LAB. MANUAL 13

MANUAL OF METHODS

OF

ANALYSIS OF FOODS

ALCOHOLIC BEVERAGES

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
MINISTRY OF HEALTH AND FAMILY WELFARE
GOVERNMENT OF INDIA
NEW DELHI
2015

ACKNOWLEDGEMENT

Deepest Sense of Gratitude and Indebtedness to all the Members of the Panel "Method of Sampling and Analysis" and Experts, who have helped, supported knowledge and insight, have led the successful completion of Revision of manuals.

Sincere Thanks to the Panel, Chairman for his valuable guidance, and encouragement and the Secretariat of this panel who have worked hard throughout the tenure of revision work.

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

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MANUAL OF METHODS FOR ANALYSIS OF ALCOHOLIC BEVERAGES

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1.0 Types of Alcoholic Beverages

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

2.0 Determination of Ethyl alcohol content

2.1 Pyknometer Method or Hydrometer Method (after distillation)

2.1.1 Apparatus:

a) 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.
b) Pyknometer: 50 ml capacity / SG Hydrometer , Short range (0.96 – 1.00).
c) Thermometer: 0-1000C.
d) Volumetric flask: 200 ml capacity

2.1.2 Procedure:

2.1.2.1 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. Distil the contents in about 35 min and collect the distillate in a 200 ml volumetric flask till the volume almost reaches the mark. Bring the distillate to room temperature and make up to volume with distilled water and mix thoroughly.

2.1.2.2

A) Find out the specific gravity of the distillate as follows:

Take a clean and dry pyknometer and weigh it empty along with the stopper at 200C (W). Fill it with the liquor sample to the brim and insert the stopper gently. Wipe the Liquid that spills out using water absorbing filter paper and weigh at 200C (W1). Next remove the liquor sample and wash it with distilled water. Fill the pyknometer with distilled water in the same manner as described above and at 200C take the weight (W2).

$$\text{Sp. gravity} = \frac{W1 - W}{W2 - W}$$

Find out the corresponding alcohol percent by volume from the table showing Sp. gravity Vs Alcohol percent (Refer Annexure I).

B) Alternatively, use a SG hydrometer to find out the specific gravity (SG) and use the following equation to convert SG to % Alcohol.

$$\%\text{Alcohol (v/v)} = 8610.6 - (16584 \times SG) + (7973.3 \times SG\ 2)$$

(One can use computer program to automate this process).

2.2 Distillation method (for products containing high volatile acids)

2.2.1 Apparatus

a) Volumetric flask, 200 ml capacity

b) Separatory funnels, 500 ml capacity

c) Distillation unit with assembly

2.2.2 Reagents

a) Sodium chloride

b) Petroleum ether 40-600C grade

c) Sodium hydroxide 0.1 N

d) Phenolphthalein indicator

2.2.3 Procedure

a) Measure 200 ml of liquor sample in a volumetric flask. Transfer to a separatory funnel and wash the volumetric flask with about 100 ml water. Add sodium chloride powder so that the solution becomes almost saturated with NaCl. Add about 100 ml of petroleum ether and shake for 2-3 min. Allow the layers to settle and transfer the lower layer to the distillation flask. Add about 20-30 ml of saturated sodium chloride solution to the petroleum ether layer and gently shake. Allow again to settle and transfer the aqueous layer to the distillation flask. Mix gently and make the solution just alkaline with NaOH solution using phenolphthalein indicator. Add little pumice stone and connect the distillation assembly via condenser to the volumetric flask. Distill gently and collect the distillate in the volumetric flask almost to the mark. Bring the contents to room temperature and make up the volume with distilled water and mix well.

b) Determine the specific gravity of the distillate as described in sec.2.1.2.2 and find out the corresponding alcohol percent by volume from the table showing Sp. gravity Vs Alcohol percent.

3.0 Determination of Residue on evaporation

3.1 Apparatus

a) Hot Air oven

b) Water bath

c) Desiccator

d) Glass bowl, 250 ml capacity

e) Volumetric flask, 200 ml

3.2 Procedure

Transfer 200 ml of alcoholic drink into a dried, weighed (W) glass bowl and evaporate on a water bath. Wipe the external sides of the bowl and keep in an air oven maintained at 100 ± 10^{o}C for 2 hrs. Cool in a desiccator and weigh the dish (W1). Repeat till constant weight is obtained. Calculate the % residual solids.

3.3 Calculation

$$\text{Residue on evaporation \% (w/v)} = \frac{W1 - W}{V} \times 100$$

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

4.0 Determination of Total acidity

4.1 Method I (For Colourless Liquors)

4.1.1 Reagents

a) Standard sodium hydroxide -0.05 N

b) Phenolphthalein indicator

4.1.2 Procedure

i) Take 50 ml of liquor sample and add about 200 ml neutral distilled water.

ii) Titrate against standard sodium hydroxide using Phenolphthalein indicator.

4.1.3 Calculation

$$\text{Total acidity as tartaric acid, gms. per 100 liters of abs. alcohol} = \frac{V \times 0.00375 \times 100 \times 1000 \times 2}{V1}$$

Where, V1 = alcohol % by volume

V = volume of std. NaOH used for titration, in ml

4.2 Method II (For Coloured Liquors such as Wine, Toddy)

4.2.1 Apparatus

a) pH Meter

b) Magnetic stirrer

c) Beaker 250 ml capacity

4.2.2 Reagents

a) Standard NaOH, 0.05 N

b) Buffer solutions of pH 4.0, 7.0 and 9.2

4.2.3 Procedure:

Calibrate and standardize the pH meter using the buffer solutions of pH 4.0, 7.0 and 9.2. Take approximately 100 ml of distilled water in a beaker and put a magnetic bead and place the beaker on a magnetic stirrer. 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 900C to remove carbon dioxide).

4.2.4 Calculation

For wines:

$$\text{Total acidity as tartaric acid gms. per liter of wine / toddy} = \frac{V \times 0.00375 \times 1000}{V1}$$

Where, V1 = Volume of wine taken for estimation

V = Volume of std. NaOH used for titration, in ml

Note: 1 ml of 0.05N NaOH is equivalent to 0.00375 g of tartaric acid.

5.0 Determination of Volatile acidity

5.1 Reagents

a) Standard Sodium Hydroxide, 0.05N

b) Phenolphthalein indicator

5.2 Procedure:

Take 50ml distillate collected during the determination of ethyl alcohol for volatile acidity determination (sec. 2.1.2.1) and titrate against std. NaOH using phenolphthalein indicator.

5.3 Calculation

a) For liquors:

$$\text{Volatile acidity as acetic acid, gms. per 100 liters of abs. alcohol} = \frac{V \times 0.003 \times 100 \times 1000 \times 2}{V1}$$

Where, V = volume of std. NaOH used for titration, in ml
V1 = alcohol % by volume

b) For wines:

$$\text{Volatile acidity as acetic acid gms. per liter of wine} = \frac{V \times 0.003 \times 1000}{V1}$$

Where, V1 = Volume of wine taken for estimation
V = volume of std. NaOH used for titration, in ml

Note: 1 ml. of 0.05N NaOH is equivalent to 0.003g of acetic acid.

6.0 Determination of Esters

6.1 Reagents

a) Standard Sodium Hydroxide, 0.1N

b) Standard Sulphuric acid, 0.1N

6.2 Procedure:

To the neutralized distillate from the volatile acidity determination (Sec. 5.2.1), add 10ml of Std. NaOH and reflux on a steam bath for 1hour. Cool and back titrate the unspent alkali against standard sulphuric acid. Carry out a blank simultaneously taking 50ml of distilled water instead of distillate in the same way. The difference in titer value in milliliters of standard sulphuric acid gives the equivalent ester.

6.3 Calculation

Esters expressed as ethyl acetate, gms. per 100 liters of abs. alcohol

$$= \frac{V \times 0.0088 \times 100 \times 1000 \times 2}{V1}$$

Where, V = difference of titer value of std.H_2SO_4 used for blank and sample, in ml

V1 = alcohol % by volume

Note: 1 ml. of 0.1N NaOH is equivalent to 0.0088g of Ethyl acetate.

7.0 Determination of Higher alcohols

7.1 Method I- Extraction / Titrimetric Method

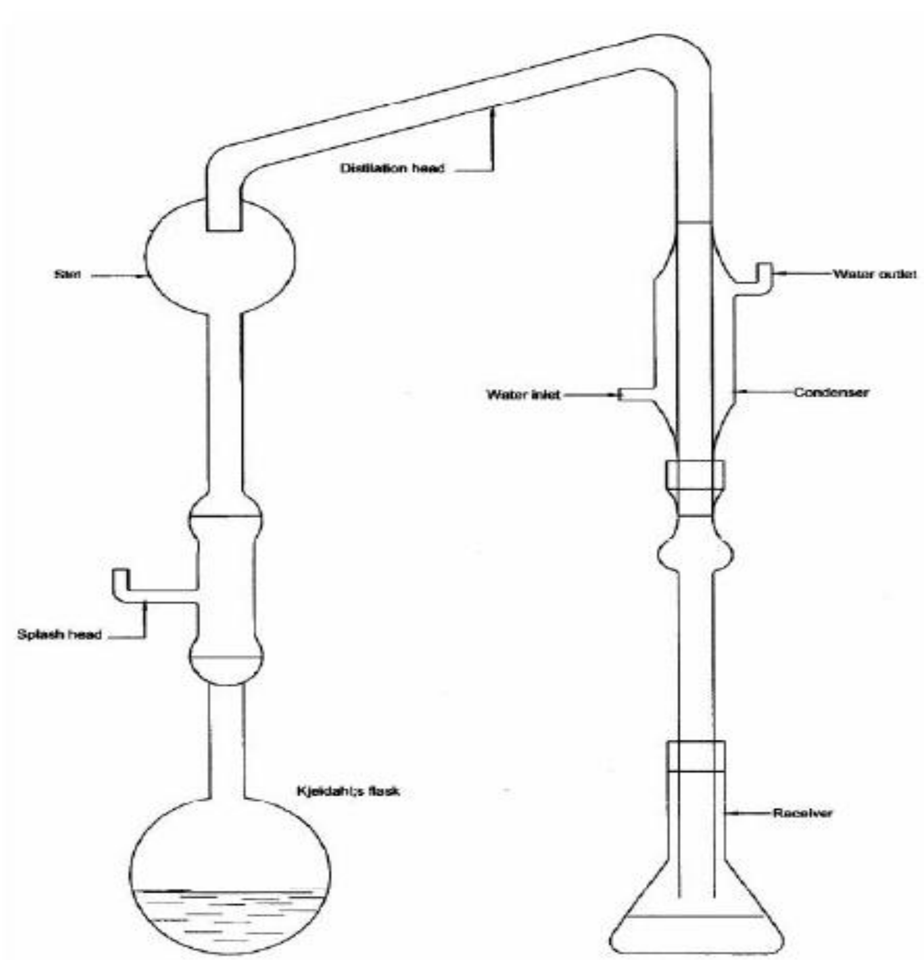
7.1.1 Apparatus

a) Separatory funnel, 250 ml

b) Volumetric flask, 1L capacity

c) Distillation assembly having Kjeldhal flask, 800 ml capacity

d) With splash head, Liebig condenser, Receiver of capacity 250 ml



7.1.2 Reagents

a) Sulphuric acid GR grade
b) Oxidizing mixture: Dissolve Potassium dichromate, 100g in 500 ml distilled water and add sulphuric acid, 100ml and make up to 1L volume with distilled water.
c) Standard NaOH, 0.1N
d) Carbon tetrachloride GR grade, distilled
e) Sodium chloride GR grade
f) Sodium sulphate, AR grade
g) Phenolphthalein indicator

7.1.3 Procedure

i) Transfer the solution obtained from the determination of esters (See sec 6.2.1) into a separatory funnel and add 50ml of distilled water.
ii) Saturate it with sodium chloride and extract four times with successive portions of 40, 30, 20 and 10ml of carbon tetrachloride.
iii) Pool all the extracts and wash 3 times with saturated sodium chloride solution and twice with saturated sodium sulphate solution. Filter the extract and add 50 ml of oxidizing mixture. Reflux for 2 hours, cool and wash the reflux with 50 ml of distilled water.
iv) Transfer it to the distillation assembly using 50ml of water. Distil about 100ml and see that no charring takes place. Titrate the distillate against standard NaOH using phenolphthalein indicator.
v) Run a blank in the same way taking 50 ml of distilled water in place of the distillate of the liquor.

7.1.4 Calculation

$$\text{Higher alcohol expressed Amyl alcohol, in gms. Per 100 liters of abs. alcohol} = \frac{V \times 0.0088 \times 100 \times 1000 \times 2}{V1 \times V2}$$

Where, V = difference of titer value of Std.alkali used for blank and sample, in ml

V1 = Volume of sample taken for estimation

V2 = alcohol % by volume

Note: 1 ml of 0.1N NaOH is equivalent to 0.0088g of Amyl alcohol

7.3 Method II- Spectrophotometric method

7.3.1 Apparatus

a) Spectrophotometer, double beam

b) Steam bath

c) Test tube, stoppered, 15 ml capacity

7.3.2 Reagents

a) p-dimethylaminobenzaldehyde solution – Dissolve 1 g in a mixture of 5ml sulphuric acid and 90 ml distilled water and transfer to a 100ml volumetric flask and make up to the mark.

b) Iso-butyl alcohol, GR grade

c) Iso-amyl alcohol, GR grade

d) Ethyl alcohol, redistilled, middle 50% fraction.

7.3.3 Preparation of Synthetic standard of higher alcohols:

i) Weigh 2 g isobutyl alcohol and 8 g iso-amyl alcohol into 1 L volumetric flask and dilute to mark with water.
ii) Pipette two 10ml portions into 100ml volumetric flasks and dilute to mark, one with water and other with ethyl alcohol.
iii) Prepare working standards for products in the range of 1.0 to 6.0 g synthetic higher alcohol per 100L 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).

7.3.4 Procedure

7.3.4.1 Preparation of sample:

a) 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. Distil the contents in about 35 min and collect the distillate in a 200 ml volumetric flask till the volume almost reaches the mark. Bring the distillate to room temperature and make up to volume with distilled water and mix thoroughly.

b) For samples containing 6 g fusel oil per 100 L, dilute the distilled sample with distilled water to concentrations of 2.0 to 5.0 g/100L.

7.3.4.2 Determination:

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). Stopper and place it in ice-bath in a rack. Pipette 1 ml p-dimethylaminobenzaldehyde solution into each tube; shake and replace in ice-bath for 3 min. With tubes retained in ice- bath, add 10 ml sulphuric acid and shake the tubes and replace in ice-bath for 3 min. Transfer the rack containing tubes into steam bath for 3 to 5 min. and bring it to room temperature. 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. Plot higher alcohol g/100 L Concentrations of Standards Vs. %T or OD. From the OD of the sample find out the concentration of Higher alcohol g/100L using the standard curve.

8.0 Determination of Aldehydes

8.1 Titrimetric method

8.1.1 Apparatus

a) Iodine flask, 250 ml capacity

b) Burette, 25/50 ml capacity

8.1.2 Reagents

a) Sodium bisulphite solution – 0.05N

b) Iodine standard solution – 0.05 N

c) Sodium thiosulphate standard – 0.05 N

d) Starch indicator – 1% solution

8.1.3 Procedure:

i) Take 50 ml of distillate of liquor (Sec. 2.1.2.1) in a 250 ml Iodine flask and add 10 ml of bisulphite solution. Keep the flask in a dark place for 30 min. with occasional shaking.

ii) Add 25 ml of standard iodine solution and back titrate excess iodine against standard thiosulphate solution using starch indicator to light green end point.

iii) Run a blank taking 50 ml of distilled water in the same way.

iv) The difference in titer value in milliliters, of sodium thiosulphate solution gives the equivalent aldehyde content.

8.1.4 Calculation:

Aldehydes expressed acetaldehyde, gms. per 100 liters of abs. alcohol =

$$\frac{V \times 0.0011 \times 100 \times 1000 \times 2}{V1}$$

Where, V1 = 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 0.0011g of Acetaldehyde.

9.0 Determination of Furfural

9.1 Apparatus

a) Nessler tubes with flat bottom tubes of thin high quality glass, 25mm in diameter and 150 mm in length and graduated at 50ml.

9.2 Reagents

a) Aniline, distilled and colourless.

b) Hydrochloric acid, sp. gr. 1.125.

9.2.1 Furfural free alcohol

a) Let alcohol containing 5g 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.

b) Let stand overnight and distill, rejecting the first 100 ml and the last 200 ml of the distillate. If this gives a coloration with aniline hydrochloride, repeat the treatment.

9.2.2 Standard furfural solution

a) Dissolve 1 g of redistilled, colourless furfural in 100 ml of the furfural free alcohol.

b) Prepare standard furfural solution by diluting 1 ml of this solution to 100 ml with50 % furfural free alcohol. 1 ml of this diluted solution contains 0.1 mg of furfural (strong furfural solution shall retain its strength but the diluted standard solution should be prepared afresh every time).

9.2.3 Procedure

a) Take 5 ml of the distillate obtained for ethanol determination, (Sec. 2.1.2.1), 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.

b) Dilute a measured portion of the distillate with 50 % furfural free alcohol to 50 ml. First add 2 ml of the colourless aniline and then 0.5 ml of hydrochloric acid. Mix and keep at 15 °C for 15 min. Compare the colour developed with standard furfural solution by using a Nessler comparator.

9.2.4 Calculation

$$\text{Furfural, grams per 100 litres of absolute alcohol} = \frac{W \times 1000 \times 100 \times 100}{V1 \times V2}$$

Where, W = is the weight in grams of the furfural present in volume used for matching the experimental solution;

V1 = volume of experimental solution used for estimation; and

V2 = alcohol, % by volume

10.0 Determination of Copper / Iron

10.1 Atomic absorption Spectrophotometric (AAS) Method

10.2 Apparatus

a) Atomic absorption Spectrophotometer (AAS) – Double beam

b) Hollow Cathode Lamp –Copper

c) (Follow operating instructions of manufacturer for the selection of optimum gas flow, wavelength settings and beam alignment.

d) Microwave Digester with Quartz tubes for digestion

e) Muffle furnace

f) Fume Hood

g) Steam bath

h) Silica crucible

i) Acetylene Ultra pure grade

10.3 Reagents

a) Nitrogen – Ultra pure grade

b) Water – triple distilled or Milli-Q /18Ω

c) Copper SRM and Iron SRM (100 µg/ml) traceable to NIST

d) Alcohol- distilled

10.3.1 Preparation of Cu / Fe working standard solutions:

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.

10.3.2 Procedure

10.3.2.1 Preparation of Ash solution:

(In case of wine samples high residue is expected and therefore, 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. Whereas, for liquor samples such as rum, gin, whisky etc., direct injection of the Millipore filtered liquor to AAS may be done to determine the quantity of copper present in the sample.)

a) Wet Ashing: Take 50 to 100 ml of wine sample in a glass bowl and evaporate to dryness. Add 5 ml of ultra pure nitric acid and transfer to the quartz tube of microwave digester using little distilled water. Pressure Digest the solution in microwave digestion apparatus for 30 min. Cool and make up to 25 ml volume.

b) Blank Solution: Prepare a blank by taking 5 ml of ultrapure nitric acid and make up to 25 ml volume.

10.3.2.2 Determination

Aspirate the blank into the AAS flame and set the instrument for zero absorbance. Aspirate the Cu/Fe Std. solutions sequentially for absorbance data acquisition. 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. Calculate the concentration in the test sample involving the dilutions made.

10.3.3 Calculation

(For directly aspirated liquor sample, dilution part will not appear in the calculation)

$$\text{Copper / Iron content in wine (in µg/ml or mg/L)} = \frac{\text{Reading (in µg) displayed x Dilution}}{\text{Weight of sample}}$$

(For Detailed Metal estimation Procedure - Refer Manual of Methods for Food additives)

11.0 Determination of Methyl alcohol

11.1 Spectrophotometric method

11.1.1 Apparatus

a) Separating funnel

b) Spectrophometer

11.1.2 Reagents

a) Potassium permanganate solution: 3.0 g KMnO4 and 15.0 ml H3PO4 shall be dissolved in 100ml water. The solution shall be prepared monthly.

b) 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.

11.1.3 Purification of chromotropic acid:

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 H2SO4 shall be added to the aqueous solution of the salt to convert it to free acid). Add 50 ml of methanol and heat to just boiling and filter. Add 100 ml isopropyl alcohol to precipitate free chromotropic acid. More isopropyl alcohol may be added to increase yield of purified acid.

11.1.4 Methanol Stock solution:

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.

11.1.5 Methanol Standard solution:

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.

11.1.6 Procedure:

i) Take 50 ml of sample in a simple still and distil, collecting about 40ml of distillate. Dilute 1 ml of distillate to 5ml with distilled water and shaken well.
ii) 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.

iii) Add to each test tube, 2 ml of KMnO 4 reagent and keep aside for 30 min.

iv) Decolourize the solution by adding a little sodium bisulphite and add 1 ml of chromotropic acid solution.

v) Mix well and add 15ml of sulphuric acid slowly with swirling and place in hot water bath maintaining 800C for 20 min. Observe the colour development from violet to red.

vi) Cool the mixture and measure the absorbance at 575 nm using 1cm cuvette cell.

11.1.7 Calculations

Calculate methanol content in g/100 Litres of absolute alcohol as follows:

$$\text{Methanol} = \frac{A2 \times C \times D \times 1000 \times 100 \times 100}{A1 \times S}$$

Where,

A2 = absorbance of sample solution

C = concentration of methanol std. solution

D = dilution factor for sample solution

A1 = absorbance of methanol std. solution

11.2 Gas chromatographic method

11.2.1 Apparatus

a) Gas Chromatograph, FID Detector, Split injection port, fixed with capillary column of HP Carbowax 20M of 25m x 0.32mm ID or SPB 20 capillary column of 30m x 0.25mm ID. N2 or He as carrier gas at a flow rate of 1.0ml/min. The detector and injector port temperatures are at 250^0 C. Oven temperature is at 45^{0}C for 4 min and then raise to100^{0}C / min at the rate of 10^0 C/min and finally at 200^{0}C for 10min at the rate of 15^{0}C/min. (Optimum operating conditions may vary with type of column used and instrumental characteristics).

b) Syringe - 10µL, Hamilton Co., or equivalent.

11.2.2 Reagents

a) Ethanol – Methanol free

b) N-Pentanol Internal standard – 0.05% w/v n-pentanol in 40% ethanol (v/v).

c) Methanol Stock solution: 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.

d) 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.

11.2.3 Procedure

i) Transfer 5 ml of sample into a 10 ml stoppered test tube and add 1ml of n- pentanol internal standard and mix well.

ii) Inject 2 µL of methanol standard solution into GC and record the chromatographic profile.

iii) Adjust the operating parameters and attenuation to obtain good resolution of the peaks.

iv) Determine the retention time of methanol and n-pentanol.

v) Inject 2 µL sample solution into GC and record the chromatogram.

11.2.4 Calculation

$$\text{Methanol, in grams /100L of Absolute alcohol} = \frac{R2 \times C \times D \times 1000 \times 100 \times 100}{R1 \times S}$$

Where,

R2 = 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

R1 = peak ratio of methanol to n-pentanol for std. solution

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

12.0 Determination of Sulphur Dioxide (for Wines only)

12.1 Modified Monier Williams Method

12.1.1 Apparatus

a) Round bottom flask – 500ml capacity connected to N2 or CO2 inlet source, coiled condenser, receiver and trap as shown in the figure.

12.1.2 Reagents

a) Hydrogen Peroxide solution – Dilute a 30 % Hydrogen peroxide solution with distilled water so as to obtain a 3 % solution of hydrogen peroxide.

b) Sodium hydroxide – 0.01N

c) Bromophenol indicator solution – Dissolve 0.1 gm 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.

d) Concentrated Hydrochloric acid – sp gr 1.16

e) Carbon dioxide gas from a cylinder.

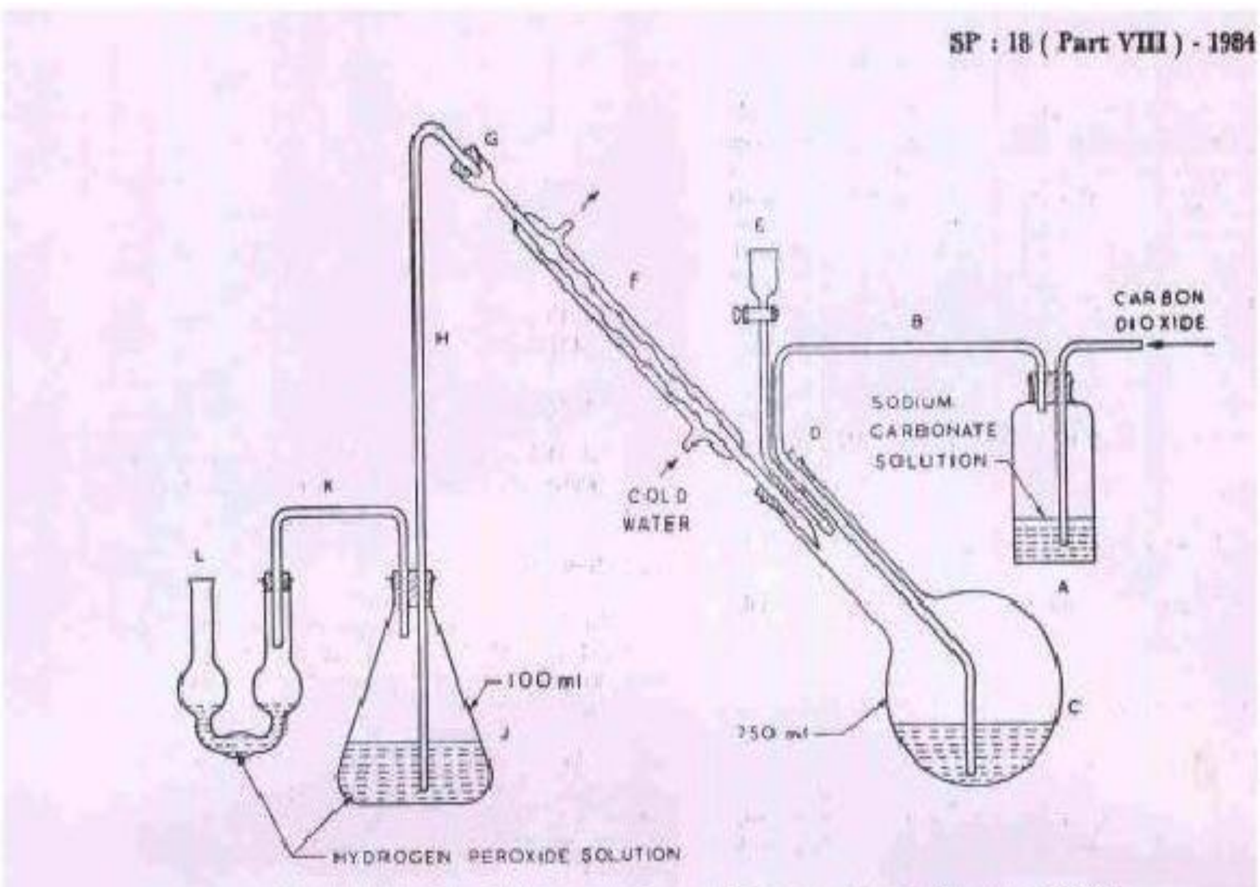


FIG. 1 ASSEMBLY OF APPARATUS FOR THE DETERMINATION OF SULPHUR DIOXIDE

12.1.3 Procedure

Transfer 25 ml of Hydrogen peroxide solution to Erlenmeyer flask (J) and 5 ml to Peligot tube (L), Assemble the apparatus as shown above. Introduce into the flask (C) 300 ml water and 20 ml of conc.HCl through the the dropping funnel (E). Run a steady current of cold water through the condenser (F). 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). Weigh accurately about 25 gm 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). Distill by heating the mixture contained in the flask (C) in a slow current of Carbon dioxide gas passed previously through the wash bottle (A) for 1 hour. 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). When the delivery tube (H) just above the Erlenmeyer flask (J) becomes hot to touch disconnect the stopper (G) immediately. Wash the delivery tube (H) and the contents of the Peligot tube (L) with water into the Erlenmeyer flask (J). 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). 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.

12.1.4 Calculation

$$\text{Sulphur Dioxide mg / kg} = \frac{32000\,(V - v)\,N}{W}$$

Where,

V = volume in ml of standard sodium hydroxide solution required for the test with sample

v = volume of standard sodium hydroxide solution required for the blank determination

N = normality of standard sodium hydroxide solution

W = weight in gm of the sample taken for test

13.0 Determination of Tannins (for Wines only)

13.1 Spectrophotometric Method

13.1.1 Reagents and Instruments

a) Preparation of Folin-Dennis reagent:

Prepare by adding 100 g Sodium tungstate (Na2WO4.2H2O), 20 g Phosphomolybdic acid and 50 ml phosphoric acid to 750 ml water and reflux for 2 hours and dilute to 1 litre.

b) Preparation of Sodium carbonate solution:

Prepare by adding 35 g anhydrous Sodium carbonate to 100 ml water at about 800C. Allow to cool overnight and seed with few crystals of sodium carbonate. Filter.

c) Preparation of standard Tannic acid solution:

Prepare fresh daily, by dissolving 100 mg Tannic acid in 1000 ml water.
(1 ml = 0.1mg of tannic acid).

d) Spectrophotometer, Double beam with a working wavelength range of 350-800nm and band width 5nm.

13.1.2 Preparation of standard curve

i) 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.

ii) Add 5 ml Folin-Dennis reagent and 10 ml sodium carbonate solution. Make up to volume. Mix well and after 30 min. determine absorbance of each standard using reagent blank.

iii) Plot absorbance against mg of tannic acid and use the graph for the determination of concentration of tannin in wine.

13.1.3 Procedure

i) Pipette 1 ml of wine into a 100 ml volumetric flask containing about 80 ml water.

ii) Add 5 ml Folin-Dennis reagent and 10 ml sodium carbonate solution. Make up to volume. Mix well and after 30 minutes, against reagent blank read the absorbance.

iii) If the absorbance is beyond 0.8, dilute the solution 1:4 times and read.

13.1.4 Calculation

Obtain the mg of tannic acid using the standard curve and calculate to express the value in g/L of wine.

14.0 Determination of Extracts in Wines

14.1 Evaporation Method

14.1.1 Apparatus

a) Pipette, 50 ml
b) Evaporating dishes, aluminium , flat bottom with lids, 75ml capacity
c) Oven- calibrated to maintain temperature of 100 ± 20C
d) Steam bath
e) Desiccators
f) Electronic balance, 0.1 mg sensitivity

14.1.2 Procedure

i) Weigh, dried and cooled aluminium dish (W1).

ii) Mix the wine sample well and draw 50 ml sample (dry wines) or 25ml sample (sweet wines) into the aluminium dish and evaporate on steam bath to almost dryness.

iii) Transfer the dish to an air oven maintained at 1000C and dry for 4-5 hours.

iv) Remove the dish and cool in a desiccator and weigh to constant weight (W2).

v) Calculate the extract in g/L of wine.

14.1.3 Calculation

$$\text{Extract, g/L} = \frac{(W2 - W1) \times 1000}{\text{Volume of sample}}$$

15.0 References

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

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

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

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

5. I.S.I.Hand book of Food Analysis (Part VIII) – 1984 page 12, Determination of Sulphur dioxide.

ANNEXURE-I

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

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

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

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

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

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

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

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

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

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

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

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

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

Sp.gr 20/20 ^{0}C	% by vol	Sp.gr 20/20 ^{0}C	% by vol
0.9077	61.36	0.9027	63.53
0.9076	61.4	0.9026	63.57
0.9075	61.44	0.9025	63.62
0.9074	61.49	0.9024	63.66
0.9073	61.53	0.9023	63.7
0.9072	61.58	0.9022	63.75
0.9071	61.62	0.9021	63.79
0.907	61.66	0.902	63.83
0.9069	61.71	0.9019	63.88
0.9068	61.75	0.9018	63.92
0.9067	61.79	0.9017	63.96
0.9066	61.84	0.9016	64
0.9065	61.88	0.9015	64.05
0.9064	61.93	0.9014	64.09
0.9063	61.97	0.9013	64.13
0.9062	62.01	0.9012	64.18
0.9061	62.06	0.9011	64.22
0.906	62.1	0.901	64.26
0.9059	62.14	0.9009	64.3
0.9058	62.19	0.9008	64.35
0.9057	62.23	0.9007	64.39
0.9056	62.27	0.9006	64.43
0.9055	62.32	0.9005	64.47
0.9054	62.36	0.9004	64.52
0.9053	62.4	0.9003	64.56
0.9052	62.45	0.9002	64.6
0.9051	62.49	0.9001	64.65
0.9	64.69	8950	66.79
0.8999	64.73	0.8949	66.83
0.8998	64.77	0.8948	66.87
0.8997	64.82	0.8947	66.92
0.8996	64.86	0.8946	66.96
0.8995	64.9	0.8945	67
0.8994	64.94	0.8944	67.04
0.8993	64.99	0.8943	67.08

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

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

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

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

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

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

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

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

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

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

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*The methods mentioned in the manual needs to be verified/ validated before they are put in use by the laboratory.

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