COMMISSION REGULATION (EC) No 2870/2000
of 19 December 2000
laying down Community reference methods for the analysis of spirits drinks

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Regulation (EEC) No 1576/89 of 29 May 1989 laying down general rules on the definition, description and presentation of spirit drinks [1], as amended by the Act of Accession of Austria, Finland and Sweden, and in particular Article 4(8) thereof,

Whereas:

(1) Article 4(8) of Regulation (EEC) No 1576/89 provides for the adoption of methods to be used for analysing spirit drinks. Reference methods should be used to ensure compliance with Regulation (EEC) No 1576/89 and Commission Regulation (EEC) No 1014/90 of 24 April 1990 laying down detailed implementing rules on the definition, description and presentation of spirit drinks [2], as last amended by Regulation (EC) No 2140/98 [3], when any official control takes place or in the event of a dispute.

(2) As far as possible, it would be useful to adopt and describe as Community analytical reference methods generally recognised methods.

(3) In order to take account of scientific advances and of differences in the equipment of official laboratories, the use of methods based on principles of measurement other than the reference methods described in the Annex hereto should be permitted under the responsibility of the laboratory director, where those methods offer adequate guarantees as regards results and in particular meet the criteria set in the Annex to Council Directive 85/591/EEC of 20 December 1985 concerning the introduction of Community methods of sampling and analysis for the monitoring of foodstuffs intended for human consumption [4] and if it can be shown that the variation in the accuracy, repeatability and reproducibility of the results obtained is within the limits of those obtained using the reference methods described in this Regulation. If that condition is met, the use of other analytical methods should be allowed. However, it is important to specify that in cases of dispute other methods may not replace reference methods.

(4) The measures provided for in this Regulation are in accordance with the opinion of the Implementation Committee for Spirit Drinks,

HAS ADOPTED THIS REGULATION:

Article 1

The Community reference methods for the analysis of spirits drinks to ensure compliance with Regulation (EEC) No 1576/89 and Regulation (EEC) No 1014/90:

— when any official control takes place, or

— in the event of a dispute,

shall be those set out in the Annex hereto.

Article 2

Notwithstanding the first indent of Article 1, other analytical methods shall be permitted, under the responsibility of the director of the laboratory, on condition that the accuracy and precision (repeatability and reproducibility) of the methods are at least equivalent to those of the relevant reference analytical methods given in the Annex.

Article 3

Where Community analytical reference methods are not laid down for the detection and quantification of substances contained in a particular spirit drink, the following methods shall be used:

(a) analytical methods which are validated to internationally recognised procedures and in particular meet the criteria set in the Annex to Directive 85/591/EEC;

(b) analytical methods conforming to the recommended standards of the International Organisation for Standardisation (ISO);

(c) analytical methods recognised by the General Assembly of the International Vine and Wine Office (OIV) and published by that Office;

(d) in the absence of a method as indicated at (a), (b) or (c), by reason of its accuracy, repeatability and reproducibility:

— an analytical method approved by the Member State concerned,

— where necessary, any other suitable analytical method.

[1] OJ L 160, 12.6.1989, p. 1.
[2] OJ L 105, 25.4.1990, p. 9.
[3] OJ L 270, 7.10.1998, p. 9.
[4] OJ L 372, 31.12.1985, p. 50.

Article 4

For the purposes of this Regulation:

(a) 'repeatability limit': shall be the value less than or equal to which the absolute difference between two test results obtained under the repeatability conditions (same operator, same apparatus, same laboratory and a short interval of time) may be expected to be with a probability of 95 % {ISO 3534-1};

(b) 'reproducibility limit': shall be the value less than or equal to which the absolute difference between two test results obtained under the reproducibility conditions (different operators, different apparatus and different laboratories), may be expected to be with a probability of 95 % {ISO 3534-1};

(c) 'accuracy': shall be the closeness of agreement between a test result and the accepted reference value {ISO 3534-1}.

Article 5

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

It shall apply from 1 January 2001.

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

Done at Brussels, 19 December 2000.

For the Commission

Franz FISCHLER

Member of the Commission

ANNEX

DESCRIPTION OF ANALYTICAL REFERENCE METHODS

I. Determination of alcoholic strength by volume
Appendix I: Preparation of distillate
Appendix II: Measurement of density of distillate
— Method A = pycnometry
— Method B = electronic densimetry
— Method C = densimetry using hydrostatic balance

II. Determination of total dry extract by gravimetry

III. Determination of volatile substances and methanol

III.1. General remarks

III.2. Volatile congeners: aldehydes, higher alcohols, ethyl acetate and methanol (gas chromatography)

III.3. Volatile acidity (p.m.)

IV. Hydrocyanic acid (p.m.)

V. Anethole (p.m.)

VI. Glycyrrhizic acid (p.m.)

VII. Chalcones (p.m.)

VIII. Total sugars (p.m.)

IX. Egg yolk (p.m.)

I. DETERMINATION OF ALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS

Introduction

The reference method includes two Appendices:

Appendix I: Preparation of distillate

Appendix II: Measurement of density of distillate

1. **Scope**

The method is suitable for the determination of the real alcoholic strength by volume of spirit drinks.

2. **Normative References**

ISO 3696:1987: Water for analytical laboratory use — Specifications and test methods.

3. **Terms and Definitions**

3.1. *Reference temperature:*

The reference temperature for the determination of alcoholic strength by volume, density and specific gravity of spirit drinks is 20 °C.

Note 1: The term 'at t °C' is reserved for all determinations (of density or alcoholic strength by volume) expressed at a temperature other than the reference temperature of 20 °C.

3.2. *Density:*

The density is the mass per unit volume in vacuo of spirit drinks at 20 °C. It is expressed in kilograms per cubic metre and its symbol is $\rho_{20\,°C}$ or ρ_{20}.

3.3. *Specific gravity:*

The specific gravity is the ratio, expressed as a decimal number, of the density of spirit drinks at 20 °C to the density of water at the same temperature. It is denoted by the symbol $d_{20\,°C/20\,°C}$ or $d_{20/20}$, or simply d when there is no possibility of confusion. The characteristic that was measured must be specified on the assay certificate using the above-defined symbols only.

Note 2: It is possible to obtain the specific gravity from the density ρ_{20} at 20 °C:

$\rho_{20} = 998{,}203 \times d_{20/20}$ or $d_{20/20} = \rho_{20}/998{,}203$

where 998,203 is the density of water at 20 °C.

3.4. *Real alcoholic strength by volume:*

The real alcoholic strength by volume of spirit drinks is equal to the number of litres of ethyl alcohol contained in 100 l of a water-alcohol mixture having the same density as the alcohol or spirit after distillation. The reference values for alcoholic strength by volume (% vol) at 20 °C versus density at 20 °C for different water-alcohol mixtures that are to be used are those given in the international table adopted by the International Legal Metrology Organisation in its Recommendation No 22.

The general equation relating the alcoholic strength by volume and density of a water-alcohol mixture at a given temperature is given on page 40 in Chapter 3 'Alcoholic strength by volume' of the Annex to Commission Regulation (EEC) No 2676/90 (OJ L 272, 3.10.1990, p. 1) or in the manual of analysis methods of the OIV (1994) (p. 17).

Note 3: For liqueurs and crèmes for which it is very difficult to measure volume accurately the sample must be weighed and the alcoholic strength is calculated first by mass.

Conversion formula:

$$\text{alcoholic strength by volume (\% vol)} = \frac{\text{ASM (\% mass)} \times P_{20}\text{ (sample)}}{P_{20}\text{ (alcohol)}}$$

where ASM = alcoholic strength by mass,

ρ_{20} (alcohol) =789,24 kg/m³

4. **Principle**

Following distillation, the alcoholic strength by volume of the distillate is determined by pycnometry, electronic densimetry, or densimetry using a hydrostatic balance.

APPENDIX I: PREPARATION OF DISTILLATE

1. **Scope**

The method is suitable for the preparation of distillates to be used to determine the real alcoholic strength by volume of spirit drinks.

2. **Principle**

The spirits are distilled to separate the ethyl alcohol and other volatile compounds from the extractive matter (substances which do not distil).

3. **Reagents and Materials**

3.1. Anti-bumping granules.

3.2. Concentrated antifoam emulsion (for crème liqueurs).

4. **Apparatus and equipment**

Usual laboratory apparatus and in particular the following.

4.1. Water bath capable of being maintained at 10 °C to 15 °C.

Water bath capable of being maintained at 20 °C (± 0,2 °C).

4.2. Class A volumetric flasks, 100 ml and 200 ml, that have been certified to 0,1 % and 0,15 % respectively.

4.3. Distillation apparatus:

4.3.1. General requirements

The distillation apparatus must meet the following specifications:

— the number of joints must be no more than the strict minimum needed to ensure the system is leak-tight,

— inclusion of a device designed to prevent priming (entrainment of the boiling liquid by the vapour) and to regularise the distillation rate of alcohol-rich vapours,

— rapid and complete condensation of the alcohol vapours,

— collection of the first distillation fractions in an aqueous medium.

The heat source must be used with a suitable heat-diffuser to prevent any pyrogenic reaction involving the extractive matter.

4.3.2. An example of a suitable distillation apparatus is shown in Figure 1 and includes the following parts:

— round-bottomed flask, 1 litre, with a standardised ground-glass joint,

— rectifying column at least 20-cm high (a Vigreux column, for example),

— elbow connector with an approximately 10-cm-long straight-rimmed condenser (a West-type condenser) fitted vertically,

— cooling coil, 40-cm long,

— drawn-out tube, taking the distillate to the bottom of a graduated collecting flask containing a small amount of water.

Note: The apparatus described above is intended for a sample of least 200 ml. However, a smaller sample size (100 ml) can be distilled by using a smaller distillation flask, provided a splashhead or some other device to prevent entrainment is used.

5. **Storage of test samples**

Samples are stored at room temperature prior to analysis.

6. **Procedure**

Preliminary remark:

Distillation may also be by the procedure published by IUPAC (1968).

6.1. Distillation apparatus verification.

The apparatus used must be capable of the following:

The distillation of 200 ml of a water-alcohol solution with known concentration close to 50 % vol must not cause a loss of alcohol of more than 0,1 % vol.

6.2. Spirit drinks with alcoholic strength below 50 % vol.

Measure out 200 ml of the spirit into a volumetric flask.

Record the temperature of this liquid, or maintain at standard temperature (20 °C).

Pour the sample into the round-bottomed flask of the distillation apparatus and rinse the volumetric flask with three aliquots each of approximately 20 ml of distilled water. Add each rinse water aliquot to the contents of the distillation flask.

Note: This 60-ml dilution is sufficient for spirits containing less than 250 g of dry extract per litre. Otherwise, to prevent pyrolysis, the volume of rinse water must be at least 70 ml if the dry extract concentration is 300 g/l, 85 ml for 400 g/l dry extract, and 100 ml for 500 g/l dry extract (some fruit liqueurs or crèmes). Adjust these volumes proportionally for different sample volumes.

Add a few anti-bumping granules (3.1) (and antifoam for crème liqueurs).

Pour 20 ml of distilled water into the original 200 ml volumetric flask that will be used to hold the distillate. This flask must then be placed in a cold water bath (4.1) (10 to 15 °C for aniseed-flavoured spirit drinks).

Distil, avoiding entrainment and charring, occasionally agitating the contents of the flask, until the level of distillate is a few millimetres below the calibration mark of the volumetric flask.

When the temperature of this distillate has been brought down to within 0,5 °C of the liquid's initial temperature, make up to the mark with distilled water and mix thoroughly.

This distillate is used for the determination of alcoholic strength by volume (Appendix II)

6.3. Spirit drinks with alcoholic strength above 50 % vol.

Measure out 100 ml of the spirit drink into a 100-ml volumetric flask and pour into the round bottomed flask of the distillation apparatus.

Rinse the volumetric flask several times with distilled water and add the washings to the contents of the round-bottomed distillation flask. Use enough water to bring the flask's contents up to approximately 230 ml.

Pour 20 ml of distilled water into a 200-ml volumetric flask that will be used to hold the distillate. This flask must then be placed in a cold water bath (4.1) (10 to 15 °C for aniseed-flavoured spirits).

Distil, agitating the contents occasionally, until the level of distillate is a few millimetres below the calibration mark of the 200-ml volumetric flask.

When the temperature of this distillate has been brought down to within 0,5 °C of the liquid's initial temperature, make up to the mark with distilled water and mix thoroughly.

This distillate is used for the determination of alcoholic strength by volume (Appendix II)

Note: The alcoholic strength by volume of the spirit drink is twice the alcoholic strength of the distillate.

APPENDIX II: MEASUREMENT OF DENSITY OF DISTILLATE

METHOD A: DETERMINATION OF REAL ALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS — MEASUREMENT BY PYCNOMETRY

A.1. Principle

The alcoholic strength by volume is obtained from the density of the distillate measured by pycnometry.

A.2. Reagents and materials

During the analysis, unless otherwise is stated, use only reagents of recognised analytical grade and water of at least grade 3 as defined in ISO 3696:1987.

A.2.1. Sodium chloride solution (2 % w/v)

To prepare 1 litre, weigh out 20 g of sodium chloride and dissolve to 1 litre using water.

A.3. Apparatus and Equipment

Usual laboratory apparatus and in particular the following:

A.3.1. Analytical balance capable of reading 0,1 mg.

A.3.2. Thermometer, with ground glass joint, calibrated in tenths of a degree from 10 to 30 °C. This thermometer must be certified or checked against a certified thermometer.

A.3.3. Pyrex-glass pycnometer of approximately 100 ml capacity fitted with a removable ground-glass thermometer (A.3.2). The pycnometer has a side tube 25 mm in length and 1 mm (maximum) in internal diameter ending in a conical ground joint. Other pycnometers as described in ISO 3507, e.g. 50 ml may be used if appropriate.

A.3.4. A tare bottle of the same external volume (to within 1 ml) as the pycnometer and with a mass equal to the mass of the pycnometer filled with a liquid of density 1,01 (sodium chloride solution A.2.1).

A.3.5. Thermally insulated jacket that fits the body of the pycnometer exactly.

Note 1: The method for determining the densities *in vacuo* of spirits calls for the use of a twin-pan balance, a pycnometer and a tare bottle of the same outside external volume to cancel out the effect of air buoyancy at any given moment. This simple technique may be applied using a single-pan balance provided that the tare bottle is weighed again to monitor changes in air buoyancy over time.

A.4. Procedure

Preliminary remarks:

The following procedure is described for the use of 100-ml pycnometer for determination of the alcoholic strength; this gives the best accuracy. However, it is also possible to use a smaller pycnometer, for example 50 ml.

A.4.1. Calibration of pycnometer

The pycnometer is calibrated by determining the following parameters:

— tare of the empty pycnometer,

— volume of the pycnometer at 20 °C,

— mass of the water-filled pycnometer at 20 °C.

A.4.1.1. Calibration using a single-pan balance:

Determine:

— the mass of the clean, dry pycnometer (P),

— the mass of the water-filled pycnometer at t °C (P1),

— the mass of the tare bottle (T0).

A.4.1.1.1. Weigh the clean, dry pycnometer (P).

A.4.1.1.2. Fill the pycnometer carefully with distilled water at ambient temperature and fit the thermometer.

Carefully wipe the pycnometer dry and place it in the thermally-insulated jacket. Agitate by inverting the container until the thermometer's temperature reading is constant.

Set the pycnometer flush with the upper rim of the side tube. Read the temperature t °C carefully and if necessary correct for any inaccuracies in the temperature scale.

Weigh the water-filled pycnometer (P1).

A.4.1.1.3. Weigh the tare bottle (T0).

A.4.1.1.4. Calculation

— Tare of the empty pycnometer = P – m

where m is the mass of air in the pycnometer.

$m = 0{,}0012 \times (P1 - P)$

Note 2: 0,0012 is the density of dry air at 20 °C at a pressure of 760 mm Hg

— Volume of the pycnometer at 20 °C:

$V_{20\,°C} = [P1 - (P - m)] \times F_t,1$

where F_t is the factor for temperature t °C taken from Table I of Chapter 1 'Density and specific gravity' of the Annex to Regulation (EEC) No 2676/90 (p. 10).

$V_{20\,°C}$ must be known to the nearest 0,001 ml.

— Mass of water in the pycnometer at 20 °C:

$M_{20\,°C} = V_{20\,°C} \times 0{,}998203$

where 0,998203 is the density of water at 20 °C.

Note 3: If necessary, the value 0,99715 of the density in air can be used and the alcoholic strength calculated with reference to the corresponding density in HM Customs and Excise tables in air.

A.4.1.2. Calibration method using a twin-pan balance:

A.4.1.2.1. Place the tare bottle on the left-hand pan and the clean, dry pycnometer with its collecting stopper on the right-hand pan. Balance them by placing weights on the pycnometer side: p grams.

A.4.1.2.2. Fill the pycnometer carefully with distilled water at ambient temperature and fit the thermometer; carefully wipe the pycnometer dry and place it in the thermally insulated jacket; agitate by inverting the container until the thermometer's temperature reading is constant.

Accurately adjust the level to the upper rim of the side tube. Clean the side tube, fit the collecting stopper; read the temperature t °C carefully and if necessary correct for any inaccuracies in the temperature scale.

Weigh the water-filled pycnometer, with p′ the weight in grams making up the equilibrium.

A.4.1.2.3. Calculation

— Tare of the empty pycnometer = p + m

where m is the mass of air in the pycnometer.

$m = 0{,}0012 \times (p - p')$

— Volume of the pycnometer at 20 °C:

$V_{20\,°C} = (p + m - p') \times F_t,1$

where F_t is the factor for temperature t °C taken from Table I of Chapter 1 'Density and specific gravity' of the Annex to Regulation (EEC) No 2676/90 (p. 10).

$V_{20\,°C}$ must be known to the nearest 0,001 ml.

— Mass of water in the pycnometer at 20 °C:

$M_{20\,°C} = V_{20\,°C} \times 0{,}998203$

where 0,998203 is the density of water at 20 °C.

A.4.2. Determination of alcoholic strength of test sample

A.4.2.1. Using a single-pan balance.

A.4.2.1.1. Weigh the tare bottle, weight T1.

A.4.2.1.2. Weigh the pycnometer with the prepared distillate (see Appendix I), P2 is its weight at t °C.

A.4.2.1.3. Calculation

— dT = T1 – T0

— Mass of empty pycnometer at moment of measuring
= P – m + dT

— Mass of the liquid in the pycnometer at t °C
= P2 – (P – m + dT)

— Density at t °C in g/ml

— $P_{t\,°C} = [P_2 – (P – m + dT)]/V_{20\,°C}$

— Express the density at t °C in kilograms per m³ by multiplying $\rho_{t\,°C}$ by 1 000, the value being known as ρ_t.

— Correct ρ_t to 20 using the table of densities ρ_T for water-alcohol mixtures (Table II of Appendix II to the OIV's manual of analysis methods (1994), pp. 17-29).

In the table find the horizontal line corresponding to temperature T in whole degrees immediately below t °C, the smallest density above ρ_t. Use the table difference found below that density to calculate the density ρ_t of the spirit at that temperature T in whole degrees.

— Using the whole temperature line, calculate the difference between density ρ′ in the table immediately above ρ_t and the calculated density ρ_t. Divide that difference by the table difference found to the right of density ρ′. The quotient provides the decimal portion of the alcoholic strength while the integer of the alcoholic strength is found at the top of the column in which density ρ′ is found (Dt, the alcoholic strength).

Note 4: Alternatively keep the pycnometer in a water bath maintained at 20 °C (± 0,2 °C) when making up to the mark.

A.4.2.1.4. Result

Using the density ρ_{20} calculate the real alcoholic strength using the alcoholic strength tables identified below:

The table giving the value of the alcoholic strength by volume (% vol) at 20 °C as a function of the density at 20 °C of water-alcohol mixtures is the international table adopted by the International Legal Metrology Organisation in its Recommendation No 22.

A.4.2.2. Method using a single-pan balance

A.4.2.2.1. Weigh the pycnometer with the distillate prepared (see part I), p″ is mass at t °C.

A.4.2.2.2. Calculation

— Mass of the liquid in the pycnometer at t °C
= p + m – p″

— Density at t °C in g/ml

$P_{t\,°C} = (p + m – p'')/V_{20\,°C}$

— Express the density at t °C in kilograms per m³ and carry out the temperature correction in order to calculate the alcoholic strength at 20 °C, as indicated above for use of the single-pan balance.

A.5. **Method performance characteristics (precision)**

A.5.1. Statistical results of the interlaboratory test

The following data were obtained from an international method performance study carried out to internationally agreed procedures [1] [2].

Year of interlaboratory test	1997
Number of laboratories	20
Number of samples	6

Samples	A	B	C	D	E	F
Number of laboratories retained after eliminating outliers	19	20	17	19	19	17
Number of outliers (laboratories)	1	—	2	1	1	3
Number of accepted results	38	40	34	38	38	34
Mean value ($\bar{x}$) % vol	23,77	40,04	40,29	39,20	42,24	57,03
	26,51 (*)			42,93 (*)	45,73 (*)	63,03 (*)
Repeatability standard (S_r) % vol	0,106	0,176	0,072	0,103	0,171	0,190
Repeatability relative standard deviation (RSD_r) (%)	0,42	0,44	0,18	0,25	0,39	0,32
Repeatability limit (r) in % vol	0,30	0,49	0,20	0,29	0,48	0,53
Reproductibility standard deviation (S_R) % vol	0,131	0,236	0,154	0,233	0,238	0,322
Reproductibility relative standard deviation (RSD_R) (%)	0,52	0,59	0,38	0,57	0,54	0,53
Reproductibility limit (R) in % vol	0,37	0,66	0,43	0,65	0,67	0,90

Sample types
A Fruit liqueur; split level (*).
B Brandy; blind duplicates.
C Whisky; blind duplicates.
D Grappa; split level (*).
E Aquavit; split level (*).
F Rum; split level (*).

METHOD B: DETERMINATION OF REAL ALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS — MEASUREMENT BY ELECTRONIC DENSIMETRY (BASED ON THE RESONANT FREQUENCY OSCILLATION OF A SAMPLE IN AN OSCILLATION CELL)

B.1. Principle

The liquid's density is determined by electronic measurement of the oscillations of a vibrating U-tube. To perform this measurement, the sample is added to an oscillating system, whose specific oscillation frequency is thus modified by the added mass.

B.2. Reagents and materials

During the analysis, unless otherwise is stated, use only reagents of recognised analytical grade and water of at least grade 3 as defined in ISO 3696:1987.

B.2.1. Acetone (CAS 666-52-4) or absolute alcohol

B.2.2. Dry air.

B.3. Apparatus and equipment

Usual laboratory apparatus and in particular the following:

B.3.1. Digital display densimeter

Electronic densimeter for performing such measurements must be capable of expressing density in g/ml to 5 decimal places.

Note 1: The densimeter should be placed on a perfectly stable stand that is insulated from all vibrations.

B.3.2. Temperature regulation

The densimeter's performance is valid only if the measuring cell is connected to a built-in temperature regulator that can achieve the same temperature stability of ± 0,02 °C or better.

Note 2: The precise setting and monitoring of the temperature in the measuring cell are very important, for an error of 0,1 °C can lead to a variation in density of the order of 0,1 kg/m^3.

B.3.3. Sample injection syringes or auto sampler.

B.4. **Procedure**

B.4.1. Calibration of the densimeter

The apparatus must be calibrated according to the instrument manufacturer's instructions when it is first put into service. It must be recalibrated regularly and checked against a certified reference standard or an internal laboratory reference solution based on a certified reference standard.

B.4.2. Determination of sample density

B.4.2.1. If required prior to measurement clean and dry the cell with acetone or absolute alcohol and dry air. Rinse the cell with the sample.

B.4.2.2. Inject the sample into the cell (using a syringe or autosampler) so that the cell is completely filled. During the filling operation make sure that all air bubbles are completely eliminated. The sample must be homogeneous and must not contain any solid particles. Any suspended matter should be removed by filtration prior to analysis.

B.4.2.3. Once the reading has stabilised, record the density ρ_{20} or the alcoholic strength displayed by the densimeter.

B.4.3. Result

When the density ρ_{20} is used, calculate the real alcoholic strength using the alcoholic strength tables identified below:

The table giving the value of the alcoholic strength by volume (% vol) at 20 °C as a function of the density at 20 °C of water-alcohol mixtures is the international table adopted by the International Legal Metrology Organisation in its Recommendation No 22.

B.5. **Method performance characteristics (precision)**

B.5.1. Statistical results of the interlaboratory test

The following data were obtained from an international method performance study carried out to internationally agreed procedures [1] [2].

Year of interlaboratory test	1997
Number of laboratories	16
Number of samples	6

Samples	A	B	C	D	E	F
Number of laboratories retained after eliminating outliers	11	13	15	16	14	13
Number of outliers (laboratories)	2	3	1	—	1	2
Number of accepted results	22	26	30	32	28	26
Mean value ($\bar{x}$) % vol	23,81	40,12	40,35	39,27	42,39	56,99
	26,52 (*)			43,10 (*)	45,91 (*)	63,31 (*)
Repeatability standard deviation (S_r) % vol	0,044	0,046	0,027	0,079	0,172	0,144
Repeatability relative standard deviation (RSD_r) (%)	0,17	0,12	0,07	0,19	0,39	0,24
Repeatability limit (r) % vol	0,12	0,13	0,08	0,22	0,48	0,40
Reproducibility standard deviation (S_R) % vol	0,054	0,069	0,083	0,141	0,197	0,205
Reproducibility relative standard deviation (RSD_R) (%)	0,21	0,17	0,21	0,34	0,45	0,34
Reproducibility limit (R) % vol	0,15	0,19	0,23	0,40	0,55	0,58

Sample types
A Fruit liqueur; split level (*).
B Brandy; blind duplicates.
C Whisky; blind duplicates.
D Grappa; split level (*).
E Aquavit; split level (*).
F Rum; split level (*).

METHOD C: DETERMINATION OF REAL ALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS — MEASUREMENT BY DENSIMETRY USING HYDROSTATIC BALANCE

C.1. **Principle**

The alcoholic strength of spirits can be measured by densimetry using a hydrostatic balance based on Archimedes' principle according to which a body immersed in a liquid receives a vertical upward thrust from the liquid equal to the weight of liquid displaced.

C.2. **Reagents and materials**

During the analysis, unless otherwise is stated, use only reagents of recognised analytical grade and water of at least grade 3 as defined in ISO 3696:1987.

C.2.1. Float cleaning solution (sodium hydroxide, 30 % w/v)

To prepare 100 ml, weigh 30 g of sodium hydroxide and make up to volume using 96 % volume ethanol.

C.3. **Apparatus and Equipment**

Usual laboratory apparatus and in particular the following:

C.3.1. Single-pan hydrostatic balance with a sensitivity of 1 mg.

C.3.2. Float with a volume of at least 20 ml, specially adapted to the balance, suspended with a thread of diameter not exceeding 0,1 mm.

C.3.3. Measuring cylinder bearing a level mark. The float must be capable of being contained completely within the volume of the cylinder located below the mark; the surface of the liquid may only be penetrated by the supporting thread. The measuring cylinder must have an internal diameter at least 6 mm larger than that of the float.

C.3.4. Thermometer (or temperature-measuring probe) graduated in degrees and tenths of a degree from 10 to 40 °C, calibrated to 0,05 °C.

C.3.5. Weights, calibrated by a recognised certifying body.

Note 1: Use of a twin-pan balance is also possible; the principle is described in Chapter 1 'Density and specific gravity' of the Annex to Regulation (EEC) No 2676/90 (p. 7).

C.4. **Procedure**

The float and measuring cylinder must be cleaned between each measurement with distilled water, dried with soft laboratory paper which does not shed fibres and rinsed with the solution whose density is to be determined. Measurements must be made as soon as the apparatus has reached stability so as to restrict alcohol loss by evaporation.

C.4.1. Calibration of the balance

Although balances usually have an internal calibration system, the hydrostatic balance must be capable of calibration with weights checked by an official certifying body.

C.4.2. Calibration of the float

C.4.2.1. Fill the measuring cylinder to the mark with double-distilled water (or water of equivalent purity, e.g. microfiltered water with a conductivity of 18,2 MΩ/cm) at a temperature between 15 and 25 °C but preferably at 20 °C.

C.4.2.2. Immerse the float and the thermometer, stir, read off the density of the liquid from the apparatus and, if necessary, correct the reading so that it is equal to that of the water at measurement temperature.

C.4.3. Control using a water-alcohol solution

C.4.3.1. Fill the measuring cylinder to the mark with a water-alcohol mixture of known strength at a temperature between 15 and 25 °C but preferably at 20 °C.

C.4.3.2. Immerse the float and the thermometer, stir, read off the density of the liquid (or the alcoholic strength if this is possible) from the apparatus. The alcoholic strength thus established should be equal to the previously determined alcoholic strength.

Note 2: This solution of known alcoholic strength can also be used to calibrate the float instead of double-distilled water.

C.4.4. Measurement of the density of a distillate (or of its alcoholic strength if the apparatus allows)

C.4.4.1. Pour the test sample into the measuring cylinder up to the graduation mark.

C.4.4.2. Immerse the float and the thermometer, stir, read off the density of the liquid (or the alcoholic strength if this is possible) from the apparatus. Note the temperature if the density is measured at t °C (ρ_t).

C.4.4.3. Correct ρ_t to 20 using the table of densities ρT for water-alcohol mixtures (Table II of Annex II to the OIV's Manual of analysis methods (1994), pp. 17-29).

C.4.5. Cleaning of float and measuring cylinder

C.4.5.1. Immerse the float in the float cleaning solution in the measuring cylinder.

C.4.5.2. Allow to soak for one hour spinning the float periodically.

C.4.5.3. Rinse with copious amounts of tap water followed by distilled water.

C.4.5.4. Dry with soft laboratory paper which does not shed fibres.

Carry out this procedure when the float is first used and then regularly as required.

C.4.6. Result

Using the density ρ_{20} calculate the real alcoholic strength using the alcoholic strength tables identified below.

The table giving the value of the alcoholic strength by volume (% vol) at 20 °C as a function of the density at 20 °C of water-alcohol mixtures is the international table adopted by the International Legal Metrology Organisation in its Recommendation No 22.

C.5. **Method performance characteristics (precision)**

C.5.1. Statistical results of the interlaboratory test

The following data were obtained from an international method performance study carried out to internationally agreed procedures [1] [2].

Year of interlaboratory test	1997
Number of laboratories	12
Number of samples	6

Samples	A	B	C	D	E	F
Number of laboratories retained after eliminating outliers	12	10	11	12	11	9
Number of outliers (laboratories)	—	2	1	—	1	2
Number of accepted results	24	20	22	24	22	18
Mean value ($\bar{x}$) % vol	23,80	40,09	40,29	39,26	42,38	57,16
	26,51 (*)			43,09 (*)	45,89 (*)	63,44 (*)
Repeatability standard deviation (S_r) % vol	0,048	0,065	0,042	0,099	0,094	0,106
Repeatability relative standard deviation (RSD_r) (%)	0,19	0,16	0,10	0,24	0,21	0,18
Repeatability limit (r) % vol	0,13	0,18	0,12	0,28	0,26	0,30
Reproducibility standard deviation (S_R) % vol	0,060	0,076	0,073	0,118	0,103	0,125
Reproducibility relative standard deviation (RSD_R) (%)	0,24	0,19	0,18	0,29	0,23	0,21
Reproducibility limit (R) % vol	0,17	0,21	0,20	0,33	0,29	0,35

Sample types
A Fruit liqueur; split level (*).
B Brandy; blind duplicates.
C Whisky; blind duplicates.
D Grappa; split level (*).
E Aquavit; split level (*).
F Rum; split level (*).

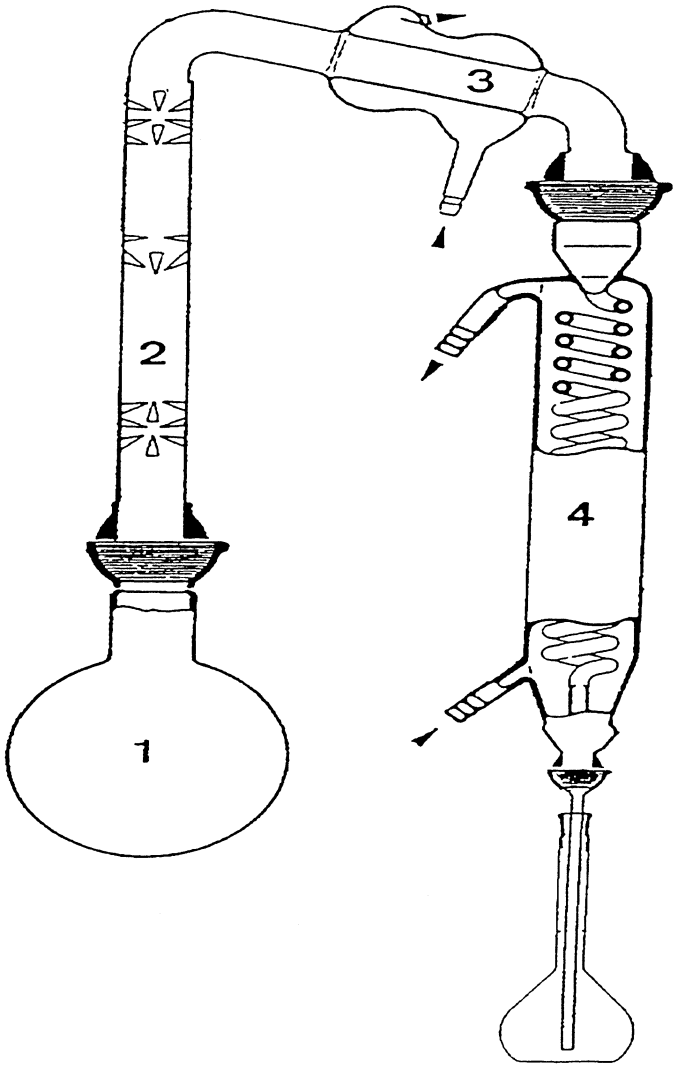

Figure 1. Distillation apparatus for measuring the real alcoholic strength by volume of spirits

1. 1-litre round-bottomed flask with standardised spherical ground-glass joint.
2. 20-cm Vigreux rectifying column.
3. 10-cm straight-rimmed West condenser.
4. 40-cm cooling coil.

II. DETERMINATION OF TOTAL DRY EXTRACT OF SPIRIT DRINKS BY GRAVIMETRY

1. Scope

Regulation (EEC) No 1576/89 provides for this method only for aquavit for which the dry extract is limited to 15 g/l.

2. Normative References

ISO 3696:1987: Water for analytical laboratory use — Specifications and test methods.

3. Definition

The total dry extract or total dry matter includes all matter that is non-volatile under specified physical conditions.

4. Principle

Weighing of the residue left by evaporation of the spirit on a boiling water bath and drying in a drying oven.

5. Apparatus and Equipment

5.1. Flat-bottomed cylindrical evaporating dish 55 mm in diameter.

5.2. Boiling water bath.

5.3. 25 ml pipette, class A.

5.4. Drying oven.

5.5. Desiccator.

5.6. Analytical balance accurate to 0,1 mg.

6. Sampling and samples

Samples are stored at room temperature prior to analysis.

7. Procedure

7.1. Pipette 25 ml of the spirit containing less than 15 g/l dry matter into a previously weighed flat-bottomed cylindrical evaporating dish 55 mm in diameter. During the first hour of evaporation the evaporating dish is placed on the lid of a boiling water bath so that the liquid will not boil, as this could lead to losses through splattering. Leave one more hour directly in contact with the steam of the boiling water bath.

7.2. Complete the drying by placing the evaporating dish in a drying oven at 105 °C ± 3 °C for two hours. Allow the evaporating dish to cool in a desiccator and weigh the evaporating dish and its contents.

8. Calculation

The mass of the residue multiplied by 40 is equal to the dry extract contained in the spirit and it must be expressed in g/l to one decimal place.

9. Method performance characteristics (precision)

9.1. Statistical results of the interlaboratory test

The following data were obtained from an international method performance study carried out to internationally agreed procedures [1] [2].

Year of interlaboratory test	1997
Number of laboratories	10
Number of samples	4

Samples	A	B	C	D
Number of laboratories retained after eliminating outliers	9	9	8	9
Number of outliers (laboratories)	1	1	2	—
Number of accepted results	18	18	16	18
Mean value ($\bar{x}$) g/l	9,0	9,1	10,0	11,8
		7,8	9,4	11,1
Repeatabilities standard deviation (S_r) g/l	0,075	0,441	0,028	0,123
Repeatabilities relative standard deviation (RSD_r) (%)	0,8	5,2	0,3	1,1
Repeatabilities limit (r) g/l	0,2	1,2	0,1	0,3
Reproductibility standard deviation (S_R) g/l	0,148	0,451	0,058	0,210
Reproductibility relative standard deviation (RSD_R) (%)	1,6	5,3	0,6	1,8
Reproductibility limit (R) g/l	0,4	1,3	0,2	0,6

Sample types
A Brandy; blind duplicaties.
B Rum; split levels.
C Grappa; split levels.
D Aquavit; split levels.

III. DETERMINATION OF VOLATILE SUBSTANCES AND METHANOL OF SPIRIT DRINKS

III.1. GENERAL REMARKS

1. Definitions

Regulation (EEC) No 1576/89 sets minimum levels of volatile compounds other than ethanol and methanol for a series of spirit drinks (rum, spirits of viticultural origin, fruit spirits, etc.). For this series of drinks only, these levels are conventionally considered to be equivalent to the sum of the concentrations of:

1. volatile acids expressed as acetic acid;
2. aldehydes expressed as ethanal by the sum of ethanal (acetaldehyde) and the ethanal fraction contained in 1,1-diethoxyethane (acetal);
3. the following higher alcohols: propan-1-ol, butan-1-ol, butan-2-ol, 2-methylpropan-1-ol, assayed by individual alcohol and 2-methylbutan-1-ol, and 3-methylbutan-1-ol assayed as individual alcohol or the sum of the two;
4. ethyl acetate.

The following are the conventional methods for measuring volatile compounds:

— the volatile acids by means of volatile acidity,

— the aldehydes (ethanal and acetal), ethyl acetate and the alcohols by means of gas chromatography (GPC).

2. Gas chromatographic analysis of volatile compounds

Gas chromatographic assays of volatile compounds other than those set out above may prove particularly interesting as a means of determining both the origin of the raw material used in the distillation and the actual conditions of distillation.

Some spirits contain other volatile components, such as aromatic compounds, which are characteristic of the raw materials used to obtain the alcohol, of the aroma of the spirit drink and of the special features of the preparation of the spirit. These compounds are important for evaluating the requirements set out in Regulation (EEC) No 1576/89.

III.2. GAS CHROMATOGRAPHIC DETERMINATION OF VOLATILE CONGENERS: ALDEHYDES, HIGHER ALCOHOLS, ETHYL ACETATE AND METHANOL

1. Scope

This method is suitable for use for the determination of 1,1-diethoxyethane (acetal), 2-methylbutan-1-ol (active amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol), methanol (methyl alcohol), ethyl ethanoate (ethyl acetate), butan-1-ol (n-butanol), butan-2-ol (sec-butanol), 2-methylpropan-1-ol (isobutyl alcohol), propan-1-ol (n-propanol) and ethanal (acetaldehyde) in spirit drinks using gas chromatography. The method uses an internal standard, for example pentan-3-ol. The concentrations of the analytes are expressed as grams per 100 litres of absolute alcohol; the alcoholic strength of the product must be determined prior to analysis. The spirit drinks that can be analysed using this method include whisky, brandy, rum, wine spirit, fruit spirit and grape marc spirit.

2. Normative References

ISO 3696:1987: Water for analytical laboratory use — Specifications and test methods.

3. Definition

Congeners are volatile substances formed along with ethanol during fermentation, distillation and maturation of spirit drinks.

4. Principle

Congeners in spirit drinks are determined by direct injection of the spirit drink, or appropriately diluted spirit drink, into a gas chromatography (GC) system. A suitable internal standard is added to the spirit drink prior to injection. The congeners are separated by temperature programming on a suitable column and are detected using a flame ionisation detector (FID). The concentration of each congener is determined with respect to the internal standard from response factors, which are obtained during calibration under the same chromatographic conditions as those of the spirit drink analysis.

5. **Reagents and materials**

Unless otherwise stated, use only reagents of a purity greater than 97 %, purchased from an ISO-accredited supplier with a certificate of purity, free from other congeners at test dilution (this may be confirmed by injection of individual congener standards at the test dilution using GC conditions as in 6.4) and only water of at least grade 3 as defined in ISO 3696. Acetal and acetaldehyde must be stored in the dark at < 5 °C, all other reagents may be stored at room temperature.

5.1. Ethanol absolute (CAS 64-17-5).

5.2. Methanol (CAS 67-56-1).

5.3. Propan-1-ol (CAS 71-23-8).

5.4. 2-methylpropan-1-ol (CAS 78-33-1).

5.5. Acceptable internal standards: pentan-3-ol (CAS 584-02-1), pentan-1-ol (CAS 71-41-0), 4-methylpentan-1-ol (CAS 626-89-1) or methyl nonanoate (CAS 1731-84-6).

5.6. 2-methylbutan-1-ol (CAS 137-32-6).

5.7. 3-methylbutan-1-ol (CAS 123-51-3).

5.8. Ethyl acetate (CAS 141-78-6).

5.9. Butan-1-ol (CAS 71-36-3).

5.10. Butan-2-ol (CAS 78-92-2).

5.11. Acetaldehyde (CAS 75-07-0).

5.12. Acetal (CAS 105-57-7).

5.13. 40 % v/v ethanol solution

To prepare 400 ml/l ethanol solution pour 400 ml ethanol (5.1) into a 1-litre volumetric flask, make up to volume with distilled water and mix.

5.14. Preparation and storage of standard solutions (procedure used for the validated method).

All standard solutions must be stored at < 5 °C and be prepared freshly on a monthly basis. Masses of components and solutions should be recorded to the nearest 0,1 mg.

5.14.1. Standard solution — A

Pipette the following reagents into a 100-ml volumetric flask, containing approximately 60-ml ethanol solution (5.13) to minimise component evaporation, make up to volume with ethanol solution (5.13) and mix thoroughly. Record the weight of the flask, each component added and the total final weight of contents.

Component	Volume (ml)
Methanol (5.2)	3,0
Propan-1-ol (5.3)	3,0
2-methylpropan-1-ol (5.4)	3,0
2-methylbutan-1-ol (5.6)	3,0
3-methylbutan-1-ol (5.7)	3,0
Ethyl acetate (5.8)	3,0
Butan-1-ol (5.9)	3,0
Butan-2-ol (5.10)	3,0
Acetaldehyde (5.11)	3,0
Acetal (5.12)	3,0

Note 1: It is preferable to add acetal and acetaldehyde last in order to minimise losses through evaporation.

5.14.2. Standard solution — B

Pipette 3 ml of pentan-3-ol, or other suitable internal standard, (5.5) into a 100-ml volumetric flask, containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, the weight of pentan-3-ol or other internal standard added and the total final weight of contents.

5.14.3. Standard solution — C

Pipette 1 ml solution A (5.14.1) and 1 ml solution B (5.14.2) into a 100-ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

5.14.4. Standard solution — D

In order to maintain analytical continuity, prepare a quality control standard using the previously prepared standard A (5.14.1). Pipette 1 ml solution A (5.14.1) into a 100-ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

5.14.5. Standard solution — E

Pipette 10 ml solution B (5.14.2) into a 100-ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

5.14.6. Standard solutions used to check the linearity of response of FID

Into separate 100-ml volumetric flasks, containing approximately 80 ml ethanol (5.13), pipette 0, 0,1, 0,5, 1,0, 2,0 ml solution A (5.14.1) and 1 ml solution B (5.14.2), make up to volume with ethanol solution (5.13) and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

5.14.7. QC standard solution

Pipette 9 ml standard solution D (5.14.4) and 1 ml of standard solution E (5.14.5) into a weighing vessel and mix thoroughly.

Record the weight of the flask, each component added and the total final weight of contents.

6. **Apparatus and equipment**

6.1. Apparatus capable of measuring the density and alcoholic strength.

6.2. Analytical balance, capable of measuring to four decimal places.

6.3. A temperature programmed gas chromatograph fitted with a flame ionisation detector and integrator or other data handling system capable of measuring peak areas or peak heights.

6.4. Gas chromatographic column(s), capable of separating the analytes such that the minimum resolution between the individual components (other than 2-methylbutan-1-ol and 3-methylbutan-1-ol) is at least 1.3.

Note 2: The following columns and GC conditions are suitable examples:

1. A retention gap 1 m × 0,32 mm i.d. connected to a CP-WAX 57 CB column 50 m × 0,32 mm i.d. 0,2 μm film thickness (stabilised polyethylene glycol) followed by a Carbowax 400 column 50 m × 0,32 mm i.d. 0,2 μm film thickness. (Columns are connected using press-fit connectors.)

Carrier gas and pressure:	Helium (135 kPa)
Column temperature:	35 °C for 17 min., 35 to 70 °C at 12 °C/min., hold at 70 °C for 25 min.
Injector temperature:	150 °C
Detector temperature:	250 °C
Injection volume:	1 µl, split 20 to 100:1

2. A retention gap 1 m × 0,32 mm i.d. connected to a CP-WAX 57 CB column 50 m × 0,32 mm i.d. 0,2 µm film thickness (stabilised polyethylene glycol). (Retention gap is connected using a press-fit connector.)

Carrier gas and pressure:	Helium (65 kPa)
Column temperature:	35 °C for 10 min., 35 to 110 °C at 5 °C/min., 110 to 190 °C at 30 °C/min., hold at 190 °C for 2 min.
Injector temperature:	260 °C
Detector temperature:	300 °C
Injection volume:	1 µl, split 55:1

3. A packed column (5 % CW 20M, Carbopak B), 2 m × 2 mm i.d.

Column temperature:	65 °C for 4 min., 65 to 140 °C at 10 °C/min., hold at 140 °C for 5 min., 140 to 150 °C at 5 °C/min., hold at 150 °C for 3 min.
Injector temperature:	65 °C
Detector temperature:	200 °C
Injection volume:	1 µl

7. **Sampling and samples.**

7.1. Laboratory sample

On receipt, the alcoholic strength of each sample is measured (6.1).

8. **Procedure (used for the validated method)**

8.1. Test portion

8.1.1. Weigh an appropriate sealed weighing vessel and record the weight.

8.1.2. Pipette 9 ml laboratory sample into the vessel and record the weight (M_{SAMPLE}).

8.1.3. Add 1 ml of standard solution E (5.14.5) and record the weight (M_{IS}).

8.1.4. Shake the test material vigorously (at least 20 inversions). Samples must be stored at less than 5 °C prior to analysis in order to minimise any volatile losses.

8.2. Blank test

8.2.1. Using a four decimal place balance (6.2), weigh an appropriate sealed weighing vessel and record the weight.

8.2.2. Pipette 9 ml 400 ml/l ethanol solution (5.13) into the vessel and record the weight.

8.2.3. Add 1 ml of standard solution E (5.14.5) and record the weight.

8.2.4. Shake the test material vigorously (at least 20 inversions). Samples must be stored at less than 5 °C prior to analysis in order to minimise any volatile losses.

8.3. Preliminary test

Inject standard solution C (5.14.3) to ensure that all of the analytes are separated with a minimum resolution of 1.3 (except 2-methylbutan-1-ol and 3-methylbutan-1-ol).

8.4. Calibration

The calibration should be checked using the following procedure. Ensure that the response is linear by successively analysing in triplicate each of the linearity standard solutions (5.14.6) containing internal standard (IS). From the integrator peak areas or peak heights for each injection calculate the ratio R for each congener and plot a graph of R versus the concentration ratio of congener to internal standard (IS), C. A linear plot should be obtained, with a correlation coefficient of at least 0,99.

$$R = \frac{\text{Peak area or height of congener}}{\text{Peak area or height of IS}}$$

$$C = \frac{\text{Concentration of congener (µg / g)}}{\text{Concentration of IS (µg / g)}}$$

8.5. Determination

Inject standard solution C (5.14.3) and 2 QC standard solutions (5.14.7). Follow with unknown samples (prepared according to 8.1 and 8.2) inserting one QC standard every 10 samples to ensure analytical stability. Inject one standard solution C (5.14.3) after every 5 samples.

9. **Calculation**

An automated system of data handling can be used, provided the data can be checked using the principles described in the method below.

Measure either peak areas or peak heights for congener and internal standard peaks.

9.1. Response factor calculation.

From the chromatogram of the injection of standard solution C (5.14.3), calculate response factors for each congener using equation (1).

$$(1)\ \text{Response factor} = \frac{\text{Peak area or height IS}}{\text{Peak area or height congener}} \times \frac{\text{Conc. congener (µg / g)}}{\text{Conc. IS (µg / g)}}$$

where:

IS = Internal Standard

Conc. congener = concentration of congener in solution C (5.14.3)

Conc. IS = concentration of internal standard in solution C (5.14.3).

9.1.2. Sample analysis

Using equation (2) below, calculate the concentration of each congener in the samples.

(2) Congener concentrations, (µg/g) =

$$\frac{\text{Peak area or height congener}}{\text{Peak area or height IS}} \times \frac{M_{IS}\ (g)}{M_{SAMPLE}\ (g)} \times \text{Conc. IS (µg / g)} \times \text{RF}$$

where:

M_{SAMPLE} = weight of sample (8.1.2);

M_{IS} = weight of internal standard (8.1.3);

Conc. IS = concentration of internal standard in solution E (5.14.5);

RF = response factor calculated using equation 1.

9.1.3. Quality control standard solution analysis

Using equation (3) below, calculate the percentage recovery of the target value for each congener in the quality control standards (5.14.7):

$$(3)\ \%\ \text{recovery of QC sample} = \frac{\text{concentration of analyte in QC standard}}{\text{concentration of analyte in solution D}} \times 100$$

The concentration of the analyte in the QC standard is calculated using equations (1) and (2) above.

9.2. Final presentation of results

Results are converted from µ/g to g per 100 litres absolute alcohol for samples using equation (4):

(4) Concentration in g per 100 literes absolute alcohol =

$$\text{Conc (µg / g)} \times \rho \times 10/(\text{strengh (\% vol)} \times 1\,000)$$

where ρ = density in kg/m³.

Results are quoted to 3 significant figures and a maximum of one decimal place e.g. 11,4 g per 100 l absolute alcohol.

10. **Quality assurance and control (used for the validated method)**

Using equation (2) above, calculate the concentration of each congener in the quality control standard solutions prepared by following the procedure as in 8.1.1 to 8.1.4. Using equation (3), calculate the percentage recovery of the target value. If the analysed results are within ± 10 % of their theoretical values for each congener, analysis may proceed. If not, an investigation should be made to find the cause of the inaccuracy and remedial action taken as appropriate.

11. **Method performance characteristics (precision)**

Statistical results of the interlaboratory test: the following tables give the values for the following compounds: ethanal, ethyl acetate, acetal, total ethanal, methanol, butan-2-ol, propan-1-ol, butan-1-ol, 2-methyl-propan-1-ol, 2 methyl-butan-1-ol, 3 methyl-butan-1-ol.

The following data were obtained from an international method performance study carried out to internationally agreed procedures.

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	ethanal

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	28	26	27	27	28
Number of outliers (laboratories)	2	4	3	3	2
Number of accepted results	56	52	54	54	56
Mean value ($\bar{x}$) µg/g	63,4	71,67	130,4	38,4	28,6
				13,8 (*)	52,2 (*)
Repeatability standard deviation (S_r) µg/g	3,3	1,9	6,8	4,1	3,6
Repeatability relative standard deviation (RSD_r) (%)	5,2	2,6	5,2	15,8	8,9
Repeatability limit (r) µg/g	9,3	5,3	19,1	11,6	10,1
Reproducibility standard deviation (S_R) µg/g	12	14	22	6,8	8,9
Reproducibility relative standard deviation (RSD_R) (%)	18,9	19,4	17,1	26,2	22,2
Reproducibility limit (R) µg/g	33,5	38,9	62,4	19,1	25,1

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	ethyl acetate

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	24	24	25	24	24
Number of outliers (laboratories)	2	2	1	2	2
Number of accepted results	48	48	50	48	48
Mean value ($\bar{x}$) µg/g	96,8	1 046	120,3	112,5	99,1
				91,8 (*)	117,0 (*)
Repeatability standard deviation (S_r) µg/g	2,2	15	2,6	2,1	2,6
Repeatability relative standard deviation (RSD_r) (%)	2,3	1,4	2,1	2,0	2,4
Repeatability limit (r) µg/g	6,2	40,7	7,2	5,8	7,3
Reproducibility standard deviation (S_R) µg/g	6,4	79	8,2	6,2	7,1
Reproducibility relative standard deviation (RSD_R) (%)	6,6	7,6	6,8	6,2	6,6
Reproducibility limit (R) µg/g	17,9	221,9	22,9	17,5	20,0

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	acetal

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	20	21	22	17	21
Number of outliers (laboratories)	4	3	2	4	3
Number of accepted results	40	42	44	34	42
Mean value ($\bar{x}$) µg/g	35,04	36,46	68,5	20,36	15,1
				6,60 (*)	28,3 (*)
Repeatability standard deviation (S_r) µg/g	0,58	0,84	1,6	0,82	1,9
Repeatability relative standard deviation (RSD_r) (%)	1,7	2,3	2,3	6,1	8,7
Repeatability limit (r) µg/g	1,6	2,4	4,4	2,3	5,3
Reproducibility standard deviation (S_R) µg/g	4,2	4,4	8,9	1,4	3,1
Reproducibility relative standard deviation (RSD_R) (%)	12,1	12,0	13,0	10,7	14,2
Reproducibility limit (R) µg/g	11,8	12,2	25,0	4,0	8,7

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	total ethanal

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	23	19	22	21	22
Number of outliers (laboratories)	1	5	2	3	2
Number of accepted results	46	38	44	42	44
Mean value ($\bar{x}$) µg/g	76,5	85,3	156,5	45,4	32,7
				15,8 (*)	61,8 (*)
Repeatability standard deviation (S_r) µg/g	3,5	1,3	6,5	4,4	3,6
Repeatability relative standard deviation (RSD_r) (%)	4,6	1,5	4,2	14,2	7,6
Repeatability limit (r) µg/g	9,8	3,5	18,3	12,2	10,0
Reproducibility standard deviation (S_R) µg/g	13	15	24,1	7,3	9,0
Reproducibility relative standard deviation (RSD_R) (%)	16,4	17,5	15,4	23,7	19,1
Reproducibility limit (R) µg/g	35,2	41,8	67,4	20,3	25,2

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	Methanol

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	26	27	27	28	25
Number of outliers (laboratories)	4	3	3	1	4
Number of accepted results	52	54	54	56	50
Mean value ($\bar{x}$) µg/g	319,8	2 245	1 326	83,0	18,6
				61,5 (*)	28,9 (*)
Repeatability standard deviation (S_r) µg/g	4,4	27	22	1,5	1,3
Repeatability relative standard deviation (RSD_r) (%)	1,4	1,2	1,7	2,1	5,6
Repeatability limit (r) µg/g	12,3	74,4	62,5	4,3	3,8
Reproducibility standard deviation (S_R) µg/g	13	99	60	4,5	2,8
Reproducibility relative standard deviation (RSD_R) (%)	3,9	4,4	4,6	6,2	11,8
Reproducibility limit (R) µg/g	35,2	278,3	169,1	12,5	7,9

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	4
Analyte	butan-2-ol

Samples	A	B	C	E
Number of laboratories retained after eliminating outliers	21	27	29	22
Number of outliers (laboratories)	4	3	1	3
Number of accepted results	42	54	58	44
Mean value ($\bar{x}$) µg/g	5,88	250,2	27,57	5,83
				14,12 (*)
Repeatability standard deviation (S_r) µg/g	0,40	2,2	0,87	0,64
Repeatability relative standard deviation (RSD_r) (%)	6,8	0,9	3,2	6,4
Repeatability limit (r) µg/g	1,1	6,1	2,5	1,8
Reproducibility standard deviation (S_R) µg/g	0,89	13	3,2	0,87
Reproducibility relative standard deviation (RSD_R) (%)	15,2	5,1	11,5	8,7
Reproducibility limit (R) µg/g	2,5	35,5	8,9	2,4

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
E Rum; split levels (*).

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	propan-1-ol

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	29	27	27	29	29
Number of outliers (laboratories)	2	4	3	2	2
Number of accepted results	58	54	54	58	58
Mean value ($\bar{x}$) µg/g	86,4	3 541	159,1	272,1	177,1
				229,3 (*)	222,1 (*)
Repeatability standard deviation (S_r) µg/g	3,0	24	3,6	2,3	3,3
Repeatability relative standard deviation (RSD_r) (%)	3,4	0,7	2,3	0,9	1,6
Repeatability limit (r) µg/g	8,3	68,5	10,0	6,4	9,1
Reproducibility standard deviation (S_R) µg/g	5,3	150	6,5	9,0	8,1
Reproducibility relative standard deviation (RSD_R) (%)	6,1	4,1	4,1	3,6	4,1
Reproducibility limit (R) µg/g	14,8	407,2	18,2	25,2	22,7

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	propan-1-ol

Samples	A	B	C
Number of laboratories retained after eliminating outliers	20	22	22
Number of outliers (laboratories)	4	4	6
Number of accepted results	40	44	44
Mean value ($\bar{x}$) µg/g	3,79	5,57	7,54
Repeatability standard deviation (S_r) µg/g	0,43	0,20	0,43
Repeatability relative standard deviation (RSD_r) (%)	11,2	3,6	5,6
Repeatability limit (r) µg/g	1,1	0,6	1,2
Reproducibility standard deviation (S_R) µg/g	0,59	0,55	0,82
Reproducibility relative standard deviation (RSD_R) (%)	15,7	9,8	10,8
Reproducibility limit (R) µg/g	1,7	1,5	2,3

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates. (*).

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	2-methylpropan-1-ol

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	28	31	30	26	25
Number of outliers (laboratories)	3	0	1	5	6
Number of accepted results	56	62	60	52	50
Mean value ($\bar{x}$) µg/g	174,2	111,7	185,0	291,0	115,99
				246,8 (*)	133,87 (*)
Repeatability standard deviation (S_r) µg/g	2,3	1,6	2,5	1,8	0,74
Repeatability relative standard deviation (RSD_r) (%)	1,3	1,4	1,3	0,7	0,6
Repeatability limit (r) µg/g	6,4	4,5	6,9	5,0	2,1
Reproducibility standard deviation (S_R) µg/g	8,9	8,9	9,7	6,0	6,2
Reproducibility relative standard deviation (RSD_R) (%)	5,1	8,0	5,2	2,2	5,0
Reproducibility limit (R) µg/g	24,9	24,9	27,2	16,9	17,4

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	2-methyl-butan-1-ol

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	25	26	25	27	25
Number of outliers (laboratories)	3	2	3	1	2
Number of accepted results	50	52	50	54	50
Mean value ($\bar{x}$) µg/g	113,0	48,3	91,6	72,1	39,5
				45,2 (*)	61,5 (*)
Repeatability standard deviation (S_r) µg/g	2,1	1,5	1,7	2,3	2,3
Repeatability relative standard deviation (RSD_r) (%)	1,9	3,1	1,8	3,9	4,5
Repeatability limit (r) µg/g	6,0	4,2	4,7	6,4	6,3
Reproducibility standard deviation (S_R) µg/g	7,4	3,8	6,6	4,7	4,5
Reproducibility relative standard deviation (RSD_R) (%)	6,6	7,9	7,2	8,1	8,8
Reproducibility limit (R) µg/g	20,8	10,7	18,4	13,3	12,5

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).

Year of interlaboratory test	1997
Number of laboratories	32
Number of samples	5
Analyte	3-methyl-butan-1-ol

Samples	A	B	C	D	E
Number of laboratories retained after eliminating outliers	23	23	24	27	21
Number of outliers (laboratories)	5	5	4	1	6
Number of accepted results	46	46	48	54	42
Mean value ($\bar{x}$) µg/g	459,4	242,7	288,4	142,2	212,3
				120,4 (*)	245,6 (*)
Repeatability standard deviation (S_r) µg/g	5,0	2,4	3,4	2,4	3,2
Repeatability relative standard deviation (RSD_r) (%)	1,1	1,0	1,2	1,8	1,4
Repeatability limit (r) µg/g	13,9	6,6	9,6	6,6	9,1
Reproducibility standard deviation (S_R) µg/g	29,8	13	21	8,5	6,7
Reproducibility relative standard deviation (RSD_R) (%)	6,5	5,2	7,3	6,5	2,9
Reproducibility limit (R) µg/g	83,4	35,4	58,8	23,8	18,7

Sample types
A Brandy; blind duplicates.
B Kirsch; blind duplicates.
C Grappa; blind duplicates.
D Whisky; split levels (*).
E Rum; split levels (*).