# COMMISSION REGULATION (EEC) No 1238/92

## of 8 May 1992

## 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 822/87 of 16 March 1987 on the common organization of the market in wine (1), as last amended by Regulation (EEC) No 1734/91 (2), and in particular Articles 35 (8), 36 (6), 38 (5), 39 (9), 41 (10) and 42 (6),

Whereas, under Council Regulation (EEC) No 2046/89 of 19 June 1989 laying down general rules for distillation operations involving wine and the by products of wine-making (3), neutral alcohol obtained by 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 these methods must be binding for all commercial transactions and control operations ; whereas, in view of the restricted opportunities for trade, a limited number of general methods permitting a rapid and sufficiently accurate analysis of the required components of the neutral alcohol should be adopted ;

Whereas the Community methods of analysis adopted should be generally recognized in order to ensure that they are supplied on a uniform basis ;

Whereas the current Community methods of analysis for neutral alcohol in the wine sector were adopted by Commission Regulation (EEC) No 3590/83 (4) ; whereas scientific progress necessitates the replacement of some methods with more suitable ones, the amendment of other methods and the introduction of new ones ; whereas, in view of the large number and complexity of these changes, all the methods of analysis should be incorporated into a new Regulation and Regulation (EEC) No 3590/83 should be replaced ;

Whereas the terms used for the repeatability and comparability of the results obtained with these methods should be defined so that the results obtained in application of the methods of analysis listed in Article 74 of Regulation (EEC) No 822/87 can be compared ;

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 2046/89 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 by the distillation operations provided for in Regulation (EEC) No 822/87.

*Article 2*

For the purposes of applying this Regulation :

(a) the repeatability shall be the value below which the absolute difference between the two single test results obtained using tests conducted under the same conditions (same operator, same apparatus, same laboratory and a short interval of time) may be expected to lie within a specified probability ;

(b) the comparability shall be the value below which the absolute difference between two single test results obtained under different conditions (different operators, different apparatus and/or different laboratories and/or different time) may be expected to lie within a specified probability.

The term 'single test result' shall be the value obtained when the standardized test method is applied fully and once to a single sample. Unless otherwise stated, the probability shall be 95 %.

*Article 3*

Regulation (EEC) No 3590/83 is hereby repealed.

*Article 4*

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

(1) OJ No L 84, 27. 3. 1987, p. 1.
(2) OJ No L 163, 26. 6. 1991, p. 6.
(3) OJ No L 202, 14. 7. 1989, p. 14.
(4) OJ No L 363, 24. 12. 1983, p. 1.

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

Done at Brussels, 8 May 1992.

*For the Commission*

Ray MAC SHARRY

*Member of the Commission*

*ANNEX*

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

## 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 quantitiy 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, dilution or washing purposes, distilled water, or demineralized water of a 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 ethanol 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.

## 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].

[1] OJ No L 262, 27. 9. 1976, p. 143.
[2] OJ No L 262, 27. 9. 1976, p. 149.

**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

4.1. 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.

**Method 3 : 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 1 ml of a 1 mmol/l potassium permanganate solution to 10 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.

4. REAGENTS

4.1. Potassium permanganate solution, 1 mmol/l. Prepare immediately before use.

4.2. Colour solution A (red)

— Weigh accurately 59,50 g $CoCl_2.6H_2O$.
— Prepare a mixture of 25 ml hydrochloric acid ($P^{20}$ = 1,19 g/ml) and 975 ml water.
— Add the cobalt chloride to some of the HCl/water mixture in a 1 000 ml volumetric flask and make up to the mark with the rest of the mixture at 20 °C.

4.3. Colour solution B (yellow)

— Weigh accurately 45,00 g $FeCl_3.6H_2O$.
— Prepare a mixture of 25 ml hydrochloric acid ($P^{20}$ = 1,19 g/ml) and 975 ml water and then proceed with the weighed quantity of ferric chloride as for colour solution A.

4.4. Colour standard solution

Pipette 13 ml of colour solution A and 5,5 ml colour solution B into a 100 ml volumetric flask and make up to the mark with water at 20 °C.

*Note :*

Colour solutions A and B may be stored in the ark at 4 °C for several months ; the colour standard should be freshly prepared from time to time.

5. APPARATUS

5.1. 100 ml Nessler tubles of colourless transparent glass, graduated to 50 ml, with ground-glass stopper, or test tubes, colourless, circa 20 mm diameter.

5.2. Pipettes, 1,2, 5, 10 and 50 ml.

5.3. Thermometer, range up to 50 °C 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, 100 and 1 000 ml with ground glass stoppers.

6. PROCEDURE

6.1.

— Pipette 10 ml of the sample into a test tube or 50 ml in a Nessler tube.
— Place in water bath at 20 °C.
— Add 1 ml or 5 ml, depending on the sample quantity used, of 1 mmol/l $KMnO_4$ solution, mix and leave in the water bath at 20 °C.
— Note the time.
— Pipette 10 ml of the colour standard into a test tube of the same diameter or 50 ml of the colour standard into a Nessler tube.
— Observe the change of colour of the sample and compare it with the colour standard against a white background from time to time.
— Note the time at which the colour of the sample becomes the same as that of the colour standard.

*Note:* Take care not to expose the sample solution to direct sunlight during the test.

7. EXPRESSION OF RESULTS

7.1. Interpretation of the time of clearing shall be the time required for the colour of the sample tube to match that of the standard tube. For a neutral alcohol that time must be at least 18 minutes at a temperature of 20 °C.

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 test 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 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.

**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

p-rosaniline hydrochloride (basic fuchsin)

sodium sulphite or anhydrous sodium metabisulphite

hydrochloric acid, density

$p^{20}$ = 1,19 g/ml

powdered active carbon

starch solution, prepared from 1 g soluble starch and 5 mg $HgI_2$ (preservative) which are suspended in a little cold water, mixed with 500 ml boiling water, boiled for 5 minutes and filtered when cold.

iodine solution, 0,05 mol/l

1-amino-ethanol $CH_3.CH(NH_2)OH$ (MW 61.08)

Schiff's reagent

— Dissolve 5,0 g powdered p-rosaniline hydrochloride with about 1 000 ml hot water in a 2 000 ml volumetric flask.

— Leave in the water bath until completely dissolved if necessary.

— Dissolve 30 g anhydrous sodium sulphite (or an equivalent quantitiy of sodium metabisulphite) in circa 200 ml water and add to the cool prosaniline solution.

— Leave to stand for amout 10 minutes.

— Add 60 ml hydrochloric acid ($p^{20}$ = 1,19 g/ml).

— Where the solution is colourless — a slight degree of brown colouration can be ignored — make up to the mark with water.

— If necessary filter with a little active carbon over a folded filter to render the solution colourless.

*Notes:*

(1) The Schiff's reagent should be prepared at least 14 days before it is used.

(2) The free $SO_2$ content in the reagent should be between 2,8 and 6,0 mmol/100 ml, the pH must be 1.

Determination of free $SO_2$

— Pipette 10 ml Schiff's reagent into a 250 ml Erlenmeyer flask.

— Add 200 ml water.

— Add 5 ml starch solution.

— Titrate with 0,05 mol/l iodine solution to starch end-point.

— If the free $SO_2$ content is outside the indicated range it should either be:

— raised with a calculated quantity of sodium metabisulphite (0,126 g $Na_2SO_3$/100 ml reagent per mmol $SO_2$ lacking), or

— lowered by bubbling air through the reagent.

Calculation of free $SO_2$ in the reagent:

mmol free $SO_2$/100 ml reagent

$$= \frac{\text{consumed ml iodine solution (0,05 mol/l)} \cdot 3{,}2 \cdot 100}{64 \cdot 10}$$

$$= \frac{\text{consumed ml iodine solution (0,05 mol/l)}}{2}$$

*Important:*

It other methods are used to prepare the Schiff's reagent, the sensitivity of the reagent should be checked so that during the test:

— there is no colouration with the aldehyde-free reference alcohol,

— the pink colouration should be visible from 0,1 g acetaldehyde per hl alcohol at 100 % vol.

(3) Purification of commercial 1-amino-ethanol

— Dissovle 5 g 1-amino-ethanol completely in circa 15 ml absolute ethanol.

— Add circa 50 ml dry diethyl ether (1-amino-ethanol precipitates).

— Leave for several hours in a refrigerator.

— Filter off the crystals and wash with dry diethyl ehter.

— Dry for three to four hours in a desiccator over sulphuric acid in a partial vacuum.

*Note :*

The cleaned 1-amino-ethanol must be white ; if not, repeat the recrystallization process.

5. APPARATUS

5.1. Colorimetric tubes, each provided with a ground-glass stopper, capacity 20 ml.

5.2. 1 ml, 2ml, 3ml, 4 ml, 5 ml and 10 ml pipettes.

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

5.4. Spectrophotometer with cuvettes of 50 mm path length.

6. PROCEDURE

6.1. Preliminary remark

When using this method to determine aldehyde content ensure that the sample's alochol content is at least 90,0 % vol. If not it must be raised by adding corresponding amounts of aldehyde-free ethanol.

6.2. Calibration curve

Weigh 1,3860 g purified and dried 1-amino-ethanol accurately on an analytical balance.

Place in a 1 000 ml volumetric flask and add aldehyde-free ethanol-free ethanol, make up to the mark at 20 °C. The solution content 1 g/l acetaldehyde.

Prepare the dilution series in two stages to produce 10 reference solutions containing 0,1 to 1,0 mg acetaldehyde per 100 ml solution.

Determine the absorbance values for these reference solutions according to 6.3 and construct graph.

6.3. Determining the aldehyde content

Pipette 5 ml of the sample into a colorimetric tube.

Add 5 ml water, mix and keep at a constant temperature of 20 °C.

At the same time make a blank using 5 ml aldehyde-free ethanol at 96 % vol, add 5 ml water and keep at a temperature of 20 °C.

Then add 5 ml Schiff's reagent to each tube, close with ground-glass stoppers and shake well.

Keep in water bath for 20 minutes at 20 ° C.

Put contents into cuvettes.

Determine absorbence values at 546 nm.

*Notes :*

(1) To determine the aldehyde figures it is necessary to check the validity of the calibration curves by comparison with test solutions ; if not, the calibration curve must be prepared again.

(2) Ensure that the blank is always colourless.

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 ethanol at 100 % vol is given by

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

where :

A is the content, in g per hl 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 concession, by the same analyst, on the same sample, under the same conditions, shall not exceed 0,1 g aldehyde per hl Ethanol at 100 % vol.

**Method 5 : Determination of higher alcohols**

1. SCOPE AND FIELD OF APPLICATION

The method determines budget 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 absorbance of the coloured products resulting from the reaction of higher alcohols and an aromatic aldehyde in hot dilute sulphuric acid (Komarowsky reaction) are determined at 560 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/m².

4.3. 2-methylpropan-1-ol.

4.4. Standard 2-methylpropan-1-ol solutions

Dilute 2-methylpropan-1-ol (4.3) with an aqueous solution of 96 % vol ethanol to give a series of standards containing 0,1, 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 6.2 of method 4.

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

5. APPARATUS

5.1. UV-VIS spectrophotometer, capable of determining absorbance of solutions at 560 nm.

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

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

5.4. Colorimetric tubes made of thick glass Pyrex or similar with ground-glass stoppers, content circa 50 ml.

6. PROCEDURE

6.1. Aldehyde content

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

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

Pipette 10 ml of each of the 2-methylpropan-1-ol standards (4.4) 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 in 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 560 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 560 nm against acetaldehyde concentration.

6.4. Sample determination

Repeat 6.2 but replacing the 10 ml 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-methalpropan-1-ol, in the sample from the calibration curve constructed under 6.2 but using the correct absorbance (7.1.1).

7.1.3. The concentration of higher alcohols, expressed as 2-methylpropan-1-ol in g per hl ethanol 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 0,2 g per hl ethanol at 100 % vol.

**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 solutions, 0,01 mol/l and 0,1 mol/l, stored so that contact with carbon dioxide is minimized.

4.2. Indigo carmine solution (A)

— Weigh 0,2 g indigo carmine.

— Dissolve in 40 ml water and make up to 100 g with ethanol.

Phenol red solution (B)

— Weigh 0,2 g phenol red.

— Dissolve in 6 ml sodium hydroxide 0,1 mol/l and make up to mark with water in a 100 ml volumetric flask.

5. APPARATUS

5.1. Burette or automatic titrater.

5.2. Pipette, 100 ml.

5.3. Round-bottom flask with ground-glass stopper, 250 ml.

5.4. Reflux condenser with ground-glass stopper.

6. PROCEDURE

— Pipette 100 ml of the sample into the 250 ml round-bottom flask.
— Add boilling stones and briefly heat to boiling in the reflux condenser.
— Add one drop of each of indicator solutions A and B to the hot solution.
— Then titrate with sodium hydroxide 0,01 mol/l until the first signs of change from greenish-yellow to violet.

7. EXPRESSION OF RESULTS

7.1. Formula and method of calculation

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

$$\frac{V \cdot 60}{T}$$

where :

V is the number of ml of 0,01 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 0,1 g per hl ethanol at 100 % vol.

**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 hydroxylamic acids. These then form coloured complexes with ferric ions in acid solution. The optical densitics of these complexes are measures 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. Hydroxylamine Hydrochloride, 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, 0,2, 0,4, 0,6, 0,8 and 1,0 g ethyl acetate per hl ester-free ethanol at 96 % vol.

5. APPARATUS

5.1. spectrophotometer with 50 mm pathlength cuvettes.

6. PROCEDURE

6.1. Calibration curve

— Weigh accurately 1.0 g ethyl acetate on an analytical balance.
— Add ester-free alcohol in a 1 000 ml volumetric flask and make up to the mark at 20 °C.
— Prepare dilution series in two stages to produce 20 reference solutions containing 0.1 to 2.0 mg ethyl acetate per 100 ml solution.
— Determine absorbence values for the reference solution in accordance with 6.2 and construct a graph.

6.2. Determination of ester content

— Pipette 10 ml of the sample into test-tubes fitted with ground-glass stoppers.
— Add 2 ml hydroxylamine hydrochloride solution.
— At the same time prepare a blank using 10 ml ester-free ethanol at 96 % vol and 2 ml hydroxylamine hydrochloride solution.
— Then add 2 ml sodium hydroxide to each solution, close the tubes with ground-glass stoppers and shake well.
— Keep for 15 minutes at 20 °C in a water bath.
— Add 2 ml hydrochloric acid to each tube, shake briefly.
— Add 2 ml ferric chloride solution, mix well.
— Pour contents into cuvettes.
— Determine absorbence values at 525 nm.

7. EXPRESSION OF RESULTS

7.1. Formula and method of calculation

Plot the optical densitie of the standards against their concentrations.

The ester content (expressed as ethyl acetate = A) corresponding to the absorbence value is read off on the graph and calculated according to the formula:

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

and given in 9 per hl ethanol at 100 % vol,

where T = alcohol content of the sample in % vol determination as described in 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, should not exceed 0,1g esters, as ethyl acetate, per hl ethanol at 100 % vol.

**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 suphurie acid and the ammonia content then determined using the conway micro-diffusion technique.

4. REAGENTS

4.1. Suphuric acid, 1 mol/l.

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

4.3. Potasium 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 falsk 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 $V_2$ ml.

7. EXPRESSION OF RESULTS

7.1. Formula and method of calculation.

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

$$\frac{(V_1 - V_2) \cdot 2\,800}{E \cdot T}$$

Where

$V_1$ is the volume, to ml, at the hydrochloric acid used to neutralize the sample.

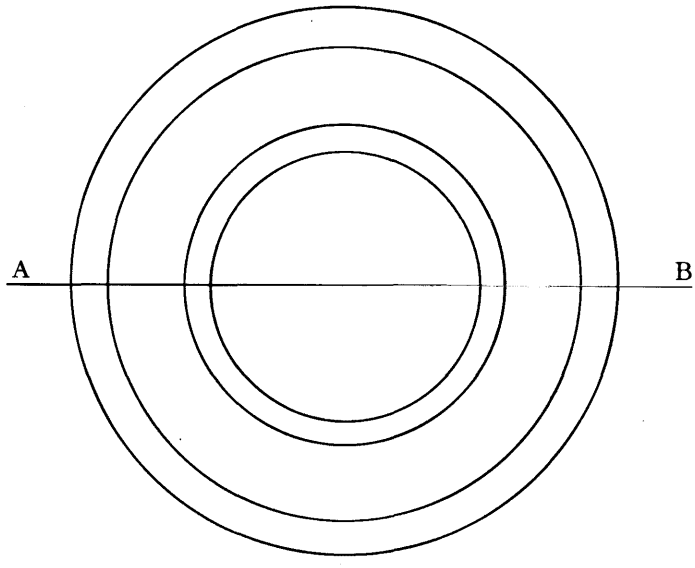
$V_2$ is the volume, in ml, of hydrochloric acid used in the blank test.

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

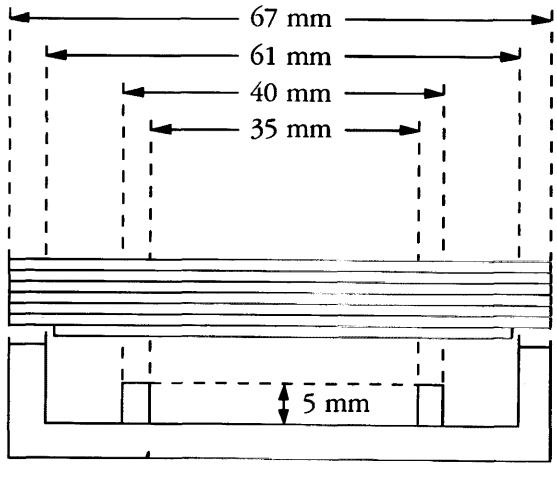
E is the quantity of sample used in ml.

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 ethanol at 100 % vol.




Top view of flask




Vertical Section on line A – B

Typical dimension given

*Figure 1*

Conway flask

**Method 9: Determination of methanol**

1. SCOPI 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 chromatographie 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 anylst, on the same sample, under the same conditions, shall not exceed 2 g methanol per hl ethanol at 100 % vol.

**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) ($m_o$). Pipette in several operations if necessary a suitable volume of sample into the dish (100 − 250 ml) ($V_o$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, to g per hl of ethanol at 100 % vol is given by :

$$\frac{(M_1 - M_o) \times 107}{V_0 \times T}$$

where :

$M_o$ is the mass, in g, of the clean dry dish,

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

$V_o$ is the volume of sample taken for drying, and

T is the alcohols 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 ethanol at 100 % vol.

## 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 tests concentration of furfural : the limit test result as determined by the method specified.

3. PRINCIPLE

The 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

Pipette 10 ml of the 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.

## Method 12 : UV test

1. SCOPE

This method determines the optical transparence of neutral alcohol.

2. PRINCIPLE

The optical transparence of the sample in the wavelength range 220 to 270 nm is measured against a defined reference substance of high optical transparence.

3. APPARATUS

3.1. UV-VIS spectrophotometer

3.2. Quartz cuvettes, 10 mn path length, same spectrum transmission.

4. REAGENTS

n-Hexane for spectroscopy.

5. PROCEDURE

— Rinse clean cuvettes with sample solution and then pour in the sample ; dry the outside of the cuvettes.
— Treat reference cuvette in the same way with n-Hexane and fill.
— Determine absorbence values and construct graph.

6. EXPRESSION OF RESULTS

The absorbence values found at 270, 240, 230 and 220 nm may not exceed the following figures : 0,02, 0,08, 0,18 and 0,3. The absorbence curve must be smooth and regular.

## Method 13 : Determination of $^{14}C$ content in ethanol

1. METHOD TO DETERMINE TYPE OF ALCOHOL

Determination of the $^{14}C$ content in ethanol permits a distinction to be made between alcohol from fossil fuels (synthesis alcohol) and alcohol from recent raw materials (fermentation alcohol).

2. DEFINITION

The $^{14}C$ content of ethanol is understood to be the $^{14}C$ content determined using the method described here.

The natural $^{14}C$ content in the atmosphere (the reference value), which is absorbed by living vegetation by assimilation, is not a constant value. The reference value is therefore determined on ethanol from raw materials of the most recent vegetation period. This annual reference value is determined each year by collaborative analyses organized by the Community Bureau of References and the Joint Research Centre, Ispra.

3. PRINCIPLE

The $^{14}C$ content of samples containing alcohol with at least 85 % mass ethanol is determined directly by liquid scintillation count.

4. REAGENTS

4.1. Toluene scintillator

5,0 g 2,5-diphenyloxazole (PPO)

0,5 g p-bis-[4-methyl-5-phenyloxazolyl(2)]-benzene (dimethyl-POPOP) in 1 litre analytical grade toluene.

Commercial, ready-to-use toluene scintillators of this composition can also be used.

4.2. $^{14}C$ standard

n-Hexadecane $^{14}C$ with an activity of about $1 \times 10^6$ dpm/g (approximately $1{,}67 \times 10^6$ cBq/g) and a guaranteed accuracy of determined activity of ± 2 % rel.

4.3. $^{14}C$-free ethanol

Synthesis alcohol from raw materials of fossil origin with at least 85 % mass ethanol, to determine the background.

4.4. Alcohol from recent raw materials of the most recent vegetation period with at least 85 % mass ethanol as reference material.

5. APPARATUS

5.1. Multi-channel liquid scintillation spectrometer with processor and automatic external standardization and display of the external standard/channel ratio (usual design : three meter channels and two external standard channels).

5.2. Low-potassium counter tubes suitable for the spectrometer, with dark screw-tops containing a polyethylene insert.

5.3. Volumetric pipettes, 10 ml.

5.4. Automatic dosing device 10 ml.

5.5. 250 ml round-bottom flask with ground-glass stopper.

5.6. Alcohol distillation apparatus with heating mantle, e.g. type Micko.

5.7. Microliter syringe 50µl.

5.8. Pycnometer funnel, pycnometers, 25 ml and 50 ml.

5.9. Thermostat with a temperature stability of ± 0,01 °C.

5.10. Official alcohol tables in accordance with the Council Directive of 27 July 1976 on the approximation of the laws of Member States relating to alcohol tables, published by the Commission of the European Communities (ISBN 92-825-0146-9).

6. PROCEDURE

6.1. Adjusting the equipment

The equipment should be adjusted according to the manufacturer's instructions. Measuring conditions are optimal when the value $E_2/B$, the quality index, is at its maximum.

E = efficiency

B = background

Only two meter channels are optimized. The third is left fully open for control purposes.

6.2. Selection of counter tubes

A larger numer of counter tubes than will later be needed are each filled with 10 ml of $^{14}C$-free synthesis ethanol and 10 ml toluene scintillator. Each is measured for at least 4 × 100 minutes. Tubes whose backgrounds vary by more than ± 1 % rel. from the mean are discarded. Only tubes new from the factory and from the same batch may be used.

6.3. Determination of the external standard/channel ratio (ESCR)

During the process of setting the channels (6.1) the ESCR is determined using the appropriate computer program when the efficiency is determined. The external standard used is $^{137}$ caesium, which is already built-in by the manufacturer.

6.4. Preparation of sample

Samples having an ethanol content of at least 85 % mass and free from impurities, which absorb at wavelengths below 450 nm can be measured. The low residue of esters and aldehydes is not a problem. After the first few ml have been discarded the sample is distilled direct into the pycnometer and the alcohol content of the sample is determined by pycnometry. The values to be determined are taken from the Official Alcohol Tables.

7. MEASUREMENT OF SAMPLES USING EXTERNAL STANDARD

7.1. Slightly extinguished samples such as those decribed in 6.4 with an ESCR of around 1.8 may be measured via the ESCR, which provides a measure of the efficiency.

7.2. Measurement

10 ml each of the samples prepared according to 6.4 is pipettes into a selected counter tube checked for background and 10 ml of toluene scintillator is added via an automatic dosing device. The samples in the tubes are homogenized by suitable rotary movements ; the liquid must not be allowed to wet the polyethylene insert in the screw-top. A tube containing $^{14}C$-free fossil ethanol is prepared in the same way to measure the background. To check the relevant annual $^{14}C$ value a duplicate of recent ethanol from the latest vegetation period is prepared, a tube being mixed with internal standard, see 8.

The control and background samples are placed at the beginning of the measurement series, which should contain no more than 10 samples for analysis. Total measuring time per sample is at least 2 × 100 minutes, with the individual samples being measured in part stages of 100 minutes so that any equipment drift or other defect can be detected. (One cycle therefore corresponds to a measuring interval of 100 minutes per sample.)

Background and control samples should be freshly prepared every four weeks.

This method requires little time and material and is particularly suitable for non-secialist laboratories processing large numbers of samples.

In the case of slightly extinguished samples (ESCR circa 1,8) the efficiency is only negligibly affected by the change in this value. If the change is within ± 5 % rel. the same efficiency can be expected. For more greatly extinguished samples, such as denatured alcohols, the efficiency can be established via the extinction correction graph. If an appropriate computer program is not available the internal standard must be used, and this gives an unambiguous result.

8. MEASURING SAMPLES USING INTERNAL STANDARD HEXADECANE$^{14}$C

8.1. Procedure

Control and background samples (recent and fossil ethanol) and the unknown material are each measured as duplicates. One sample of the duplicate is prepared in a non-selected tube and an accurately dosed quantity (30 µl) of hexadecane$^{14}$C is added as internal standard (added activity around 26 269 dpm/gC approximately 43 782 cBq/gC). For the sample preparation and measuring time of the other samples see 7.2, but the measuring time for the samples with the internal standard can be reduced to about five minutes by presetting at $10^5$ pulses. One duplicate each of background and control samples is used per measuring series; these are placed at the beginning of the measuring series.

8.2. Handling the internal standard and counter tubes

To prevent contamination when measuring with the internal standard these must be stored and handled well away from the area where the samples for analysis are prepared and measured. After measurement the tubes checked for background may be re-used. The screw-tops and tubes containing the internal standard are disposed of.

9. EXPRESSION OF THE RESULTS

9.1. The unit of activity of a radio-active substance is the becquerel; 1 Bq = 1 decay/sec.

Indication of specific radio-activity is expressed as becquerels relative to one gram carbon = Bq/gC.

To obtain more practical results it is best to express the results in centi-bequerels = cBq/gC.

The descriptions and formulae used in the literature, based on dpm, may be retained for the time being. To obtain corresponding figures in cBq merely multiply the dpm figure by $\frac{100}{60}$.

9.2. Expression of results with external standard

$$\text{cBq/g C} = \frac{(\text{cpm}_{pr} - \text{cpm}_{NE}) \cdot 1{,}918 \cdot 100}{V \cdot F \cdot Z \cdot 60}$$

9.3. Expression of results with internal standaard

$$\text{cBq/g C} = \frac{(\text{cpm}_{pr} - \text{cpm}_{NE}) \cdot \text{dpm}_{IS} \cdot 1{,}918 \cdot 100}{(\text{cpm}_{IS} - \text{cpm}_{pr}) \cdot V \cdot F \cdot 60}$$

9.4. Abbreviations

$\text{cpm}_{pr}$ = is the mean sample count rate over the total measuring time.
$\text{cpm}_{NE}$ = is the mean background pulse rate calculated in the same way.
$\text{cpm}_{IS}$ = is the amount of added internal standard added (calibration radioactivity dpm).
$\text{dpm}_{IS}$ = is the quantity of internal standard added (calibration radioactivity dpm).
V = is the volume of the samples used in ml.
F = is the content in grammes pure alcohol per ml corresponding to its concentration.
Z = is the efficiency corresponding to the ESCR value.
1,918 = is the number of grammes alcohol per gramme carbon.

10. RELIABILITY OF THE METHOD

10.1. Repeatability (r)

$r = 0{,}632$ cBq/g C; $S_{(r)} = \pm 0{,}223$ cBq/g C

10.2. Comparability (R)

$R = 0{,}821$ cBq/g C; $S_{(R)} = \pm 0{,}290$ cBq/g C.