S) and ammonia (NH_3). 3. Electrodes - Combined electrode: The electrodes marketed for this specific purpose are generally of the type-combined electrode. The useful part of the electrode consists of a cylindrical membrane and a ground in diaphragm made of Teflon. The reference electrolyte is an ethanol solution at 95% vol. of lithium chloride (LiCl) to 1 mol/L. Its alcoholic strength should be close to that of the alcoholic beverage to be analysed. Immerse the electrode tip when not used continuously, in an ethanol solution of lithium chloride to 1 mol/1, unless otherwise specified by the manufacturer of the electrode. 4. Stirring device: magnetic stirrer and bar, for example. 5. Cleaning supplies: Joseph paper, etc.
Materials and Reagents	1. Alcoholic beverages 2. Deionised or distilled water-Free from carbon dioxide and metal ions, with a maximum conductivity of 200 µS/m@ 20 °C 3. Potassium acid tartrate 4. Thymol 5. Potassium hydrogen phthalate 6. Potassium dihydrogen phosphate 7. Dipotassium phosphate 8. Decahydrated Borax, ($B_4O_7Na_2.10\ H_2O$) 9. Standard buffer solution: With reference to standard NFT 01012 "pH measurement - standard solutions for calibration of a pH meter" (i) pH buffer solution: 3.57 at 20 °C (ii) pH buffer solution: 4.00 at 20 °C (iii) pH buffer solution: 6.88 at 20 °C (iv) pH buffer solution: 9.22 at 20 °C
Preparation of reagents	1. pH buffer solution - 3.57 at 20 °C: Saturated solution of potassium acid tartrate. Solution containing at least 5.7 g/l of potassium acid tartrate (HOOC $C_2H_4O_2COOK$) at 20 °C. This solution can be kept for two months in the presence of 0.1 g of thymol per 200 mL. (3.57 at 20 °C); (3.56 at 25 °C); (3.55 at 30 °C). 2. pH buffer solution - 4.00 at 20 °C: 0.05 M solution of potassium hydrogen phthalate. Solution containing 10.211 g/1 of potassium hydrogen phthalate at 20 °C (maximum storage time: 2 months). (3.999 at 15 °C); (4.003 at 20 °C);

	(4.008 at 25 °C); (4.015 at 30 °C). 3. pH buffer solution - 6.88 at 20 °C: Solution containing Potassium dihydrogen phosphate (KH_2PO_4 - 3.402 g), and Dipotassium phosphate, (K_2HPO_4 - 4.354 g) and Water q.s.p 1 L (Shelf life: 2 months). (6.90 at 15 °C); (6.88 at 20 °C); (6.86 at 25 °C); (6.85 at 30 °C). 4. pH buffer solution - 9.22 at 20 °C: Solution containing Decahydrated Borax, ($B_4O_7Na_2$.10 H_2O... 3.810 g) Water q.s.p 1 L. (pH: 9.22 at 20 °C) P.S.: Basic buffer solutions are quickly altered by the carbon dioxide in the surrounding air, and it is therefore necessary to renew the solution for each calibration). Note: market-available reference buffer solutions can also be used (according to the DIN 19266 standard and NBS, for example).
Method of Analysis	Calibration of the measurement chain 1. Two standard solutions are needed to calibrate the pH meter. Their pH should, if possible, be located on either side of the presumed pH value of the test solution; if this is not possible, one of them must not differ by more than one unit pH from the presumed value. 2. Zero setting the measurement chain (pH): Operate in accordance with the instructions provided with the apparatus used. Rinse the electrodes with the first standard buffer solution by pouring the liquid along them. 3. Introducing a sufficient volume of the same standard solution into the measuring vessel (it should be clean and dry) and immerse the electrodes. 4. Adjust the indication of the pH meter on the pH value of the standard solution taking into account its temperature (if necessary). 5. Remove the electrodes and discard the standard solution contained in the measuring vessel. Setting the slope of the electrode 6. Rinse the electrodes with distilled or deionised water and then with the second standard buffer solution introduce a sufficient volume of the same standard buffer solution and immerse the electrodes. If the result matches the known value of the pH of the standard solution, the unit is in working condition and is properly calibrated. Calibration Check 7. Use a buffer solution with an intermediate pH value in relation those used for calibration. pH measurements 8. Once the device has been calibrated, rinse the electrodes and the measuring vessel, first with deionised or distilled water, then with the test solution by proceeding as above. 9. Homogenize the test solution, introduce a sufficient volume in the measuring vessel. 10. Immerse the electrodes. 11. Lightly stir the test solution. 12. Verify that the indication given by the pH meter is stable and record it.
Calculation with units of expression	EXPRESSION OF RESULTS 1. In the operating conditions described above, the accuracy of the determination is ± 0.02 pH units. 2. The results are expressed in units of pH, at a temperature of 20 °C, in the form pH at 20 °C = xx, xx

Reference	Compendium of International Methods of Spirituous Beverages of Vitivinicultural Origin, International Organisation Of Vine And Wine, Edition 2019, pH, Method No OIV-MA-BS-13
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Anethole - Gas Chromatography determination of Trans-anethole in Spirit Drinks of Viti-vinicultural origin		
Method No.	FSSAI 13.041:2021	**Revision No. & Date**	0.0
Scope	Anethole (also known as anise camphor) is an organic compound that is widely used as a flavouring substance. It is a derivative of phenylpropene, a type of aromatic compound that occurs widely in nature, This method is suitable for the determination of trans-anethole in aniseed flavoured spirit drinks using capillary gas chromatography.		
Caution	1. Trans-anethole: May cause an allergic skin reaction. Avoid breathing dust /fume /gas/mist/vapors/spray. 2. Estragole: Harmful if swallowed, Acute oral toxicity. Recently estragole carcinogenicity is reported.		
Principle	Concentration of the trans-anethole in spirit is determined by gas chromatography (GC). The same quantity of an internal standard, e.g. 4-allylanisole (estragole) (when estragole is not naturally present in the sample), is added to the test sample and to a trans-anethole reference solution of known concentration, both of which are then diluted with a 45% ethanol solution and injected directly into the GC system. An extraction is necessary before sample preparation during analysis for liqueurs that contain large amounts of sugars.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. A capillary gas chromatograph fitted with a flame ionisation detector (FID) and integrator or other data handling system capable of measuring peak areas, and with an automatic sampler or the necessary equipment for manual sample injection. 3. Split/splitless injector. 4. Capillary column: Length - 50 m; Internal diameter - 0.32 mm; Film thickness - 0.2 µm; Stationary phase – Free Fatty Acid Phase (FFAP-modified TPA polyethylene glycol cross-linked porous Polymer .		
Materials and Reagents	1. Alcoholic beverages. 2. Trans-anethole (>98% pure; stored at 4 °C) - Trans-anethole will need to be "thawed" from its crystalline state before use, but in this case its temperature should never exceed 35 °C. 3. Estragole (>98% pure; stored at 4 °C). 4. Water of at least grade 3 as defined by ISO 3696. 5. Ethanol 96%		
Preparation of reagents	1. Ethanol 45%: Add 560 g of distilled water to 378 g of ethanol 96% vol. Preparation of standard solutions 2. Standard solution A -Stock solution of trans-anethole (concentration - 2 g/L): Weigh 40 mg of trans-anethole in a 20 mL volumetric flask. Add some 96% ethanol and make up to volume with 45% vol. ethanol, mix thoroughly. 3. Internal standard solution B - Stock solution of internal standard, e.g. estragole (concentration- 2 g/L): Weigh 40 mg of estragole in a 20 mL volumetric flask. Add some 96% vol. ethanol make up to volume with 45% vol. ethanol, mix thoroughly. 4. All standard solutions should be stored at room temperature (15-35 °C) away from light in aluminium containers or in tinted (amber) glass reagent bottles.		

	The stopper should preferably be fitted with an aluminium seal. The stock solutions must be freshly prepared each week. Solutions used to check the linearity response of the FID 5. The linearity response of the FID must be checked for the analysis taking into account a range of concentrations of trans-anethole in spirits from 0 g/L up to 0.25 g/L. (In the procedure of analysis, the unknown samples of spirits to be analysed are diluted 10 times). 6. For the conditions of the analysis described in the method, stock solutions corresponding to concentrations of 0, 0.05, 0.1, 0.15, 0.2, and 0.25 g/L of trans-anethole in the sample to be analysed are prepared as follows: take 0.5, 1, 1.5, 2, and 2.5 mL of stock solution A and pipette in separate 20 mL volumetric flasks; pipette into each flask 2 mL of internal standard solution B and make up to volume with 45% vol. ethanol, mix thoroughly. 7. The blank solutions are used as the 0 g/L solution. 8. **Standard solution C:** Take 2 mL of standard solution A and pipette into a 20 mL volumetric flask then add 2 mL of internal standard solution B and make up to volume with 45% vol. ethanol, mix thoroughly.
Sample Preparation	Preparation of unknown samples 1. Pipette 2 mL sample into a 20 mL volumetric flask then add 2 mL of internal standard solution B and make up to volume with 45% vol. ethanol mix thoroughly. 2. Blank - Pipette 2 mL of internal standard solution B into a 20 mL volumetric flask and make up to volume with 45% vol. ethanol, mix thoroughly.
Method of Analysis	1. The column type and dimensions, and the GC conditions, should be such that anethole and the internal standard are separated from each other and from any interfering substances. 2. Typical conditions for the column: i. Carrier gas: analytical helium. ii. Flow rate: 2 mL/min iii. Injector temperature: 250 °C. iv. Detector temperature: 250 °C. v. Oven temperature conditions: isothermal, 180 °C, run time 10 min vi. Injection volume: 1 µL, split 1:40 3. Samples should be stored at room temperature, away from light and cold. Procedure 4. Sample screening for estragole. 5. To ensure that there is no estragole naturally present in the sample, a blank analysis should be carried out without the addition of any internal standard. 6. If estragole is naturally present, then another internal standard must be chosen (for instance menthol). Pipette 2 mL sample into a 20 mL volumetric flask and make up to volume with 45% vol. ethanol (4.4), mix thoroughly. 7. Linearity test Prior to the commencement of the analysis the linearity of the response of the FID should be checked by successively analysing in triplicate each of the linearity standard solutions. 8. From the integrator peak areas for each injection plot a graph of their mother solution concentration in g/L versus the ratio R for each. R = trans-anethole peak area divided by the estragole peak area. 9. A linear plot should be obtained.

	10. Determination: Inject the blank solution, followed by standard solution C, followed by one of the linearity standards which will act as a quality control sample (this may be chosen with reference to the probable concentration of trans-anethole in the unknown), followed by 5 unknowns; insert a linearity (quality control) sample after every 5 unknown samples, to ensure analytical stability.
Calculation with units of expression	Measure peak areas (using an integrator or other data system) for trans-anethole and internal standard peaks. 1. Response factor (RFi) calculation The response factor is calculated as follows $$RF_i = \left(\frac{C_i}{area_i}\right) \times \left(\frac{area_{is}}{C_{is}}\right)$$ Where: C_i is the concentration of trans-anethole in the standard solution A. C_{is} is the concentration of internal standard in the standard solution B. $area_i$ is the area of the trans-anethole peak $area_{is}$ is the area of the internal standard peak RF_i is calculated from the 5 samples of standard solution C 2. Analysis of the linearity response test solutions Inject the linearity response test solutions. 3. Analysis of the sample Inject the unknown sample solution (head – sample preparation) RESULTS The formula for the calculation of the concentration of trans-anethole is the following: $c_i = C_{is} \times \left(\frac{area_i}{area_{is}}\right) \times RF_i$ where: c_i is the unknown trans-anethole concentration C_{is} is the concentration of internal standard in the unknown $Area_i$ is the area of the trans-anethole peak $Area_{is}$ is the area of the internal standard peak RF_i is the response coefficient (calculated as in pt. no. 8 - Method of Analysis) The trans-anethole concentration is expressed as grams per litre, to one decimal place. QUALITY ASSURANCE AND CONTROL The chromatograms should be such that anethole and the internal standard are separated from each other and from any interfering substances. The RF_i value is calculated from the results for the 5 injections of solution C. If the coefficient of variation (CV % = (standard deviation/mean) *100)) is within plus or minus 1%, the RF_i average value is acceptable. The calculation above should be used to calculate the concentration of trans-anethole in the sample selected for the quality control from the linearity control solutions. If the mean calculated results from analysis of the linearity solution selected for Internal Quality Control sample (IQC) are within plus or minus 2.5% of their theoretical value, then the results for the unknown samples can be accepted.
Reference	ISO 3696: 1987 Water for analytical laboratory use - Specifications and test methods
Approved by	Scientific Panel on Methods of Sampling and Analysis

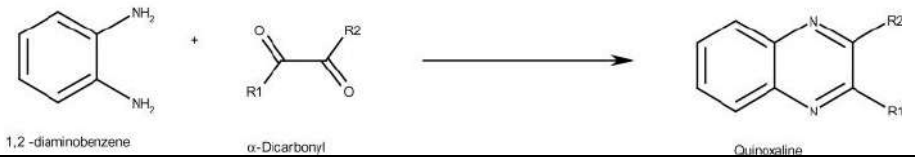
FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Trans-anethole in Spirit Drinks containing large amount of Sugar by GC Analysis**		
Method No.	FSSAI 13.042:2021	**Revision No. & Date**	0.0
Scope	Extraction of alcohol from spirit drinks containing a large amount of sugar, in order to be able to determine the trans-anethole concentration using capillary gas chromatography.		
Caution	1. Trans-anethole: May cause an allergic skin reaction. Avoid breathing dust /fume/gas/mist/vapors/spray. 2. Estragole: Harmful if swallowed, Acute oral toxicity. Recently estragole carcinogenicity is reported. 3. Ammonium sulphate: Causes irritation to skin, eyes and respiratory tract. May be harmful if swallowed. Avoid contact with eyes, skin and clothing. 4. Sodium phosphate, dibasic, dodecahydrate: Contact can irritate the skin and eyes. Breathing Sodium Phosphate Dibasic can irritate the nose and throat causing coughing and wheezing. High and repeated exposure can cause a skin rash.		
Principle	Take an aliquot of the liqueur sample and add the internal standard, at a concentration similar to that of the analyte (trans-anethole) in the liqueur. Add sodium phosphate dodecahydrate and anhydrous ammonium sulphate. Shake well the resulting mixture and chill to develop two layers, and remove the upper alcohol layer. Take an aliquot of this alcohol layer and dilute with 45 % ethanol solution. Analyse the resulting solution using gas chromatography as described in FSSAI 13.041:2021		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Equipment as described in FSSAI 13.041:2021		
Materials and Reagents	1. Alcoholic beverages. 2. Ammonium sulphate, anhydrous, (Purity >99%) 3. Sodium phosphate, dibasic, dodecahydrate, (Purity >99%) 4. Materials and Reagents as described in FSSAI 13.041:2021		
Sample Preparation	Sample screening for estragole 1. To ensure that there is no estragole naturally present in the sample, a blank extraction and analysis should be carried out without the addition of any internal standard. If estragole is naturally present, then another internal standard must be chosen. Extraction *2.* Pipette 5 mL of 96% ethanol into a conical flask, weigh into this flask 50 mg of internal standard, and add 50 mL of the sample. Add 12 g of ammonium sulphate, anhydrous, and 8.6 g of dibasic sodium phosphate, dodecahydrate. Stopper the conical flask. 3. Shake the flask for at least 30 min. A mechanical shaking device may be used, but not a Teflon coated magnetic stirring bar, as the Teflon will absorb some of the analyte. Note that the added salts will not dissolve completely. 4. Place the stoppered flask in a refrigerator (T< 5 °C) for at least two hours. 5. After this time, there should be two distinct liquid layers and a solid residue. The alcohol layer should be clear; if not replace in the refrigerator until a clear separation is achieved. 6. When the alcohol layer is clear, carefully take an aliquot (e.g. 10 mL),		

	without disturbing the aqueous layer, place in an amber vial and close securely. Preparation of the extracted sample to be analysed 7. Allow extract to reach room temperature. Take 2 mL of the alcohol layer of the extracted sample and pipette into a 20 mL volumetric flask, make up to volume with 45% ethanol, mix thoroughly.
Procedure	Analyse as described in FSSAI 13.041:2021
Calculation with units of expression	Follow the procedure as outlined in FSSAI 13.041:2021 CALCULATION OF RESULTS Use the following formula to calculate the results $C_i = \left(\frac{m_{is}}{V}\right) \times \left(\frac{area_i}{area_{is}}\right) \times RF_i$ Where: m_{is} is the weight of internal standard taken (in milligrams) V is the volume of unknown sample (50 mL) RF_i is the response factor (21.0) $area_i$ is the area of the trans-anethole peak $area_{is}$ is the area of the internal standard peak The results are expressed in grams per litre, to one decimal place.
Reference	Compendium of International Methods of Spirituous Beverages of Vitivinicultural Origin by International Organisation of Vine and Wine, Edition 2019, Anethole. Determination of trans-anethole by GC, Method No. OIV-MA-BS-15 : R2009
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of the Principal Compounds Extracted from Wood during Ageing of Spirit Drinks of Viti-vinicultural origin		
Method No.	FSSAI 13.043:2021	**Revision No. & Date**	0.0
Scope	The present method pertains to the determination of furfural, 5-hydroxyl methylfurfural, 5-methylfurfural, vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, gallic, ellagic, vanillic, and syringic acids, and scopoletin, by high performance liquid chromatography.		
Caution	1. Methanol: Methanol is highly flammable and toxic. Direct ingestion of more than 10 mL can cause permanent blindness by destruction of the optic nerve, poisoning of the central nervous system, coma and possibly death. These hazards are also true if methanol vapors are inhaled. It is best to avoid direct exposure. 2. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation.		
Principle	Determination by high-performance liquid chromatography (HPLC), with detection by ultraviolet spectrophotometry at several wavelengths, and by spectrofluorimetry.		
Apparatus	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. A high-performance liquid chromatograph capable of functioning in binary gradient mode and equipped with: 3. A spectrophotometric detector capable of measuring at wavelengths from 280 to 313 nm. It is however preferable to work with a multiple wavelength detector with a diode array or similar, in order to confirm the purity of the peaks. 4. A spectrofluorimetric detector – excitation wavelength: 354 nm, emission wavelength: 446 nm (for the trace determination of scopoletin; which is also detectable at 313 nm by spectrophotometry). 5. An injection device capable of introducing 10 or 20 µL of the test sample. 6. A high-performance liquid chromatography column, RP C18 type, 5µm maximum particle size. 7. Syringes for HPLC. 8. Device for membrane-filtration of small volumes. 9. Integrator-computer or recorder with performance compatible with the entire apparatus, and in particular, it must have several acquisition channels.		
Materials and Reagents	1. Alcoholic beverages. The reagents must be of analytical quality. The water used must be distilled water or water of at least equivalent purity. 2. Microfiltered water with a resistivity of 18.2 M Ω. 3. 96% vol. alcohol. 4. HPLC-quality methanol (Solvent B). 5. Acetic acid. Reference standards of 99% minimum purity 6. Furfural. 7. 5-Hydroxymethyl furfural. 8. 5-Methylfurfural.		

	9. Vanillin. 10. Syringaldehyde. 11. Coniferaldehyde. 12. Sinapaldehyde. 13. Gallic acid. 14. Ellagic acid 15. Vanillic acid. 16. Syringic acids. 17. Scopoletin.
Preparation of reagents	1. HPLC-quality methanol (Solvent B). 2. Acetic acid diluted with Microfiltered water (with a resistivity of 18.2 M Ω) to 0.5% vol. (Solvent A). 3. Mobile phases: Solvent A (0.5% acetic acid) and solvent B (pure methanol). Filter through a membrane (porosity 0.45 µm). 4. Degas in an ultrasonic bath, if necessary. Reference solution - the standard substances are dissolved in a 50% vol. aqueous-alcoholic solution. 5. Furfural: 5 mg/L. 6. 5-Hydroxymethyl furfural: 10 mg/L. 7. 5-Methylfurfural 2 mg/L. 8. Vanillin: 5 mg/L. 9. Syringaldehyde: 10 mg/L. 10. Coniferaldéhyde: 5 mg/L. 11. Sinapaldehyde: 5 mg/L. 12. Gallic acid: 10 mg/L. 13. Ellagic acid: 10 mg/L. 14. Vanillic acid: 5 mg/L. 15. Syringic acid: 5 mg/L. 16. Scopoletin: 0.5 mg/L.
Sample Preparation	Preparation of the samples for injection - The reference solution and the spirit drink are filtered if necessary through a membrane with a maximum pore diameter of 0.45 µm.
Method of Analysis	Chromatographic operating conditions: 1. Carry out the analysis at ambient temperature. 2. Flow rate – 0.6 mL/min 3. Gradient (given as an example only) Time: 0 min 50 min 70 min 90 min solvent A (water-acid): 100% 60% 100% 100% solvent B (methanol): 0% 40% 0% 0% Note that in certain cases this gradient should be modified to avoid co-elutions. Determination 4. Inject the reference standards separately, then mixed. 5. Adapt the operating conditions so that the resolution factors of the peaks of all the compounds are equal to at least 1. 6. Inject the sample as prepared in FSSAI 13.010:2021, after filtering it through a membrane. 7. Measure the area of the peaks in the reference solution and the spirit drink and calculate the concentrations.

Calculation with units of expression	Calculate the concentration of each constituent by compare the peak areas of respective constituent in reference solution and spirit drinks. Express the concentration of each constituent in mg/L.
Reference	Compendium of International Methods of Spirituous Beverages Of Vitivinicultural Origin by International Organisation Of Vine And Wine, Edition 2019, Principal compounds extracted from wood during ageing Method No. OIV-MA-BS-16
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of α-dicarbonyl Compounds in Spirituous Beverages of Viti-vinicultural Origin by Gas Chromatography after derivation by 1,2 diaminobenzene**		
Method No.	FSSAI 13.044:2021	**Revision No. & Date**	0.0
Scope	The principal α-Dicarbonyl compounds found in wine spirits are: Glyoxal, Methylglyoxal, Diacetyl and Pentane-2,3-dione. Their molecular formulas are: (i) Glyoxal: OCH−CHO (ethanedial) (ii) Methylglyoxal: CH3−CO−CHO (2-oxopropanal) (iii) Diacetyl: CH3−CO−CO−CH3 (butane-2,3-dione) (iv) Pentane-2,3-dione: CH3−CH2−CO−CO−CH3 (v) Hexane-2,3-dione: CH3−CH2−CH2−CO−CO−CH3 The principal α-dicarbonyl compounds of wine (hexane-2,3-dione is not naturally present in wine but it is used as internal standard). Dicarbonyl compounds are important because of their sensory impact Applicability: This method applies to spirituous beverages of viti-vinicultural origin, for a content of carbonyl compounds included between 0.05 mg/L and 20 mg/L.		
Caution	1. 1,2-Diaminobenzene: Toxic if swallowed. Harmful in contact with skin or if inhaled. May cause an allergic skin reaction. 2. Sulphuric acid: Concentrated sulfuric acid is extremely corrosive and can cause serious burns when not handled properly. This chemical is unique because it not only causes chemical burns, but also secondary thermal burns as a result of dehydration. This dangerous chemical is capable of corroding skin, paper, metals, and even stone in some cases. If sulfuric acid makes direct contact with the eyes, it can cause permanent blindness. If ingested, this chemical may cause internal burns, irreversible organ damage, and possibly death. 3. Acetic acid: Acetic acid can be a hazardous chemical if not used in a safe and appropriate manner. This liquid is highly corrosive to the skin and eyes and, because of this, must be handled with extreme care. Acetic acid can also be damaging to the internal organs if ingested or in the case of vapor inhalation. 4. Sodium hydroxide: Sodium hydroxide is strongly irritating and corrosive. It can cause severe burns and permanent damage to any tissue that it comes in contact with. Sodium hydroxide can cause hydrolysis of proteins, and hence can cause burns in the eyes which may lead to permanent eye damage. 5. Dichloromethane: Higher levels of dichloromethane inhalation can lead to headache, mental confusion, nausea, vomiting, dizziness and fatigue. Skin Exposure - Redness and irritation may occur if skin comes in contact with liquid dichloromethane and, if it remains on the skin for an extended period of time, it may lead to skin burns.		
Principle	The method is based on the formation of quinoxaline derivatives from α-dicarbonyl compounds with 1,2-diaminobenzene  1,2 -diaminobenzene α-Dicarbonyl Quinoxaline		

	Formation of 1,2-Diaminobenzene Dicarbonyl Quinoxaline derivative Formation of derivatives. The reaction takes place in the spirituous beverage diluted four-fold, pH 8 and after a reaction time of 3 h at 60 °C. The analysis of the derivatives is then carried out after extraction of the derivatives by dichloromethane and analysis by gas chromatography with detection by mass spectrometry (GC-MS) or using a specific detector of nitrogenous compounds.
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Gas chromatography with detection by mass spectrometry (GC-MS) or using a special nitrogenised compound detector. 3. Moderately polar, polyethylene glycol capillary column (such as the Carbowax 20M, BP21) with the following dimensions (as an example):50 m X 0.32 mm X 0.25 μm. 4. Data acquisition system. 5. pH measuring apparatus. 6. Magnetic stirrer. 7. Oven which can be set to 60 °C. 8. 30 mL screw-cap flasks. 9. Micro syringes.
Materials and Reagents	1. Alcoholic beverages. 2. Glyoxal (in a 40% solution). 3. Methylglyoxal (in a 40% solution). 4. Diacetyl (> 99% pure). 5. Pentane-2,3-dione (> 97% pure). 6. Hexane-2,3-dione (> 90% pure). 7. 1,2-Diaminobenzene in the form of powder, > 97% pure. 8. Water for HPLC (according to standard EN ISO 3696). 9. Ethanol (HPLC grade). 10. Sodium hydroxide. 11. Acetic acid - pure crystallisable. 12. Dichloromethane. 13. Anhydrous sodium sulphate. 14. Sulphuric acid.
Preparation of reagents	1. 50% vol. hydroalcoholic solution - Mix 50 mL of pure ethanol for HPLC, with 50 mL of water. 2. Solution of internal standard hexane-2,3-dione at 2.0 g/L. Place 40 mg of hexane-2,3-dione in a 30 mL flask, dilute in 20 mL of 50% vol. hydroalcoholic solution, stir until complete dissolution. 3. Sodium Hydroxide (0.1M): Dissolve sodium hydroxide (4 g) in 100 mL water. 4. Sulphuric acid 2M (H_2SO_4 2M): Dilute concentrated sulphuric acid (11 mL) to 100 mL with water.
Samples Preparation	Dilute the spirituous beverage four-fold in water.
Method of Analysis	1. Place 10 mL of spirituous beverage (diluted four-fold) in a 30 mL flask. 2. Bring to pH 8 while stirring, with sodium hydroxide 0.1 M. 3. Add 5 mg of 1,2-diaminobenzene. 4. Add 10 μL of hexane-2,3-dione (internal standard@ at 2.0 g/L). 5. Close the flask using a screw-cap fitted with a Teflon-faced seal. Stir until

	the reagent has completely disappeared. 6. Place in the oven at 60 °C for 3 h. 7. Cool. Analysis Extraction of quinoxalines 8. Bring the reaction medium to pH 2 using sulphuric acid 2M. 9. Extract 2 times using 5 mL of dichloromethane by magnetic stirring for 5 min. 10. Elutriate the lower phase each time. 11. Mix all the solvent phases. 12. Dry on approximately 1 g of anhydrous sodium sulphate. 13. Elutriate. Chromatographic analysis (given as an example) 14. Detection: For GC-MS analysis, a Hewlett Packard HP 5890 gas-phase chromatograph was coupled with a chemstation and an HP 5970 mass spectrometer (electron impact 70eV, 2.7 kV), Note: A specific detector of the nitrogenous compounds can be used. 15. Column. The column is a BP21 (SGE, 50 m x 0.32 mm x 0.25 μm). 16. Temperatures. The temperatures of the injector and the detector are 250 °C and 280 °C respectively; the oven is kept at 60 °C for 1 min. then programmed to increase at the rate of 2 °C/min. to 220 °C, and the final isotherm lasting 20 min. 17. Injection. The volume injected is 2 μL and the closing time of the injector valves (splitless) is 30 s. 18. Gases as per the instructions from manufacturer. Analysis of quinoxalines formed. 19. Separation: The chromatogram of the derivatives by 1,2-diaminobenzene of a wine according to the ion selection method (SIM). 20. Identification of the peaks - GC-MS was used to identify the dicarbonyl compounds derived from the wine spirit based on the total ionic current method (scan) which is used to obtain the mass spectra of quinoxaline derivatives and to compare them with those memorised in the spectra library. 21. The retention times were compared with those for pure compounds treated in the same way. 22. The principal ions of the mass spectra for the obtained dicarbonyl compound derivatives are provided below. 23. Proportioning. The quantitative determination of the dicarbonyl compounds is carried out with the SIM method, by selecting ions M/Z = 76, 77, 103, 117, 130, 144, 158 and 171. 24. The ions M/Z = 76 and 77 are used for the quantification. 25. The others as qualifiers, i.e. (i) glyoxal: ions M/Z = 103 and 130, (ii) methylglyoxal: ions M/Z = 117 and 144, (iii) diacetyl: ions M/Z = 117 and 158, (iv) pentan-2,3-dione: ions M/Z = 171 and (v) hexane-2,3-dione: ions M/Z = 158 and 171. Characteristics of the method: 26. Some internal validation was defined but these are not a formal validation as per the protocol governing the planning, the implementing and the

	interpreting of the performance studies relating to the analysis methods. Repeatability. 27. The repeatability of the GC-MS-SIM method displays variation coefficients ranging between 2 and 5% for the four dicarbonyl compounds. Recovery rate. 28. The quantities added to a wine were recovered within a below 6% deviation from expected results. Linearity. 29. Linear correlations were obtained in concentration domains ranging between 0.05 to 20 mg/L. Detection limit. 30. The detection limit of most of the derived dicarbonylated products is 0.05 mg/L.
Calculation with units of expression	Mass spectra (ion m/z (intensity of the molecular ion in relation to that of the basic peak) of derivatives of dicarbonyl compounds by 2,3 diaminobenzene are provided below: **Dicarbonylated Derivative Mass spectrum** (principal compound ions and abundance) 1. Glyoxal Quinoxaline: 130(100), 103 (56.2), 76(46.8), 50(20.2)75 (10.4), 131 (9.4). 2. Methylglyoxal 2-Methyl quinoxaline: 144 (100), 117 (77.8), 76(40.5), 77 (23.3)50 (21.9), 75 (11.3), 145(10.3). 3. Diacetyl 2,3-Dimethyl quinoxaline: 117 (100), 158 (75.6), 76(32.3), 77(23.1)50 (18.3), 75 (10.4). 4. Pentane-2,3-dione 2-Ethyl-3-methylquinoxaline 171 (100), 172 (98), 130(34.1), 75 (33.3),77 (21), 50 (19.4), 144(19), 143 (14.1),103 (14). 5. Hexane-2,3-dione 2,3-Diethylquinoxaline 158 (100), 171 (20.1), 76(13.7), 77 (12.8), 159 (11.4),157 (10.8), 50 (8.1).
Reference	Compendium of International Methods of Spirituous Beverages Of Vitivinicultural Origin by International Organisation Of Vine And Wine, Edition 2019, Analysis of α-diacarbonyl compounds by gaschromatography after derivation by 1,2-Diaminobenzene, Method, Method No. OIV-MA-BS-17
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Propan-2-ol by Gas Chromatography**		
Method No.	FSSAI 13.045:2021	**Revision No. & Date**	0.0
Scope	This assay is not part of the official determinations provided by the international regulations, but is quite often requested since propan-2-ol is not a natural constituent of fermented beverages of vinous origin. It may be added to the alcohol during its denaturation. The presence (or more accurately lack thereof) of this compound must be verifiable. In addition it may be present in various alcoholic beverages.		
Caution	1. Propan-2-ol: Highly flammable liquid and vapor. May cause drowsiness or dizziness. Causes serious eye irritation. Symptoms/effects after inhalation-exposure to high concentrations- coughing. 2. Pentan-1-ol: This substance is a flammable liquid and vapour, is harmful if inhaled, causes skin irritation and may cause respiratory irritation		
Principle	The separation of propan-2-ol from ethanol is carried out by means of gas chromatography.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Gas chromatograph equipped with a flame ionization detector. 3. Classic stainless steel column 6 m long and with an internal diameter of 2 mm and Stationary phase - for example, coated with diglycerol at 5% on Chromosorb P 60-80 mesh (0.22 to 0.32 mm). Note: It is also possible to use a mixture of phases known as the ESD: Erythritol, sorbitol, diglycerol respectively at 1%, 2.5%, and 5% weight of the support (it can be used in other phases: porapak, poraplot, etc.) 4. Nitrogen R * carrier gas (Air Liquid standard). 5. Oven: Isothermal temperature 80 °C. 6. The settings of the various gas flows must be performed to obtain proper performance of the chromatograph.		
Materials and Reagents	1. Alcoholic beverages. 2. Propan-2-ol. 3. Pentan-1-ol		
Sample Preparation	1. For a qualitative test, the sample of the alcoholic beverage can be injected directly into the gas chromatograph (1 to 2 µL). 2. For accurate dosing is possible to use an internal standard separated from the other alcohols such as pentan-1-ol. 3. Pentan-1-ol content must be the same order of magnitude as that of the propan-2-ol.		
Method of Analysis	Assay: 1. Depending on whether the purpose is to detect the presence of the propan-2-ol or measure it, a reference solution of propan-2-ol must be injected into the pure alcohol, its content depending on the required dose (in principle several grams per litre). 2. For accurate dosing the internal calibration method will be applied using pentan-1-ol.		
Calculation with units of expression	The concentrations of propan-2-ol will be calculated using the traditional method in gas chromatography with the use of an internal standard (c.f. volatile substances) and expressed in g/hL of alcohol at 100% vol.		

Reference	Compendium of International Methods of Spirituous Beverages Of Vitivinicultural Origin by International Organisation Of Vine And Wine, Edition 2019, Propan-2-ol by GC, Method No. OIV-MA-BS-20
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Absorbance Test in UV light of Neutral Alcohol		
Method No.	FSSAI 13.046:2021	**Revision No. & Date**	0.0
Scope	This method can be used to determine the optical permeability of neutral alcohol liable to enter into the composition of certain alcoholic beverages.		
Caution	Hexane: Chronic exposure can cause more severe damage to the nervous system. If swallowed, it may cause severe abdominal pain and impact the respiratory system, resulting in shortness of breath, coughing, burning of the mouth, throat or chest, and even chemical pneumonitis.		
Principle	The optical permeability of the sample in the wavelength range from 220 to 270 nm is measured against a defined reference substance with high optical permeability.		
Apparatus /Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. UV-visible spectrophotometer. 3. Quartz cells 10 mm thick, with identical spectral transmission.		
Materials and Reagents	1. Alcoholic beverages. 2. Hexane for spectroscopy.		
Method of analysis	1. Rinse the tanks clean beforehand with a sample solution and then fill with the sample, dry the tanks outside. 2. Treat the reference cell (n) with hexane in the same way and fill it. 3. Determine the absorbance value and build the graph.		
Calculation with units of expression	The absorbance values recorded at 270, 240, 230 and 220 nm should not exceed the following values: 0.02, 0.08, 0.18 and 0.3. The absorbance curve must be smooth and regular.		
Reference	Compendium of International Methods of Spirituous Beverages of Vitivinicultural Origin by International Organisation Of Vine And Wine, Edition 2019, Ultraviolet light test for neutral alcohol, Method No. OIV-MA-BS-21		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Ethyl Carbamate**		
Method No.	FSSAI 13.047:2021	**Revision No. & Date**	0.0
Scope	Ethyl carbamate (EC), also known as urethane, is a compound found in fermented foods and beverages. It's also a known carcinogen. The formation and distribution of ethyl carbamate (urethane) occurs during pot still distillation. When copper was present, during and subsequent to distillation, formation of ethyl carbamate was time-dependent. The degree of formation was maximised between pH 4 and 6.		
Caution	1. Ethyl carbamate: May be harmful if swallowed. May cause cancer. May cause harm to breast-fed children. May cause damage to the nervous system. 2. Dichloromethane: Higher levels of dichloromethane inhalation can lead to headache, mental confusion, nausea, vomiting, dizziness and fatigue. Skin Exposure - Redness and irritation may occur if skin comes in contact with liquid dichloromethane and, if it remains on the skin for an extended period of time, it may lead to skin burns.		
Principle	The assay is performed by direct injection of the drink into a gas chromatograph coupled to a mass spectrometer operating under the principle of electron impact, in "Selected Ion Monitoring (SIM)" acquisition mode.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Gas chromatography with detection by mass spectrometry (GC-MS). 3. Capillary column of the Carbowax 20 M (50 m x 0.22 mm) type, film thickness 0.2 µm. 4. Data acquisition system.		
Materials and Reagents	1. Alcoholic beverages. 2. Propyl carbamate (Reference and internal standard). 3. Ethyl carbamate (Reference). 4. Ethanol. 5. Distilled water. 6. Ether. 7. Sodium sulphate. 8. Porus polymer (of Extrelut type). 9. Dichloro methane		
Preparation of reagents	Internal standard Dissolve propyl carbamate (100 mg/L) in a 50% vol. hydroalcoholic solution. (Check that the alcohol used is free of ethyl carbamate).		
Sample Preparation	Addition of the internal standard 1. At 5 mL of the alcoholic beverage, add 50 µL of the solution of propyl carbamate (at 100 mg/L) which results in 1 mg/L in the sample. Note: this final quantity of the internal standard in the sample can be modulated according to the ethyl carbamate content in the medium to be analyzed. 2. In the case of sweet alcoholic beverages (over 10 g/L), after adding the internal standard it is preferable to extract the ethyl carbamate as per the following methods 3. Method 1: Extract the ethyl carbamate with ether after saturating the medium with excess sodium sulphate to fix the water (or) 4. Method 2: Fixing the carbamates (ethyl carbamate and them internal standard) on a porous polymer (of Extrelut type) followed by elution with		

	dichloromethane.
Method of analysis	1. Capillary column of the Carbowax 20 M (50 mx 0.22 mm) type, film thickness 0.2 μm. 2. Temperature programming from 60 to 200 °C, 3 °C per minute. 3. Data acquisition method of the mass spectrometer: Selected Ion Monitoring (SIM), MZ = 62, 74, 84. 4. The chromatograms are re-processed with the single ion M/Z = 62. The other ions are used to confirm peak purity by taking into account the ratio of their respective intensities. *Note:* Certain NP or Hall sensors can be used. PREPARATION OF THE REFERENCE SOLUTION 5. According to the alcoholic beverage to be analyzed, prepare a solution of ethyl carbamate at 50 μg/L or 400 μg/L or more if necessary. 6. 5 mL of the reference solution are added by 50 μL of the internal standard solution (propyl carbamate at 100 mg/L). 7. The solution is injected using the Splitless mode (valve closure for 20 to 30 seconds) by 2 μL of the prepared solution into the chromatograph after being properly adjusted.
Calculation with units of expression	The ethyl carbamate is expressed in μg/L of the spirit.
Reference	Compendium of International Methods of Spirituous Beverages of Vitivinicultural Origin by International Organisation of Vine And Wine, Edition 2019, Ethyl carbamate, Method No. OIV-MA-BS-25
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Colour Intensity**		
Method No.	FSSAI 13.048:2021	**Revision No. & Date**	0.0
Scope	Alcoholic beverage of a natural "golden yellow" colour.		
Principle	Colour intensity is determined by measuring the absorbance at 445 nm for an optical length of 1 cm thick (for traditional alcoholic beverages).		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. A spectrophotometer enabling measurements at different wavelengths. 3. Glass tanks with an optical path length of 1 cm and 0.2 cm.		
Materials and Reagents	1. Alcoholic beverages		
Method of analysis	1. Measure the absorbance at the wavelength 445 nm of the alcoholic beverage placed in a glass tank with an optical path length of 1 cm by setting the zero of the absorbance scale compared with distilled water. Remarks. 2. It is possible to measure the absorbance at any wavelength for alcoholic beverages naturally aged in wood and/or supplemented by caramel and/or supplemented by "woody" brandies because in all cases the absorption curves are continuous, without any maximum, or even a significant change in slope. 3. Taking into account the maximum perceived by human vision it would be preferable to perform the measurement at 530 nm. 4. The hue or hue gamut between two alcoholic beverages can be expressed, in certain cases, by measuring absorbance at 620 nm. 5. Theoretically the sample should not be filtered if it is a product intended for direct consumption, but care should be taken to ensure that the sample is free of particles that are not a priori contained in the alcoholic beverage, especially those resulting from corking. Alcoholic beverage containing synthetic dyes. 6. First, the absorption maximum should be measured, and then the wavelength corresponding to the selected maximum, if necessary using a tank with an optical path length of 0.2 cm.		
Calculation with units of expression	Express the colour intensity by the absorbance measured under the conditions specified above, indicating the size of the colorimeter tank, and the chosen wavelength.		
Reference	Compendium of International Methods of Spirituous Beverages of Vitivinicultural Origin by International Organisation of Vine And Wine, Edition 2019, Colour intensity, Method No. OIV-MA-BS-26		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Calcium by Atomic Absorption Spectrophotometric (AAS) Method**		
Method No.	FSSAI 13.049:2021	**Revision No. & Date**	0.0
Scope	Calcium present in the alcoholic beverages is determined.		
Caution	1. Hydrochloric acid: Hydrochloric acid is a hazardous liquid which must be used with care. The acid itself is corrosive, and concentrated forms release acidic mists that are also dangerous. If the acid or mist come into contact with the skin, eyes, or internal organs, the damage can be irreversible or even fatal in severe cases.		
Principle	Calcium is determined by atomic absorption spectrophotometry with a reductive air acetylene flame using a calcium hollow-cathode lamp, wavelength of 422.7 nm, on the dealcoholised alcoholic beverage, concentrated 2 times. The measurement is performed in the presence of lanthanum chloride referred to as the "matrix modifier".		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Volumetric flasks -25, 50, 100, 1000 mL. 3. Volumetric pipettes -1, 2, 3, 4, 10, 50 mL. 4. Test tube- 100 mL. 5. Beaker -250 mL. 6. Tablet bottle - 20 mL. 7. Atomic absorption Spectrophotometer (sample setting for Varian 575 model). 8. Reducing air-acetylene flame, flow rates: air: 7.5 $V_{min.}$ 9. C2 H2: 4.0 V_{min}. 10. Calcium hollow-cathode lamp with calcium; Wavelength: 422.7 nm, slit (slit): 0.2 nm, lamp intensity: 5 mA.		
Materials and Reagents	1. Ultrapure demineralised water resistivity 18.2 M Ω. 2. Stock solution 1 g/l of Calcium: (e.g. Titrisol Merck). 3. Hydrochloric acid d = 1.18 (35% minimum). 4. Lanthanum chloride ($LaCl_3.6H_2O$)		
Preparation of reagents	1. Solution of 100 mg/L of calcium: Place 10 mL of stock solution in a 100 mL flask, fill to volume with demineralised water. 2. Lanthanum Chloride Solution, 25 g/L: Weigh 63.6 g of lanthanum chloride ($LaCl_3.6H_2O$) in a 1000 mL flask, add approximately 500 mL of demineralised water, then to the test tube 50 mL of hydrochloric acid. After solubilisation, allow to cool and fill to volume with demineralised water. 3. Calibration range: 2, 4, 6, 8 mg/L of calcium: Place successively 1.0, 2.0, 3.0, 4.0 mL of the solution at 100 mg/L Calcium in four 50 mL vials, add 10 mL of the solution of lanthanum chloride, and fill to volume with demineralised water. Perform a blank test without calcium in the same conditions.		
Sample Preparation	1. The calcium content in alcoholic beverages is often very low, it is therefore necessary to concentrate the sample by evaporating the alcohol. Pipette 50 mL of the alcoholic beverage into a 250 mL beaker. Evaporate the alcohol in a water bath to about one volume of 10 mL. 2. Leave to cool. 3. Pour the concentrate into a vial of 25 mL, rinse the beaker and fill to volume		

	with demineralised water. 4. Place 4 mL of this solution to be determined prepared in a clean, dry tablet bottle with 1 mL of lanthanum chloride solution. 5. Cork it and stir.
Method of analysis	1. Successively present the calibration solutions, the blank solution, and the samples. 2. Note the corresponding absorbance.
Calculation with units of expression	1. Establish the calibration curve absorbance = f (concentration in mg/L calcium) by the least squares method. 2. Deduce the concentration of calcium in mg/L taking into account the concentration factor.
Reference	Compendium of International Methods of Spirituous Beverages Of Vitivinicultural Origin by International Organisation of Vine And Wine, Edition 2019, Determination of calcium by atomic absorption, Method No. OIV-MA-BS-29
Approved by	Scientific Panel on Methods of Sampling and Analysis

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India	**Determination of Lead by Atomic Absorption Spectrophotometric (AAS) Method**		
Method No.	FSSAI 13.050:2021	**Revision No. & Date**	0.0
Scope	Lead present in the alcoholic beverages is determined.		
Caution	1. Phosphoric acid: Phosphoric acid can be very hazardous in the case of skin contact, eye contact, and ingestion. It can also cause irritation if vapors are inhaled. Repeated or prolonged exposure to phosphoric acid mist can lead to chronic eye irritation, severe skin irritation, or prolonged respiratory tract issues. 2. Nitric acid: May be fatal if inhaled. Causes severe eye and skin burns. Causes severe respiratory and digestive tract burns. Strong oxidizer. Contact with other material may cause a fire. Acute pulmonary edema or chronic obstructive lung disease may occur from inhalation of the vapors of nitric acid. Corrosive to metal. Target Organs: Lungs, eyes, skin, mucous membranes.		
Principle	Lead is determined directly in the alcoholic beverage, using a lead hollow-cathode lamp by flameless atomic absorption spectrometry, using a matrix modifier.		
Apparatus / Instruments	1. General Glassware and apparatus (Refer 2.0 at page no. 2). 2. Atomic absorption spectrophotometer equipped with a graphite oven, a nonselective absorption corrector and a multi-potentiometric recorder. 3. Lead hollow-cathode lamp. 4. Micropipettes with special tips for atomic absorption measurements.		
Materials and Reagents	All the reagents must be of analytical purity and, in particular must be lead-free. 1. The water used must be double-distilled in a borosilicate glass apparatus or with water of equivalent purity. 2. Phosphoric acid ($\rho 20 = 1.71$ g/mL). 3. Nitric acid ($\rho 20 = 1.38$ g/mL) 4. Lead solution to 1 g/L.		
Preparation of reagents	1. Phosphoric acid solution: Dilute phosphoric acid (6 mL) to 100 mL with water. 2. Lead solution to 1 g/L (Use a standard commercial solution): This solution can be obtained by dissolving 1.600 g of lead nitrate II, Pb $(NO_3)_2$ in nitric acid diluted to 1% (v/v) and adjusting the volume to 1 L. Keep the solution in a borosilicate glass bottle with a ground glass stopper. 3. Nitric acid solution diluted to 1% (v/v) (solution of phosphoric and nitric acids): The solution is obtained by diluting the phosphoric acid solution at 6% with the nitric acid solution at 1%.		
Sample Preparation	1. Add to the test sample of the alcoholic beverage an equal volume of the solution of phosphoric and nitric acids. Determine its absorbance If it is greater than 0.6, dilute the alcoholic beverage (a dilution of 1/5 is sufficient in most cases). 2. Prepare the test solution by adding to the test sample of the diluted alcoholic beverage an equal volume of the solution of phosphoric and nitric acids.		
Method of analysis	1. Preparation of the solutions in the calibration range: Using the control solution of lead, prepare dilutions in which 50% of the final volume is the		

<table>
<tr><td></td><td>solution of phosphoric and nitric acids The concentration scale of the range depends on the sensitivity of the apparatus. For example, prepare solutions containing 10 - 20 - 30 micrograms of lead per litre.
2. Determination
2.1 Oven program.
<table><tr><th>Step</th><th>Temperature (°C)</th><th>Time (s)</th><th>Nitrogen (L/min)</th><th>Reading</th></tr><tr><td>1</td><td>75</td><td>2</td><td>3</td><td></td></tr><tr><td>2</td><td>95</td><td>20</td><td>3</td><td></td></tr><tr><td>3</td><td>140</td><td>15</td><td>3</td><td></td></tr><tr><td>4</td><td>300</td><td>8</td><td>3</td><td></td></tr><tr><td>5</td><td>450</td><td>7</td><td>3</td><td></td></tr><tr><td>6</td><td>480</td><td>10</td><td>3</td><td></td></tr><tr><td>7</td><td>900</td><td>20</td><td>3</td><td></td></tr><tr><td>8</td><td>900</td><td>1</td><td>0</td><td></td></tr><tr><td>9</td><td>2250</td><td>0.7</td><td>0</td><td>L</td></tr><tr><td>10</td><td>2250</td><td>1</td><td>0</td><td>L</td></tr><tr><td>11</td><td>2250</td><td>2</td><td>3</td><td></td></tr></table>
3. Measurements: Select wavelength 283.3 nm. Set to zero the absorbance scale with double distilled water. Using a micropipette or an automatic sampler, inject into the programmed oven 3 times 5 lt. of each solution in the calibration range and of the solution of the sample to be analysed.
4. Record the measured absorbances. Calculate the mean absorbance value based on the results for the three injections. The absorbances are measured in height of peaks.</td></tr>
<tr><td>Calculation with units of expression</td><td>Plot the changes in absorbance versus the concentrations of lead in solutions of the calibration range. The relationship is linear. Record the mean value of the absorbance of the sample solution on the calibration curve and determine then concentration C of lead.
The lead concentration in micrograms per litre of alcoholic beverage is equal to:
$C \times F$
F = dilution factor.</td></tr>
<tr><td>Reference</td><td>Compendium of International Methods of Spirituous Beverages of Vitivinicultural Origin by International Organisation of Vine And Wine, Edition 2019, Determination of lead by atomic absorption, Method No. OIV-MA-BS-32</td></tr>
<tr><td>Approved by</td><td>Scientific Panel on Methods of Sampling and Analysis</td></tr>
</table>

	Determination of Ochratoxin A in Wine and other Fermented Alcoholic Beverages		
Method No.	FSSAI 13.051:2024	**Revision No. & Date**	0.0
Scope	Applicable to the determination of ochratoxin A in Wine and Other Fermented alcoholic Beverages at >0.06 ng/L.		
Caution	OTA is toxic as well as carcinogenic in nature, use nitrile gloves while handling these substances. Prior to sample extract disposal, the solutions must be treated with 5–6% sodium hypochlorite. All glass ware exposed to the residues of these toxins must be rinsed with methanol and 1% sodium hypochlorite solution and then washed.		
Principle	Wine and beer are diluted with a solution containing polyethylene glycol and NaHCO3, and the diluted solutions are filtered and cleaned up on an immunoaffinity column. OTA is eluted with methanol and quantified by reversed-phase liquid chromatography (HPLC) with fluorometric detection.		
Apparatus/Instruments	1. Microbalance (Measuring to within ± 0.01 mg). 2. Glass vials - 4 mL. (Note: Certain types of vials might lead to losses of OTA during evaporation. To avoid this, silanization can be used. Prepare vials by filling them with silanizing reagent and leave this reagent in vials for 1 min. Rinse vials twice with a solvent [toluene, acetone, or hexane] followed by water [twice], and dry vials). 3. Volumetric flasks - 5 mL, with accuracy of at least ± 0.5%. 4. Vacuum manifold - To accommodate immunoaffinity columns. 5. Reservoirs and attachments -To fit immunoaffinity columns. 6. Glass microfiber filters - Whatman GF/A, or equivalent. 7. Immunoaffinity columns - Containing antibodies against OTA with a total binding capacity of ≥100 ng OTA and a recovery of ≥85% when a diluted wine solution containing 100 ng OTA is applied. 8. Solvent evaporator. 9. Syringe and microliter pipet(s).—250 µL. 10. HPLC system equipped with pump (Isocratic; delivering constant flow rate of 1.0 mL/min.), Injection system (Syringe-loading injection valve with 100 µL injection loop, or equivalent. 11. HPLC analytical column - Stainless steel (150×4.6 mm id) packed with 5 µm C18 reversed-phase material. 12. Reversed-phase guard column (i.e., 20×4.6 mm id, 0.5µm particle size) or guard filter (i.e., 0.5µm, Rheodyne); Columns of different dimensions may be used, if they adequately resolve the OTA peak from all other peaks. 13. Fluorescence detector - Fitted with a flow cell and set at 333 nm (excitation) and 460 nm (emission) indicating a peak from ≥0.02 ng of OTA. 14. Data collection system. 15. UV spectrophotometer.		
Materials and Reagents	1. Polyethylene glycol (PEG) - PEG 8000. 2. Methanol - HPLC grade. 3. Acetonitrile - HPLC grade.		

	4. Water - HPLC grade. 5. Glacial acetic acid - 99% purity. 6. Toluene - Analytical grade. 7. Ochratoxin A (OTA).—Crystalline form, film, or solution (stored in the dark at 4°C).
Preparation of Reagents	1. Diluting solution (1% PEG + 5% $NaHCO_3$, pH 8.3) - Dissolve PEG (10 g) and $NaHCO_3$ (50 g) in water (950 mL) and dilute to 1 L with water. 2. Washing solution (2.5% NaCl + 0.5% $NaHCO_3$, pH 8.1) - Dissolve NaCl (25 g), and $NaHCO_3$ (5 g) in 950 mL water and dilute to 1 L with water. 3. HPLC mobile phase [Water–acetonitrile–glacial acetic acid (99 + 99 + 2, v/v/v; pH 3.2)] - Mix 990 mL water, 990 mL and 20 mL acetic acid, filter through 0.45μm filter and degas (e.g., with He). 4. Solvent mixture [Toluene–acetic acid (99 + 1, v/v)] - Mix 99 parts, by volume of toluene with 1 part by volume of acetic acid. 5. OTA stock solution - Dissolve OTA (1 mg) or the contents of 1 ampule (if OTA has been obtained as a film) in the solvent mixture, (4), to prepare a solution containing OTA at approximately 20–30 μg/mL. To determine the exact concentration, record the absorption curve between 300 and 370 nm in a 1 cm quartz cell with the solvent mixture (4) as the reference. Identify the maximum absorption, and calculate the mass concentration of OTA, C_{OTA}, in μg/mL, using the following equation: $C_{OTA} = A_{max} \times M \times 100/\varepsilon \times \delta$ Where A_{max} is the absorption determined at the maximum of the absorption curve (at 333 nm); M is the relative molecular mass of OTA (M = 403.8 g/mol); ε is the relative molar absorption coefficient of OTA in the solvent mixture (4), (ε = 544 m^2/mol); and δ is the path length of the quartz cell in cm. This solution is stable at –18°C for ≥4 years. 6. OTA standard solution [2 μg/mL in toluene–acetic acid (99 + 1, v/v)]. Dilute stock solution (5), with solvent mixture (4) to obtain a standard solution with a mass concentration of OTA of 2 μg/mL. Store standard solution at +4°C. **7.** Calibration solutions - Pipet 0.5 mL standard solution containing OTA at 2 μg/mL, into a glass vial, and evaporate the solvent under a stream of N. Re-dissolve contents of vial in 10 mL HPLC mobile phase, which has been filtered through a 0.45μm filter. This gives a solution containing OTA at 100 ng/mL. Take 6 different volumes (30, 100, 300, 1000, 2000, 3000 μL) of this solution in separate 5mL volumetric flasks and Dilute each standard solution to volume (5 mL) with filtered HPLC mobile phase (3) to obtain solutions with following concentrations (0.6, 2.0, 6.0, 20, 40 and 60 ng/mL). Inject 100 μL of each calibration solution (containing 0.06, 0.20, 0.60, 2.00, 4.00 and 6.00 ng respectively) into the HPLC system.
Sample Preparation	1. Cool beer at +4°C for 30 min to prevent fast foam formation. 2. Degas by sonicating for 1 h. 3. Pour 10 mL of alcoholic beverage into a 100 mL conical flask. 4. Add 10 mL diluting solution. Mix vigorously. Filter through glass microfiber filter, if solution is cloudy solutions or if solid residue is formed after dilution.

	5. Connect the immunoaffinity column to the vacuum manifold and attach the reservoir to the immunoaffinity column. Add 10 mL (equivalent to 5 mL alcoholic beverage) diluted solution to the reservoir, and pass solution through the immunoaffinity column at a flow rate of about 1 drop/s. Do not permit the immunoaffinity column to run dry. 6. Wash the immunoaffinity column with 5 mL washing solution and then with 5 mL water at a flow rate of 1–2 drops/s. Dry the column by passing air through it. 7. Elute OTA into the vial by passing 2 mL methanol at a flow rate of 1 drop/s. 8. Evaporate the eluate to dryness at 50°C under N. Re-dissolve eluate immediately in 250 µL HPLC mobile phase and store at +4°C until HPLC analysis.
Method of analysis	1. Set flow rate of the mobile phase at 1.0 mL/min. 2. Inject 100 µL reconstituted extract (equivalent to 2 mL alcoholic beverage) into the HPLC system. 3. Quantify OTA by comparing OTA peak area with the relevant calibration curve. If the content of OTA in the test solutions fall outside the calibration range, dilute test extracts. 4. Prepare a calibration curve at the beginning of every day of analysis and whenever chromatographic conditions change.
Calculation with units of expression	Determine from the calibration curve the amount of OTA (in ng) in the aliquot of test solution injected into the HPLC system. Calculate the concentration of OTA (C_{OTA}; in ng/mL) from the following equation: $C_{OTA} = M_A \times (2/V_1) \times (V_3/V_2)$ Where M_A is the mass of OTA (in ng) in the aliquot injected on column, determined from the calibration graph; *2* is the dilution factor; V_1 is the volume of solution taken for analysis (10 mL); V_2 is the volume of test solution injected on column (100 µL); V_3 is the volume of solution used to dissolve the dried eluate (250 mL).
Reference	Angelo Visconti, Michelangelo Pascale, And Gianluca Centonze; Determination of Ochratoxin A in Wine and Beer by Immunoaffinity Column Cleanup and Liquid Chromatographic Analysis with Fluorometric Detection: Collaborative Study; Journal of AOAC International. 84, (6), 2001; 1818-1827.
Approved by	Scientific Panel on Methods of Sampling and Analysis

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

Sp.gr @20°C	% by Vol
0.99	7.15
0.9899	7.23
0.9898	7.31
0.9897	7.39
0.9896	7.47
0.9895	7.55
0.9894	7.63
0.9893	7.71
0.9892	7.79
0.9891	7.87
0.989	7.95
0.9889	8.03
0.9888	8.11
0.9887	8.19
0.9886	8.27
0.9885	8.35
0.9884	8.44
0.9883	8.52
0.9882	8.6
0.9881	8.68
0.988	8.76
0.9879	8.84

Sp.gr @20°C	% by Vol
0.9878	8.93
0.9877	9.01
0.9876	9.09
0.9875	9.17
0.9874	9.26
0.9873	9.34
0.9872	9.42
0.9871	9.51
0.987	9.59
0.9869	9.67
0.9868	9.75
0.9867	9.84
0.9866	9.92
0.9865	10
0.9864	10.09
0.9863	10.17
0.9862	10.25
0.9861	10.34
0.986	10.42
0.9859	10.5
0.9858	10.59
0.9857	10.67

Sp.gr @20°C	% by Vol
0.9856	10.75
0.9855	10.84
0.9854	10.92
0.9853	11
0.9852	11.09
0.9851	11.17
0.985	11.26
0.9849	11.34
0.9848	11.43
0.9847	11.51
0.9848	11.59
0.9845	11.68
0.9844	11.76
0.9843	11.85
0.9842	11.93
0.9841	12.02
0.984	12.1
0.9839	12.19
0.9838	12.28
0.9837	12.36
0.9836	12.45
0.9835	12.53

Sp.gr @20°C	% by Vol
0.9834	12.62
0.9833	12.71
0.9832	12.8
0.9831	12.88
0.983	12.97
0.9829	1306
0.9828	13.14
0.9827	13.23
0.9826	13.32
0.9825	13.41
0.9824	13.49
0.9823	13.58
0.9822	13.67
0.9821	13.76
0.982	13.85
0.9819	13.94
0.9818	14.02
0.9817	14.11
0.9816	14.2
0.9815	14.29
0.9814	14.38
0.9813	14.47
0.9812	14.56
0.9811	14.65
0.981	14.74

Sp.gr @20°C	% by Vol
0.9809	14.83
0.9808	14.92
0.9807	15.01
0.9806	15.1
0.9805	15.19
0.9804	15.28
0.9803	15.37
0.9802	15.46
0.9801	15.54
0.98	15.64
0.9799	15.73
0.9798	15.82
0.9797	15.91
0.9796	16
0.9795	16.09
0.9794	16.18
0.9793	16.27
0.9792	16.36
0.9791	16.45
0.979	16.54
0.9789	16.64
0.9788	16.73
0.9787	16.82
0.9786	16.91
0.9785	17

Sp.gr @20°C	% by Vol
0.9784	17.1
0.9783	17.19
0.9782	17.28
0.9781	17.38
0.978	17.47
0.9779	17.56
0.9778	17.66
0.9777	17.75
0.9776	17.84
0.9775	17.94
0.9774	18.03
0.9773	18.12
0.9772	18.22
0.9771	18.31
0.977	18.41
0.9769	18.5
0.9768	18.6
0.9767	18.69
0.9766	18.79
0.9765	18.88
0.9764	18.98
0.9763	19.07
0.9762	19.17
0.9761	19.26
0.976	19.36

Sp.gr @20°C	% by Vol
0.9759	19.45
0.9758	19.55
0.9757	19.64
0.9756	19.74
0.9755	19.83
0.9754	19.93
0.9753	20.02
0.9752	20.12
0.9751	20.21
0.975	20.3
0.9749	20.4
0.9748	20.49
0.9747	20.59
0.9746	20.68
0.9745	20.77
0.9744	20.87
0.9743	20.96
0.9742	21.05
0.9741	21.15
0.974	21.24
0.9739	21.33
0.9738	21.42
0.9737	21.52
0.9736	21.61
0.9735	21.7

Sp.gr @20°C	% by Vol
0.9734	21.79
0.9733	21.89
0.9732	21.98
0.9731	22.07
0.973	22.16
0.9729	22.25
0.9728	22.34
0.9727	22.43
0.9726	22.52
0.9725	22.62
0.9724	22.71
0.9723	22.8
0.9722	22.89
0.9721	22.98
0.972	23.07
0.9719	23.16
0.9718	23.25
0.9717	23.34
0.9716	23.43
0.9715	23.52
0.9714	23.61
0.9713	23.7
0.9712	23.79
0.9711	23.88
0.971	23.97

Sp.gr @20°C	% by Vol
0.9709	24.06
0.9708	24.15
0.9707	24.24
0.9706	24.33
0.9705	24.42
0.9704	24.51
0.9703	24.59
0.9702	24.68
0.9701	24.77
0.97	24.86
0.9699	24.95
0.9698	25.04
0.9697	25.12
0.9696	25.21
0.9695	25.3
0.9694	25.39
0.9693	25.48
0.9692	25.56
0.9691	25.65
0.969	25.74
0.9689	25.83
0.9688	25.91
0.9687	26
0.9686	26.09
0.9685	26.17

Sp.gr @20°C	% by Vol
0.9684	26.26
0.9683	26.35
0.9682	26.43
0.9681	26.52
0.968	26.61
0.9679	26.69
0.9678	26.78
0.9677	26.86
0.9676	26.95
0.9675	27.04
0.9674	27.12
0.9673	27.21
0.9672	27.29
0.9671	27.38
0.967	27.46
0.9669	27.55
0.9668	27.63
0.9667	27.72
0.9666	27.8
0.9665	27.89
0.9664	27.97
0.9663	28.05
0.9662	28.14
0.9661	28.22
0.966	28.31

Sp.gr @20°C	% by Vol
0.9659	28.39
0.9658	28.47
0.9657	28.56
0.9656	28.64
0.9655	28.73
0.9654	28.81
0.9653	28.89
0.9652	28.98
0.9651	29.06
0.965	29.14
0.9649	29.22
0.9648	29.31
0.9647	29.39
0.9646	29.47
0.9645	29.55
0.9644	29.64
0.9643	29.72
0.9642	29.8
0.9641	29.88
0.964	29.96
0.9639	30.04
0.9638	30.12
0.9637	30.20
0.9636	30.29
0.9635	30.37

Sp.gr @20°C	% by Vol
0.9634	30.45
0.9633	30.53
0.9632	30.61
0.9631	30.69
0.963	30.77
0.9629	30.85
0.9628	30.92
0.9627	31
0.9626	31.08
0.9625	31.16
0.9624	31.24
0.9623	31.32
0.9622	31.4
0.9621	31.47
0.962	31.55
0.9619	31.63
0.9618	31.71
0.9617	31.78
0.9616	31.86
0.9615	31.94
0.9614	32.01
0.9613	32.09
0.9612	32.17
0.9611	32.24
0.961	32.32

Sp.gr @20°C	% by Vol
0.9609	32.39
0.9608	32.47
0.9607	32.54
0.9606	32.62
0.9605	32.69
0.9604	32.77
0.9603	32.84
0.9602	32.92
0.9601	32.99
0.96	33.07
0.9599	33.14
0.9598	33.22
0.9597	33.29
0.9596	33.36
0.9595	33.44
0.9594	33.51
0.9593	33.59
0.9592	33.66
0.9591	33.73
0.959	33.8
0.9589	33.88
0.9588	33.95
0.9587	34.02
0.9586	34.09
0.9585	34.16

Sp.gr @20°C	% by Vol
0.9584	34.24
0.9583	34.31
0.9582	34.38
0.9581	34.45
0.958	34.52
0.9579	34.59
0.9578	34.66
0.9577	34.73
0.9576	34.8
0.9575	34.88
0.9574	34.95
0.9573	35.02
0.9572	35.09
0.9571	35.16
0.957	35.23
0.9569	35.3
0.9568	35.37
0.9567	35.43
0.9566	35.5
0.9565	35.57
0.9564	35.64
0.9563	35.71
0.9562	35.78
0.9561	35.85
0.956	35.92

Sp.gr @20°C	% by Vol
0.9559	35.99
0.9558	36.05
0.9557	36.12
0.9556	36.19
0.9555	36.26
0.9554	36.33
0.9553	36.39
0.9552	36.46
0.9551	36.53
0.955	36.6
0.9549	36.66
0.9548	36.73
0.9547	36.8
0.9546	36.87
0.9545	36.93
0.9544	37
0.9543	37.07
0.9542	37.13
0.9541	37.2
0.954	37.27
0.9539	37.33
0.9538	37.4
0.9537	37.46
0.9536	37.53
0.9535	37.6

Sp.gr @20°C	% by Vol
0.9534	37.66
0.9533	37.73
0.9532	37.79
0.9531	37.86
0.953	37.92
0.9529	37.99
0.9528	38.05
0.9527	38.12
0.9526	38.18
0.9525	38.25
0.9524	38.31
0.9523	38.38
0.9522	38.44
0.9521	38.51
0.952	38.57
0.9519	38.63
0.9518	38.7
0.9517	38.76
0.9516	38.83
0.9515	38.89
0.9514	38.95
0.9513	39.02
0.9512	39.08
0.9511	39.14
0.951	39.21

Sp.gr @20°C	% by Vol
0.9509	39.27
0.9508	39.33
0.9507	39.4
0.9506	39.46
0.9505	39.52
0.9504	39.58
0.9503	39.65
0.9502	39.71
0.9501	39.77
0.95	39.83
0.9499	39.9
0.9498	39.96
0.9497	40.02
0.9496	40.08
0.9495	40.15
0.9494	40.21
0.9493	40.27
0.9492	40.33
0.9491	40.39
0.949	40.46
0.9489	40.52
0.9488	40.58
0.9487	40.64
0.9486	40.70
0.9485	40.76

Sp.gr @20°C	% by Vol
0.9484	40.82
0.9483	40.88
0.9482	40.95
0.9481	41.01
0.948	41.07
0.9479	41.13
0.9478	41.19
0.9477	41.25
0.9476	41.31
0.9475	41.37
0.9474	41.43
0.9473	41.49
0.9472	41.55
0.9471	41.61
0.947	41.67
0.9469	41.73
0.9468	41.79
0.9467	41.85
0.9466	41.91
0.9465	41.97
0.9464	42.03
0.9463	42.09
0.9462	42.15
0.9461	42.21
0.946	42.27

Sp.gr @20°C	% by Vol
0.9459	42.32
0.9458	42.38
0.9457	42.44
0.9456	42.5
0.9455	42.56
0.9454	42.62
0.9453	42.68
0.9452	42.74
0.9451	42.8
0.945	42.85
0.9449	42.91
0.9448	42.97
0.9447	43.03
0.9446	43.09
0.9445	43.15
0.9444	43.2
0.9443	43.26
0.9442	43.32
0.9441	43.38
0.944	43.43
0.9439	43.49
0.9438	43.55
0.9437	43.61
0.9436	43.66
0.9435	43.72

Sp.gr @20°C	% by Vol
0.9434	43.78
0.9433	43.84
0.9432	43.89
0.9431	43.95
0.943	44.01
0.9429	44.06
0.9428	44.12
0.9427	44.18
0.9426	44.23
0.9425	44.29
0.9424	44.35
0.9423	44.4
0.9422	44.46
0.9421	44.52
0.942	44.57
0.9419	44.63
0.9418	44.69
0.9417	44.74
0.9416	44.8
0.9415	44.86
0.9414	44.91
0.9413	44.97
0.9412	45.02
0.9411	45.08
0.941	45.13

Sp.gr @20°C	% by Vol
0.9409	45.19
0.9408	45.24
0.9407	45.3
0.9406	45.36
0.9405	45.41
0.9404	45.47
0.9403	45.52
0.9402	45.58
0.9401	45.63
0.94	45.69
0.9399	45.74
0.9398	45.8
0.9397	45.85
0.9396	45.9
0.9395	45.96
0.9394	46.01
0.9393	46.07
0.9392	46.12
0.9391	46.18
0.939	46.23
0.9389	46.28
0.9388	46.34
0.9387	46.39
0.9386	46.45
0.9385	46.5

Sp.gr @20°C	% by Vol
0.9384	46.55
0.9383	46.61
0.9382	46.66
0.9381	46.72
0.938	46.77
0.9379	46.82
0.9378	46.88
0.9377	46.93
0.9376	46.98
0.9375	47.04
0.9374	47.09
0.9373	47.14
0.9372	47.2
0.9371	47.25
0.937	47.3
0.9369	47.36
0.9368	47.41
0.9367	47.46
0.9366	47.52
0.9365	47.57
0.9364	47.62
0.9363	47.68
0.9362	47.73
0.9361	47.78
0.936	47.84

Sp.gr @20°C	% by Vol
0.9359	47.89
0.9358	47.94
0.9357	47.99
0.9356	48.05
0.9355	48.1
0.9354	48.15
0.9353	48.2
0.9352	48.26
0.9351	48.31
0.935	48.36
0.9349	48.41
0.9348	48.47
0.9347	48.52
0.9346	48.57
0.9345	48.62
0.9344	48.67
0.9343	48.73
0.9342	48.78
0.9341	48.83
0.934	48.88
0.9339	48·93
0.9338	48.99
0.9337	49.04
0.9336	49.09
0.9335	49.14

Sp.gr @20°C	% by Vol
0.9334	49.19
0.9333	49.24
0.9332	49.3
0.9331	49.35
0.933	49.4
0.9329	49.45
0.9328	49.5
0.9327	49.55
0.9326	49.6
0.9325	49.65
0.9324	49.71
0.9323	49.76
0.9322	49.81
0.9321	49.86
0.932	49.91
0.9319	49.96
0.9318	50.01
0.9317	50.06
0.9316	50.11
0.9315	50.16
0.9314	50.21
0.9313	50.26
0.9312	50.31
0.9311	50.36
0.931	50.41

Sp.gr @20°C	% by Vol
0.9309	50.46
0.9308	50.51
0.9307	50.56
0.9306	50.62
0.9305	50.67
0.9304	50.72
0.9303	50.77
0.9302	50.82
0.9301	50.87
0.93	50.92
0.9299	50.97
0.9298	51.02
0.9297	51.07
0.9296	51.12
0.9295	51.16
0.9294	51.21
0.9293	51.26
0.9292	51.31
0.9291	51.36
0.929	51.41
0.9289	51.46
0.9288	51.51
0.9287	51.56
0.9286	51.61
0.9285	51.66

Sp.gr @20°C	% by Vol
0.9284	51.71
0.9283	51.76
0.9282	51.81
0.9281	51.86
0.928	51.91
0.9279	51.96
0.9278	52.01
0.9277	52.06
0.9276	52.11
0.9275	52.16
0.9274	52.21
0.9273	52.26
0.9272	52.31
0.9271	52.35
0.927	52.4
0.9269	52.45
0.9268	52.5
0.9267	52.55
0.9266	52.6
0.9265	52.65
0.9264	52.7
0.9263	52.75
0.9262	52.8
0.9261	52.84
0.926	52.89

Sp.gr @20°C	% by Vol
0.9259	52.94
0.9258	52.99
0.9257	53.04
0.9256	53.09
0.9255	53.14
0.9254	53.19
0.9253	53.23
0.9252	53.28
0.9251	53.33
0.925	53.38
0.9249	53.43
0.9248	53.48
0.9247	53.52
0.9246	53.57
0.9245	53.62
0.9244	53.67
0.9243	53.72
0.9242	53.77
0.9241	53.82
0.924	53.86
0.9239	53.91
0.9238	53.96
0.9237	54.01
0.9236	54.06
0.9235	54.1

Sp.gr @20°C	% by Vol
0.9234	54.15
0.9233	54.2
0.9232	54.25
0.9231	54.3
0.923	54.35
0.9229	54.39
0.9228	54.44
0.9227	54.49
0.9226	54.54
0.9225	54.59
0.9224	54.63
0.9223	54.68
0.9222	54.73
0.9221	54.78
0.922	54.82
0.9219	54.87
0.9218	54.92
0.9217	54.97
0.9216	55.01
0.9215	55.06
0.9214	55.11
0.9213	55.16
0.9212	55.2
0.9211	55.25
0.921	55.3

Sp.gr @20°C	% by Vol
0.9209	55.35
0.9208	55.39
0.9207	55.44
0.9206	55.49
0.9205	55.54
0.9204	55.58
0.9203	55.63
0.9202	55.68
0.9201	55.72
0.92	55.77
0.9199	55.82
0.9198	55.87
0.9197	55.91
0.9196	55.96
0.9195	56.01
0.9194	56.05
0.9193	56.1
0.9192	56.15
0.9191	56.19
0.919	56.24
0.9189	56.29
0.9188	56.33
0.9187	56.38
0.9186	56.43
0.9185	56.47

Sp.gr @20°C	% by Vol
0.9184	56.52
0.9183	56.57
0.9182	56.61
0.9181	56.66
0.918	56.71
0.9179	56.75
0.9178	56.8
0.9177	56.85
0.9176	56.9
0.9175	56.94
0.9174	56.99
0.9173	57.04
0.9172	57.08
0.9171	57.13
0.917	57.17
0.9169	57.22
0.9168	57.27
0.9167	57.31
0.9166	57.36
0.9165	57.41
0.9164	57.46
0.9163	57.5
0.9162	57.55
0.9161	57.59
0.916	57.64

Sp.gr @20°C	% by Vol
0.9159	57.69
0.9158	57.73
0.9157	57.78
0.9156	57.82
0.9155	57.87
0.9154	57.91
0.9153	57.96
0.9152	58
0.9151	58.05
0.915	58.1
0.9149	58.14
0.9148	58.19
0.9147	5823
0.9146	58.28
0.9145	58.32
0.9144	58.37
0.9143	58.41
0.9142	58.46
0.9141	58.5
0.914	58.55
0.9139	58.59
0.9138	58.64
0.9137	58.68
0.9136	58.73
0.9135	58.77

Sp.gr @20°C	% by Vol
0.9134	58.82
0.9133	58.86
0.9132	58.91
0.9131	58.95
0.913	59
0.9129	59.04
0.9128	59.09
0.9127	59.13
0.9126	59.18
0.9125	59.22
0.9124	59.27
0.9123	59.31
0.9122	59.36
0.9121	59.4
0.912	59.45
0.9119	59.49
0.9118	59.54
0.9117	59.58
0.9116	59.63
0.9115	59.67
0.9114	59.72
0.9113	59.76
0.9112	59.8
0.9111	59.85
0.911	59.89

Sp.gr @20°C	% by Vol
0.9109	59.94
0.9108	59.98
0.9107	60.03
0.9106	60.07
0.9105	60.12
0.9104	60.16
0.9103	60.21
0.9102	60.25
0.9101	60.3
0.91	60.34
0.9099	60.38
0.9098	60.43
0.9097	60.47
0.9096	60.52
0.9095	60.56
0.9094	60.61
0.9093	60.65
0.9092	60.69
0.9091	60.74
0.909	60.78
0.9089	60.83
0.9088	60.87
0.9087	60.92
0.9086	60.96
0.9085	61

Sp.gr @20°C	% by Vol
0.9084	61.05
0.9083	61.09
0.9082	61.14
0.9081	61.18
0.908	61.22
0.9079	61.27
0.9078	61.31
0.9077	61.36
0.9076	61.4
0.9075	61.44
0.9074	61.49
0.9073	61.53
0.9072	61.58
0.9071	61.62
0.907	61.66
0.9069	61.71
0.9068	61.75
0.9067	61.79
0.9066	61.84
0.9065	61.88
0.9064	61.93
0.9063	61.97
0.9062	62.01
0.9061	62.06
0.906	62.1

Sp.gr @20°C	% by Vol
0.9059	62.14
0.9058	62.19
0.9057	62.23
0.9056	62.27
0.9055	62.32
0.9054	62.36
0.9053	62.4
0.9052	62.45
0.9051	62.49
0.905	62.53
0.9049	62.58
0.9048	62.62
0.9047	62.66
0.9046	62.71
0.9045	62.75
0.9044	62.8
0.9043	62.84
0.9042	62.88
0.9041	62.93
0.904	62.97
0.9039	63.01
0.9038	63.06
0.9037	63.10
0.9036	63.14
0.9035	63.19

Sp.gr @20°C	% by Vol
0.9034	63.23
0.9033	63.27
0.9032	63.31
0.9031	63.36
0.903	63.4
0.9029	63.44
0.9028	63.49
0.9027	63.53
0.9026	63.57
0.9025	63.62
0.9024	63.66
0.9023	63.7
0.9022	63.75
0.9021	63.79
0.902	63.83
0.9019	63.88
0.9018	63.92
0.9017	63.96
0.9016	64
0.9015	64.05
0.9014	64.09
0.9013	64.13
0.9012	64.18
0.9011	64.22
0.901	64.26

Sp.gr @20°C	% by Vol
0.9009	64.3
0.9008	64.35
0.9007	64.39
0.9006	64.43
0.9005	64.47
0.9004	64.52
0.9003	64.56
0.9002	64.6
0.9001	64.65
0.9	64.69
0.8999	64.73
0.8998	64.77
0.8997	64.82
0.8996	64.86
0.8995	64.9
0.8994	64.94
0.8993	64.99
0.8992	65.03
0.8991	65.07
0.899	65.11
0.8989	65.16
0.8988	65.2
0.8987	65.24
0.8986	65.28
0.8985	65.32

Sp.gr @20°C	% by Vol
0.8984	65.37
0.8983	65.41
0.8982	65.45
0.8981	65.49
0.898	65.54
0.8979	65.58
0.8978	65.62
0.8977	65.66
0.8976	65.7
0.8975	65.75
0.8974	65.79
0.8973	65.83
0.8972	65.87
0.8971	65.91
0.897	65.96
0.8969	66
0.8968	66.04
0.8967	66.08
0.8966	66.12
0.8965	66.17
0.8964	66.21
0.8963	66.25
0.8962	66.29
0.8961	66.33
0.896	66.37

Sp.gr @20°C	% by Vol
0.8959	66.42
0.8958	66.46
0.8957	66.5
0.8956	66.54
0.8955	66.58
0.8954	66.62
0.8953	66.67
0.8952	66.71
0.8951	66.75
0.8950	66.79
0.8949	66.83
0.8948	66.87
0.8947	66.92
0.8946	66.96
0.8945	67
0.8944	67.04
0.8943	67.08
0.8942	67.12
0.8941	67.16
0.894	67.21
0.8939	67.25
0.8938	67.29
0.8937	67.33
0.8936	67.37
0.8935	67.41

Sp.gr @20°C	% by Vol
0.8934	67.45
0.8933	67.49
0.8932	67.54
0.8931	67.58
0.893	67.62
0.8929	67.66
0.8928	67.7
0.8927	67.74
0.8926	67.78
0.8925	67.82
0.8924	67.87
0.8923	67.91
0.8922	67.95
0.8921	67.99
0.892	68.43
0.8919	68.07
0.8918	68.11
0.8917	68.15
0.8916	68.19
0.8915	68.24
0.8914	68.28
0.8913	68.32
0.8912	68.36
0.8911	68.4
0.891	68.44

Sp.gr @20°C	% by Vol
0.8909	68.48
0.8908	68.52
0.8907	68.56
0.8906	68.6
0.8905	68.65
0.8904	68.69
0.8903	68.73
0.8902	68.77
0.8901	68.81
0.89	68.85
0.8899	68.89
0.8898	68.93
0.8897	68.97
0.8896	69.01
0.8895	69.05
0.8894	69.09
0.8893	69.13
0.8892	69.17
0.8891	69.22
0.889	69.26
0.8889	69.34
0.8887	69.38
0.8886	69.42
0.8885	69.46
0.8884	69.5

Sp.gr @20°C	% by Vol
0.8883	69.54
0.8882	69.58
0.8881	69.62
0.888	69.66
0.8879	69.7
0.8878	69.74
0.8877	69.78
0.8876	69.82
0.8875	69.86
0.8874	69.9
0.8873	69.94
0.8872	69.98
0.8871	70.02
0.887	70.06
0.8869	70.1
0.8868	70.14
0.8867	70.18
0.8866	70.22
0.8865	70.26
0.8864	70.3
0.8863	70.34
0.8862	70.38
0.8861	70.42
0.886	70.46
0.8859	70.5

Sp.gr @20°C	% by Vol
0.8858	70.54
0.8857	70.58
0.8856	70.62
0.8855	70.66
0.8854	70.7
0.8853	70.74
0.8852	70.78
0.8851	70.82
0.885	70.86
0.8849	70.9
0.8848	70.94
0.8847	70.98
0.8846	71.02
0.8845	71.06
0.8844	71.1
0.8843	71.14
0.8842	71.18
0.8841	71.22
0.884	71.26
0.8838	71.34
0.8837	71.38
0.8836	71.42
0.8835	71.46
0.8834	71.5
0.8833	71.54

Sp.gr @20°C	% by Vol
0.8832	71.58
0.8831	71.61
0.883	71.65
0.8829	71.69
0.8828	71.73
0.8827	71.77
0.8826	71.81
0.8825	71.85
0.8824	71.89
0.8823	71.93
0.8822	71.97
0.8821	72.01
0.882	72.05
0.8819	72.09
0.8818	72.12
0.8817	72.16
0.8816	72.2
0.8815	72.24
0.8814	72.28
0.8813	72.32
0.8812	72.36
0.8811	72.4
0.881	72.44
0.8809	72.48
0.8808	72.52

Sp.gr @20°C	% by Vol
0.8807	72.56
0.8806	72.59
0.8805	72.63
0.8804	72.67
0.8803	72.71
0.8802	72.75
0.8801	72.79
0.88	72.83
0.8799	72.87
0.8798	72.91
0.8797	72.95
0.8796	72.99
0.8795	73.02
0.8794	73.06
0.8793	73.1
0.8792	73.14
0.8791	73.18
0.879	73.22
0.8789	73.26
0.8788	73.3
0.8787	73.33
0.8786	73.37
0.8785	73.41
0.8784	73.45
0.8783	73.49

Sp.gr @20°C	% by Vol
0.8782	73.53
0.8781	73.57
0.878	73.61
0.8779	73.64
0.8778	73.68
0.8777	73.72
0.8776	73.76
0.8775	73.8
0.8774	73.84
0.8773	73.87
0.8772	73.91
0.8771	73.95
0.877	73.99
0.8769	74.03
0.8768	74.07
0.8767	74.11
0.8766	74.14
0.8765	74.18
0.8764	74.22
0.8763	74.26
0.8762	74.3
0.8761	74.34
0.876	74.37
0.8759	74.41
0.8758	74.45

Sp.gr @20°C	% by Vol
0.8757	74.49
0.8756	74.53
0.8755	74.57
0.8754	74.6
0.8753	74.64
0.8752	74.68
0.8751	74.72
0.875	74.76
0.8749	74.8
0.8748	74.83
0.8747	74.87
0.8746	74.91
0.8745	74.95
0.8744	74.99
0.8743	75.03
0.8742	75.06
0.8741	75.1
0.874	75.14
0.8739	75.18
0.8738	75.22
0.8737	75.25
0.8736	75.29
0.8735	75.33
0.8734	75.37
0.8733	75.41

Sp.gr @20°C	% by Vol
0.8732	75.44
0.8731	75.48
0.873	75.52
0.8729	75.56
0.8728	75.6
0.8727	75.63
0.8726	75.67
0.8725	75.71
0.8724	75.75
0.8723	75.78
0.8722	75.82
0.8721	75.86
0.872	75.9
0.8719	75.93
0.8718	75.97
0.8717	76.01
0.8716	76.05
0.8715	76.09
0.8714	76.12
0.8713	76.16
0.8712	76.2
0.8711	76.24
0.871	76.27
0.8709	76.31
0.8708	76.35

Sp.gr @20°C	% by Vol
0.8707	76.39
0.8706	76.42
0.8705	76.46
0.8704	76.5
0.8703	76.54
0.8702	76.57
0.8701	76.61
0.87	76.65
0.8699	76.68
0.8698	76.72
0.8697	76.76
0.8696	76.8
0.8695	76.83
0.8694	76.87
0.8693	76.91
0.8692	76.94
0.8691	76.98
0.869	77.02
0.8689	77.06
0.8688	77.09
0.8687	77.13
0.8686	77.17
0.8685	77.2
0.8684	77.24
0.8683	77.28

Sp.gr @20°C	% by Vol
0.8682	77.32
0.8681	77.35
0.868	77.39
0.8679	77.43
0.8678	77.46
0.8677	77.5
0.8676	77.54
0.8675	77.57
0.8674	77.61
0.8673	77.65
0.8672	77.68
0.8671	77.72
0.867	77.76
0.8669	77.79
0.8668	77.83
0.8667	77.87
0.8666	77.9
0.8665	77.94
0.8664	77.98
0.8663	78.01
0.8662	78.45
0.8661	78.09
0.8643	78.12
0.8659	78.16
0.8658	78.2

Sp.gr @20°C	% by Vol
0.8657	78.23
0.8656	78.27
0.8655	78.31
0.8654	78.34
0.8653	78.38
0.8652	78.42
0.8651	78.45
0.865	78.49
0.8649	78.52
0.8648	78.56
0.8647	78.6
0.8646	78.63
0.8645	78.67
0.8644	78.71
0.8643	78.74
0.8642	78.78
0.8641	78.82
0.864	78.85
0.8639	78.89
0.8638	78.93
0.8637	78.96
0.8636	79
0.8635	79.03
0.8634	79.07
0.8633	79.11
0.8632	79.14
0.8631	79.18
0.863	79.22
0.8629	79.25
0.8628	79.29
0.8627	79.32
0.8626	79.36
0.8625	79.4
0.8624	79.43
0.8623	79.47

Sp.gr @20°C	% by Vol
0.8622	79.5
0.8621	79.54
0.862	79.58
0.8619	79.61
0.8618	79.65
0.8617	79.68
0.8616	79.72
0.8615	79.76
0.8614	79.79
0.8613	79.83
0.8612	79.86
0.8611	79.9
0.861	79.94
0.8609	79.97
0.8608	80.01
0.8607	80.04
0.8606	80.08
0.8605	80.12
0.8604	80.15
0.8603	80.19
0.8602	80.22
0.8601	80.26
0.86	80.29
0.8599	80.33
0.8598	80.36
0.8597	80.4
0.8596	80.44
0.8595	80.47
0.8594	80.51
0.8593	80.54
0.8592	80.58
0.8591	80.61
0.859	80.65
0.8589	80.68
0.8588	80.72
0.8587	80.76
0.8586	80.79
0.8585	80.83

Sp.gr @20°C	% by Vol
0.8584	80.86
0.8583	80.9
0.8582	80.93
0.8581	80.97
0.858	81
0.8579	81.04
0.8578	81.07
0.8577	81.11
0.8576	81.14
0.8575	81.18
0.8574	81.21
0.8573	81.25
0.8572	81.28
0.8571	81.32
0.857	81.35
0.8569	81.39
0.8568	81.43
0.8567	81.46
0.8566	81.5
0.8565	81.53
0.8564	81.57
0.8563	81.6
0.8562	81.64
0.8561	81.67
0.856	81.71
0.8559	81.74
0.8558	81.78
0.8557	81.81
0.8556	81.85
0.8555	81.88
0.8554	81.92
0.8553	81.95
0.8552	81.99
0.8551	82.02
0.85	83.78
0.8499	83.82
0.8498	83.85
0.8497	83.88

Sp.gr @20°C	% by Vol
0.8496	83.92
0.8495	83.95
0.8494	83.99
0.8493	84.02
0.8492	84.05
0.8491	84.09
0.849	84.12
0.8489	84.15
0.8488	84.19
0.8487	84.22
0.8486	84.26
0.8485	84.29
0.8484	84.32
0.8483	84.36
0.8482	84.39
0.8481	84.42
0.848	84.46
0.8479	84.49
0.8478	84.53
0.8477	84.56
0.8476	84.59
0.8475	84.63
0.8474	84.66
0.8473	84.69
0.8472	84.73
0.8471	84.76
0.847	84.79
0.8469	84.83
0.8468	84.86
0.8467	84.90
0.8466	84.93
0.8465	84.96
0.8464	85.00
0.8463	85.03
0.8462	85.06
0.8461	85.10
0.846	85.13
0.8459	85.16

Sp.gr @20°C	% by Vol
0.8458	85.2
0.8457	85.23
0.8456	85.26
0.8455	8530
0.8454	85.33
0.8453	85.36
0.8452	85.40
0.8451	85.43
0.845	85.46
0.8449	85.49
0.8448	85.53
0.8447	85.56
0.8446	85.59
0.8445	85.63
0.8444	85.66
0.8443	85.69
0.8442	85.73
0.8441	85.76
0.8440	85.79
0.8439	85.82
0.8438	85.86
0.8437	85.89
0.8436	85.92
0.8435	85.95
0.8434	85.99
0.8433	86.02
0.8432	86.05
0.8431	86.08
0.843	86.12
0.8429	86.15
0.8428	86.18
0.8427	86.22
0.8426	86.25
0.8425	86.28
0.8424	86.31
0.8423	86.35
0.8422	86.38
0.8421	86.41

Sp.gr @20°C	% by Vol
0.842	86.44
0.8419	86.48
0.8418	86.51
0.8417	86.54
0.8416	86.57
0.8415	86.61
0.8414	86.64
0.8413	86.67
0.8412	86.7
0.8411	86.74
0.841	86.77
0.8409	86.8
0.8408	86.83
0.8407	86.87
0.8406	86.9
0.8405	86.93
0.8404	86.96
0.8403	87
8402	87.03
0.8401	87.06
0.84	87.09
0.8399	87.13
0.8398	87.16
0.8397	87.19
0.8396	87.22
0.8395	87.26
0.8394	87.29
0.8393	87.32
0.8392	87.35
0.8391	87.38
0.839	87.42
0.8389	87.45
0.8388	87.48
0.8387	87.51
0.8386	87.55
0.8385	87.58
0.8384	87.61
0.8383	87.64

Sp.gr @20°C	% by Vol
0.8382	87.67
0.8381	87.71
0.838	87.74
0.8379	87.77
0.8378	87.8
0.8377	87.83
0.8376	87.86
0.8375	87.90
0.8374	87.93
0.8373	87.96
0.8372	87.99
0.8371	88.02
0.837	88.06
0.8369	88.09
0.8368	88.12
0.8367	88.15
0.8366	88.18
0.8365	88.21
0.8364	88.24
0.8363	88.28
0.8362	88.31
0.8361	88.34
0.836	88.37
0.8359	88.4
0.8358	88.43
0.8357	88.47
0.8356	88.5
0.8355	88.53
0.8354	88.56
0.8353	88.59
0.8352	88.62
0.8351	88.65
0.835	88.68
0.8349	88.72
0.8348	88.75
0.8347	88.78
0.8346	88.81
0.8345	88.84

Sp.gr @20°C	% by Vol
0.8344	88.87
0.8343	88.9
0.8342	88.93
0.8341	88.96
0.834	89
0.8339	89.03
0.8338	89.06
0.8337	89.09
0.8336	89.12
0.8335	89.15
0.8334	89.18
0.8333	89.21
0.8332	89.24
0.8331	89.27
0.833	89.3
0.8329	89.33
0.8328	89.37
0.8327	89.4
0.8326	89.43
0.8325	89.46
0.8324	89.49
0.8323	89.52
0.8322	89.55
0.8321	89.58
0.832	89.61
0.8319	89.64
0.8318	89.67
0.8317	89.7
0.8316	89.73
0.8315	89.76
0.8314	89.79
0.8313	89.82
0.8312	89.85
0.8311	89.88
0.831	89.91
0.8309	89.94
0.8308	89.97
0.8307	90

Sp.gr @20°C	% by Vol
0.8306	90.04
0.8305	90.07
0.8304	90.1
0.8303	90.13
0.8302	90.16
0.8301	90.19
0.825	91.69
0.8249	91.72

ANNEXURE-II

CONCENTRATION (IN WEIGHT %) OF ETHANOL-WATER MIXTURE VS. SPECIFIC GRAVITY AT VARIOUS TEMPERATURE

wt % Ethanol	Temperature (degC)				wt % Ethanol	Temperature (degC)			
	20	25	30	35		20	25	30	35
0	0.99823	0.99708	0.99568	0.99406	50	0.91384	0.90985	0.90580	0.90168
1	0.99636	0.9952	0.99379	0.99217	51	0.91160	0.90760	0.90353	0.89940
2	0.99453	0.99336	0.99194	0.99031	52	0.90936	0.90534	0.90125	0.89710
3	0.99275	0.99157	0.99014	0.98849	53	0.90711	0.90307	0.89896	0.89479
4	0.99103	0.98984	0.98839	0.98672	54	0.90485	0.90079	0.89667	0.89248
5	0.98938	0.98817	0.98679	0.98501	55	0.90258	0.89850	0.89437	0.89016
6	0.9878	0.98656	0.98507	0.98335	56	0.90031	0.89621	0.89206	0.88784
7	0.98627	0.98500	0.98347	0.98172	57	0.89803	0.89392	0.88975	0.88552
8	0.98478	0.98346	0.98189	0.98009	58	0.89574	0.89162	0.88744	0.88319
9	0.98331	0.98193	0.98031	0.97846	59	0.89344	0.88931	0.88512	0.88085
10	0.98187	0.98043	0.97875	0.97685	60	0.89113	0.88699	0.88278	0.87851
11	0.98047	0.97897	0.97723	0.97527	61	0.88882	0.88446	0.88044	0.87615
12	0.97910	0.97753	0.97573	0.97371	62	0.88650	0.88233	0.87809	0.87379
13	0.97775	0.97661	0.97424	0.97216	63	0.88417	0.87998	0.87574	0.87142
14	0.97643	0.97472	0.97278	0.97063	64	0.88183	0.87763	0.87337	0.86905
15	0.97514	0.97334	0.97133	0.96911	65	0.87948	0.87527	0.87100	0.86667
16	0.97387	0.97199	0.96990	0.9676	66	0.87713	0.87291	0.86863	0.86429
17	0.97259	0.97062	0.96844	0.96607	67	0.87477	0.87054	0.86625	0.86190
18	0.97129	0.96923	0.96697	0.96452	68	0.87241	0.86817	0.86387	0.85950
19	0.96997	0.96782	0.96547	0.96294	69	0.87004	0.86579	0.86148	0.85710
20	0.96864	0.96639	0.96395	0.96134	70	0.86766	0.86340	0.85908	0.85470
21	0.96729	0.96495	0.96242	0.95973	71	0.86527	0.86100	0.85667	0.85228
22	0.96592	0.96348	0.96087	0.95809	72	0.86287	0.85859	0.85426	0.84986
23	0.96453	0.96199	0.95929	0.95643	73	0.86047	0.85618	0.85184	0.84743
24	0.96312	0.96048	0.95769	0.95476	74	0.85806	0.85376	0.84941	0.84500
25	0.96168	0.95895	0.95607	0.95306	75	0.85564	0.85134	0.84698	0.84257
26	0.96020	0.95738	0.95442	0.95133	76	0.85322	0.84891	0.84455	0.84013
27	0.95867	0.95576	0.95272	0.94995	77	0.85079	0.84647	0.84211	0.83768
28	0.95710	0.95410	0.95098	0.95774	78	0.84835	0.84403	0.83966	0.83523
29	0.95548	0.95241	0.94922	0.94590	79	0.84590	0.84158	0.83720	0.83277
30	0.95382	0.95067	0.94741	0.94403	80	0.84344	0.83911	0.83473	0.83029
31	0.95212	0.94890	0.94557	0.94214	81	0.84096	0.83664	0.83224	0.82780
32	0.95038	0.94709	0.94370	0.94021	82	0.83848	0.83415	0.82974	0.82530
33	0.94860	0.94525	0.94180	0.93825	83	0.83599	0.83164	0.82724	0.82279
34	0.94679	0.94337	0.93986	0.93626	84	0.83348	0.82913	0.82473	0.82027
35	0.94494	0.94146	0.93790	0.93425	85	0.83095	0.82660	0.82220	0.81774
36	0.94306	0.93952	0.93591	0.93221	86	0.82840	0.82405	0.81965	0.81519
37	0.94114	0.93756	0.93390	0.93016	87	0.82583	0.82148	0.81708	0.81262
38	0.93919	0.93556	0.93186	0.92808	88	0.82323	0.81888	0.81448	0.81003
39	0.93720	0.93353	0.92979	0.92597	89	0.82062	0.81626	0.81186	0.80742
40	0.93518	0.93148	0.92770	0.92385	90	0.81797	0.81362	0.80922	0.80478
41	0.93314	0.92940	0.92558	0.92170	91	0.81529	0.81094	0.80655	0.80211
42	0.93107	0.92729	0.92344	0.91952	92	0.81257	0.80823	0.80384	0.79941
43	0.92897	0.92516	0.92128	0.91733	93	0.80983	0.80549	0.80111	0.79669
44	0.92685	0.92301	0.91910	0.91513	94	0.80705	0.80272	0.79835	0.79393
45	0.92472	0.92085	0.91692	0.91291	95	0.80424	0.79991	0.79555	0.79114
46	0.92257	0.91868	0.91472	0.91069	96	0.80138	0.79706	0.79271	0.78831
47	0.92041	0.91649	0.91250	0.90845	97	0.79846	0.79415	0.78991	0.78542
48	0.91823	0.91426	0.91028	0.90621	98	0.79547	0.79117	0.78684	0.78247
49	0.91604	0.91208	0.90805	0.90396	99	0.79543	0.78814	0.78382	0.77946
					100	0.78934	0.78506	0.78075	0.77641

NOTE: Numbers obtained from Table 3-110 (Pg.3.89) "Perry's Chemical Engineers' Handbook", 6th Ed.

RAPID ANALYTICAL FOOD TESTING (RAFT) KIT/ EQUIPMENT

Alternate Rapid kits/equipment may be used to get quick results for screening and surveillance purposes, provided the kit/equipment is approved by FSSA(I). Details of the rapid food testing kit/ equipment approved by FSSA(I) are available at https://www.fssai.gov.in/cms/raft.php

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