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(Acts whose publication is obligatory)

COMMISSION REGULATION (EEC) No 3590/83
of 13 December 1983
determining the Community methods applicable in the wine sector for the analysis of neutral alcohol

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Regulation (EEC) No 337/79 of 5 February 1979 on the common organization of the market in wine (1), as last amended by Regulation (EEC) No 1595/83 (2), and in particular Articles 11 (5), 12a (5), 15 (9), 39 (8), 40 (6) and 41 (7) thereof,

Whereas, under Council Regulation (EEC) No 2179/83 of 25 July 1983 laying down general rules for distillation operations involving wine and the by-products of wine making (3), neutral alcohol obtained by the distillation operations in the wine-growing sector must be as defined in the Annex to that Regulation on the basis of criteria relating to its composition; whereas Community methods of analysis should be adopted in order to check whether the criteria have been complied with;

Whereas, to the extent possible, the Community methods of analysis adopted should be generally recognized methods and should be published in all the official languages of the Community in order to ensure that they are applied on a uniform basis;

Whereas suitable transitional methods should be adopted to take into account neutral alcohol already produced by distillation operations adopted in respect of the 1983/84 wine-growing year;

Whereas the measures provided for in this Regulation are in accordance with the opinion of the Management Committee for Wine,

HAS ADOPTED THIS REGULATION:

Article 1

1. The Community methods for the analysis of neutral alcohol as defined in the Annex to Regulation (EEC) No 2179/83 shall be as set out in the Annex to this Regulation.

2. The methods of analysis specified in paragraph 1 shall apply to neutral alcohol obtained, as from the date of entry into force of this Regulation, by the distillation operations provided for in Regulation (EEC) No 337/79.

In the case of neutral alcohol obtained before the date laid down in the first subparagraph, by distillation operations adopted in respect of the 1983/84 wine-growing year, Member States shall use the methods of analysis normally used on their territory.

Article 2

This Regulation shall enter into force on the third day following its publication in the *Official Journal of the European Communities*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 13 December 1983.

For the Commission
Paul DALSAGER
Member of the Commission

(1) OJ No L 54, 5. 3. 1979, p. 1.
(2) OJ No L 163, 22. 6. 1983, p. 48.
(3) OJ No L 212, 3. 8. 1983, p. 1.

ANNEX

COMMUNITY METHODS APPLICABLE IN THE WINE SECTOR FOR ANALYSIS OF NEUTRAL ALCOHOL

1. INTRODUCTION

1. PREPARATION OF THE SAMPLE FOR ANALYSIS

1.1. General

The volume of laboratory sample intended for analysis must normally be 1,5 l unless a larger quantity is required for a specific determination.

1.2. Sample preparation

The sample shall be made homogeneous before analysis.

1.3. Preservation

The prepared sample shall always be kept in an air-tight and moisture-tight container and stored so that deterioration is prevented; in particular seals of cork, rubber and plastic should not come into direct contact with the alcohol and the use of sealing wax is expressly prohibited.

2. REAGENTS

2.1. Water

2.1.1. Wherever mention is made of water for solution, dilution or washing purposes, distilled water, or demineralized water of at least equivalent purity shall be used.

2.1.2. Wherever reference is made to 'solution' or 'dilution', without further indication of a reagent, an aqueous solution is intended.

2.2. Chemicals

All chemicals shall be of analytical reagent quality except where otherwise specified.

3. EQUIPMENT

3.1. List of equipment

The list of equipment contains only those items with a specialized use and items with a particular specification.

3.2. Analytical balance

Analytical balance means a balance with a sensitivity of 0,1 mg or better.

4. EXPRESSION OF RESULTS

4.1. Results

The result stated in the analytical report is the mean value obtained from at least two determinations, the repeatability (r) of which is satisfactory.

4.2. Calculation of results

Except where otherwise specified, the results shall be calculated as g per hl of alcohol at 100 % vol.

4.3. Number of significant figures

The result shall not contain more significant figures than are justified by the precision of the method of analysis used.

2. METHOD 1: DETERMINATION OF ALCOHOL CONTENT

The alcoholic strength by volume of the alcohol shall be determined in accordance with existing national provisions or, in the event of dispute, by means of alcoholometers or hydrometers as defined in Council Directive 76/765/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to alcoholometers and alcohol hydrometers (1).

It shall be expressed as percentage by volume, as prescribed by Council Directive 76/766/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to alcohol tables (2).

3. METHOD 2: EVALUATION OF COLOUR AND/OR CLARITY

1. SCOPE AND FIELD OF APPLICATION

The method enables the colour and/or clarity of neutral alcohol to be assessed.

2. DEFINITION

The colour and/or clarity: the colour and/or clarity as assessed by the procedure specified.

3. PRINCIPLE

The colour and clarity are assessed visually by comparison with water against a white background and a black background respectively.

4. APPARATUS

Glass cylinders, colourless, at least 40 cm in height.

5. PROCEDURE

Place two glass cylinders (4) on the white background or black background and fill one cylinder with sample to a depth of approximately 40 cm and the other with water to the same depth.

Observe the sample from above i.e. through the length of the cylinder, and compare it with the comparison cylinder.

6. INTERPRETATION

Assess the colour and/or clarity of the sample when observed as set out in 5.

4. METHOD: DETERMINATION OF PERMANGANATE CLEARING TIME

1. SCOPE AND FIELD OF APPLICATION

The method determines the permanganate clearing time of neutral alcohol.

2. DEFINITION

The permanganate clearing time, as determined by the method specified, is the number of minutes required for the colour of the sample to match that of the colour standard after adding 2 ml of a 0,2 g/l potassium permanganate solution to 50 ml of the sample.

3. PRINCIPLE

The time for the colour of the sample, after addition of potassium permanganate, to match that of a colour standard is determined and defined as the permanganate clearing time.

(1) OJ No L 262, 27. 9. 1976, p. 143.
(2) OJ No L 262, 27. 9. 1976, p. 149.

4. REAGENTS

4.1. **Potassium permanganate solution, 0,2 g/l.** Prepare immediately before use.

4.2. **Cobalt chloride solution.** Weigh accurately 5,0 g of cobalt (II) chloride hexahydrate ($Co\ Cl_2 \cdot 6\ H_2O$) into a 100-ml volumetric flask and dissolve in a little water. Add 2,5 ml of concentrated hydrochloric acid and then make up to the mark with water.

4.3. **Uranyl nitrate solution.** Weigh accurately 4,0 g of uranyl nitrate hexahydrate ($UO_2(NO_3)_2 \cdot 6H_2O$) into a 100 ml volumetric flask and dissolve in a little water. Add 2,5 ml of concentrated hydrochloric acid and then make up to the mark with water.

4.4. **Colour reference standard.** Pipette 5,0 ml of the cobalt chloride solution (4.2) and 7,0 ml of the uranyl nitrate solution (4.3) into a 50-ml volumetric flask and make up to the mark with water.

5. APPARATUS

5.1. Nessler tubes of 100 ml, colourless transparent glass graduated at 50 ml, with ground glass stoppers.

5.2. Pipette, 2 ml.

5.3. Thermometer, range up to 50 °C, graduated in 0,1 or 0,2 °C.

5.4. Analytical balance.

5.5. Water bath, thermostatically controlled at 20 ± 0,5 °C.

5.6. Volumetric flasks, 50 and 100 ml with ground glass stoppers.

6. PROCEDURE

6.1. Rinse a tube (5.1) with the laboratory sample, fill to the mark with the sample and then place in a water bath (5.5) at a constant temperature of 20 ± 0,5 °C. After 20 minutes remove the tube from the bath, add 2 ml of potassium permanganate solution (4.1) from the pipette (5.2). Note the time. Stopper the tube immediately. Shake well and place the tube in the bath again.

6.2. Observe the change of colour of the solution and compare the colour by taking the tube out of the bath from time to time and placing it against a white background next to a second tube filled to the mark with the colour standard (4.4). Take care not to expose the test solution during the test to the action of direct sunlight. Note the time at which the colour of the test solution becomes equal to that of the colour standard.

7. EXPRESSION OF RESULTS

7.1. **Interpretation**

The time of clearing shall be the time required for the colour of the sample tube to match that of the standard tube.

7.2. **Repeatability**

The difference in the times of two tests, carried out simultaneously or in rapid succession, by the same analyst, on the same sample, under the same conditions, shall not exceed two minutes.

8. NOTES

8.1. Traces of manganese dioxide have a catalyzing effect on the reaction; ensure that pipettes and Nessler tubes are used which have been scrupulously cleaned and reserved exclusively for the purpose. Clean them with hydrochloric acid and rinse thoroughly with water; no brown coloration shall be visible on the glass apparatus.

8.2. The quality of the water used to prepare the dilute permanganate solution (4.1) shall be carefully monitored; it must not consume any permanganate. If the required quality cannot be obtained, distilled water should be brought to the boil and a small quantity of permanganate added in order to obtain a very slightly pink coloration. This should then be cooled and used for the dilution.

8.3. For some samples the decoloration may occur without passing through the exact shade of the reference solution.

8.4. The permanganate test may be distorted when the alcohol sample for analysis has not been stored in a perfectly clean glass flask, sealed with either a ground glass stopper which has been rinsed with alcohol or another stopper encased in tin or aluminium.

5. METHOD 4: DETERMINATION OF ALDEHYDES

1. SCOPE AND FIELD OF APPLICATION

The method determines aldehydes, expressed as acetaldehyde, in neutral alcohol.

2. DEFINITION

The aldehyde content: the content of aldehydes, expressed as acetaldehyde, as determined by the method specified.

3. PRINCIPLE

The colour obtained after the reaction of the sample with Schiff's reagent is compared with standard solutions having a known acetaldehyde content.

4. REAGENTS

4.1. Schiff's reagent

Place 1 500 ml of water in a 3 000-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 five to 10 minutes. Then add 40 ml of 3 mol/l sulphuric acid solution. Mix well, stopper and allow to stand overnight. Use activated carbon, if necessary, to render the solution in the flask colourless. Store away from the light in a coloured bottle.

Determine the sulphur dioxide content in the reagent in the following manner: pipette 10 ml of the decolourized Schiff's reagent into a 250-ml conical flask, add 200 ml of water and 5 ml of starch solution. Titrate the free sulphur dioxide to a starch end-point with standard 0,05 mol/l iodine solution (0,1 N). The sulphur dioxide content shall be in the range 2,8 to 4,8 millimoles of sulphur dioxide per 100 ml of Schiff's reagent. If it is outside this range the content should be either raised, by adding a calculated amount of sodium metabisulphite, or lowered by bubbling air through the reagent, as appropriate.

4.2. Standard acetaldehyde solutions

Accurately weigh 1,386 ± 0,0001 g of purified aldehyde ammonia (see Note 1) and dissolve it in 1 000 ml of 96 % vol aldehyde-free ethanol (see Note 2). The solution then contains 1 g of acetaldehyde per litre. Dilute the solution to obtain a concentration of 0,1 g acetaldehyde per litre. Further dilute with 96 % vol aldehyde-free ethanol to obtain standard solutions containing 0,002, 0,003, 0,005, 0,007, 0,009 and 0,01 g aldehyde per litre.

Note 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 Buchner funnel, immediately wash with diethyl ether and then dry for three to four hours in a vacuum desiccator over concentrated sulphuric acid. The aldehyde ammonia purified in this way should be colourless. If it is not, the purification should be repeated.

Note 2

Aldehyde-free ethanol should be prepared by boiling 500 ml of absolute ethanol under reflux for two hours with 5 g *m*-phenylenediamine and then distilling it, rejecting the first 50 ml and the last 50 ml of the distillate. Adjust the concentration to 96 % vol by addition of the appropriate volume of water and then mixing.

5. APPARATUS

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

5.2. Pipette 5 ml.

5.3. Water bath, thermostatically controlled at 20 ± 0,5 °C.

5.4. Spectrophotometer with cuvettes of pathlength between 10 and 50 mm.

6. PROCEDURE

6.1. Pipette 5 ml of the sample into a colorimetric tube (5.1), 5 ml of each of the standard solutions (4.2) and 5 ml of water alone as a reference into a series of colorimetric tubes (5.1). Dilute the contents of all the tubes with water to 10 ml. Add to each tube the Schiff's reagent (4.1) up to the 14-ml mark. Stopper the tubes, mix the solutions thoroughly by inversion (simultaneously if possible) and place them in the water bath (5.3) at 20 ± 0,5 °C for 20 minutes.

6.2. Transfer the solutions to a series of appropriate pathlength spectrophotometer cuvettes placing the reference solution prepared above in the reference cuvette.

Determine the optical density of each standard solution and the sample solution at 546 nm after exactly 20 minutes.

7. EXPRESSION OF RESULTS

7.1. **Formula and method of calculation**

Construct a graph of optical density against concentration of acetaldehyde and determine the concentration in the sample by reference to this plot.

The content of aldehydes, expressed as acetaldehyde, in g/hl alcohol at 100 % vol is given by

$$\frac{10{,}000\ A}{T}$$

where:

A is the content, in g per litre, of acetaldehyde in the sample solution as determined by reference to the standard curve.

T is the alcoholic strength by volume of the sample as determined by method 1.

7.2. **Repeatability**

The difference between the results of two determinations, carried out simultaneously or in rapid succession, by the same analyst, on the same sample, under the same conditions, shall not exceed 0,1 g aldehyde per hl alcohol at 100 % vol.

6. METHOD 5: DETERMINATION OF HIGHER ALCOHOLS

1. SCOPE AND FIELD OF APPLICATION

The method determines higher alcohols, expressed as 2-methylpropan-1-ol, in neutral alcohol.

2. DEFINITION

The higher alcohol content: the content of higher alcohols, expressed as 2-methylpropan-1-ol as determined by the method specified.

3. PRINCIPLE

The absorbances of the coloured products resulting from the reaction of higher alcohols and an aromatic aldehyde in hot sulphuric acid (Komarowsky reaction) are determined at 492 nm, corrected for the presence of any aldehyde in the sample and then compared with that produced by 2-methylpropan-1-ol reacting under the same conditions.

4. REAGENTS

4.1. **Salicyl aldehyde solution,** 1 % mas. Prepare by adding 1 g of salicyl aldehyde to 99 g of ethanol at 96 % vol (which is free of fusel oil).

4.2. **Sulphuric acid,** concentrated, density 1,84 g/cm³.

4.3. **2-methylpropan-1-ol.**

4.4. **Standard 2-methylpropan-1-ol solutions.**

Dilute 2-methylpropan-1-ol (4.3) with 96 % vol ethanol to give a series of standards containing 0,2, 0,4, 0,6 and 1,0 g of 2-methylpropan-1-ol per hl of solution.

4.5. **Standard acetaldehyde solutions.**

Prepare the standard acetaldehyde solutions as described in section 4.2 of method 4.

4.6. **Ethanol 96 % vol** free from higher alcohols and aldehydes.

5. APPARATUS

5.1. Solution spectrophotometer, capable of determining absorbance of solutions at 492 nm.

5.2. Spectrophotometer cuvettes, 10, 20 or 50 mm pathlengths.

5.3. Water bath, thermostatically controlled at 20 ± 0,5 °C.

5.4. Cylinders.

6. PROCEDURE

6.1. **Aldehyde content**

Determine the aldehyde content, expressed as acetaldehyde, in the sample using method 4.

6.2. **Calibration curve: 2-methylpropan-1-ol**

Pipette 10 ml of each of the 2-methylpropan-1-ol standards (4.4) and 10 ml ethanol (4.6) into 50-ml glass cylinders each fitted with ground glass stoppers. Pipette 1 ml of the salicyl aldehyde solution (4.1) into the cylinders and then 20 ml of sulphuric acid (4.2). Mix the contents thoroughly by carefully tilting the cylinders backwards and forwards several times (care being taken to lift the stopper occasionally). Leave for 10 minutes at room temperature and then put in the water bath (5.3) at 20 ± 0,5 °C. After 20 minutes pour the contents into a series of spectrophotometer cuvettes.

Exactly 30 minutes after adding the sulphuric acid determine the absorbance of the solutions at 492 nm using water in the reference cuvette of the spectrophotometer.

Construct a calibration curve of absorbance against 2-methylpropan-1-ol concentration.

6.3. **Calibration curve – aldehydes**

Repeat 6.2 but replacing the 10 ml of each of the 2-methylpropan-1-ol standards by 10 ml of each of the acetaldehyde standards.

Construct a calibration curve of absorbance at 492 nm against acetaldehyde concentration.

6.4. **Sample determination**

Repeat 6.2 put replacing the 10 ml of each of the 2-methylpropan-1-ol standards by 10 ml of the sample.

Determine the absorbance of the sample.

7. EXPRESSION OF RESULTS

7.1. **Formula and method of calculation**

7.1.1. Correct the absorbance of the sample by subtracting the value of the absorbance corresponding to the aldehyde concentration in the sample (obtained from the calibration curve constructed under 6.3).

7.1.2. Determine the concentration of higher alcohols, expressed as 2-methylpropan-1-ol, in the sample from the calibration curve constructed under 6.2 but using the corrected absorbance (7.1.1).

7.1.3. The concentration of higher alcohols, expressed as 2-methylpropan-1-ol in g per hl alcohol at 100 % vol, is given by:

$$\frac{A \times 100}{T}$$

where:

A is the concentration of higher alcohols in the sample as calculated in 7.1.2.

T is the alcoholic strength by volume of the sample as determined by method 1.

7.2. **Repeatability**

The difference between the results of two determinations, when carried out simultaneously or in rapid succession, by the same analyst, on the same sample, under the same conditions, shall not exceed 0,25 g per hl alcohol at 100 % vol.

7. METHOD 6: DETERMINATION OF TOTAL ACIDITY

1. SCOPE AND FIELD OF APPLICATION

The method determines the total acidity, expressed as acetic acid, of neutral alcohol.

2. DEFINITION

The total acidity content, expressed as acetic acid: the content of total acidity, expressed as acetic acid, as determined by the method specified.

3. PRINCIPLE

The sample, after de-gassing, is titrated against standard sodium hydroxide solution and the acidity calculated as acetic acid.

4. REAGENTS

4.1. **Sodium hydroxide solution, 0,01 mol/l,** stored so that contact with carbon dioxide is minimized.

4.2. **Phenol red indicator solution, 1 g/l:** prepare by dissolving solid indicator in sufficient sodium hydroxide solution (4.1) and dilute with water. Use when a pH meter is not employed.

5. APPARATUS

5.1. pH meter, with an accuracy of within 0,1 pH units.

5.2. Electrodes, either a combined glass electrode or single class and reference electrodes together with suitable clamps to hold the electrodes.

6. PROCEDURE

6.1. De-gas an aliquot of the sample by heating just to boiling and cooling rapidly or by bubbling nitrogen through the sample.

6.2. Neutralize about 50 ml of previously boiled and cooled water in a beaker or conical flask. Pipette 50 ml of de-gassed sample into the water and titrate with standard sodium hydroxide (4.1) to:

(a) phenol red end-point using prepared indicator (4.2), or

(b) pH 7,8 using pH meter and electrodes (5.1 and 5.2). Let the volume of titrant used be V_0 ml.

7. EXPRESSION OF RESULTS

7.1. **Formula and method of calculation**

The content of total acidity, expressed as acetic acid, in g per hl alcohol at 100 % vol is given by:

$$1{,}2\ V_0 \times \frac{100}{T}$$

where:

V_0 is the number of ml of 0,1 mol/l sodium hydroxide required for neutralization.

T is the alcoholic strength by volume of the sample as determined by method 1.

7.2. **Repeatability**

The difference between the results of two determinations, carried out simultaneously or in rapid succession, by the same analyst, on the same sample, under the same conditions, shall not exceed 0,3 g per hl alcohol at 100 % vol.

8. METHOD 7: DETERMINATION OF ESTERS

1. SCOPE AND FIELD OF APPLICATION

The method determines esters, expressed as ethyl acetate, in neutral alcohol.

2. DEFINITION

The ester content: the content of esters, expressed as ethyl acetate, as determined by the method specified.

3. PRINCIPLE

Esters react quantitatively with hydroxylamine hydrochloride in alkaline solution to form hydroxamic acids. These then form coloured complexes with ferric iron in acid solution. The optical densities of these complexes are measured at 525 nm.

4. REAGENTS

4.1. **Hydrochloric acid, 4 mol/l.**

4.2. **Ferric chloride solution,** 0,37 **mol/l** in 1 mol/l hydrochloric acid.

4.3. **Hydroxylammonium chloride solution, 2 mol/l.** Store in a refrigerator.

4.4. **Sodium hydroxide solution, 3,5 mol/l.**

4.5. **Ethyl acetate standard solutions** containing 0, 0,4, 0,6, 0,8 and 1,0 g ethyl acetate per hl in 50 % vol ester-free aqueous ethanol.

5. APPARATUS

5.1. Solution spectrophotometer with 50 mm pathlength cuvettes.

6. PROCEDURE

6.1. **Preparation of sample**

Determine the strength of the sample (using method 1) and bring and aliquot to 50 % vol by the addition of water.

6.2. Pipette 10,0 ml aliquots of the sample and of each standard, including the 0,0 g/hl as a reference, into test tubes.

Add 2,0 ml of hydroxylammonium chloride solution (4.3) to each tube followed by 2,0 ml of the hydrochloric acid (4.1) to the reference tube only.

Add 2,0 ml of the sodium hydroxide solution (4.4) to all the tubes with mixing.

After 15 minutes add, with mixing, 2,0 ml of hydrochloric acid (4.1) to all tubes except the reference, and 2 ml of ferric chloride solution (4.2) to all the tubes.

6.3. Transfer each solution to the 50 mm pathlength solution spectrophotometer cuvettes and measure the optical density at 525 nm using the reference solution in the reference cuvette.

7. EXPRESSION OF RESULTS

7.1. **Formula and method of calculation**

Plot the optical densities of the standards against their concentration. Determine the content of ester in the sample by reference to the graph.

Multiply the value obtained by 2,0 to obtain total esters, expressed as ethyl acetate, in g per hl alcohol at 100 % vol in the sample.

7.2. **Repeatability**

The difference between the results of two determinations, carried out simultaneously or in rapid succession, by the same analyst, on the same sample, under the same conditions, should not exceed 0,2 g esters, as ethyl acetate, per hl alcohol at 100 % vol.

9. METHOD 8: DETERMINATION OF VOLATILE NITROGEN BASES

1. SCOPE AND FIELD OF APPLICATION

The method determines volatile nitrogen bases, expressed as nitrogen, in neutral alcohol.

2. DEFINITION

The volatile nitrogen bases content: the content of volatile nitrogen bases, expressed as nitrogen, as determined by the method specified.

3. PRINCIPLE

The sample is evaporated to a small volume in the presence of sulphuric acid and the ammonia content then determined using the Conway micro-diffusion technique.

4. REAGENTS

4.1. **Sulphuric acid, 1 mol/l.**

4.2. **Boric acid indicator solution.** Dissolve 10 g of boric acid, 8 mg of bromocresolgreen and 4 mg of methyl red in 30 % vol propan-2-ol and make up to 1 000 mls with 30 % vol propan-2-ol.

4.3. **Potassium hydroxide solution, 500 g/l;** carbon dioxide free.

4.4. **Hydrochloric acid, 0,02 mol/l.**

5. APPARATUS

5.1. Evaporating dish, of sufficient capacity to accept 50 ml of sample.

5.2. Water bath.

5.3. Conway flask with tightly fitting lid; see figure 1 for description and suggested dimensions.

5.4. Micro-burette, capacity 2 to 5 ml, graduated in 0,01 ml.

6. PROCEDURE

6.1. Pipette 50 mls of the sample (with an anticipated nitrogen content of less than 0,2 g per hl sample take 200 ml of sample) into a glass dish, add 1 ml of 1 mol/l sulphuric acid (4.1), place the dish (5.1) on a water bath (5.2) and evaporate until there is about 1 ml remaining.

6.2. Pipette 1 ml of the boric acid indicator solution (4.2) into the inner chamber of the Conway flask (5.3) and wash the residue liquid from the evaporation process (6.1) into the outer chamber. Slightly tilt the Conway flask and add about 1 ml of the potassium hydroxide solution (4.3) to the outer chamber as quickly as possible but as far from the majority of the liquid in the outer chamber as possible. Immediately seal the Conway flask by covering with a tightly fitting lid smeared with grease.

6.3. Mix the two solutions in the outer chamber taking care that there is no liquid spillage from either chamber to the other. Allow to stand for two hours.

6.4. Titrate the ammonia in the inner chamber against 0,02 mol/l hydrochloric acid (4.4) using a micro-burette (5.4) to neutralization. The volume of acid used should be between 0,2 and 0,9 ml; let the volume of acid used be V_1 ml.

6.5. Carry out a blank titration by repeating sections 6.1 to 6.4 but replacing the 50 ml of sample in section 6.1 by the same volume of water. Let the volume of hydrochloric acid used be V_0 ml.

7. EXPRESSION OF RESULTS

7.1. **Formula and method of calculation**

The content of volatile nitrogen bases, in g per hl alcohol at 100 % vol, calculated and expressed as nitrogen, is given by:

$$0{,}56\,(V_1 - V_0) \times \frac{100}{T}$$

Where:

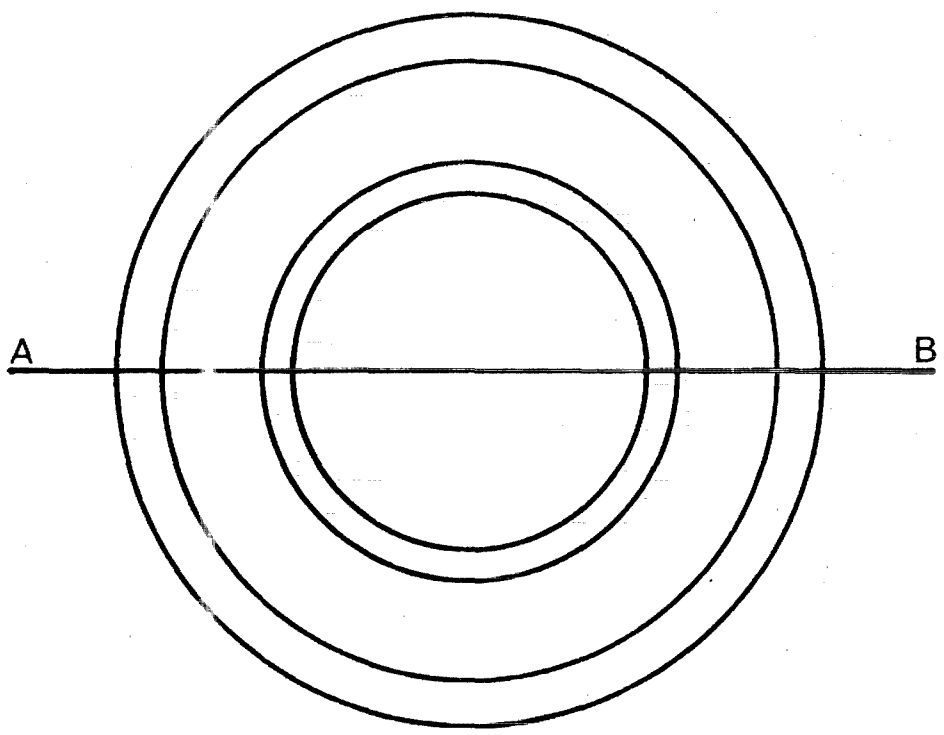
V_1 is the volume, in ml, of the hydrochloric acid used to neutralize the sample.

V_0 is the volume, in ml, of hydrochloric acid used in the blank test.

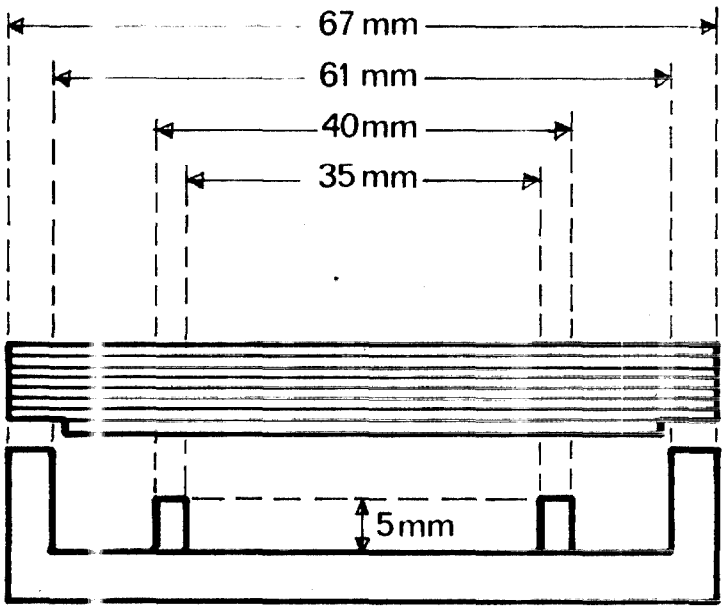
T is the alcoholic strength by volume of the sample as determined by method 1.

7.2. **Repeatability**

The difference between the results of two determinations, carried out simultaneously or in rapid succession, by the same analyst, on the same sample, under the same conditions, shall not exceed 0,05 g per hl alcohol at 100 % vol.



Top view of flask



Vertical Section on line A – B

Typical dimension given

Figure 1.

Conway flask

10. METHOD 9: DETERMINATION OF METHANOL

1. SCOPE AND FIELD OF APPLICATION

The method determines the content of methanol in neutral alcohol.

2. DEFINITION

The methanol content: the content of methanol as determined by the method specified.

3. PRINCIPLE

The methanol concentration is determined by direct injection of sample into a gas liquid chromatography apparatus.

4. PROCEDURE

Any GLC method is suitable provided that the gas chromatographic column and conditions employed are capable of achieving a clear separation between methanol, acetaldehyde, ethanol and ethyl acetate. The limit of detection of methanol in ethanol shall be less than 2 g/hl.

5. REPEATABILITY

The difference between the results of two determinations, carried out simultaneously or in rapid succession, by the same analyst, on the same sample, under the same conditions, shall not exceed 2 g methanol per hl alcohol at 100 % vol.

11. METHOD 10: DETERMINATION OF DRY RESIDUE

1. SCOPE AND FIELD OF APPLICATION

The method determines the dry residue content of neutral alcohol.

2. DEFINITION

The dry residue content: the dry matter content as determined by the method specified.

3. PRINCIPLE

An aliquot of the sample is dried at 103 °C and the residue determined gravimetrically.

4. APPARATUS

4.1. Water bath, boiling.

4.2. Evaporating dish of suitable capacity.

4.3. Desiccator, containing freshly activated silica gel (or an equivalent desiccant) with a moisture content indicator.

4.4. Analytical balance.

4.5. Oven, thermostatically controlled at 103 ± 2 °C.

5. PROCEDURE

Accurately weigh, to the nearest 0,1 mg, a clean dry evaporating dish (4.2) (M_0). Pipette a suitable volume of sample into the dish (100 – 250 ml) (V_0 ml). Place the dish with sample on the boiling water bath (4.1) and allow to dry. Place in the oven (4.5) at 103 ± 2 °C for 30 minutes and then transfer dish with residue into a desiccator (4.3). Allow the dish to cool for 30 minutes and then weigh, to the nearest 0,1 mg, the dish with residue (M_1).

6. EXPRESSION OF RESULTS

6.1. **Formula and method of calculation**

The content of dry residue, in g per hl of alcohol at 100 % vol is given by:

$$\frac{(M_1 - M_0) \times 10^7}{V_0 \times T}$$

where:

M_0 is the mass, in g, of the clean dry dish,

M_1 is the mass, in g, of the dish and residue after drying,

V_0 is the volume of sample taken for drying, and

T is the alcoholic strength by volume of the sample as determined by method 1.

6.2 **Repeatability**

The difference between the results of two determinations, carried out simultaneously or in rapid succession, by the same analyst, on the same sample, under the same conditions, shall not exceed 0,5 g per hl of alcohol at 100 % vol.

12. METHOD 11: LIMIT TEST FOR THE ABSENCE OF FURFURAL

1. SCOPE AND FIELD OF APPLICATION

The method detects furfural in neutral alcohol.

2. DEFINITION

The detection of the limit test concentration of furfural: the limit test result as determined by the method specified.

3. PRINCIPLE

Diluted alcohol sample is mixed with aniline and glacial acetic acid. The presence of furfural is indicated by a salmon pink colour appearing in the solution within 20 minutes of mixing.

4. REAGENTS

4.1. Aniline, freshly distilled.

4.2. Acetic acid, glacial.

5. APPARATUS

Tubes, fitted with ground glass stoppers.

6. PROCEDURE

Dilute the sample so that the alcohol concentration is 50 % vol.

Pipette 10 ml of the diluted sample into a tube (5); add 0,5 ml of aniline and 2 ml of glacial acetic acid. Shake the tube and contents to mix.

7. EXPRESSION OF RESULTS

7.1. **Limit test interpretation**

If the time of development of any salmon pink colouration in the tube is less than 20 minutes the test is positive and the sample contains furfural.

7.2. **Observations**

The results of two limit tests, carried out simultaneously or in rapid succession, by the same analyst, on the same sample, under the same conditions, shall be identical.