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INTERNATIONAL ORGANIZATION FOR STANDARDIZATION

ISO RECOMMENDATION

R 1388

ETHANOL FOR INDUSTRIAL USE

METHODS OF TEST

1st EDITION

June 1970

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BRIEF HISTORY

The ISO Recommendation R 1388, *Ethanol for industrial use – Methods of test,* was drawn up by Technical Committee ISO/TC 47, *Chemistry,* the Secretariat of which is held by the Ente Nazionale Italiano di Unificazione (UNI).

Work on this question led to the adoption of Draft ISO Recommendation No. 1388, which was circulated to all the ISO Member Bodies for enquiry in February 1968. It was approved, subject to a few modifications of an editorial nature, by the following Member Bodies :

Austria
Belgium
Brazil
Chile
Cuba
Czechoslovakia
Germany
Hungary
India
Iran
Italy
Japan
Korea, Rep. of
Netherlands
New Zealand
Poland
Romania
South Africa, Rep. of
Spain
Sweden
Switzerland
Thailand
Turkey
United Kingdom
U.S.S.R.

The following Member Bodies opposed the approval of the Draft :

France
Portugal

This Draft ISO Recommendation was then submitted by correspondence to the ISO Council, which decided to accept it as an ISO RECOMMENDATION.

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ISO Recommendation R 1388 June 1970

ETHANOL FOR INDUSTRIAL USE

METHODS OF TEST

INTRODUCTION

This ISO Recommendation gives general test methods for ethanol for industrial use, and results of test methods are given, where appropriate, as percentages by mass.

In some countries, however, certain impurities are calculated as milligrammes per 100 ml or per litre of 100 % ethanol. To convert the results obtained as percentages by mass by the methods in this ISO Recommendation to this basis, they should be multiplied by the factors $\frac{10^5 \rho}{P}$ and $\frac{10^6 \rho}{P}$ respectively,

where

ρ is the density of the sample at 20 °C, expressed in grammes per millilitre;

P is the percentage, by mass, of ethanol in the test portion.

1. SCOPE

This ISO Recommendation describes methods of test for ethanol for industrial use.

2. SAMPLE*

The laboratory sample should be of sufficient size for carrying out all the analyses. It should be preserved in a clean, dry and airtight glass-stoppered bottle of such a size that it is nearly filled by the sample and, if indicated by certain tests, the bottle should be kept cold and stored in the dark.

If it has been necessary to seal the container, care should be taken to avoid contaminating the contents in any way.

3. DETERMINATION OF DENSITY AT 20 °C

Use the method described in ISO Recommendation R 758, *Method for the determination of density of liquids at 20 °C.*

NOTE. – Other methods or different conditions may be used in each country, if required by the local Excise regulations.

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* Sampling of chemical products will form the subject of a further ISO Recommendation.

4. DETERMINATION OF RESIDUE ON EVAPORATION ON A WATER BATH

Use the method described in ISO Recommendation R 759, *Method for the determination of residue on evaporation on a water bath.*

NOTE. – If a residue of less than 0.001 % is to be determined, a 250 ml sample should be used, added to the basin in small increments.

5. DETECTION OF ALKALINITY AND DETERMINATION OF ACIDITY BY PHENOLPHTHALEIN

5.1 Principle

Titration with sodium hydroxide solution of a solution of the sample, after removal of carbon dioxide by boiling, using phenolphthalein as indicator.

5.2 Reagents

Distilled water or water of equivalent purity should be used in the test.

5.2.1 *Sodium hydroxide,* 0.1 N standard volumetric solution.

5.2.2 *Phenolphthalein,* 5 g/l ethanolic solution.

Dissolve 0.5 of phenolphthalein in 100 ml of 95 % (V/V) ethanol and make faintly pink by the addition of dilute sodium hydroxide solution.

5.3 Apparatus

Ordinary laboratory apparatus and

5.3.1 *Conical flask,* of borosilicate glass, capacity 500 ml, provided with a stopper carrying a soda-lime tube.

5.3.2 *Microburette,* graduated in 0.02 ml, or smaller divisions.

5.4 Procedure

Place 100 ml of water and a few pieces of clean porous porcelain into the flask (5.3.1) and boil gently for 5 minutes to eliminate carbon dioxide. Cool slightly and add 100 ml of the laboratory sample. Boil gently for a further 10 minutes.

At the end of this period close the neck of the flask with the stopper carrying the soda-lime tube and allow to cool. When cold remove the stopper, add 0.5 ml of the phenolphthalein solution (5.2.2) and examine for alkalinity; if not alkaline, titrate the mixture with the sodium hydroxide solution (5.2.1) until the pink colour remains for 15 seconds.

5.5 Expression of results

Acidity, expressed as acetic acid (CH_3COOH), is given, as a percentage by mass, by the following formula :

$$\frac{0.006\,V}{\rho}$$

where

V is the volume, in millilitres, of the sodium hydroxide solution (5.2.1) used;

ρ is the density, in grammes per millilitre, of the sample at 20 °C.

6. ESTIMATION OF CARBONYL COMPOUNDS PRESENT IN SMALL AMOUNTS

(between 0.00025 and 0.0025 % of carbonyl compounds, calculated as acetaldehyde)

NOTE. – Although this method is used in commercial practice, it is not satisfactory for all types of carbonyl compounds.

6.1 Principle

Conversion in acid medium of the carbonyl compounds present with 2,4-dinitrophenylhydrazine into the corresponding 2,4-dinitrophenylhydrazones. In alkaline medium, these have a red colour, which is compared visually with colours produced by standard solutions, or its optical density is measured and the carbonyl compounds content found by reference to a calibration curve.

6.2 Reagents

6.2.1 *Ethanol,* purified.

Prepare by boiling 500 ml of ethanol under reflux for 2 hours with 5 g of 2,4-dinitrophenylhydrazine and a few drops of hydrochloric acid, (6.2.3) and distilling the mixture, rejecting the first and last 50 ml portions of distillate. The distillate should be colourless and this may be expected to contain not more than 0.0001 % (m/m) of carbonyl compounds calculated as acetaldehyde (CH_3CHO).

6.2.2 *2,4-dinitrophenylhydrazine,* saturated solution in the ethanol (6.2.1).

6.2.3 *Hydrochloric acid,* ρ 1.19 (g/ml), solution approximately 38 % (m/m).

6.2.4 *Potassium hydroxide,* 100 g/l solution in a 70 % (V/V) aqueous solution of ethanol (6.2.1).

6.2.5 *Standard ethanolic solution of carbonyl compounds.*

Dissolve 1.200 g of acetophenone in the ethanol (6.2.1) and dilute to 100 ml with the same ethanol. Dilute 10 ml of the solution to 100 ml with the same ethanol.

1 ml of this solution corresponds to 440 μg of carbonyl compounds calculated as acetaldehyde (CH_3CHO).

6.3 Apparatus

Ordinary laboratory apparatus and

6.3.1 *Water bath,* controlled at 50 °C.

6.3.2 *Spectrophotometer* set at 445 nm or *photoelectric absorptiometer* with a filter having miximum transmission at 445 nm, or *colorimetric tubes* of equal diameter, capacity about 20 ml, ground glass stoppered.

6.4 Procedure

6.4.1 *Instrumental procedure*

6.4.1.1 To 1 ml of the laboratory sample, contained in a test tube with ground glass stopper, add 1 ml of the 2,4-dinitrophenylhydrazine solution (6.2.2) and one drop of the hydrochloric acid solution (6.2.3).

Heat for 30 minutes in the water bath (6.3.1), allow to cool, add 5 ml of the potassium hydroxide solution (6.2.4) and, after 5 minutes, read the optical density of the solution using cells of the same thickness as used below in preparing the calibration curve, and using for comparison a solution containing the purified ethanol (6.2.1) in a blank test.

6.4.1.2 Prepare a calibration curve by treating in a similar manner 1 ml portions of solutions made by diluting 0.125, 0.25, 0.50, 0.75, 1.00 and 1.25 ml of the standard carbonyl compounds solution (6.2.5) to 25 ml with the ethanol (6.2.1).

6.4.1.3 If the optical density of the sample solution obtained in accordance with clause 6.4.1.1 is greater than that covered by the calibration curve, dilute the sample solution with an appropriate quantity of the ethanol (6.2.1) and repeat the procedure. (Take this dilution into account when calculating the results.)

6.4.2 *Visual procedure*

6.4.2.1 In a series of stoppered colorimetric tubes (6.3.2) place 1 ml each of the laboratory sample and of solutions made by diluting 0.125, 0.25, 0.50, 0.75, 1.00 and 1.25 ml of the standard carbonyl compounds solution (6.2.5) to 25 ml with the ethanol (6.2.1).

6.4.2.2 To each tube add 1 ml of the 2,4-dinitrophenylhydrazine solution (6.2.2) and one drop of the hydrochloric acid solution (6.2.3), and heat in the water bath (6.3.1) for 30 minutes. Allow to cool, add 5 ml of the potassium hydroxide solution (6.2.4) to each tube and after 5 minutes compare the colour in the tube containing the sample solution with those in the tubes containing the standards. In the event of doubt as to which of two standards is to be chosen as a match for the sample solution, take the mean of the two values as the match.

6.4.2.3 If the colour of the sample solution is deeper than that of the standards, dilute the sample with an appropriate quantity of the ethanol (6.2.1) and repeat the procedure. (Take this dilution into account when calculating the results.)

6.5 Expression of results

6.5.1 *Instrumental procedure.* Calculate the percentage by mass of carbonyl compounds in the test sample from the calibration curve and the density of the test sample.

6.5.2 *Visual procedure.* Carbonyl compounds, calculated as acetaldehyde (CH_3CHO), is given, as a percentage by mass, by the following formula :

$$\frac{0.00176\ V}{\rho}$$

where

V is the volume, in millilitres, of the standard carbonyl solution (6.2.5) used in preparing the standard dilution giving a colour most closely matching that of the sample;

ρ is the density, in grammes per millilitre, of the sample at 20 °C.

NOTE. – For the highest accuracy the instrumental procedure is to be preferred.

7. ESTIMATION OF CARBONYL COMPOUNDS PRESENT IN LARGER AMOUNTS

(above 0.05 % of carbonyl compounds, calculated as acetaldehyde)

NOTE. – Although this method is used in commercial practice, it is not satisfactory for all types of carbonyl compounds.

7.1 Principle

Liberation of hydrochloric acid by the reaction between hydroxylammonium chloride and any aldehyde or ketone present leading to the formation of an oxime.

Titration of this acid with sodium hydroxide solution, using bromophenol blue as indicator.

7.2 Reagents

Distilled water or water of equivalent purity should be used in the test.

7.2.1 *Sodium hydroxide,* 0.1 N standard volumetric solution.

7.2.2 *Hydrochloric acid,* 0.1 N standard volumetric solution.

7.2.3 *Bromophenol blue,* 2 g/l, ethanolic solution.

Dissolve 0.2 g of bromophenol blue in 1.5 ml of the sodium hydroxide solution (7.2.1) and dilute to 100 ml with 95 % (V/V) ethanol.

7.2.4 *Hydroxylammonium chloride reagent.*

Dissolve 4 g of hydroxylammonium chloride in 20 ml of water and dilute to 200 ml with 95 % (V/V) ethanol. Heat under reflux on a boiling-water bath for 30 minutes, cool and add 5 ml of bromophenol blue solution (7.2.3) together with just sufficient dilute sodium hydroxide solution to impart a dichroic green colour to the liquid.

7.3 Apparatus

Ordinary laboratory apparatus and

7.3.1 *Conical flasks,* glass-stoppered, capacity 150 ml.

7.4 Procedure

Measure 50 ml of the laboratory sample into one of the flasks (7.3.1) and add 0.25 ml of the bromophenol blue solution (7.2.3). Into a second flask measure 50 ml of the hydroxylammonium chloride reagent (7.2.4).

To the solution in the first flask add, drop by drop, the sodium hydroxide solution (7.2.1) or hydrochloric acid solution (7.2.2) until the colour matches that in the second flask.

Add to each of the flasks 25 ml of the hydroxylammonium chloride reagent (7.2.4) and stopper the second flask. Loosely stopper the first flask and heat it on a boiling-water bath for 10 minutes. Cool it, and titrate the contents with the sodium hydroxide solution (7.2.1) until as near a match as possible is obtained with the colour in the second flask.

7.5 Expression of results

Carbonyl compounds, calculated as acetaldehyde (CH_3CHO), is given, as a percentage by mass, by the following formula :

$$\frac{0.0088\,V}{\rho}$$

where

V is the volume, in millilitres, of sodium hydroxide solution (7.2.1) used;

ρ is the density, in grammes per millilitre, of the sample at 20 °C.

8. DETERMINATION OF ALDEHYDES

8.1 Principle

Comparison of the colour obtained by the reaction of aldehydes with Schiff reagent, with standards containing known amounts of acetaldehyde.

8.2 Reagents

Distilled water or water of equivalent purity should be used in the test.

8.2.1 *Schiff reagent.*

Place 1500 ml of water in a 3000 ml conical flask, add 4.500 ± 0.005 g of *p*-rosaniline hydrochloride (basic fuchsin), and swirl to dissolve. Add 9.60 ± 0.05 g of sodium metabisulphite ($Na_2S_2O_5$), mix and allow to stand for 5 to 10 minutes, then add 40 ml of 6 N sulphuric acid solution. Mix well, stopper and allow to stand overnight. Use activated carbon if necessary, to render the solution in the flask colourless.

Determine the sulphur dioxide content in the reagent in the following manner : Pipette 10 ml of the decolourized Schiff reagent into a 250 ml conical flask, add 20 ml of water and 5 ml of starch solution and titrate the free sulphur dioxide to a starch end point with standard 0.1 N iodine solution. The SO_2 content should be in the range of 2.8 to 4.8 millimoles of SO_2 per 100 ml of Schiff reagent. If it falls outside this optimum range, the SO_2 content should be raised by adding a calculated amount of sodium metabisulphite, or lowered by bubbling air through the reagent.

8.2.2 *Standard acetaldehyde solutions.*

Weigh 1.386 g of purified aldehyde ammonia (see Note 1) to an accuracy of 0.0002 g and dissolve it in 1000 ml of 95 % (V/V) aldehyde-free ethanol (see Note 2). This solution contains 1 g of acetaldehyde per litre. Dilute it to obtain a solution of 0.1 g acetaldehyde per litre. By further dilution with 95 % (V/V) aldehyde-free ethanol prepare standard solutions containing 0.002, 0.003, 0.005, 0.007, 0.009 and 0.01 g of acetaldehyde per litre.

NOTES

1. Commercial aldehyde ammonia should be purified as follows : Dissolve 5 g of aldehyde ammonia in a small quantity of absolute ethanol and then precipitate it by adding twice the volume of dry diethyl ether. Filter the crystals through a Büchner funnel, immediately wash with diethyl ether and then dry for 3 to 4 hours in a vacuum desiccator over concentrated sulphuric acid. The aldehyde ammonia purified in this way should be quite colourless. If it is not, the purification should be repeated.

2. Aldehyde-free ethanol should be prepared by boiling 500 ml of absolute ethanol under reflux for 2 hours with 5 g of *m*-phenylenediamine, then distilling it, rejecting the first 50 ml and the last 50 ml of the distillate. Adjust the concentration to 95 % (V/V) by addition of the appropriate volume of water and then mixing.

8.3 Apparatus

Ordinary laboratory apparatus and

8.3.1 *Colorimetric tubes*, each provided with a ground glass stopper, capacity about 20 ml, with two marks, at 10 ml and at 14 ml.

8.3.2 *Graduated pipettes*, capacity 5 ml and 10 ml, 0.02 ml graduations.

8.4 Procedure

8.4.1 Pipette 3 ml of the laboratory sample into one of the colorimetric tubes (8.3.1) and 3 ml of each of the standard solutions into the remaining tubes. Dilute the contents of all the tubes with water to 10 ml. Add to each tube the Schiff reagent (8.2.1) up to the 14 ml mark. Stopper the tubes, mix the solutions thoroughly (simultaneously, if possible) and place them in a rack for 25 minutes.

8.4.2 In dispersed daylight compare the colour of the test solution with those of the standards.

NOTE. – If the colour of the test solution is deeper than that of the most concentrated standard, repeat the determination using a sample diluted ten-fold with the aldehyde-free ethanol used in preparing the standard solutions. Apply an appropriate correction to the formula for calculation of result.

8.5 Expression of results

Aldehydes, calculated as acetaldehyde (CH_3CHO), is given, as a percentage by mass, by the following formula :

$$\frac{A}{10\,\rho}$$

where

A is the concentration, in grammes per litre, of acetaldehyde in the standard solution giving a colour closest to that of the test solution;

ρ is the density, in grammes per millilitre, of the sample at 20 °C.

9. TEST FOR MISCIBILITY WITH WATER

9.1 Principle

Addition of water to the test portion while shaking under the conditions of the test, and examination for any opalescence or cloudiness produced thereby.

9.2 Reagent

Distilled water or water of equivalent purity should be used in the test.

9.3 Apparatus

Ordinary laboratory apparatus and

9.3.1 *Two 100 ml Nessler cylinders,* similar in every respect.

9.4 Procedure

To the agreed volume of the sample contained in one of the cylinders, (9.3.1) add water to bring the volume to 100 ml, mix thoroughly and adjust the temperature to 20 °C.

Examine vertically for opalescence against a black background with side illumination, using as a standard the other cylinder containing 100 ml of water.

10. DETERMINATION OF METHANOL

(for methanol contents between 0.01 and 0.20 %)

10.1 Principle

Dilution of a test portion with water and oxidation with a solution of potassium permanganate in phosphoric acid, any methanol being transformed into formaldehyde; comparison of the intensity of the violet colour produced by reaction of the aldehyde with chromotropic acid against standards, or absorptiometric measurement.

10.2 Reagents

Distilled water or water of equivalent purity should be used in the test.

10.2.1 *Potassium permanganate solution in phosphoric acid.*

Dissolve 30 g of potassium permanganate in a mixture of 150 ml of phosphoric acid, ρ 1.75 (g/ml), and 700 ml of water. Dilute with water to 1000 ml.

10.2.2 *Sodium metabisulphite,* 100 g/l solution.

Dissolve 10 g of sodium metabisulphite ($Na_2S_2O_5$), in water and dilute to 100 ml.

10.2.3 *Chromotropic acid* solution.

Dissolve 0.1 g of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulphonic acid) in 10 ml of water, then, while cooling, add 90 ml of 90 % (m/m) sulphuric acid. The solution should be freshly prepared.

It is important that the chromotropic acid used should be very pure; if the chromotropic acid gives more than a negligible colour in the blank test, it should be purified or repurified as follows :

Dissolve 10 g of chromotropic acid, or its salt, in 25 ml of water. If the salt is used, add 2 ml of sulphuric acid ρ 1.84 (g/ml), to convert it to the free acid. Add 50 ml of methanol, heat just to boiling and filter. Add 100 ml of isopropyl alcohol to precipitate the free chromotropic acid.

NOTE. – If satisfactory results are not obtained, the sulphuric acid should be examined to ensure that nitric acid is not present to a greater extent than one part per million. If, after purification, the result of the blank is unacceptable, the chromotropic acid should be rejected.

10.2.4 *Standard methanol solutions.*

Prepare a 5 % (V/V) aqueous solution of ethanol, free from methanol.

Add to 1.00 ml of absolute methanol a quantity of methanol-free ethanol equivalent to 99 ml of anhydrous ethanol and dilute to 250 ml with water. Further dilute 25.0 ml of this solution to 200 ml with water. The resulting solution contains 1.0 % (V/V) of methanol with respect to the total alcohols present.

Take the following volumes of the diluted solution of methanol in ethanol and dilute to 100 ml with the 5 % aqueous ethanol solution : 1.00, 2.50, 5.00, 10.0 and 20.0 ml.

These standards thus contain respectively 0.010, 0.025, 0.050, 0.10 and 0.20 % (V/V) of methanol calculated on the total alcohols.

10.3 Apparatus

Ordinary laboratory apparatus and

10.3.1 *Colorimetric tubes,* capacity about 20 ml, preferably ground glass stoppered, or *spectrophotometer* set at approximately 570 nm or *photoelectric absorptiometer* with a filter having a maximum transmission at 570 nm.

10.4 Procedure

10.4.1 Dilute a volume of the laboratory sample equivalent to 5 ml of anhydrous alcohols to 100 ml with water in a one-mark volumetric flask.

10.4.2 Take 2 ml of the sample solution (10.4.1) and of each of the methanol standards (10.2.4) and to each add 1 ml of the potassium permanganate solution (10.2.1) and, after 15 minutes, 0.6 ml of the sodium metabisulphite solution (10.2.2). To the colourless solutions add, while cooling with ice, 10 ml of the chromotropic acid solution (10.2.3) and heat for 20 minutes in a water bath at 70 ± 2 °C.

10.4.3 *Visual procedure.* If the solutions are to be compared visually, note which methanol standard is closest in colour to the sample (in case of doubt take the lower concentration as matching).

10.4.4 *Instrumental procedure.* If a spectrophotometer or a photoelectric absorptiometer is used, prepare a calibration curve by measuring the optical densities of the methanol standards, and plotting these against methanol contents, and measure the optical density of the sample solution.

NOTE. – For the highest accuracy the instrumental procedure is to be preferred.

10.5 Expression of results

10.5.1 *Visual procedure.* Multiply the methanol content (as defined in clause 10.2.4) in the standard most nearly matching the sample by the factor $\frac{5}{V}$, where V is the volume, in millilitres, of the test portion, and express it as the percentage by volume of methanol in the sample.

10.5.2 *Instrumental procedure.* Multiply the methanol content (as defined in clause 10.2.4) read from the calibration curve by the factor $\frac{5}{V}$, where V is the volume, in millilitres, of the test portion, and express it as the percentage by volume of methanol in the sample.

11. DETERMINATION OF METHANOL

(for methanol contents between 0.10 and 1.50 %)

11.1 Principle

Dilution of a test portion with water and oxidation with a solution of potassium permanganate in phosphoric acid, any methanol being transformed into formaldehyde which is then determined colorimetrically using Schiff reagent.

11.2 Reagents

Distilled water or water of equivalent purity should be used in the test.

11.2.1 *Potassium permanganate, solution in phosphoric acid.*

Dissolve 3 g of potassium permanganate and 15 ml of phosphoric acid, ρ 1.75 (g/ml) in 100 ml of water.

11.2.2 *Oxalic acid, sulphuric acid solution.*

Dissolve 5 g of oxalic acid in 100 ml of 50 % (V/V) sulphuric acid.

11.2.3 *Schiff reagent.*

Place 1500 ml of water in a 3000 ml conical flask, add 4.500 ± 0.005 g of *p*-rosaniline hydrochloride (basic fuchsin), and swirl to dissolve. Add 9.60 ± 0.05 g of sodium metabisulphite ($Na_2S_2O_5$), mix and allow to stand for 5 to 10 minutes, then add 40 ml of 6 N sulphuric acid solution. Mix well, stopper and allow to stand overnight. Use activated carbon, if necessary, to render the solution in the flask colourless.

Determine the sulphur dioxide content of the reagent in the following manner : Pipette 10 ml of the decolourized Schiff reagent into a 250 ml conical flask, add 20 ml of water and 5 ml of starch solution and titrate the free sulphur dioxide to a starch end point with standard 0.1 N iodine solution. The SO_2 content should be in the range of 2.8 to 4.8 millimoles of SO_2 per 100 ml of Schiff reagent. If it falls outside this optimum range, the SO_2 content should be raised by adding a calculated amount of sodium metabisulphite, or lowered by bubbling air through the reagent.

11.2.4 *Standard methanol solutions.*

Prepare a 10 % (V/V) aqueous solution of ethanol, free from methanol.

Add to 2.00 ml of absolute methanol a quantity of methanol-free ethanol equivalent to 98 ml of anhydrous ethanol and dilute to 1000 ml with water. The resulting solution contains 2.0 % (V/V) of methanol with respect to the total alcohols present.

Take the following volumes of the diluted solution of methanol in ethanol and dilute to 100 ml with the 10 % aqueous ethanol : 5.00, 10.0, 25.0, 50.0 and 75.0 ml.

These standards thus contain respectively 0.10, 0.20, 0.50, 1.00 and 1.50 % (V/V) of methanol calculated on the total alcohols.

11.3 Apparatus

Ordinary laboratory apparatus and

11.3.1 *Colorimetric tubes,* capacity about 20 ml, preferably ground glass stoppered.

11.4 Procedure

11.4.1 Dilute a volume of the laboratory sample equivalent to 10 ml of anhydrous alcohols to 100 ml with water in a one-mark volumetric flask. Pipette 5 ml of this solution into one of the colorimetric tubes (11.3.1) and 5 ml of each of the standard solutions (11.2.4) into the remaining tubes. Add to each tube 2 ml of the potassium permanganate solution (11.2.1), mix well and allow to stand for 10 minutes in a water bath maintained at 20 ± 1 °C. Subsequently add 2 ml of oxalic acid solution (11.2.2) to each tube and mix again. To the colourless solutions, free from manganese precipitate, add 5 ml of Schiff reagent (11.2.3), mix well, and let stand for 1 hour.

11.4.2 Examine the tubes vertically and note which methanol standard is closest in colour to the solution containing the sample (in case of doubt take the lower concentration as matching).

11.5 Expression of results

Multiply the methanol content (as defined in clause 11.2.4) in the standard most nearly matching the test sample by the factor $\frac{10}{V}$ where V is the volume, in millilitres, of the test portion, and express it as the percentage by volume of methanol in the sample.

12. DETERMINATION OF ESTERS

12.1 Principle

Saponification of any esters present by boiling with standard volumetric sodium hydroxide solution, and determination of the excess by titration with standard hydrochloric acid solution.

12.2 Reagents

Distilled water or water of equivalent purity, freshly boiled and cooled, should be used in the test.

12.2.1 *Sodium hydroxide,* 0.1 N standard volumetric solution.

12.2.2 *Hydrochloric acid,* 0.1 N standard volumetric solution.

12.2.3 *Phenolphthalein,* ethanolic solution.

Dissolve 0.5 g of phenolphthalein in 100 ml of 95 % (V/V) ethanol and make faintly pink by the addition of dilute sodium hydroxide solution.

12.3 Apparatus

Ordinary laboratory apparatus and

12.3.1 *500 ml conical flask* of borosilicate glass, with ground glass joint, fitted with a water-cooled reflux condenser.

12.4 Preparation of apparatus

Clean the 500 ml flask fitted with the condenser by introducing ethanol, water and sodium hydroxide solution, heating and then washing out carefully.

12.5 Procedure

12.5.1 Measure into the flask 50 ml of the laboratory sample or 100 ml if less than 0.005 % of esters, calculated as ethyl acetate, is expected, add 20 ml of water and 0.5 ml of the phenolphthalein solution (12.2.3), then neutralize (usually by adding the sodium hydroxide solution (12.2.1), until the pink colour persists for 15 seconds).

12.5.2 Add 10.0 ml of the sodium hydroxide solution (12.2.1). Fix the condenser and heat in a boiling-water bath for 1 hour.

12.5.3 Fit a soda-lime tube to the condenser and cool the flask in water. Wash down the inside of the condenser with two 10 ml portions of water. Disconnect the flask and wash the joint with a further 10 ml of water.

12.5.4 Add the hydrochloric acid solution (12.2.2) from a burette until the pink colour is discharged and titrate the excess acid with the sodium hydroxide solution (12.2.1) until the pink colour persists for 15 seconds.

12.5.5 Carry out a blank test on the neutral titrated liquid, proceeding as above commencing at the addition of the 10 ml of the sodium hydroxide solution (12.2.1) specified in clause 12.5.2.

12.6 Expression of results

Esters, calculated as ethyl acetate ($CH_3COOC_2H_5$), is given, as a percentage by mass, by the following formula :

$$\frac{0.88\,[(V_2 - V_4) - (V_1 - V_3)]}{V\rho}$$

where

V_1 is the volume, in millilitres, of the hydrochloric acid solution (12.2.2) added in the test;

V_2 is the volume, in millilitres, of the hydrochloric acid solution (12.2.2) added in the blank test;

V_3 is the volume, in millilitres, of the sodium hydroxide solution (12.2.1) required by the test;

V_4 is the volume, in millilitres, of the sodium hydroxide solution (12.2.1) required by the blank test;

V is the volume, in millilitres, of the test portion;

ρ is the density, in grammes per millilitre, of the sample at 20 °C.

13. DETERMINATION OF WATER CONTENT

Use one of the methods described in ISO Recommendation R 760, *Determination of water by the Karl Fischer method.*

14. DETERMINATION OF HYDROCARBONS

14.1 Principle

Fractionation in presence of water, addition of sodium chloride to the distillate, and measurement of the volume of insoluble hydrocarbons.

14.2 Reagents

Distilled water or water of equivalent purity should be used in the test.

14.3 Apparatus

Ordinary laboratory apparatus and

14.3.1 *Hempel fractionating column,* height of filling 230 mm as illustrated in Figure 1.

14.3.2 *Liebig condenser,* jacket length minimum 400 mm.

14.3.3 *Condenser adaptor.*

14.3.4 *Round-bottomed flask,* capacity 4000 ml.

14.3.5 *Receiver for determination of hydrocarbons* as illustrated in Figure 2.

14.4 Procedure

Measure 1500 ml of the laboratory sample into the flask (14.3.4) and add 1500 ml of water. Fit the flask to the Hempel fractionating column (14.3.1) and attach the side arm of the column to the Liebig condenser (14.3.2), on the end of which the adaptor (14.3.3) is fitted. Heat the flask, so as not to allow the distillation rate to exceed 30 drops per minute and collect the distillate in the receiver (14.3.5). When the collected distillate reaches the mark on the constricted part of the receiver (13 ml of distillate), stop the distillation. Add sufficient saturated sodium chloride solution to the receiver to raise the liquid level to between the 1 ml and 2 ml graduations.

Mix the contents of the receiver by rolling it around, taking care however not to turn it upside down. Allow it to stand until the liquid settles in layers. Subsequently, bring the remaining drops of the hydrocarbon mixture on the walls into the upper layer by rolling the receiver and read the volume of hydrocarbon mixture on the graduated scale.

14.5 Expression of results

From the volume of the upper liquid layer read the hydrocarbon content of the sample as a percentage by volume, using the graph given in Figure 3.

15. TEST FOR THE ABSENCE OF FURFURAL

15.1 Principle

Reaction of any furfural present with aniline and acetic acid to give a red colour.

15.2 Reagents

15.2.1 *Aniline,* freshly distilled.

15.2.2 *Acetic acid,* glacial.

15.3 Apparatus

Ordinary laboratory apparatus.

15.4 Procedure

To the agreed volume of laboratory sample contained in a test tube, add 10 drops of the aniline (15.2.1) and 1 ml of the acetic acid (15.2.2).
Note if any red coloration, indicating the presence of furfural, appears immediately, or after a short time.

16. DETERMINATION OF PERMANGANATE TIME

NOTE. – This test should be carried out as soon as possible after receipt of the sample which should be stored in a cool dark place.

16.1 Definition

The permanganate time is defined as the number of minutes required for the colour of the sample to match that of the colour standard after adding 1 ml of the 0.2 g/l potassium permanganate solution to 50 ml of the sample.

16.2 Reagents

Distilled water or water of equivalent purity should be used in the test.

16.2.1 *Potassium permanganate,* 0.2 g/l solution, prepared immediately before use.

Water used in preparing the solution should be boiled for 30 minutes with sufficient diluted potassium permanganate solution to give a stable faint pink coloration, and then cooled.

16.2.2 *Cobalt (II) chloride-uranyl nitrate colour standard.*

Weigh accurately 2.50 g of cobalt (II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) and 2.80 g of uranyl nitrate hexahydrate [$UO_2(NO_3)_2 \cdot 6H_2O$] into a 1000 ml one-mark volumetric flask, dissolve in water, add 10 ml of 2 N nitric acid solution, dilute to the mark and mix thoroughly.

16.3 Apparatus

Ordinary laboratory apparatus and

16.3.1 *Two 100 ml cylinders* of colourless, transparent glass graduated at 50 ml, with ground glass stoppers.

16.3.2 *Burette,* 10 ml, graduated in 0.05 ml divisions.

16.3.3 *Thermometer,* range to 50 °C, graduated in 0.1 or 0.2 °C.

16.4 Procedure

16.4.1 Rinse one of the cylinders (16.3.1) with the laboratory sample, then fill it to the mark with the laboratory sample and place in a water bath at a constant temperature of 15 ± 0.2 °C. After 15 minutes take the cylinder from the bath, and using the burette (16.3.2) add 1 ml of the potassium permanganate solution (16.2.1) . Note the time. Stopper the cylinder immediately. Shake well and place the cylinder in the bath again.

16.4.2 Observe the change of colour of the solution and compare the colour by taking the cylinder out of the bath from time to time, and placing it against a white background, next to the second cylinder (16.3.1) filled to the mark with the colour standard (16.2.2). Take care not to expose the test solution during the test to the direct action of sunlight. Note the time at the moment at which the colour of the test solution becomes equal to that of the colour standard.