# RESOLUTION OIV/OENO 379/2009

# UPDATE OF THE OIV INTERNATIONAL COMPENDIUM OF METHODS OF ANALYSIS OF SPIRIT DRINKS OF VITIVINICULTURAL ORIGIN – PART 1

THE GENERAL ASSEMBLY

CONSIDERING article 2 paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

IN VIEW OF the actions of the 2009-2012 OIV Strategic plan, particularly those focused on reorganising publications related to the vitivinicultural methods of analysis

CONSIDERING the works of the Methods of Analysis sub-commission

IN VIEW OF the 1994 publication of the Compendium of International Methods of Analysis of spirituous beverages, alcohol and the aromatic fraction of beverages

GIVEN the evolution of methods and availability of inter-laboratory validation parameters since 1994, which are already recognised by international authorities bodies, the following methods shall be described and retained as Type II methods of analysis;

CONSIDERING that certain methods of analysis are no longer used and should be removed from the Compendium of International Methods of Analysis of spirituous beverages, alcohol and the aromatic fraction of beverages,

For the purpose of the present resolution, the following terms are defined as such:

- (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}.

DECIDES to revise the 1994 publication of the « Compendium of International



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Methods of Analysis of spirituous beverages, alcohol and the aromatic fraction of beverages » while retaining and describing the following methods as Type II methods of analysis. The title of the compendium shall be renamed as « Compendium of International Methods of Analysis of Spirit Drinks of Vitivinicultural Origin »

DECIDES that the methods listed in the Compendium of International Methods of Analysis of spirituous beverages, alcohol and aromatic fractions of beverages shall, if necessary, be modified accordingly.

# REFERENCE METHOD FOR THE DETERMINATION OF ALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS OF VITI-VINCULTURAL ORIGIN: General Remarks

## Introduction

The reference method includes two Annexes:

Annex I - Preparation of distillate

Annex II - Measurement of density of distillate by three methods A, B, and C

## 1. Scope

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

## 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.



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## 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 $\rho_{20}$.

## 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 $\rho_{20}$ 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.

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:

$$Alcoholic\ strength\ by\ volume\ (\%\ vol) = \frac{ASM\ (\%\ mass) \times \rho_{20}(Sample)}{\rho_{20}(alcohol)}$$

where ASM = alcoholic strength by mass,

$\rho_{20}$ (alcohol) = 789.24 kg/m$^3$



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## 4. Principle

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

## 5. Bibliography

1. Commission Regulation (EC) N° 2870/2000 of 19 December 2000 laying down Community reference methods for the analysis of spirits drinks, *OJEC of 29 December 2000, L333/20*
2. P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003

# Annex I: REFERENCE METHOD FOR THE DETERMINATION OFALCOHOLIC STRENGTH BY VOLUME OF SPIRIT DRINKS OF VITI-VINCULTURAL ORIGIN : 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)



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# 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. As an example, a suitable distillation apparatus would include 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



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flask, provided a splash-head 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

## 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 - 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 (Annex 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 °C 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 (Annex II)

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

## Annex IIA: Determination of real alcoholic strength by volume of spirit drinks of viti-vinicultural origin - Measurement by pycnometry

**Type II method**

**Year: 2009**

## 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 sodium chloride and dissolve to 1 litre using water.



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## 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.



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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 x (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:

- V20 °C = [P1 - (P - m)] x Ft
- where Ft is the factor for temperature t °C taken from Table I below.
- V20 °C must be known to the nearest 0.001 ml.

Mass of water in the pycnometer at 20 °C :

- M20 °C = V20 °C x 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. ( p )

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. (p' )

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:

- $V_{20\,°C} = (p + m - p') \times F_t$
- where $F_t$ is the factor for temperature t °C taken from Table I below.
- $V_{20\,°C}$ 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 Annex 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

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

Express the density at t °C in kilograms per $m^3$ by multiplying $\rho_{t\,°C}$ by 1000, the value being known as $\rho_t$.

Correct $\rho_t$ to 20 using the table of densities $\Delta T$ for water-alcohol mixtures in the Manual of Analysis Methods for Wines of the OIV.

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

Using the whole temperature line, calculate the difference between density $\rho'$ in the table immediately above $\rho_t$ and the calculated density $\rho_t$. Divide that difference by the table difference found to the right of density $\rho'$. 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 $\rho'$ is found (Dt, the alcoholic strength).



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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

- $\rho_{t\,°C} = (p + m - p")/V_{20\,°C}$

Express the density at t °C in kilograms per $m^3$ 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 on a variety of spirit drinks to internationally agreed procedures.

Year of interlaboratory test: 1997

Number of laboratories: 20

Number of samples: 6

| Samples | A | B | C |
|---|---|---|---|
| Number of laboratories retained after eliminating outliers | 19 | 20 | 17 |
| Number of outliers (Laboratories) | 1 | - | 2 |
| Number of accepted results | 38 | 40 | 34 |
| *Mean value* ($\bar{x}$)% vol. | 23.77 | 40.04 | 40.29 |
| | 26.51* | | |
| Repeatability standard deviation ($s_r$) % vol. | 0.106 | 0.176 | 0.072 |
| Repeatability relative standard deviation ($RSD_r$) (%) | 0.42 | 0.44 | 0.18 |
| Repeatability limit ( r ) % vol. | 0.30 | 0.49 | 0.20 |
| Reproducibility standard deviation ($s_R$) % vol. | 0.131 | 0.236 | 0.154 |
| Reproducibility relative standard deviation ($RSD_R$) (%) | 0.52 | 0.59 | 0.38 |
| Reproducibility limit ( R ) % vol. | 0.37 | 0.66 | 0.43 |

Sample types

A Fruit liqueur; split level*

B Brandy; blind duplicates

C Whisky; blind duplicates

| Samples | D | E | F |
|---|---|---|---|
| Number of laboratories retained after eliminating outliers | 19 | 19 | 17 |
| Number of outliers (Laboratories) | 1 | 1 | 3 |
| Number of accepted results | 38 | 38 | 34 |



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| | | | |
|---|---|---|---|
| *Mean value* ($\bar{x}$)% vol. | 39.20 | 42.24 | 57.03 |
| | 42.93* | 45.73* | 63.03* |
| Repeatability standard deviation ($s_r$) % vol. | 0.103 | 0.171 | 0.190 |
| Repeatability relative standard deviation ($RSD_r$) (%) | 0.25 | 0.39 | 0.32 |
| Repeatability limit ( r ) % vol. | 0.29 | 0.48 | 0.53 |
| Reproducibility standard deviation ($s_R$) % vol. | 0.233 | 0.238 | 0.322 |
| Reproducibility relative standard deviation ($RSD_R$) (%) | 0.57 | 0.54 | 0.53 |
| Reproducibility limit ( R ) % vol. | 0.65 | 0.67 | 0.90 |

Sample types
D grappa; split level*
E aquavit; split level*
F rum; split level*





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TABLE I

**F factors by which the mass of water contained in the Pyrex pycnometer at t °C has to be multiplied in order to calculate the pycnometer volume at 20 °C**

| t °C | F | t °C | F | t °C | F | t °C | F | t °C | F | t °C | F | t °C | F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10,0 | 1,000398 | 13,0 | 1,000691 | 16,0 | 1,001097 | 19,0 | 1,001608 | 22,0 | 1,002215 | 25,0 | 1,002916 | 28,0 | 1,003704 |
| ,1 | 1,000406 | ,1 | 1,000703 | ,1 | 1,001113 | ,1 | 1,001627 | ,1 | 1,002238 | ,1 | 1,002941 | ,1 | 1,003731 |
| ,2 | 1,000414 | ,2 | 1,000714 | ,2 | 1,001128 | ,2 | 1,001646 | ,2 | 1,002260 | ,2 | 1,002966 | ,2 | 1,003759 |
| ,3 | 1,000422 | ,3 | 1,000726 | ,3 | 1,001144 | ,3 | 1,001665 | ,3 | 1,002282 | ,3 | 1,002990 | ,3 | 1,003787 |
| ,4 | 1,000430 | ,4 | 1,000738 | ,4 | 1,001159 | ,4 | 1,001684 | ,4 | 1,002304 | ,4 | 1,003015 | ,4 | 1,003815 |
| 10,5 | 1,000439 | 13,5 | 1,000752 | 16,5 | 1,001175 | 19,5 | 1,001703 | 22,5 | 1,002326 | 25,5 | 1,003041 | 28,5 | 1,003843 |
| ,6 | 1,000447 | ,6 | 1,000764 | ,6 | 1,001191 | ,6 | 1,001722 | ,6 | 1,002349 | ,6 | 1,003066 | ,6 | 1,003871 |
| ,7 | 1,000456 | ,7 | 1,000777 | ,7 | 1,001207 | ,7 | 1,001741 | ,7 | 1,002372 | ,7 | 1,003092 | ,7 | 1,003899 |
| ,8 | 1,000465 | ,8 | 1,000789 | ,8 | 1,001223 | ,8 | 1,001761 | ,8 | 1,002394 | ,8 | 1,003117 | ,8 | 1,003928 |
| ,9 | 1,000474 | ,9 | 1,000803 | ,9 | 1,001239 | ,9 | 1,001780 | ,9 | 1,002417 | ,9 | 1,003143 | ,9 | 1,003956 |
| 11,0 | 1,000483 | 14,0 | 1,000816 | 17,0 | 1,001257 | 20,0 | 1,001800 | 23,0 | 1,002439 | 26,0 | 1,003168 | 29,0 | 1,003984 |
| ,1 | 1,000492 | ,1 | 1,000829 | ,1 | 1,001273 | ,1 | 1,001819 | ,1 | 1,002462 | ,1 | 1,003194 | ,1 | 1,004013 |
| ,2 | 1,000501 | ,2 | 1,000842 | ,2 | 1,001290 | ,2 | 1,001839 | ,2 | 1,002485 | ,2 | 1,003222 | ,2 | 1,004042 |
| ,3 | 1,000511 | ,3 | 1,000855 | ,3 | 1,001306 | ,3 | 1,001859 | ,3 | 1,002508 | ,3 | 1,003247 | ,3 | 1,004071 |
| ,4 | 1,000520 | ,4 | 1,000868 | ,4 | 1,001323 | ,4 | 1,001880 | ,4 | 1,002531 | ,4 | 1,003273 | ,4 | 1,004099 |
| 11,5 | 1,000530 | 14,5 | 1,000882 | 17,5 | 1,001340 | 20,5 | 1,001900 | 23,5 | 1,002555 | 26,5 | 1,003299 | 29,5 | 1,004128 |
| ,6 | 1,000540 | ,6 | 1,000895 | ,6 | 1,001357 | ,6 | 1,001920 | ,6 | 1,002578 | ,6 | 1,003326 | ,6 | 1,004158 |
| ,7 | 1,000550 | ,7 | 1,000909 | ,7 | 1,001374 | ,7 | 1,001941 | ,7 | 1,002602 | ,7 | 1,003352 | ,7 | 1,004187 |
| ,8 | 1,000560 | ,8 | 1,000923 | ,8 | 1,001391 | ,8 | 1,001961 | ,8 | 1,002625 | ,8 | 1,003379 | ,8 | 1,004216 |
| ,9 | 1,000570 | ,9 | 1,000937 | ,9 | 1,001409 | ,9 | 1,001982 | ,9 | 1,002649 | ,9 | 1,003405 | ,9 | 1,004245 |
| 12,0 | 1,000580 | 15,0 | 1,000951 | 18,0 | 1,001427 | 21,0 | 1,002002 | 24,0 | 1,002672 | 27,0 | 1,003432 | 30,0 | 1,004275 |
| ,1 | 1,000591 | ,1 | 1,000965 | ,1 | 1,001445 | ,1 | 1,002023 | ,1 | 1,002696 | ,1 | 1,003458 | | |
| ,2 | 1,000601 | ,2 | 1,000979 | ,2 | 1,001462 | ,2 | 1,002044 | ,2 | 1,002720 | ,2 | 1,003485 | | |
| ,3 | 1,000612 | ,3 | 1,000993 | ,3 | 1,001480 | ,3 | 1,002065 | ,3 | 1,002745 | ,3 | 1,003513 | | |
| ,4 | 1,000623 | ,4 | 1,001008 | ,4 | 1,001498 | ,4 | 1,002086 | ,4 | 1,002769 | ,4 | 1,003540 | | |
| 12,5 | 1,000634 | 15,5 | 1,001022 | 18,5 | 1,001516 | 21,5 | 1,002107 | 24,5 | 1,002793 | 27,5 | 1,003567 | | |
| ,6 | 1,000645 | ,6 | 1,001037 | ,6 | 1,001534 | ,6 | 1,002129 | ,6 | 1,002817 | ,6 | 1,003594 | | |
| ,7 | 1,000656 | ,7 | 1,001052 | ,7 | 1,001552 | ,7 | 1,002151 | ,7 | 1,002842 | ,7 | 1,003621 | | |
| ,8 | 1,000668 | ,8 | 1,001067 | ,8 | 1,001570 | ,8 | 1,002172 | ,8 | 1,002866 | ,8 | 1,003649 | | |
| ,9 | 1,000679 | ,9 | 1,001082 | ,9 | 1,001589 | ,9 | 1,002194 | ,9 | 1,002891 | ,9 | 1,003676 | | |

# Annexe IIB: 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 oscillating cell)

## Type II method



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**Year: 2009**

# 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 0.0001 g/mL.

## B.3.3. Sample injection syringes, auto sampler, or other equivalent system.

# 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, autosampler, or other equivalent system) 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 $\rho_{20}$ or the alcoholic strength displayed by the densimeter.

### B.4.3. Result

When the density $\rho_{20}$ 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 (Table IVa).

## 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 on a variety of spirit drinks to internationally agreed procedures.

Year of interlaboratory test: 1997

Number of laboratories: 16

Number of samples: 6





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| Samples | A | B | C |
|---|---|---|---|
| Number of laboratories retained after eliminating outliers | 11 | 13 | 15 |
| Number of outliers (Laboratories) | 2 | 3 | 1 |
| Number of accepted results | 22 | 26 | 30 |
| *Mean value* ($\bar{x}$)*% vol.* | 23.81 | 40.12 | 40.35 |
| | 26.52* | | |
| Repeatability standard deviation ($s_r$) % vol. | 0.044 | 0.046 | 0.027 |
| Repeatability relative standard deviation ($RSD_r$) (%) | 0.17 | 0.12 | 0.07 |
| Repeatability limit ( r ) % vol. | 0.12 | 0.13 | 0.08 |
| Reproducibility standard deviation ($s_R$) % vol. | 0.054 | 0.069 | 0.083 |
| Reproducibility relative standard deviation ($RSD_R$) (%) | 0.21 | 0.17 | 0.21 |
| Reproducibility limit ( R ) % vol. | 0.15 | 0.19 | 0.23 |

Sample types

A Fruit liqueur; split level*

B Brandy; blind duplicates

C Whisky; blind duplicates

| Samples | D | E | F |
|---|---|---|---|
| Number of laboratories retained after eliminating outliers | 16 | 14 | 13 |
| Number of outliers (Laboratories) | - | 1 | 2 |
| Number of accepted results | 32 | 28 | 26 |





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| | | | |
|---|---|---|---|
| *Mean value ($\bar{x}$)% vol.* | 39.27 | 42.39 | 56.99 |
| | 43.10* | 45.91* | 63.31* |
| Repeatability standard deviation ($s_r$) % vol. | 0.079 | 0.172 | 0.144 |
| Repeatability relative standard deviation ($RSD_r$) (%) | 0.19 | 0.39 | 0.24 |
| Repeatability limit ( r ) % vol. | 0.22 | 0.48 | 0.40 |
| Reproducibility standard deviation ($s_R$) % vol. | 0.141 | 0.197 | 0.205 |
| Reproducibility relative standard deviation ($RSD_R$) (%) | 0.34 | 0.45 | 0.34 |
| Reproducibility limit ( R ) % vol. | 0.40 | 0.55 | 0.58 |

Sample types
D Grappa; split level*
E Aquavit; split level*
F Rum; split level*

# Annexe IIC: Determination of real alcoholic strength by volume of spirit drinks - measurement by densimetry using hydrostatic balance

**Type II method**
**Year 2009**

## 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.





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## 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 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 the Manual of Analysis Methods for Wines of the OIV.

## 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.



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## 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 M□/cm) at a temperature between 15 °C 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 °C and 25 °C but preferably at 20 °C.

C.4.2.1. 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 ($□_t$).

C.4.4.3. Correct $□_t$ to 20 using the table of densities □T for water-alcohol mixtures in the Manual of Analysis Methods for Wines of the OIV.

## 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.



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### C.4.6. Result

Using the density ¤$_{20}$ 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 on a variety of spirit drinks to internationally agreed procedures.

Year of interlaboratory test: 1997

Number of laboratories: 12

Number of samples: 6

| Samples | A | B | C |
|---|---|---|---|
| Number of laboratories retained after eliminating outliers | 12 | 10 | 11 |
| Number of outliers (Laboratories) | - | 2 | 1 |
| Number of accepted results | 24 | 20 | 22 |
| *Mean value* ($\bar{x}$)*% vol.* | 23.80 | 40.09 | 40.29 |
| | 26.51* | | |
| Repeatability standard deviation ($s_r$) % vol. | 0.048 | 0.065 | 0.042 |
| Repeatability relative standard deviation ($RSD_r$) (%) | 0.19 | 0.16 | 0.10 |
| Repeatability limit ( r ) % vol. | 0.13 | 0.18 | 0.12 |

| | | | |
|---|---|---|---|
| Reproducibility standard deviation ($s_R$) % vol. | 0.060 | 0.076 | 0.073 |
| Reproducibility relative standard deviation ($RSD_R$) (%) | 0.24 | 0.19 | 0.18 |
| Reproducibility limit ( R ) % vol. | 0.17 | 0.21 | 0.20 |

Sample types

A Fruit liqueur; split level*

B Brandy; blind duplicates

C Whisky; blind duplicates

| Samples | D | E | F |
|---|---|---|---|
| Number of laboratories retained after eliminating outliers | 12 | 11 | 9 |
| Number of outliers (Laboratories) | - | 1 | 2 |
| Number of accepted results | 24 | 22 | 18 |
| *Mean value* ($\bar{x}$)*% vol.* | 39.26 | 42.38 | 57.16 |
| | 43.09* | 45.89* | 63.44* |
| Repeatability standard deviation ($s_r$) % vol. | 0.099 | 0.094 | 0.106 |
| Repeatability relative standard deviation ($RSD_r$) (%) | 0.24 | 0.21 | 0.18 |
| Repeatability limit ( r ) % vol. | 0.28 | 0.26 | 0.30 |
| Reproducibility standard deviation ($s_R$) % vol. | 0.118 | 0.103 | 0.125 |
| Reproducibility relative standard deviation ($RSD_R$) (%) | 0.29 | 0.23 | 0.21 |
| Reproducibility limit ( R ) % vol. | 0.33 | 0.29 | 0.35 |

Sample types





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D Grappa; split level*

E Aquavit; split level*

F Rum; split level*

# METHOD FOR THE DETERMINATION OF TOTAL DRY EXTRACT OF SPIRIT DRINKS OF VITI-VINICULTURAL ORIGIN - GRAVIMETRIC METHOD

**Type II method**

**Year: 2009**

## 1. Scope

This method is suited to the determination of the total dry extract in spirit drinks of viti-vinicultural origin whichcontain less than 15 g/L of dry matter.

## 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 stainless-steel cylindrical capsule, of sufficient dimensions to avoid loss of liquid when evaporating.

5.2 Boiling water bath.

5.3 25 ml pipette, class A.

5.4 Drying oven.

5.5 Dessicator.



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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 drink into a previously-weighed cylindrical capsule (5.1) . 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 dessicator 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 on a variety of spirit drinks to internationally agreed procedures.

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 |
| Repeatability standard deviation ($s_r$) g/l | 0.075 | 0.441 | 0.028 | 0.123 |
| Repeatability relative standard deviation ($RSD_r$) (%) | 0.8 | 5.2 | 0.3 | 1.1 |
| Repeatability limit ( r ) g/l | 0.2 | 1.2 | 0.1 | 0.3 |
| Reproducibility standard deviation ($s_R$) g/l | 0.148 | 0.451 | 0.058 | 0.210 |
| Reproducibility relative standard deviation ($RSD_R$) (%) | 1.6 | 5.3 | 0.6 | 1.8 |
| Reproducibility limit ( R ) g/l | 0.4 | 1.3 | 0.2 | 0.6 |

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

# 10. Bibliography

1. Commission Regulation (EC) N° 2870/2000 of 19 December 2000 laying down Community reference methods for the analysis of spirits drinks, OJEC of 29 December 2000, L333/20
2. P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003





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# DETERMINATION OF THE PRINCIPAL VOLATILE substances oF SPIRIT DRINKS OF VITI-VINICULTURAL ORIGIN

**Type II method**

**Year: 2009**

## 1. Scope

This method is suitable for the determination of the following compounds by gas chromatography in spirit drinks of viti-vinicultural origin: ethanal (acetaldehyde), both free and total (obtained from the sum of ethanal and the fraction of ethanal contained in 1,1-diéthoxyéthane), ethyl ethanoate (ethyl acetate), 1,1-diethoxyethane (acetal), methanol (methyl alcohol), butan-2-ol (sec-butanol), propan-1-ol (n-propanol), 2-methylpropan-1-ol (isobutyl alcohol), butan-1-ol (n-butanol), 2-methylbutan-1-ol (active amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol).

## 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, or its distillate, 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.

Note: 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.



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# 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 should be stored according to the supplier's instructions.

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), 4-méthylpentan-2-ol (CAS 108-11-2), 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 suggested for the validated method: the calibration ranges should be adapted to the nature of the different types of products analysed by each laboratory).

All standard solutions must be stored at <5 °C and be prepared freshly on a monthly basis, if necessary. 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



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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 - It is preferable to add acetal and acetaldehyde last in order to minimise losses through evaporation. The solutions may be prepared individually, and the final solution and dilutions prepared subsequently.

### 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



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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 and an effective quality control, prepare a quality control standard using the previously prepared standard A (5.14.1) or, preferably, prepare a control standard as indicated for standard A, but using different batches or suppliers of reagents. 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.



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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.

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, as a guide, at least 1.3, if a simple visual examination of the chromatogram is not sufficient.

NOTE - The following columns and GC conditions are given as suitable examples:

| | |
|---|---|
| 1 | A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 □m film thickness (stabilised polyethylene glycol) followed by a Carbowax 400 column 50 m x 0.32 mm i.d. 0.2 □m film thickness. (Columns are connected using press-fit connectors.)<br>Carrier gas and pressure:Helium (135 kPa)<br>Column temperature: 35 °C for 17 min., 35 °C to 70 °C at 12 °C/min., hold at 70 C for 25 min.<br>Injector temperature: 150 □C<br>Detector temperature: 250 □C<br>Injection volume: 1 □l, split 20 to 100:1 |
| 2 | A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 □m film thickness (stabilised polyethylene glycol). (Retention gap is connected using a press-fit connector.)<br>Carrier gas and pressure: Helium (65 kPa)<br>Column temperature: 35 °C for 10 min., 35 °C to 110 °C at 5 °C/min., 110 °C to 190 °C at 30 °C/min., hold at 190 °C for 2 min.<br>Injector temperature: 260 □C<br>Detector temperature: 300 □C<br>Injection volume: 1 □l, split 55:1 |
| 3 | A packed column (5% CW 20M, Carbopak B), 2 m x 2 mm i.d.<br>Column temperature: 65 °C for 4 min., 65 °C to 140 °C at 10 °C/min., hold at 140 °C for 5 min., 140 °C to 150 °C at 5 °C/min., hold at 150 °C for 3 min.<br>Injector temperature: 65 □C<br>Detector temperature: 200 □C |
| | Injection volume: 1 □l |



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# 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, and given as an example; the exact procedure, and in particular the calibration range, should be adapted to the nature of the spirit drinks analysed and to the procedures validated by each laboratory)

## 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 for



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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.

$$R = \frac{Peak\ area\ of\ congener}{Peak\ are\ of\ IS}$$

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

### 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 and to good gas-chromatographic practice (calculation of response factors and/or establishment of calibration curves).

Measure peak areas 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).

$$(1) Response\ factor = \frac{Peak\ are\ of\ IS}{Peak\ are\ of\ congener} \times \frac{Conc.\ congener\ (\mu g/g)}{Conc.\ IS\ (\mu g/g)}$$

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).

## 9.2. Sample analysis

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

$$(2)\,Congener\ concentrations\left(\frac{\mu g}{g}\right) = \frac{Peak\ are\ of\ congener}{Peak\ are\ of\ IS} \times \frac{M_{IS}(g)}{M_{sample}(g)} \times Conc.\,IS\ (\mu g/g) \times RF$$

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.

## 9.3. 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):

$$(3)\%\ Recovery\ of\ QC\ sample = \frac{concentration\ of\ analyte\ in\ QC\ standard}{concentration\ of\ analyte\ in\ solution\ D} \times 100_{<0}$$

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

## 9.4. Final presentation of results

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

$$(4)\ Concentration\ in\ g\ per\ 100\ litres\ absolute\ alcohol = Conc\ (\mu g/g) \times \rho \times 10/(strength\ (\%vol.) \times 1000)$$

where □ = density in kg/m³.

Results are quoted to a maximum of 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)

The following data were obtained from an international method performance study carried out on a variety of spirit drinks 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 |
| $Mean\ value\ (\bar{x})\mu g/g$ | 63.4 | 71.67 | 130.4 | 38.4<br>13.8* | 28.6<br>52.2* |

| | | | | | |
|---|---|---|---|---|---|
| Repeatability standard deviation ($s_r$) □g/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 ) □g/g. | 9.3 | 5.3 | 19.1 | 11.6 | 10.1 |
| Reproducibility standard deviation ($s_R$) □g/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 ) □g/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* $(\bar{x})\mu g/g$ | 96.8 | 1046 | 120.3 | 112.5<br>91.8* | 99.1<br>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 |





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|---|---|---|---|---|---|
| *Mean value* $(\bar{x})\mu g/g$ | 35.04 | 36.46 | 68.5 | 20.36<br>6.60* | 15.1<br>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 |





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|---|---|---|---|---|---|
| *Mean value* $(\bar{x})\mu g/g$ | 76.5 | 85.3 | 156.5 | 45.4<br>15.8* | 32.7<br>61.8* |
| Repeatability standard deviation ($s_r$) ¤g/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 ) ¤g/g. | 9.8 | 3.5 | 18.3 | 12.2 | 10.0 |
| Reproducibility standard deviation ($s_R$) ¤g/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 ) ¤g/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* $(\bar{x})\mu g/g$ | 319.8 | 2245 | 1326 | 83.0. 61.5* | 18.6. 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* $(\bar{x})\mu g/g$ | 5.88 | 250.2 | 27.57 | 5.83<br>14.12* |
| Repeatability standard deviation ($s_r$) □g/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 ) □g/g. | 1.1 | 6.1 | 2.5 | 1.8 |
| Reproducibility standard deviation ($s_R$) □g/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 ) □g/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* $(\bar{x})\mu g/g$ | 86.4 | 3541 | 159.1 | 272.1<br>229.3* | 177.1<br>222.1* |



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|---|---|---|---|---|---|
| Repeatability standard deviation ($s_r$) □g/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 ) □g/g. | 8.3 | 68.5 | 10.0 | 6.4 | 9.1 |
| Reproducibility standard deviation ($s_R$) □g/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 ) □g/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: 3
Analyte: butan-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 |
| $Mean\ value\ (\bar{x})\mu g/g$ | 3.79 | 5.57 | 7.54 |





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|---|---|---|---|
| Repeatability standard deviation ($s_r$) □g/g | 0.43 | 0.20 | 0.43 |
| Repeatability relative standard deviation ($RSD_r$) (%) | 11.2 | 3.6 | 5.6 |
| Repeatability limit ( r ) □g/g. | 1.1 | 0.6 | 1.2 |
| Reproducibility standard deviation ($s_R$) □g/g | 0.59 | 0.55 | 0.82 |
| Reproducibility relative standard deviation ($RSD_R$) (%) | 15.7 | 9.8 | 10.8 |
| Reproducibility limit ( R ) □g/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
Analye: 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* ($\bar{x}$)$\mu g/g$ | 174.2 | 111.7 | 185.0 | 291.0<br>246.8* | 115.99<br>133.87* |
| Repeatability standard deviation ($s_r$) □g/g | 2.3 | 1.6 | 2.5 | 1.8 | 0.74 |



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| | | | | | |
|---|---|---|---|---|---|
| Repeatability relative standard deviation ($RSD_r$) (%) | 1.3 | 1.4 | 1.3 | 0.7 | 0.6 |
| Repeatability limit ( r ) □g/g. | 6.4 | 4.5 | 6.9 | 5.0 | 2.1 |
| Reproducibility standard deviation ($s_R$) □g/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 ) □g/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 |
| $Mean\ value\ (\bar{x})\mu g/g$ | 113.0 | 48.3 | 91.6 | 72.1<br>45.2* | 39.5<br>61.5* |





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| | | | | | |
|---|---|---|---|---|---|
| Repeatability standard deviation ($s_r$) □g/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 ) □g/g. | 6.0 | 4.2 | 4.7 | 6.4 | 6.3 |
| Reproducibility standard deviation ($s_R$) □g/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 ) □g/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 |
| $Mean\ value\ (\bar{x})\mu g/g$ | 459.4 | 242.7 | 288.4 | 142.2<br>120.4* | 212.3<br>245.6* |

| | | | | | |
|---|---|---|---|---|---|
| Repeatability standard deviation ($s_r$) □g/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 ) □g/g. | 13.9 | 6.6 | 9.6 | 6.6 | 9.1 |
| Reproducibility standard deviation ($s_R$) □g/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 ) □g/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*

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2. P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003

# ANETHOLE. GAS CHROMATOGRAPHIC DETERMINATION OF TRANS□ANETHOLE IN SPIRIT DRINKS OF VITI-VINICULTURAL ORIGIN

**Type II method**





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**Year: 2009**

# 1. SCOPE

This method is suitable for the determination of trans-anethole in aniseed-flavoured spirit drinks using capillary gas chromatography.

# 2. NORMATIVE REFERENCES

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

# 3. PRINCIPLE

The trans□anethole concentration of the spirit is determined by gas chromatography (GC). The same quantity of an internal standard, e.g. 4□allylanisole (estragole) when estragole is not naturally present in the sample, is added to the test sample and to a trans□anethole reference solution of known concentration, both of which are then diluted with a 45% ethanol solution and injected directly into the GC system.

An extraction is necessary before sample preparation and analysis for liqueurs that contain large amounts of sugars.

# 4. REAGENTS AND MATERIALS

During the analysis use only reagents of a purity of at least 98 %. Water of at least grade 3 as defined by ISO 3696 should be used.

Reference chemicals should be stored cold (ca. 4°C), away from light, in aluminium containers or in tinted (amber) glass reagent bottles. The stoppers should preferably be fitted with an aluminium seal. Trans-anethole will need to be "thawed" from its crystalline state before use, but in this case its temperature should never exceed 35°C.

4.1. Ethanol 96 % vol. (CAS 64-17-5)

4.2. 1-methoxy-4- (1-propenyl) benzene; (trans-anethole) (CAS 4180-23-8)

4.3. 4-allylanisole, (estragole) (CAS 140-67-0), suggested internal standard (IS)

4.4. Ethanol 45 % vol.

Add 560 g of distilled water to 378 g of ethanol 96 % vol.

4.5. Preparation of standard solutions

All standard solutions should be stored at room temperature (15-35°C) away from light in aluminium containers or in tinted (amber) glass reagent bottles. The stopper



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should preferably be fitted with an aluminium seal.

Trans-anethole and 4-allylanisole are practically insoluble in water, and it is therefore necessary to dissolve the trans-anethole and 4-allylanisole in some 96 % ethanol (4.1) before the addition of 45 % ethanol (4.4).

The stock solutions must be freshly prepared each week.

### 4.5.1. Standard solution A

Stock solution of trans□anethole (concentration: 2 g/L)

Weigh 40 mg of trans-anethole (4.2) in a 20 mL volumetric flask (or 400 mg in 200 mL, etc.). Add some 96 % ethanol (4.1) and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

### 4.5.2. Internal standard solution B

Stock solution of internal standard, e.g. estragole (concentration: 2 g/L)

Weigh 40 mg of estragole (4.3) in a 20 mL volumetric flask (400 mg in 200 mL etc.). Add some 96 % vol. ethanol (4.1) make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

### 4.5.3. Solutions used to check the linearity response of the FID

The linearity response of the FID must be checked for the analysis taking into account a range of concentrations of trans-anethole in spirits from 0 g/L up to 2.5 g/L. In the procedure of analysis, the unknown samples of spirits to be analysed are diluted 10 times (8.3). For the conditions of the analysis described in the method, stock solutions corresponding to concentrations of 0, 0.05, 0.1, 0.15, 0.2, and 0.25 g/L of trans-anethole in the sample to be analysed are prepared as follows: take 0.5, 1, 1.5, 2, and 2.5 mL of stock solution A (4.5.1) and pipette in separate 20 mL volumetric flasks; pipette into each flask 2 mL of internal standard solution B (4.5.2) and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

The blank solutions (8.4) is used as the 0 g/L solution.

### 4.5.4. Standard solution C

Take 2 mL of standard solution A (4.5.1) and pipette into a 20 mL volumetric flask then add 2 mL of internal standard solution B (4.5.2) and make up to volume with 45% vol. ethanol (4.4), mix thoroughly.



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# 5. APPARATUS AND EQUIPMENT

5.1. A capillary gas chromatograph fitted with a flame ionisation detector (FID) and integrator or other data handling system capable of measuring peak areas, and with an automatic sampler or the necessary equipment for manual sample injection.

5.2. Split/splitless injector

5.3. Capillary column, for example:

Length: 50 m

Internal diameter: 0.32 mm

Film thickness: 0.2 µm

Stationary phase: FFAP □ modified TPA polyethylene glycol cross□linked porous polymer

5.4. Common laboratory equipment: A grade volumetric glassware, analytical balance (precision: ±0.1 mg).

# 6. CHROMATOGRAPHY CONDITIONS

The column type and dimensions, and the GC conditions, should be such that anethole and the internal standard are separated from each other and from any interfering substances. Typical conditions for the column given as an example in 5.3 are:

6.1. Carrier gas: analytical helium.

6.2. Flow rate: 2 mL/min

6.3. Injector temperature: 250°C.

6.4. Detector temperature: 250°C.

6.5. Oven temperature conditions: isothermal, 180°C, run time 10 minutes

6.6. Injection volume: 1µL, split 1:40

# 7. SAMPLES

Samples should be stored at room temperature, away from light and cold.

# 8. PROCEDURE



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### 8.1. Sample screening for estragole

To ensure that there is no estragole naturally present in the sample, a blank analysis should be carried out without the addition of any internal standard. If estragole is naturally present then another internal standard must be chosen (for instance menthol).

Pipette 2 mL sample into a 20 mL volumetric flask and make up to volume with 45% vol. ethanol (4.4), mix thoroughly.

### 8.2. Preparation of unknown samples

Pipette 2 mL sample into a 20 mL volumetric flask then add 2 mL of internal standard solution B (4.5.2) and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

### 8.3. Blank

Pipette 2 mL of internal standard solution B (4.5.2) into a 20 mL volumetric flask and make up to volume with 45 % vol. ethanol (4.4), mix thoroughly.

### 8.4. Linearity test

Prior to the commencement of the analysis the linearity of the response of the FID should be checked by successively analysing in triplicate each of the linearity standard solutions (4.5.3).

From the integrator peak areas for each injection plot a graph of their mother solution concentration in g/L versus the ratio R for each.

R = trans-anethole peak area divided by the estragole peak area.

A linear plot should be obtained.

### 8.5. Determination

Inject the blank solution (8.3), followed by standard solution C (4.5.4), followed by one of the linearity standards (4.5.3) which will act as a quality control sample (this may be chosen with reference to the probable concentration of trans-anethole in the unknown), followed by 5 unknowns (8.2); insert a linearity (quality control) sample after every 5 unknown samples, to ensure analytical stability.

## 9. CALCULATION OF RESPONSE FACTOR

Measure peak areas (using an integrator or other data system) for trans-anethole and internal standard peaks.

## 9.1. Response factor ($RF_i$) calculation

The response factor is calculated as follows

- $RF_i = (C_i / area_i)*(area_{is} / C_{is})$

Where:

$C_i$ is the concentration of trans-anethole in the standard solution A (4.5.1.)

$C_{is}$ is the concentration of internal standard in the standard solution B (4.5.2.)

$area_i$ is the area of the trans-anethole peak

$area_{is}$ is the area of the internal standard peak

$RF_i$ is calculated from the 5 samples of solution C (4.5.4)

## 9.2. Analysis of the linearity response test solutions

Inject the linearity response test solutions (4.5.3).

## 9.3. Analysis of the sample

Inject the unknown sample solution (8.2)

# 10. CALCULATION OF RESULTS

The formula for the calculation of the concentration of trans-anethole is the following:

- $c_i = C_{is} * (area_i/area_{is})*RF_i$

where:

$c_i$ is the unknown trans-anethole concentration

$C_{is}$ is the concentration of internal standard in the unknown (4.5.2)

$Area_i$ is the area of the trans-anethole peak

$Area_{is}$ is the area of the internal standard peak

$RF_i$ is the response coefficient (calculated as in 9.1)

The trans-anethole concentration is expressed as grams per litre, to one decimal place.

# 11. QUALITY ASSURANCE AND CONTROL

The chromatograms should be such that anethole and the internal standard are separated from each other and from any interfering substances. The $RF_i$ value is calculated from the results for the 5 injections of solution C (4.5.4). If the coefficient of variation (CV % = (standard deviation/mean)*100)) is within plus or minus 1 %, the $RF_i$ average value is acceptable.

The calculation above should be used to calculate the concentration of trans-anethole in the sample selected for the quality control from the linearity control solutions (4.5.3).

If the mean calculated results from analysis of the linearity solution selected for Internal Quality Control sample (IQC) are within plus or minus 2.5 % of their theoretical value, then the results for the unknown samples can be accepted.

# 12. TREATMENT OF SPIRITS SAMPLE CONTAINING LARGE AMOUNT OF SUGAR AND OF LIQUEUR SAMPLE PRIOR TO GC ANALYSIS

Extraction of alcohol from spirit drink containing a large amount of sugar, in order to be able to determine the trans-anethole concentration using capillary gas chromatography.

## 12.1. PRINCIPLE

An aliquot of the liqueur sample is taken and to this is added the internal standard, at a concentration similar to that of the analyte (trans-anethole) in the liqueur. To this are added sodium phosphate dodecahydrate and anhydrous ammonium sulphate. The resulting mixture is well shaken and chilled, two layers develop, and the upper alcohol layer is removed. An aliquot of this alcohol layer is taken and diluted with 45 % ethanol solution (4.4) (Note: no internal standard is added at this stage, because it has already been added). The resulting solution is analysed in gas chromatography.

## 12.2. REAGENTS AND MATERIALS

During the extraction use only reagents of a purity greater than 99 %.

12.2.1. Ammonium sulphate, anhydrous, (CAS 7783-20-2)

12.2.2. Sodium phosphate, dibasic, dodecahydrate, (CAS 10039-32-4)



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## 12.3. APPARATUS AND EQUIPMENT

Conical flasks, separating flasks, refrigerator.

## 12.4. PROCEDURE

### 12.4.1. Sample screening for estragole

To ensure that there is no estragole naturally present in the sample, a blank extraction (12.6.2) and analysis should be carried out without the addition of any internal standard. If estragole is naturally present then another internal standard must be chosen.

### 12.4.2. Extraction

Pipette 5 mL of 96 % ethanol (4.1) into a conical flask, weigh into this flask 50 mg of internal standard (4.3), and add 50 mL of the sample. Add 12 g of ammonium sulphate, anhydrous (12.2.1), and 8.6 g of dibasic sodium phosphate, dodecahydrate (12.2.2). Stopper the conical flask.

Shake the flask for at least 30 minutes. A mechanical shaking device may be used, but not a Teflon coated magnetic stirring bar, as the Teflon will absorb some of the analyte. Note that the added salts will not dissolve completely.

Place the stoppered flask in a refrigerator ($T < 5^{o}C$) for at least two hours.

After this time, there should be two distinct liquid layers and a solid residue. The alcohol layer should be clear; if not replace in the refrigerator until a clear separation is achieved.

When the alcohol layer is clear, carefully take an aliquot (e.g. 10 mL), without disturbing the aqueous layer, place in an amber vial and close securely.

### 12.4.3. Preparation of the extracted sample to be analysed

Allow extract (12.4.2) to reach room temperature.

Take 2 mL of the alcohol layer of the attemperated extracted sample and pipette into a 20 mL volumetric flask, make up to volume with 45 % ethanol (4.4), mix thoroughly.

## 12.5. DETERMINATION

Follow the procedure as outlined in 8.5.



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## 12.6. CALCULATION OF RESULTS

Use the following formula to calculate the results

- $C_i = ( m_{is} / V)^* (area_i / area_{is}) ^*RF_i$

Where:

$m_{is}$ is the weight of internal standard (4.3.) taken (12.4.2) (in milligrams)

V is the volume of unknown sample (50 mL)

$RF_i$ is the response factor (9.1.)

$area_i$ is the area of the trans-anethole peak

$area_{is}$ is the area of the internal standard peak

The results are expressed in grams per litre, to one decimal place.

## 12.7. QUALITY CONTROL AND ASSURANCE

Follow the procedure as outlined in 11 above.

# 13. METHOD PERFORMANCE CHARACTERISTICS (PRECISION)

Statistical results of the interlaboratory test: the following tables give the values for anethole.

The following data were obtzined from an international method performance study carried out on a variety of spirit drinks to internationally agreed procedures.

Year of interlaboratory test: 1998

Number of laboratories: 16

Number of samples: 10

Analyte: anethole

Pastis :

| Samples | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| Number of laboratories retained after eliminating outliers | 15 | 15 | 15 | 13 | 16 | 16 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Number of outliers (laboratories) | 1 | 1 | 1 | 3 | - | - |
| Number of accepted results | 30 | 30 | 30 | 26 | 16 | 16 |
| Mean value g/L | 1.477 | 1.955 | 1.040 | 1.833 | 1.741 | 1.754 |
| Repeatibility standard deviation ($S_r$ g/L | 0.022 | 0.033 | 0.034 | 0.017 | - | - |
| Repeatability relative Standard deviation($RSD_r$) (%) | 1.5 | 1.7 | 1.8 | 0.9 | - | - |
| Repeatability limit (r) g/L | 0.062 | 0.093 | 0.096 | 0.047 | - | - |
| Reproducibility standard deviation ($s_R$) g/L | 0.034 | 0.045 | 0.063 | 0.037 | 0.058 | 0.042 |
| Reproducibility relative Standard deviation ($RSD_R$) (%) | 2.3 | 2.3 | 3.2 | 2.0 | 3.3 | 2.4 |
| Reproducibility limit (R) g/L | 0.094 | 0.125 | 0.176 | 0.103 | 0.163 | 0.119 |

Sample types:

A pastis, blind duplicates

B pastis, blind duplicates

C pastis, blind duplicates

D pastis, blind duplicates

E pastis, single sample

F pastis, single sample

Other aniseed-flavoured spirit drinks:

| Samples | G | H | I | J |
|---|---|---|---|---|
| Number of laboratories retained after eliminating outliers | 16 | 14 | 14 | 14 |
| Number of outliers (Laboratories) | - | 2 | 1 | 1 |
| Number of accepted results | 32 | 28 | 28 | 28 |
| Mean value g/L | 0.078<br>0.530* | 1.742 | 0.351 | 0.599 |
| Repeatability standard deviation ($S_r$) g/L | 0.020 | 0.012 | 0.013 | 0.014 |
| Repeatability relative standard deviation ($RSD_r$) (%) | 3.1 | 0.7 | 3.8 | 2.3 |
| Repeatability limit ( r ) g/L | 0.056 | 0.033 | 0.038 | 0.038 |
| Reproducibility standard deviation ($S_R$) g/L | 0.031 | 0.029 | 0.021 | 0.030 |
| Repeatability relative standard deviation ($RSD_R$) (%) | 4.8 | 1.6 | 5.9 | 5.0 |
| Reproducibility limit (R) g/L | 0.088 | 0.080 | 0.058 | 0.084 |

Sample types:

G ouzo, split levels (*)

H anis, blind duplicates

I aniseed-flavoured liqueur, duplicates

J aniseed-flavoured liqueur, duplicates

# 14. Bibliography

1. Commission Regulation (EC) N° 2091/2002 of 26 November 2002 amending Regulation (EC) No 2870/2000 laying down Community reference methods for the analysis of spirits drinks, OJEC of 27 November 2002, L322/11

2. P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003





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