COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS FOR SPIRITUOUS BEVERAGES AND ALCOHOLS

INTERNATIONAL ORGANISATION
OF VINE AND WINE

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Foreword

Given the evolution of OIV analyses methods , the Enology Commission and Sub-commission of methods of analyses decided to revise the 1994 publication of the « Compendium of International Methods of Analysis of spirituous beverages, alcohol and the aromatic fraction of beverages » and to renamed as « Compendium of International Methods of Analysis of Spirituous beverages of Vitivinicultural Origin »

This compendium, established by the Sub-commission of methods of analyses and approved in 2009 by the 7th General Assembly of the International organisation of Wine and Vine, gathers the principles methods able to be applied to the spirituous beverages of vitinicultural origin analyses

Method OIV-MA-BS-01 : R2017

Type II method

Reference method for the determination of alcoholic strength by volume of spirit drinks of viti-vinicultural origin: General remarks

(OIV/OENO 379/2009;

OIV-OENO 587-2017:

OIV-OENO 588-2017)

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 3696:1987Water 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 $\rho_{2.}$

3.3. Apparent alcoholic strength:

The apparent alcoholic strength of alcohols and spirituous beverages is equal to the number of litres of ethyl alcohol contained in 100 litres of an aqueous-alcoholic mixture with the same density as that of the alcohol or spirituous beverage. Therefore, the apparent alcoholic strength is directly deduced from the density of the product, without distillation. The apparent alcoholic strength is expressed in % vol.

3.4. 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 d20 °C/20 °C or d20/20, 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 ρ20 at 20 °C:

ρ_{20} = 998.203 x $d_{20/20}$ or $d_{20/20} = \rho_{20} / 998.203$ where 998.203 is the density of water at 20 °C.

3.5. Real alcoholic strength by volume:

The real alcoholic strength by volume, or alcohol by volume (ABV), 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:

$$\textit{Alcoholic strength by volume (\%vol)} = \frac{ASM\ (\%mass) x\ p_{20}\ (sample)}{p_{20}\ (alcohol)}$$

where ASM = alcoholic strength by mass,

ρ_{20} (alcohol) = 789.24 kg/m^3

3.6. Density – Alcoholic Strength Correspondence

The reference values for the alcoholic strength (% vol.) at 20 °C, defined in 3.3 and 3.5, versus density at 20 °C for different aqueous-alcoholic mixtures that are to be used are those given in the international table adopted by the International Organization of Legal Metrology in its recommendation N° 22.

3.7. Obscuration:

Obscuration is defined as the difference between the real alcoholic strength by volume and the apparent alcoholic strength, expressed in % vol.

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

Method OIV-MA-BS-02 : R2009

Type II method

Reference method for the determination of alcoholic strength by volume of spirit drinks of viti-vinicultural origin: Preparation of the distillate

(OIV/OENO 379/2009)

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. 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 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.

Method OIV-MA-BS-03: R2009

Type II method

Reference method for the determination of real alcoholic strength by volume of spirit drinks of viti-vinicultural origin: Measurement by pycnometry

(OIV/OENO 379/2009)

1. Principle

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

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.

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.

3. Apparatus and Equipment

Usual laboratory apparatus and in particular the following.

3.1. Analytical balance capable of reading 0.1 mg.

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.

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.

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).

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.

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.

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.

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).

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

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).

4.1.1.3. Weigh the tare bottle (T0).

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:
  - $V_{20°c}$ = [P1 - (P - m)] x 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 :
  - $M_{20°C} = V_{20°c}$ 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.

4.1.2. Calibration method using a twin-pan balance:

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)

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')

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 x (p - p')
- Volume of the pycnometer at 20 °C:
  - $V_{20°c}$ = (p + m - p') x 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:
  - $M_{20°C} = V_{20°c}$ x 0.998203
  - where 0.998203 is the density of water at 20 °C.

4.2. Determination of alcoholic strength of test sample

4.2.1. Using a single-pan balance.

4.2.1.1. Weigh the tare bottle, weight T1

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

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 ρ_t.
- Correct ρ_t to 20 using the table of densities ρT 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 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.

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.

4.2.2. Method using a single-pan balance

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

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.

5. Method performance characteristics (Precision)

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
Mean value ($\overline{X}$) % 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*

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		

Method OIV-MA-BS-04 : R2009

Type II method

Reference method for the determination of real alcoholic strength by volume of spirit drinks of viti-vinicultural origin: measurement by electronic densimetry (based on the resonant frequency oscillation of a sample in an oscillating cell)

(OIV/OENO 379/2009)

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.

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.

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

2.2. Dry air.

3. Apparatus and Equipment

Usual laboratory apparatus and in particular the following.

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.

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.

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

4. Procedure

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.

4.2. Determination of sample density

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.

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.

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

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 (Table IVa).

5. Method performance characteristics (Precision)

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

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 26.52*	40.12	40.35
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
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*

Method OIV-MA-BS-05 : R2009

Type II method

Reference method for the determination of real alcoholic strength by volume of spirit drinks of viti-vinicultural origin: Measurement by densimetry using hydrostatic balance

(OIV/OENO 379/2009)

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.

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.

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.

3. Apparatus and Equipment

Usual laboratory apparatus and in particular the following.

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

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.

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.

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.

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.

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.

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.

4.2. Calibration of the float

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 °C and 25 °C but preferably at 20 °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.

4.3. Control using a water-alcohol solution

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.

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.

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

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

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).

4.4.3. Correct ρ_t to 20 using the table of densities ρT for water-alcohol mixtures in the Manual of Analysis Methods for Wines of the OIV.

4.5. Cleaning of float and measuring cylinder

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

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

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

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.

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.

5. Method performance characteristics (Precision)

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 26.51*	40.09	40.29
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

D Grappa; split level*

E Aquavit; split level*

F Rum; split level*

Method OIV-MA-BS-06 : R2009

Type II method

Density of alcohols and alcoholic beverages method for determining electronic densimetry (principle based on measuring the period of oscillation)

(OENO 6/94;

OIV/OENO 382A/2009)

1. Introduction

This method for determining the density of neutral alcohols and alcoholic beverages is based on the change in oscillation frequency in relation to the change in mass based on calibration with two fluids of known density.

Electronic densimeters with digital displays are commercially available to perform this determination.

2. Object and scope of application

The purpose of this document is to describe a method for determining the density of alcohols and alcoholic beverages at atmospheric pressure.

The application of the method is restricted to products with a vapour pressure of less than 800 hectoPascal (600 mmHg) and a viscosity of less than approximately 15,000 m$m^2$2/s (I mm^2/s = 1 cSt) at the test temperature.

With reference to the currently applicable regulations, the test temperature is set to: 20°C.

3. Density

The density of a liquid at a given temperature is equal to its mass divided by its volume:

$$\rho = \frac{m}{V}$$

It is expressed in kilograms per cubic meter (kg/m^3) at a temperature of 20 degrees Celsius (°C) for alcohols and alcoholic beverages.

Note: electronic densimeters display results expressed in grams per cubic centimetre which may be converted into kilograms per cubic meter.

4. Principle

4.1. A liquid sample of a few millilitres is introduced into a vibrating measuring tube.

Measuring the period of oscillation of the tube containing the sample determines the density of the sample at the test temperature, using a previously calibrated apparatus.

4.2. Principle of vibrating measuring tube.

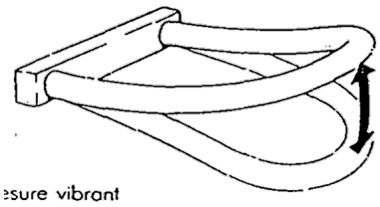


Figure 1: Vibrating measuring tube

Electronic densimeters operate according to the vibrating measuring tube principle (fig. 1): the fluid is introduced into a U-shaped tube and subjected to electromagnetic excitation (fig. 2 and fig. 3).

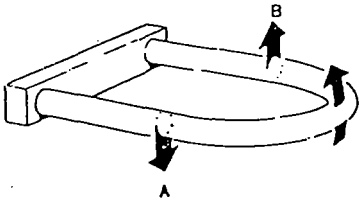


Figure 2: Reactive forces exerted by the fluid

The induced period of oscillation is thus proportional to the total mass subject to vibration and can be used to determine the density of the sample based on the following equation:

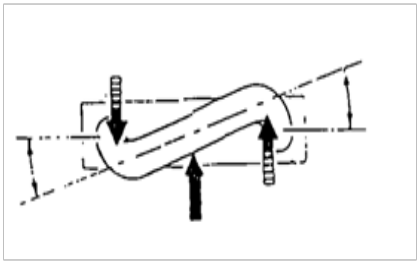


Figure 3: Resulting torsion

$$T = 2\pi\sqrt{(M + pV)/C}$$

- where T = induced period of vibration
- M = mass of the empty tube
- V = volume of the oscillated sample
- C = spring constant

- p = density of the sample

4.3. Detailed principle.

The density of the liquids is determined by the electronic measurement of the oscillations of a vibrating U-shaped tube.

To carry out this measurement, the sample is introduced into an oscillating system, whose specific frequency is thus modified by the mass of the substance introduced.

The system comprises an undamped U-shaped vibration tube, subject to electronic excitation. The two straight sections of the U-shaped tube act like a spring mechanism. The oscillatory movements occur perpendicular to the U. The filling capacity, V, is delimited by the two fastening points. If the oscillator contains the volume established as V, the latter vibrates within and with the tube. It is accepted that the mass is proportional to the density. Since filling the tube beyond the fastening point does not affect the measurement, it is possible to perform a continuous flow measurement.

By maintaining a constant temperature over the entire system, the density will be calculated based on the period assuming a hollow container having mass M suspended by a spring, with a spring constant c. The hollow container will be filled with a volume of liquid of density p. The natural frequency of this vibratory system is:

$$F=1/2\sqrt{\frac{Vc}{M}+pV}$$

$$\mathrm{T}=2\sqrt{(\mathrm{VM}+\frac{\mathrm{pV}}{\mathrm{c}})}*\mathrm{p}=\mathrm{T2}*(\mathrm{c}/4)^2*(\mathrm{V}-\mathrm{M})/\mathrm{M}$$

- $A = (c/4)^2 \cdot V$
- et $B=M/V$
- which gives $p = A \cdot T2 - B$
- (p = rho).

Constants A and B are the oscillator's spring constants, i.e. the mass of the empty tube and the tube's filling capacity. A and B are therefore system constants specific to each oscillator. They can be deduced from the measurement of two periods when the oscillator is filled with substances of known densities.

5. Apparatus

5.1. Digital display densimeter.

This appliance comprises the following elements:

- a glass measuring cell containing the measuring tube, a constant temperature chamber to be connected to an external circulating thermostatic bath, and a thermowell. The chamber can also be thermostated using an integrated device with a semiconductor element which uses the PELTIER effect,
- a system for oscillating the measuring tube and for measuring the period of oscillation,
- a clock,
- a calculator and digital display.

5.2. Temperature control.

The densimeter's performance standards can only be met if the measuring cell is connected to a thermostatic bath, ensuring a temperature stability better than ± 0.02 °C, or if the densimeter has an integrated thermal control device which can achieve the same temperature stability.

5.3. Sample injection syringes.

At least 2 ml polypropylene or glass syringes with tips that fit to the cell inlet. An adapter with PTFE cones is required in order to avoid the deterioration of the tip of the measuring cell.

The electronic densimeter can also be equipped with the appropriate autosampler for the apparatus.

5.4. Temperature measurement.

Temperature measurement is carried out on the cell with a temperature probe whose sensing device (a platinum resistance probe compliant with class A of standard NF C 42.330), which is mounted with 4 wires, is introduced into the thermowell provided for this purpose in the cell. The probe is combined with an electronic temperature transmitter whose readout has a resolution of 0.01 °C. The probe and transmitter must first have been be calibrated in an approved calibration centre in order to ensure temperature measurement with an uncertainty less than or equal to ± 0.05°C.

The probe and transmitter must be periodically checked.

6. Products

6.1. Reference fluids.

During tests, the fluids must maintain their density characteristics; therefore, they must not be made of mixtures of products with different vapour pressures; their molecular composition and purity must be known. Their viscosity must be lower than 2 mm^2/s.

Reference fluids must be chosen so that the densities encompass those of the products to be measured. The difference in density between the 2 reference fluids at the same temperature must be higher than 0.01 g/m^3.

The reference fluid densities determined at a temperature of 20 °C with an uncertainty of less than ± 0.05 °C must be known with an uncertainty of less than ± 0.05 kg/m^3.

When measuring the density of alcohols and alcoholic beverages, the following must be used, under the conditions previously described:

- hydro-alcoholic solutions whose density is exclusively determined using the pycnometric method (reference method).
- recently prepared, degassed double distilled water, or water of equivalent analytical purity,
- dry air.

6.2. Cleaning products.

- chromic acid,
- organic solvents: ethanol 96 vol%, pure acetone.

6.3. Drying.

- Pure acetone, dry air

7. Period measurements in ambient air

Prior to the commissioning and calibration of the densimeter, it is essential to ensure the reproducibility of the measurement in the ambient air so that this measurement can be used to quickly check the cleanliness of the cell and consistency of the densimeter before every density measurement.

It should be possible to perform measurements of periods in the ambient air with a reproducibility of $\pm 10^{-5}$ in relative values over the period for the same barometric pressure and the same temperature.

With some densimeters, the resonant period in the ambient air varies depending on the position of the temperature probe in the thermowell. For these densimeters, either the measuring cell must be replaced, or the temperature probe must be permanently attached, or its position in the thermowell must be accurately determined in order to achieve the reproducibility conditions described above.

NOTE: The use of polluted or excessively humid air may negatively influence the measurements. When these characteristics are combined in the test room, it is advisable to make the drying air flow through a purifier/dryer.

8. Apparatus calibration

8.1. General.

The apparatus must be calibrated upon initial commissioning. It must be recalibrated if a deviation in the air measurement is observed (see section 9.20) and, in any case, every three months.

8.2. During initial calibration, it is necessary to calculate the values of constants A and B, determined by measuring the periods of oscillation (T1 and T2) respectively obtained using two reference fluids.

8.2.1. Place the display selector on the period measurement (T) position. Rinse the cell with acetone. Dry it with dry air generated by the pump that is integrated to the densimeter. When the reading is almost stable, stop the air supply, wait for thermal equilibrium and record the period of oscillation (Ta) obtained with an ambient air temperature of 20 °C. This process helps to check the cleanliness of the cell and stability of the apparatus at every calibration or determination of sample density.

8.2.2. Calibration measurement using the first reference fluid. Use a syringe to fill the cell through its bottom port with the standard liquid until it comes flush with the top port. Leave the syringe in place. While performing this, check the filling quality by visually checking for any air bubbles, even tiny ones. When thermal equilibrium has been reached, record the reading for the period of oscillation (T1). If the thermal control is compliant with the required accuracy, the value of T1 must not vary by more than ± 20 nanoseconds (2 resolution points).

8.2.3. Calibration measurement using the second reference fluid. Empty the cell with the syringe by drawing from the bottom port. Rinse the cell with acetone. Dry it with dry air generated by the pump that is integrated to the densimeter. To do so, connect the air outlet to the top port of the cell, start

the pump and allow it to operate until the reading for T2 is almost constant; stop the pump, and when thermal equilibrium is reached, record the reading for the period of oscillation (T2) which corresponds to the air. If the reading for T2 matches the value obtained during previous tests carried out with a properly cleaned cell and at the same temperature, the cell can be considered clean and dry.

Carry out calibration using the second standard by repeating the steps in paragraph 8.22 and record the reading for the period of oscillation (T2) corresponding to the second reference fluid.

8.2.4. Based on the T1 and T2 values measured and the known values for the densities of both reference fluids, calculate constants A and B using the following equations:

- $A = T1^2 - T2^2 / p1 - p2$
- $B = T2^2 - A \cdot p2$

where

- T1 is the observed period of oscillation with the cell containing the first reference fluid (in ms).
- T2 is the observed period of oscillation with the cell containing the second reference fluid (in ms).
- p1 is the density of the first reference fluid at the test temperature (in g/cm^3),
- p2 is the density of the second reference fluid at the test temperature (in g/cm^3).

Depending on the procedure for the densimeter being used:

8.2.5. Enter constants A and B using the digital display at the top of the apparatus. In order to make sure they have been correctly memorised, display the values by placing the selector on the "A" and "B" positions.

8.2.6. Place the selector on the measurement position. The densimeter should immediately display the densities of the samples introduced into the measuring cell.

NOTE: Some electronic densimeter models automatically calculate the calibration constants.

8.3. Checking the calibration.

In order to validate the operation, measure a reference solution whose density value is within the calibration range used.

Reference substances, verified by a metrology agency, are commercially available.

The calibration is validated if the result of the measurement of the reference substance density complies with the accuracy class of the electronic densimeter being used.

9. Procedure

9.1. Preparation of test apparatus.

- Place the densimeter on a perfectly stable support, isolated from any vibrations.
- Connect the densimeter to the circulating constant temperature bath using flexible rubber pipes or insulating tubes. Fill the water bath according to the manufacturer's

instructions and add a product to prevent the formation of algae.

Set the bath temperature to reach and maintain the requisite test temperature on the densimeter.

- Accurately setting and controlling the temperature in the measuring cell are very important parameters, as a 0.1 °C error can result in a variation in density in the order of 0.1 kg/m^3.
- The following rules must be observed:
- the measuring cell must be maintained at a constant temperature for 6 hours before the test.
- the maximum temperature variation measured by the temperature probe of the measuring cell must not exceed ± 0.02 °C.
- the pipe flow, length and insulation between the thermostatic bath and the cell are to be adjusted to ensure the stability of the cell temperature.

9.2. Checking period measurements in the ambient air.

- Clean, rinse and dry the cell.
- Carry out a measurement in the ambient air. Check that the period measured does not deviate by more than i0- in relative values from the period determined under the conditions described in section 7. If deviation occurs, clean the cell again with a lukewarm chromatic acid solution (warning: this product can cause serious burns), which is the most effective cleaning agent. If the deviation persists after several cleaning operations, repeat the calibration process.

9.3. Density measurement.

- Filter the sample first, if necessary.
- Illuminate the cell.

If only a small quantity of sample is available, use the syringe to introduce the quantity needed so that the liquid to be measured reaches the top port of the clean and dry cell. While filling the cell, make sure all air bubbles are completely removed; the sample must be homogeneous and must not contain any solid particulates.

- Leave the syringe in place on the bottom port of the cell.

NOTE: Introducing dark coloured samples into the cell does not help to establish the absence of air bubbles or solid particulates with certainty.

Switch off the lamp immediately after introducing the sample, as the heat it produces influences the measurement temperature.

9.4. Calculation and expression of results.

9.4.1. Densimeter with an integrated calculator.

After a few minutes, the density value stabilises, indicating that the equilibrium temperature of the measurement has been reached. If the measurement temperature does not differ by more than ± 0.01 °C from a temperature of 20 °C, record the reading.

If needed, convert the obtained result into kg/m^3.

9.4.2. Densimeter without a calculator.

Allow the reading for the period of oscillation (T) to stabilise within one unit from the fourth decimal place. If the measurement temperature does not differ by more than ± 0.01 °C from a temperature of 20 °C, record the reading.

Calculate the density of the sample in kg/m3 using the following formula:

$$p = \frac{1000}{A} * (T2 - B)$$

where

- T is the period of oscillation for the sample measured (in ms).
- A and B are the constants defined during the calibration prescribed in paragraph 8.

9.4.3. Viscosity correction.

If the liquid whose density is being measured has a viscosity higher than 2 mm^2/s, correction is required to take the viscosity into account, using the formula provided by the manufacturer of the densimeter.

10. Test report

The test report must indicate:

- the method used,
- the result and mode of expression of results,
- the specific details and any unforeseen events recorded during the measurement,
- operations not included in the method.

APPENDIX A: TABLE 1 AIR DENSITY

Air density, expressed in g/cm^3, varies with the pressure P expressed in mbar and the temperature expressed in °C.

A t °C and p Ton-, calculate the density using the following formula:

$$density\ t,p = \frac{0{,}0012930}{1 + 0;00367t} * \frac{P}{760}$$

Values are given for contents of 0.03 vol% of CO_2 in the air; values change by ± 1/19000 with each variation of ± 0.0001 of the CO_2 volume.

Composition of dry air at ground level:

	N_2	O_2	A	CO_2	Ne	He	Kr	X	H_2
Volume in %	78.09	20.95	0.93	0,03	0.0018	0.0005	0.041	0.068	0.045
Mass in %	75.52	23.15	1.28	0.05	0.0013	0.047	0.043	0.044	0.084

Units are g/ml
Pressure (H) in centimetres of mercury

les unités sont des g/ml
Presion (H) en centimètres de mercure

T °C	72.0	73.0	74.0	75.0	76.0	77.0	proport. parts parties proport.
10	0.001182	0.001198	0.001215	0.001231	0.001247	0.001264	17
11	178	193	210	227	243	259	cm
12	173	190	206	222	239	255	0,1 2
13	169	186	202	218	234	251	0,2 3
14	165	181	198	214	230	246	0,3 5
							0,4 7
15	0.001161	0.001177	0.001193	0.001210	0.001226	0.001242	0,5 8
16	157	173	189	205	221	238	0,6 10
17	153	169	185	201	217	233	0,7 12
18	149	165	181	197	213	229	0,8 14
19	145	161	177	193	209	225	0,9 15
							16
20	0.001141	0.001157	0.001173	0.001189	0.001205	0.001221	cm
21	137	153	169	185	201	216	0,1 2
22	134	149	165	181	197	212	0,2 3
23	130	145	161	177	193	208	0,3 5
24	126	142	157	173	189	204	0,4 6
							0,5 8
25	0.001122	0.001138	0.001153	0.001169	0.001185	0.001200	0,6 10
26	118	134	149	165	181	196	0,7 11
27	115	130	146	161	177	192	0,8 13
28	111	126	142	157	173	188	0,9 14
29	107	123	138	153	169	184	15
30	10.001104	0.001119	0.001134	0.001150	0.001165	0.001180	0,1 1
							0,9 13

Proportional parts 17:

Dry air density at 20° and 760 mm of mercury: 1.204 mg/ml

DENSITY OF PURE DEAERATED WATER

0 °C : 0.99987	3,98 °C : 1.0000	5 °C : 0.99999	10 °C : 0.9973
15 °C : 0.99913	18 °C : 0.99862	20 °C : 0.99823	25 °C : 0.99707
30 °C : 0.99567	35 °C : 0.99406	38 °C : 0.99299	40 °C : 0.99224
45 °C : 0.99025	50 °C : 0.98807	55 °C : 0.98573	60 °C : 0.98324
65 °C : 0.98059	70 °C : 0.97781	75 °C : 0.97489	80 °C : 0.97183
85 °C : 0.96865	90 °C : 0.96534	95 °C : 0.96192	100 °C : 0.95838

Water density according to the temperature: ρ (g/cm³) (1)

t (°C)	0,0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9
0	0,999 839	0,999 846	0,999 852	0,999 859	0,999 865	0,999 871	0,999 877	0,999 882	0,999 888	0,999 893
1	0,999 898	0,999 903	0,999 908	0,999 913	0,999 917	0,999 921	0,999 925	0,999 929	0,999 933	0,999 936
2	0,999 940	0,999 943	0,999 946	0,999 949	0,999 952	0,999 954	0,999 956	0,999 959	0,999 961	0,999 962
3	0,999 964	0,999 966	0,999 967	0,999 968	0,999 969	0,999 970	0,999 971	0,999 971	0,999 972	0,999 972
4	0,999 972	0,999 972	0,999 972	0,999 971	0,999 971	0,999 970	0,999 969	0,999 968	0,999 967	0,999 965
5	0,999 964	0,999 962	0,999 960	0,999 958	0,999 956	0,999 954	0,999 951	0,999 949	0,999 946	0,999 943
6	0,999 940	0,999 937	0,999 934	0,999 930	0,999 926	0,999 923	0,999 919	0,999 915	0,999 910	0,999 906
7	0,999 901	0,999 897	0,999 892	0,999 887	0,999 882	0,999 877	0,999 871	0,999 866	0,999 860	0,999 854
8	0,999 848	0,999 842	0,999 836	0,999 829	0,999 823	0,999 816	0,999 809	0,999 802	0,999 795	0,999 788
9	0,999 781	0,999 773	0,999 765	0,999 758	0,999 750	0,999 742	0,999 734	0,999 725	0,999 717	0,999 708
10	0,999 699	0,999 691	0,999 682	0,999 672	0,999 663	0,999 654	0,999 644	0,999 635	0,999 625	0,999 615
11	0,999 605	0,999 595	0,999 584	0,999 574	0,999 563	0,999 553	0,999 542	0,999 531	0,999 520	0,999 509
12	0,999 497	0,999 486	0,999 474	0,999 462	0,999 451	0,999 439	0,999 426	0,999 414	0,999 402	0,999 389
13	0,999 377	0,999 364	0,999 351	0,999 338	0,999 325	0,999 312	0,999 299	0,999 285	0,999 272	0,999 258
14	0,999 244	0,999 230	0,999 216	0,999 202	0,999 188	0,999 173	0,999 159	0,999 144	0,999 129	0,999 114
15	0,999 099	0,999 084	0,999 069	0,999 054	0,999 038	0,999 022	0,999 007	0,998 991	0,998 975	0,998 958
16	0,998 943	0,998 926	0,998 910	0,998 894	0,998 877	0,998 860	0,998 843	0,998 826	0,998 809	0,998 792
17	0,998 775	0,998 757	0,998 740	0,998 722	0,998 704	0,998 686	0,998 668	0,998 650	0,998 631	0,998 614
18	0,998 595	0,998 577	0,998 558	0,998 539	0,998 520	0,998 502	0,998 482	0,998 463	0,998 444	0,998 425
19	0,998 405	0,998 385	0,998 366	0,998 346	0,998 326	0,998 306	0,998 286	0,998 265	0,998 245	0,998 224
20	0,998 204	0,998 183	0,998 162	0,998 141	0,998 120	0,998 099	0,998 078	0,998 057	0,998 035	0,998 014
21	0,997 992	0,997 971	0,997 949	0,997 927	0,997 905	0,997 883	0,997 860	0,997 838	0,997 816	0,997 793
22	0,997 770	0,997 747	0,997 725	0,997 702	0,997 679	0,997 656	0,997 632	0,997 609	0,997 585	0,997 562
23	0,997 538	0,997 515	0,997 491	0,997 467	0,997 443	0,997 419	0,997 394	0,997 370	0,997 345	0,997 321
24	0,997 296	0,997 272	0,997 247	0,997 222	0,997 197	0,997 172	0,997 146	0,997 121	0,997 096	0,997 070
25	0,997 045	0,997 019	0,996 993	0,996 967	0,996 941	0,996 915	0,996 889	0,996 863	0,996 836	0,996 810
26	0,996 783	0,996 757	0,996 730	0,996 703	0,996 676	0,996 649	0,996 622	0,996 595	0,996 568	0,996 540
27	0,996 513	0,996 485	0,996 458	0,996 430	0,996 402	0,996 374	0,996 346	0,996 318	0,996 290	0,996 262
28	0,996 233	0,996 205	0,996 176	0,996 148	0,996 119	0,996 090	0,996 061	0,996 032	0,996 003	0,995 974
29	0,995 945	0,995 915	0,995 886	0,995 856	0,995 827	0,995 797	0,995 767	0,995 737	0,995 707	0,995 677
30	0,995 647	0,995 617	0,995 586	0,995 556	0,995 526	0,995 495	0,995 464	0,995 433	0,995 403	0,995 372
31	0,995 341	0,995 310	0,995 278	0,995 247	0,995 216	0,995 184	0,995 153	0,995 121	0,995 090	0,995 058
32	0,995 026	0,994 997	0,994 962	0,994 930	0,994 898	0,994 865	0,994 833	0,994 801	0,994 768	0,994 735
33	0,994 703	0,994 670	0,994 637	0,994 604	0,994 571	0,994 538	0,994 505	0,994 472	0,994 438	0,994 405
34	0,994 371	0,994 338	0,994 304	0,994 270	0,994 236	0,994 202	0,994 168	0,994 134	0,994 100	0,994 066
35	0,994 032	0,993 997	0,993 963	0,993 928	0,993 893	0,993 859	0,993 824	0,993 789	0,993 754	0,993 719
36	0,993 684	0,993 648	0,993 613	0,993 578	0,993 543	0,993 507	0,993 471	0,993 436	0,993 400	0,993 364
37	0,993 328	0,993 292	0,993 256	0,993 220	0,993 184	0,993 148	0,993 111	0,993 075	0,993 039	0,993 002
38	0,992 965	0,992 928	0,992 891	0,992 855	0,992 818	0,992 780	0,992 743	0,992 706	0,992 669	0,992 631
39	0,992 594	0,992 557	0,992 519	0,992 481	0,992 444	0,992 406	0,992 368	0,992 330	0,992 292	0,992 254
40	0,992 215	0,992 177	0,992 139	0,992 100	0,992 062	0,992 023	0,991 985	0,991 946	0,991 907	0,991 868
41	0,991 830	0,991 791	0,991 751	0,991 712	0,991 673	0,991 634	0,991 594	0,991 555	0,991 515	0,991 476
42	0,991 436	0,991 396	0,991 357	0,991 317	0,991 277	0,991 237	0,991 197	0,991 157	0,991 116	0,991 076
43	0,991 036	0,990 995	0,990 955	0,990 914	0,990 873	0,990 833	0,990 792	0,990 751	0,990 710	0,990 669
44	0,990 628	0,990 587	0,990 546	0,990 504	0,990 463	0,990 421	0,990 380	0,990 338	0,990 297	0,990 255
45	0,990 213	0,990 171	0,990 129	0,990 087	0,990 045	0,990 003	0,989 961	0,989 919	0,989 876	0,989 834
46	0,989 792	0,989 749	0,989 706	0,989 664	0,989 621	0,989 578	0,989 535	0,989 492	0,989 449	0,989 406
47	0,989 363	0,989 320	0,989 276	0,989 233	0,989 190	0,989 146	0,989 103	0,989 059	0,989 015	0,988 971
48	0,988 928	0,988 884	0,988 840	0,988 796	0,988 752	0,988 707	0,988 663	0,988 619	0,988 574	0,988 530
49	0,988 485	0,988 441	0,988 396	0,988 352	0,988 307	0,988 262	0,988 217	0,988 172	0,988 127	0,988 082
50	0,988 037	0,987 992	0,987 946	0,987 901	0,987 844	0,987 810	0,987 764	0,987 719	0,987 673	0,987 627
51	0,987 581	0,987 536	0,987 490	0,987 444	0,987 398	0,987 351	0,987 305	0,987 259	0,987 213	0,987 166

Water density according to the temperature: ρ (g/cm^3) (1) (end)

t (°C)	0,0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9
52	0,987 120	0,987 073	0,987 027	0,986 980	0,986 933	0,986 886	0,986 840	0,986 793	0,986 746	0,986 699
53	0,986 652	0,986 604	0,986 557	0,986 510	0,986 463	0,986 415	0,986 368	0,986 320	0,986 272	0,986 225
54	0,986 177	0,986 129	0,986 081	0,986 033	0,985 985	0,985 937	0,985 889	0,985 841	0,985 793	0,985 745
55	0,985 696	0,985 648	0,985 599	0,985 551	0,985 502	0,985 454	0,985 405	0,985 356	0,985 307	0,985 258
56	0,985 219	0,985 160	0,985 111	0,985 062	0,985 013	0,984 963	0,984 914	0,984 865	0,984 815	0,984 766
57	0,984 716	0,984 666	0,984 617	0,984 567	0,984 517	0,984 467	0,984 417	0,984 367	0,984 317	0,984 267
58	0,984 217	0,984 167	0,984 116	0,984 066	0,984 016	0,983 965	0,983 914	0,983 864	0,983 813	0,983 762
59	0,983 712	0,983 661	0,983 610	0,983 559	0,983 508	0,983 457	0,983 406	0,983 354	0,983 303	0,983 252
60	0,983 200	0,983 149	0,983 097	0,983 046	0,982 994	0,982 943	0,982 891	0,982 839	0,982 787	0,982 735
61	0,982 683	0,982 631	0,982 579	0,982 527	0,982 475	0,982 422	0,982 370	0,982 318	0,982 265	0,982 213
62	0,982 160	0,982 108	0,982 055	0,982 002	0,981 949	0,981 897	0,981 844	0,981 791	0,981 738	0,981 685
63	0,981 631	0,981 578	0,981 525	0,981 472	0,981 418	0,981 365	0,981 311	0,981 258	0,981 204	0,981 151
64	0,981 097	0,981 043	0,980 989	0,980 935	0,980 881	0,980 827	0,980 773	0,980 719	0,980 665	0,980 611
65	0,980 557	0,980 502	0,980 443	0,980 393	0,980 339	0,980 284	0,980 230	0,980 175	0,980 120	0,980 065
66	0,980 011	0,979 956	0,979 901	0,979 846	0,979 791	0,979 736	0,979 680	0,979 625	0,979 570	0,979 515
67	0,979 459	0,979 403	0,979 348	0,979 293	0,979 237	0,979 181	0,979 126	0,979 070	0,979 014	0,978 958
68	0,978 902	0,978 846	0,978 790	0,978 734	0,978 678	0,978 621	0,978 565	0,978 509	0,978 452	0,978 396
69	0,978 339	0,978 283	0,978 226	0,978 170	0,978 113	0,978 056	0,977 999	0,977 942	0,977 885	0,977 828
70	0,977 771	0,977 714	0,977 657	0,977 600	0,977 543	0,977 485	0,977 428	0,977 370	0,977 313	0,977 255
71	0,977 198	0,977 140	0,977 082	0,977 025	0,976 967	0,976 909	0,976 851	0,976 793	0,976 735	0,976 677
72	0,976 619	0,976 561	0,976 503	0,976 444	0,976 386	0,976 327	0,976 269	0,976 211	0,976 152	0,976 093
73	0,976 035	0,975 976	0,975 917	0,975 858	0,975 800	0,975 741	0,975 682	0,975 623	0,975 564	0,975 504
74	0,975 445	0,975 386	0,975 327	0,975 267	0,975 208	0,975 148	0,975 089	0,975 029	0,974 970	0,974 910
75	0,974 850	0,974 791	0,974 731	0,974 671	0,974 611	0,974 551	0,974 491	0,974 431	0,974 371	0,974 311
76	0,974 250	0,974 190	0,974 130	0,974 069	0,974 009	0,973 948	0,973 888	0,973 827	0,973 767	0,973 706
77	0,973 645	0,973 584	0,973 524	0,973 463	0,973 402	0,973 341	0,973 280	0,973 218	0,973 157	0,973 096
78	0,973 025	0,972 974	0,972 912	0,972 851	0,972 789	0,972 728	0,972 666	0,972 605	0,972 543	0,972 481
79	0,972 419	0,972 356	0,972 296	0,972 234	0,972 172	0,972 110	0,972 048	0,971 986	0,971 923	0,971 861
80	0,971 799	0,971 737	0,971 674	0,971 612	0,971 549	0,971 487	0,971 424	0,971 361	0,971 299	0,971 236
81	0,971 173	0,971 110	0,971 048	0,970 985	0,970 922	0,970 859	0,970 796	0,970 732	0,970 669	0,970 606
82	0,970 543	0,970 479	0,970 416	0,970 353	0,970 289	0,970 226	0,970 162	0,970 098	0,970 035	0,969 971
83	0,969 907	0,969 843	0,969 772	0,969 715	0,969 652	0,969 587	0,969 523	0,969 459	0,969 395	0,969 331
84	0,969 267	0,969 202	0,969 138	0,969 073	0,969 009	0,968 944	0,968 880	0,968 815	0,968 751	0,968 686
85	0,968 621	0,968 556	0,968 491	0,968 427	0,968 362	0,968 297	0,968 232	0,968 166	0,968 101	0,968 036
86	0,967 971	0,967 906	0,967 840	0,967 775	0,967 709	0,967 641	0,967 578	0,967 513	0,967 447	0,967 381
87	0,967 316	0,967 250	0,967 184	0,967 118	0,967 052	0,966 986	0,966 920	0,966 854	0,966 788	0,966 722
88	0,966 656	0,966 589	0,966 523	0,966 457	0,966 390	0,966 324	0,966 257	0,966 191	0,966 124	0,966 057
89	0,965 991	0,965 924	0,965 857	0,965 790	0,965 723	0,965 656	0,965 589	0,965 522	0,965 455	0,965 388
90	0,965 321	0,965 254	0,965 187	0,965 119	0,965 052	0,964 984	0,964 917	0,964 849	0,964 782	0,964 714
91	0,964 647	0,964 579	0,964 511	0,964 443	0,964 376	0,964 308	0,964 240	0,964 172	0,964 104	0,964 036
92	0,963 967	0,963 899	0,963 831	0,963 763	0,963 694	0,963 626	0,963 558	0,963 489	0,963 421	0,963 352
93	0,963 284	0,963 215	0,963 146	0,963 077	0,963 009	0,962 940	0,962 871	0,962 802	0,962 733	0,962 664
94	0,962 595	0,962 526	0,962 457	0,962 387	0,962 318	0,962 249	0,962 180	0,962 110	0,962 041	0,961 971
95	0,961 902	0,961 832	0,961 762	0,961 693	0,961 623	0,961 553	0,961 483	0,961 414	0,961 344	0,961 274
96	0,961 204	0,961 134	0,961 064	0,960 993	0,960 923	0,960 853	0,960 783	0,960 712	0,960 642	0,960 572
97	0,960 501	0,960 431	0,960 360	0,960 289	0,960 219	0,960 148	0,960 077	0,960 006	0,959 936	0,959 865
98	0,959 794	0,959 723	0,959 652	0,959 581	0,959 510	0,959 438	0,959 367	0,959 296	0,959 225	0,959 153
99	0,959 082	0,959 010	0,958 939	0,958 867	0,958 796	0,958 724	0,958 653	0,958 581	0,958 509	0,958 431
100	0,958 365									

(1) Tenths of degrees are shown in the column headers, e.g. at 4.5 °C, ρ = 0.999 970

Method OIV-MA-BS-08 : R2009

Type IV method

ABV by near infrared spectroscopy in spirit drinks of viti-viniculture origin

(OENO 6/94;

OIV/OENO 382A/2009)

1. Introduction

This method of determining the real alcoholic strength by volume of alcoholic beverages and distillates is based on the physical principle of the spectral analysis of materials with absorption bands in the near infrared range.

Ethanol has this characteristic.

The spectral data of the sample being tested are compared with those obtained during an initial calibration covering the entire measurement range.

Spectrometers employing this principle are commercially available to perform this determination.

2. Object and scope of application

The purpose of this document is to describe a method for determining the real alcoholic strength of alcoholic beverages and distillates handled at atmospheric pressure.

The application of the method is restricted to products with a viscosity of less than around 15,000 mm^2/S (1 mm^2/S = 1 cSt) at the test temperature.

The analysis of alcoholic beverages whose composition is estimated to be close may nevertheless require a separate initial calibration for each of them.

With reference to the currently applicable regulations, the test temperature is set to 20°C.

3. Definition

Real alcoholic strength by volume (See pycnometry).

4. Principle

4.1. Physical principle

According to quantum theory, a molecule is capable of absorbing light energy according to Planck's formula:

$$\Delta = \frac{h \,.\, C}{E2 - E1}$$

Where:

- h = Planck constant
- C = speed of light
- E1 = fundamental energy state of the electron
- E2 = excited energy state of the electron,

From which it follows that the energy absorbed is proportional to the frequency of the incident light.

Near-infrared spectroscopy is a physical method of analysis based on the absorption of hv photons with very little energy that can be used to change the vibrational energy of molecules.

The number of v' waves is proportional to the v "frequencies" and hence to the hv energy of the photon.

The observed transitions correspond to vibrations of clearly identified groups of atoms.

They result in non-separable rays clustered into "bands" (band spectra), which can often be used for functional analysis.

In practice, only three transitions can be observed:

- transition from v = 0 to v = 1 with a high intensity,
- transition from v = 0 to v = 2 with a low intensity,
- transition from v = 0 to v = 3 with a negligible intensity,
- v = vibrational quantum number.

The corresponding three spectral bands are approximately at v (fundamental), 2v (first harmonic), 3v (second harmonic) frequencies.

The fundamental vibrations can be observed in the mid-infrared range, while the harmonics are only visible in the near infrared range (700 - 2500 nanometres).

In addition to the harmonics, combination bands can be observed, when several vibrations interact, resulting in bands whose frequency is the sum or difference of multiples of the fundamental frequencies.

$$v\,\text{comb.} = n1v1 \pm n2v2 \pm n3v3 \pm \cdots$$

Spectroscopy in the near infrared range spans the entire electromagnetic spectrum from 780 nm to 3000 nm. In this range, it is possible to study the transitions, harmonics and low-energy electronic combinations of the stretching and deformation vibrations of hydrogen

bonds (C - H, N - H, O - H); the latter have high frequencies and are suitable for quantitative analysis applications.

Although it does not necessarily enable the characterisation of a complete structure, a near infrared spectrum provides useful information about the hydrogen clustering of a molecule.

Accordingly, near IR spectroscopy can be used first of all for the quantitative determination of components comprising clusters such as C - H, O - H or N - H, i.e. including water, alcohols, phenols , etc., preferably for the characterization of molecular structures.

Generally, the C - H cluster is characterized by stretching fundamental bands between: 3.0 and 3.6 microns, stretching bands of the first harmonics, between 1.6 and 1.8 microns and stretching bands of the second harmonics between 1.1 and 1.2 microns.

Because of the influence of other functional clusters of the molecule, these bands are liable to undergo shifts.

The vibrations of N - H secondary movements are characterized by stretching fundamental bands at 2.9 microns, the first harmonics at 1.5 microns and the second harmonics at 1.0 microns. The N - H cluster has a highly characteristic band of combinations around 2.2 microns.

The O-H cluster has

- a fundamental stretching band at 2.8 microns,
- the first harmonics at 1.4 microns and
- the second harmonics at 1.0 microns;

in addition, there is

- a combination band around 2 microns.

Bands of this type are used for the quantitative determination of various organic components, both monomeric and polymeric.

Absorption bands in the near infrared range

2500 nm	_____ C-H	Combinations
2200 nm	_____ O-H N-H	Combinations
1800 nm	_____ C-H	First harmonics
1600 nm	_____ N-H H-O	First harmonics
1420 nm	_____ C-H	Combinations
1300 nm	_____ C-H	Combinations
1100 nm		

4.2. Principle de measurement

4.2.1. A sample of a few millilitres of liquid is introduced into a measuring cell thermostated at the test temperature.

4.2.2. The sample is exposed to infrared radiation whose wavelengths have been previously selected by a primary calibration specific to the analyte.

With regard to ethanol, these wavelengths are generally four to five in number.

4.2.3. The beam of the light source is located through a collimator and a "chopper" directly on a filter wheel, selected automatically by the microprocessor.

The monochromatic light is then directed either to the measuring cell or to the reference by a tilting mirror.

The infrared rays penetrate the sample, interact with its components and are then reflected to the detector(s).

4.2.4. The spectral data of the sample are processed by the microprocessor integrated with the spectroscope and compared with calibration curves that have been determined and stored beforehand.

The regression equation is of the type:

$$\%C = F_0 + F_1R_1 + F_2R_2 + \cdots + F_nR_n$$

Where

- % C = percentage strength by volume,
- F_0 - Fn = constants corresponding to ethanol,
- R_1 - Rn = spectral reflection values measured at wavelengths from 1 to n.

After about a minute, the result is displayed by the apparatus. It is directly expressed as a percentage strength by volume.

5. Apparatus

5.1. NIR Spectroscopes

Two variants of apparatus suitable for measuring the real alcoholic strength of alcoholic beverages are available on the market.

They differ mainly by the type of measuring cell used.

Transflectance principle: the bottom of the measuring cell is fitted with a reflector which reflects the light beam having passed through the sample; the light can then interact again with the sample. This combination of transmission and reflection is called transflectance.

Transmission principle: the measuring cell is mounted vertically. The sample is directly crossed by the ray of light.

5.2. The apparatus consists of the following items:

- sample pumping system,
- light source,
- wavelength selection appliance,
- thermostated measuring cell,
- detectors converting light energy into an electrical signal,
- computer system to process the signals and display the results.

5.2.1. Sample pumping system.

The sample is injected into the measuring cell using a peristaltic pump. Some spectroscopes can be fitted with an automatic sample changer.

5.2.2. Light source.

Thermal light sources are mainly used. In tungsten filament lamps, the most commonly used, the filament is heated to 2100°C by the transformation of electrical energy. The polychromatic light obtained has a spectrum ranging between 320 nm and 2500 nm. It is necessary to control very precisely the intensity of the light in order to obtain repeatable measurements.

5.2.3. Wavelength selection appliance

Three basic principles are commonly applied: interference filters, tilting filters, and grating monochromators.

Interference filters are constituted by a layer of a semi-transparent material, such as magnesium fluoride, placed between two semi-reflecting layers. Under these conditions, the incident light interferes inside the filter and only certain wavelengths, depending on the thickness of the transparent layer, pass through the appliance.

Market-available appliances include three to twenty filters whose central wavelengths have been carefully selected.

The filters are attached onto a thermostated wheel, whose rotation is controlled by microprocessor.

Tilting filter systems take into account the fact that the wavelength selected by an interference filter depends on the angle of incidence between the ray of light and the filter. By varying this angle, it is possible to select different wavelengths around the central value. A large number of spectral measurements can thus be obtained with only a few filters. The correspondence between the position of the wheel carrying the filters and the measurement wavelength is, however, difficult to establish.

Grating monochromators are polished mirrors on which numerous parallel grooves are etched. The grooves diffract light and act as light sources that are phase-shifted in relation to each other. This causes light interference, as in the case of filters, and can be used to select wavelengths by turning the grate. Spectral measurements can be taken every 2 nm between 1100 and 2500 nm, providing 700 measurement points for each sample.

5.2.4. Thermostated measuring cell.

The optical walls are made of quartz.

Temperature control of the chamber comprising the cell is obtained by means of an integrated semiconductor element which uses the Peltier effect.

5.2.5. Detectors.

The detectors most commonly used are made of lead sulphide. These are semiconductors whose resistance decreases as the incident light

intensity increases. They operate in the spectral range from 1000 nm to 2500 nm.

5.2.6. Data processing and display.

The spectral data are first of all collected in the form of continuous electrical signals. In general, the raw data includes successive measurements of the light intensity of the source (I0) and that of the sample (I). These data are converted analogously into absorbance values [log (I0/I)] and digitized.

On the simplest appliances, digitized spectral data are not stored but are used to predict the response variable, which is immediately displayed.

On other systems, the spectrometer is coupled to a microcomputer which can be used to store spectral information, manage data files, and carry out the mathematical and statistical analyses.

6. Products

6.1. Products to clean the measuring cell

Cell cleaning is recommended at the end of each series of measurements and can be performed using the following solutions:

- Aqueous solution of sodium hypochlorite (NaClO), at market-available concentration, diluted to one tenth,
- Laboratory glassware cleaning agent, suitably diluted.

Cleaning should be followed by prolonged rinsing with freshly prepared, distilled or demineralised water.

6.2. Calibration substances.

The calibration substances must be chosen such that:

- The values of their alcoholic strengths by volume cover those of the products to be measured,
- they are based on a common matrix, except for their alcohol content, with identical compositional characteristics. The nature and composition of the matrix are essential in ensuring the reliability of the NIR spectroscopy technique.

Given the large number of samples required for calibration, their alcoholic strength by volume may be determined beforehand using:

- the reference method,
- the areometric method using EC Class I alcoholmeters, - the electronic densimetry method.

(see the description of these methods).

Remark: The determination of alcoholic strength by volume according to one of these two methods is, where appropriate, performed after distilling the sample.

The analytical application in the near infrared range resulting from this calibration will have at best an accuracy equivalent to that of the method used.

7. Calibration of apparatus

7.1. The implementation of a quantitative analysis technique in the near infrared range involves several levels of calibration:

- initial calibration in order to select, according to statistical calculations, a variable number of significant wavelengths of the characteristic (ethanol) to be analyzed,

- periodic re-calibration to verify the reliability of the calibration equation,
- routine calibration to correct the bias of the reference curve. It should be performed before each series of measurements.

7.2. Initial calibration.

This operation requires the use of a spectroscope in the near IR capable of performing measurements in a series of twenty successive wavelengths.

The connection between the spectral data and the characteristics that are to be predicted by near NIR spectroscopy are often difficult to determine.

The spectral bands overlap to a considerable degree and it is usually impossible to establish an analytical application of the single measurement of the height of a significant peak. On the contrary, a calibration procedure must be used that is quite complex to implement. The calibration is only valid for the determination of the alcoholic strength by volume of alcoholic beverages with strictly identical compositional characteristics. There are six steps in the process.

7.2.1. Establish a set of representative samples and analyze them using the reference method.

The collection of standard solutions must include thirty to fifty individual samples and cover the entire concentration range encountered in practice.

In addition, they must be divided into concentration classes of approximately the same size.

7.2.2. The collection is divided into two separate lots: one is used for calibration, the second for verification purposes.

7.2.3. Carry out the spectral measurement of the calibration collection: each standard solution must be analyzed twice in succession (double sampling).

7.2.4. The alcohol concentration values obtained by the reference method are entered on a microcomputer equipped with software for statistical computing.

The values are then correlated with the spectral measurements.

7.2.5. A multi-linear regression program is used to establish the following relation on the calibration samples:

$$C = a_0 + a_1 r_1 + a_2 r_2 + a_3 r_3 + \cdots$$

where

- C, is the characteristic being measured,
- a_0, a_1, a_2, ... : are the regression coefficients,
- r_1, r_2, r_3, ... : are the spectral reflectance measurements at wavelengths: L_1, L_2, L_3 ...

Two to ten wavelengths are selected from among those that are available, based on statistical criteria. The residual error of calibration is calculated: it must be small compared with the standard deviation of the characteristic being studied.

$$\sqrt{\sum_{i=1} \frac{n(di - d)^2}{(n - k - 1)}}$$

where

- d_i = difference between the concentrations obtained by the reference method and those obtained by NIR spectroscopy
- d = average di
- n = number of samples used for calibration
- k = number of calibration wavelengths.

7.2.6. Carry out the spectral measurement of the verification collection and apply the equation with the values obtained.

Compare the residual error of verification with that for the calibration: they should be close.

Standard deviation of prediction:

$$\sqrt{\sum_{i=1} \frac{n(di - d)^2}{(n-1)}}$$

where

- n = number of standard solutions used to verify the calibration,
- di = difference between the concentrations obtained by the reference method and those obtained by NIR spectroscopy,
- d = average di.

When the wavelengths have been selected and the calibration has been performed, and the results are recognized as being statistically consistent, the analytical method can be routinely applied.

7.3. Periodic recalibration.

Aging of the electronic components, repairs, parts replacement, or other abnormalities, require periodic recalibration of the equipment.

Similarly, the transfer of the calibration process from one appliance to another requires periodic recalibration.

Recalibration involves adjusting the bias and sometimes the slope of the initial calibration equation.

This procedure does not affect the selection of wavelengths.

In practice, in order to limit the sources of error, it is preferable to analyze the spectrum of ten representative samples covering the entire calibration range. These standard substances are recognized beforehand using the reference method.

In this case the multilinear calibration equation becomes a simple linear equation:

$$Y + K + mX = F_0 + m\,[F_1 log 1/R(\lambda 1) + F_2 log 1/r(\lambda 2) + \ldots]$$

where

- F_0, is the bias,
- m, is the slope.

7.4. Calibration of routine bias correction.

This correction must be made before any series of measurements, and at least once a day.

Using NIR spectroscopy, analyse a standard solution whose alcoholic strength by volume has been determined beforehand using the reference method.

The bias value is adjusted by assigning it the difference obtained between the measurement of the reference method and that of the spectroscopic method. The difference can be negative or positive.

8. Procedure

8.1. Preparation of test apparatus.

Place the spectroscope:

- on a perfectly stable support, isolated from any vibrations.
- away from direct sunlight,
- free from corrosive vapours, magnetic fields, and large variations in temperature.

After connecting the apparatus to a power source, allow it to warm for at least thirty minutes.

Fill the thermostat unit of the measuring cell with a coolant in accordance with the manufacturer's instructions. Set the temperature in order to reach and maintain the requisite test temperature.

8.2. Measurement of alcoholic strength by volume.

8.2.1. If necessary, select the spectroscopic method corresponding to the alcoholic beverage to be analyzed.

8.2.2. Check the cleanliness of the measuring cell:

- no particles in the cell,
- if necessary, clean the window using a brush and a soft cloth dampened with ethyl alcohol.

8.2.3. Calibrate the bias correction in accordance with the method described in point: 7.40.

8.2.4. Filter the sample first, if necessary.

8.2.5. Rinse at length the measuring cell with the alcoholic beverage to be tested.

Filter if necessary.

8.2.6. Carry out the determination. After approximately one minute, the result is displayed on the easy-to-read display.

8.2.7. Carry out five determinations in a row for the same sample: (the use of this analytical technique allows measurements to be obtained in a very short period of time).

The value of the alcoholic strength by volume of the sample is based on the calculation of the average for the five determinations.

Note: The five determinations must result in homogeneous values, in all cases covering the range of accuracy of the reference method used.

In the opposite case, carry out a second complete series of measurements after checking the cleanliness of the measuring cell and if necessary re-calibrating the bias of the calibration curve.

8.2.8. Check the relevance of the measurement accuracy; determinations performed in series should include the periodic analysis of a standard solution recognized by the reference method.

The cycle is to be respected, under the conditions described above, involves the analysis of a standard after five determinations.

8.2.9. Clean and rinse the measuring cell at length, at the end of the analysis.

CALIBRATION OF AN INFRALYSER FOR THE DETERMINATION OF THE ALCOHOLIC STRENGTH BY VOLUME ON BRANDIES OR LIQUEUR WINES

1. Selection of filters

As an example, the filter selections listed below can be used to measure the alcoholic strength by volume in the following alcoholic beverages:

1.1. Wine brandies aged in wooden casks

1.1.1. First combination.

- 2310 nm
- 1778 nm
- 2100 nm
- 1680 nm

1.1.2. Second combination.

- 2310 nm
- 2230 nm
- 1769 nm
- 1940 nm
- 1680 nm

1.2. "Pastis" aniseed-flavoured alcoholic beverage.

- 2270 nm

- 2230 nm
- 1769 nm
- 1940 nm
- 1680 nm

Use of an infralyser for WINE BRANDIES

Select the following filters: 4, 13, 14 and 20.

Four levels of alcoholic strength by volume are calibrated on raw brandies:

1 : 37.5 to 43 % vol.

2 : 42.5 to 47.5 % vol.

3 : 57.5 to 62 % vol.

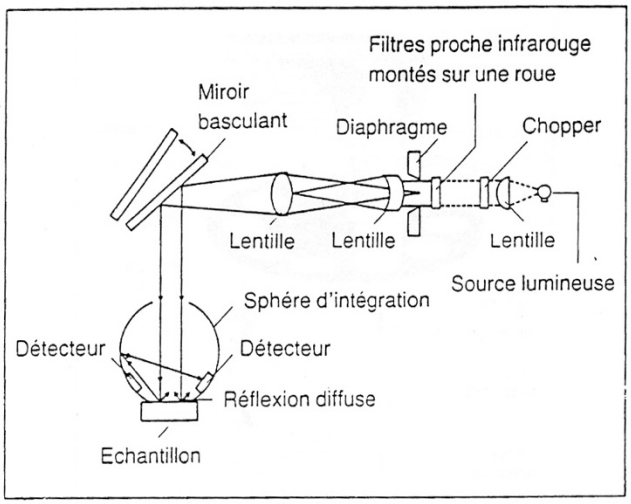
4 : 67.5 to 72.5 % vol.

For each level, and by increments of 0.5% vol., 10 to 11 determinations of the alcoholic strength by volume based on the pycnometric method are used to calibrate the infralyser

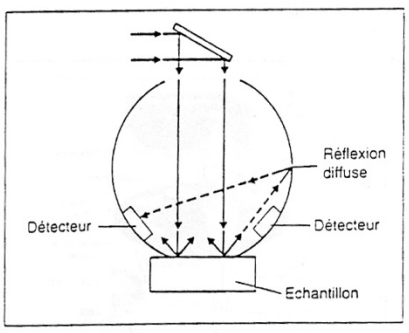
2. Bibliography

1. Calibration of an infralyser for the determination of the alcoholic strength by volume on brandies or liqueur wines.
2. Station viticole, Cognac National Interprofessionnel Bureau

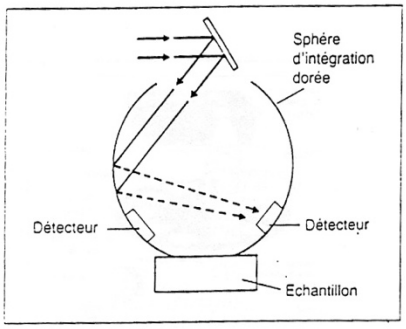
CONCEPTION DES SPECTROSCOPES DANS LE PROCHE I.R.
UTILISANT LE PRINCIPE DE MESURE PAR TRANSFLEXION



SCHEMA DE PRINCIPE

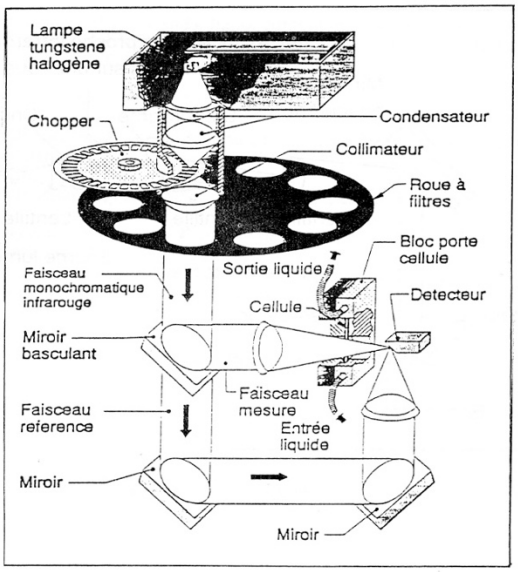


Mesure de l'échantillon



Mesure de la référence

CONCEPTION DES SPECTROSCOPES DANS LE PROCHE I.R.
UTILISANT LE PRINCIPE DE MESURE PAR TRANSFLEXION



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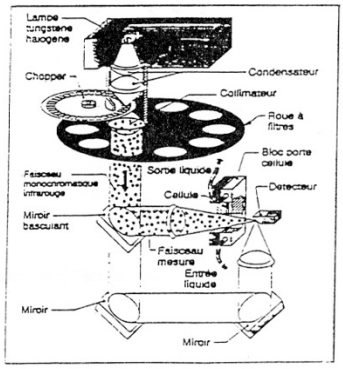
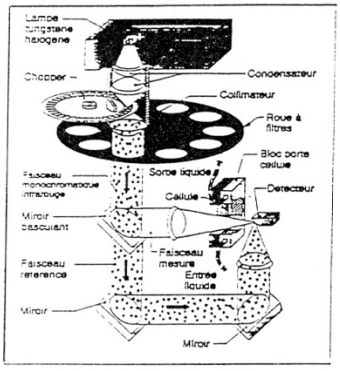


Mesure de l'échantillon



Mesure de référence

80

Total dry extract: Gravimetric method

Method OIV-MA-BS-09 : R2009

Type II method

Method for the determination of total dry extract of spirit drinks of viti-vinicultural origin: gravimetric method

(OIV/OENO 379/2009)

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.

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 ($\overline{\times}$) 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

Total dry extract: Usual calculation method

Method OIV-MA-BS-10 : R2009

Type IV method

Determination of total dry extract by the usual calculation method in spirit drinks of viti-vinicultural

(OENO 6/94;

OIV/OENO 382A/2009)

1. Principle

The total dry extract can be calculated indirectly based on the value of the density of the "residue without alcohol" or the alcoholic beverage from which the alcohol has been removed and has been returned to the original volume by adding water. This dry extract is expressed as the amount of sucrose which, dissolved in one litre of solution, has the same density.

This quantity is given by the Plato table (table 1).

2. Procedure

Density 20/20, dr, of the "residue without alcohol" is calculated by Tabarie's Formula:

$$dr = ds - da + 1{,}000$$

- where ds = density of the alcoholic beverage at 20°C compared with water at 20°C
- da = density at 20°C of the same hydroalcoholic mixture as the alcoholic beverage, relative to water at 20°C.

dr can also be calculated based on the densities at 20°C ρv of the alcoholic beverage ρa the hydroalcoholic mixture of the same degree by the formula

Total dry extract: Usual calculation method

$$dr = 1{,}0018(\rho v - \rho a) + 1{,}00$$

Record the density dr of the de-alcoholised medium in Table 1 to obtain the weight of total dry extract in grams per litre.

Table 1 for the calculation of solids content

Relative density to 2 decimal points	Third decimal point of relative density									
	0	1	2	3	4	5	6	7	8	9
	Grams of extract per litre									
1,00	0,0	2,6	5,1	7,7	10,3	12,9	15,4	18,0	20,6	23,2
1,01	25,8	28,4	31,0	33,6	36,2	38,8	41,3	43,9	46,5	49,1
1,02	51,7	54,3	56,9	59,5	62,1	64,7	67,3	69,9	72,5	75,1
1,03	77,7	80,3	82,9	85,5	88,1	90,7	93,3	95,9	98,5	101,1
1,04	103,7	106,3	109,0	111,6	114,2	116,8	119,4	122,0	124,6	127,2
1,05	129,8	132,4	135,0	137,6	140,3	142,9	145,5	148,1	150,7	153,3
1,06	155,9	158,6	161,2	163,8	166,4	169,0	171,6	174,3	176,9	179,5
1,07	182,1	184,8	187,4	190,0	192,6	195,2	197,8	200,5	203,1	205,8
1,08	208,4	211,0	213,6	216,2	218,9	221,5	224,1	226,8	229,4	232,0
1,09	234,7	237,3	239,9	242,5	245,2	247,8	250,4	253,1	255,7	258,4
1,10	261,0	263,6	266,-	268,-	271,5	274,2	276,8	279,5	282,1	284,8
1,11	287,4	290,-	292,7	295,3	298,-	300,-	303,3	305,-	308,-	311,2
1,12	313,9	316,5	319,2	321,8	324,5	327,1	329,8	332,4	335,1	337,8
1,13	340,4	343,0	345,7	348,3	351,0	353,7	356,3	359,	361,6	364,

1,14	366,9	369,6	372,3	375,0	377,6	380,3	382,9	385,6	388,3	390,9
1,15	393,6	396,2	398,9	401,6	404,3	406,9	409,6	412,3	415,0	417,6
1,16	420,3	423,0	425,7	428,3	431,0	433,7	436,4	439,0	441,7	444,4
1,17	447,1	449,8	452,4	455,2	457,8	460,5	463,2	465,9	468,6	471,3
1,18	473,9	476,6	479,3	482,0	484,7	487,4	490,1	492,8	495,5	498,2
1,19	500,9	503,5	506,2	508,9	511,6	514,3	517,0	519,7	522,4	525,1
1,20	527,8	-	-	-	-	-	-	-	-	-

Interpolation table

Fourth decimal point of relative density	Grams of extract per litre	Fourth decimal point of relative density	Grams of extract per litre	Fourth decimal point of relative density	Grams of extract per litre
1	0,3	4	1,0	7	1,8
2	0,5	5	1,3	8	2,1
3	0,8	6	1,6	9	2,3

Method OIV-MA-BS-11 : R2009

Type II method

Determination of sugars in spirit drinks of viti-vinicultural origin

(OIV/OENO 380/2009)

Introduction

Spirit drinks of viti-vinicultural origin may be sweetened by various compounds, and in certain legislations the concentrations of sweetener are subject to minimum or maximum levels.

1. Scope

This method is suitable for the determination of the glucose, fructose, and sucrose contents of spirit drinks of viti-vinicultural origin. It is not suitable for spirit drinks containing dairy products or eggs.

2. Normative References

ISO 3696:1897 Waters for analytical use - Specifications and test methods.

3. Principle

High performance liquid chromatography (HPLC) to determine the glucose, fructose, and sucrose concentrations.

This method is described as an example. It uses an alkylamine stationary phase and differential refractometry detection. Other columns/detectors may be used, for example anion exchange resins as the stationary phase.

4. Reagents and Materials

4.1. Glucose (CAS 50-99-7), at least 99 % pure.

4.2. Fructose (CAS 57-48-7), at least 99 % pure.

4.3. Sucrose (CAS 57-50-1), at least 99 % pure.

4.4. Pure acetonitrile (CAS 75-05-8) for HPLC analysis.

Acetonitrile is a highly flammable liquid. It is toxic by inhalation, in contact with skin and if swallowed. It is irritating to eyes.

4.5. Distilled or demineralised water, preferably micro-filtered.

4.6. Solvents (example)

The elution solvent is prepared beforehand by mixing:

- 75 parts by volume of acetonitrile (4.4),
- 25 parts by volume of distilled or demineralised water (4.5).

Pass helium through at a slow rate for 5 - 10 minutes prior to use to degas.

If the water being used has not been micro-filtered, it is advisable to pass the solvent through a filter for organic solvents with a pore size less than or equal to 0.45 μm.

4.7. Ethanol, absolute (CAS 64-17-5).

4.8. Ethanol solution (5 %, v/v).

4.9. Preparation of stock standard solution (20 g/L)

Weigh 2 g each of the sugars to be analysed (4.1 to 4.3), transfer them without loss to a 100 mL volumetric flask. Adjust to 100 mL with a 5 % vol. alcohol solution (4.8), shake and store at around +4 °C. Prepare a new stock solution once a week if necessary.

4.10. Preparation of working standard solutions (2.5, 5.0, 7.5, 10.0 and 20.0 g/L)

Dilute the stock solution, 20 g/L, (4.9) appropriately with a 5% vol. alcohol solution (4.8) to give five working standards of 2.5, 5.0, 7.5, 10.0 and 20.0 g/L. Filter with a filter of a pore size less than or equal to 0.45 µm (5.3.).

5. Apparatus and Equipment (as an example - other systems that provide equivalent performance can be used)

Standard laboratory apparatus, "A" grade volumetric glassware and, in particular, the following:

5.1. HPLC system capable of achieving baseline resolution of all of the sugars.

5.1.1. High-performance liquid chromatograph with a six-way injection valve fitted with a 10 µL loop or any other device, whether automatic or manual, for the reliable injection of micro-volumes.

5.1.2. Pumping system enabling one to achieve and maintain a constant or programmed rate of flow with great precision.

5.1.3. Differential refractometer.

5.1.4. Computational integrator or recorder, the performance of which is compatible with the rest of the set-up.

5.1.5. Pre-column:

It is recommended that a suitable pre-column is attached to the analytical column.

5.1.6. Column (example):

Material: stainless steel or glass

Internal diameter: 2-5 mm

Length: 100-250 mm (depending on the packing particle size), for example 250 mm if the particles are 5 µm in diameter

Stationary phase: cross-linked silica with radicals containing the alkylamine functional group, maximum particle size 5 µm.

5.1.7. Chromatography conditions (example):

Elution solvent (4.6), flow rate: 1 mL/minute

Detection: Differential refractometry

To make certain that the detector is perfectly stable, it may be advisable to switch it on a few hours before use. The reference cell must be filled with the elution solvent.

5.2. Analytical balance accurate to 0.1 mg.

5.3. Filtration equipment for small volumes using a 0.45 µm membrane.

6. Sample storage

On receipt, samples are to be stored at room temperature prior to analysis.

7. Procedure

7.1. PART A: Sample Preparation

7.1.1. Shake the sample.

7.1.2. Filter the sample through a filter with a pore size less than or equal to 0.45 µm (5.3).

7.2. PART B: HPLC

7.2.1. Determination

Inject 10 µL of the standard solutions (4.10) and samples (7.1.2.). Perform the analysis under suitable chromatography conditions, for example those described above.

7.2.2. Should any peak of a sample have a greater area (or height) than the corresponding peak in the most concentrated standard, then the sample should be diluted with distilled or demineralised water and re-analysed.

8. Calculation

Compare the two chromatograms obtained for the standard solution and spirit. Identify the peaks by their retention times. Measure their areas (or heights) to calculate the concentrations by the external standard method. Take into account any dilutions made to the sample.

The final result by convention is the sum of sucrose, glucose, and fructose, in g/L.

9. Method performance characteristics (Precision)

The following data were obtained in 2000 from an international method-performance study carried out on a variety of spirit drinks, following internationally-agreed procedures.

Key to the tables below:

nLT	Number of laboratories (2 results per laboratory)
nL	Number of laboratories to calculate precision values
r	repeatability limit
Sr	repeatability standard deviation
RSDr	repeatability standard deviation expressed in % of the level
R	reproducibility limit
SR	reproducibility standard deviation

RSDR	reproducibility standard deviation expressed in % of the level
PRSDR	RSDR predicted with the Horwitz formula (%)
HoR	HorRat value = RSDR / PRSDR

9.1. Glucose

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	24	92,4	5,4	1,9	2,1	13	4,8	5,2	1,8
Liqueur 2	24	23	93,2	9,7	3,5	3,7	28	10	11	3,8

9.2. Fructose

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	22	87	3,2	1,2	1,3	8,5	3,0	3,5	1,2
Liqueur 2	24	21	93	6,6	2,3	2,5	22	7,7	8,3	2,9

9.3. Saccharose

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	24	174	12	4,2	2,4	24	8,7	5,0	1,9
Liqueur 2	24	18	320	12	4,3	1,3	45	16	5,0	2,1
Liqueur 3	24	18	349	22	8,0	2,3	30	11	3,1	1,3
Pastis	24	19	11	0,2	0,1	0,8	2,2	0,8	7,3	1,9
Ouzo	24	19	24	2,1	0,8	3,1	2,6	0,9	3,8	1,1
Kirsch	24	20	103	6,1	2,2	2,1	12	4,2	4,0	1,4

9.4. Sucres totaux

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	21	353	8,7	3,1	0,9	41	15	4,2	1,8
Liqueur 2	24	18	510	16	5,6	1,1	41	15	2,9	1,3
Liqueur 3	24	18	349	22	8,0	2,3	30	11	3,1	1,3

Pastis	24	20	11	0,4	0,1	1,2	2,2	0,8	7,3	1,8
Ouzo	24	19	24	2,1	0,8	3,1	2,6	0,9	3,8	1,1
Kirsch	24	2	103	6,1	2,2	2,1	12	4,2	4,0	1,4

10. Bibliography

1. R. Wittkowski, A. Bertrand, P. Brereton, C. Guillou, 2000. PROJECT SMT4-CT96-2119, Validation of analytical methods of analysis for spirit drinks. REPORT NO. 02/09 - WORKSTREAM 10.
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.

Method OIV-MA-BS-12 : R2009

Type II method

Determination of the acidities of spirit drinks of viti-vinicultural origin

(OIV/OENO 380/2009)

1. Scope

This method is suitable for the determination of the volatile, total, and fixed acidities of spirit drinks of viti-vinicultural origin.

2. Normative References

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

3. Definitions

3.1. Volatile acidity is made up of acetic and higher volatile aliphatic acids that are present in spirit drinks.

3.2. Total acidity is the sum of titratable acidities.

3.3. Fixed acidity is the acidity of the residue left after evaporating the spirit drink to dryness.

4. Principle

The total acidity is determined by direct titration of the sprit drink. The fixed acidity is determined by titration of the aqueous solution obtained after dissolving the residue from evaporation of the spirit drink. The volatile acidity is calculated by deducting the fixed acidity from the total acidity.

5. Reagents and Materials

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

5.1. 0.05 M sodium hydroxide solution

5.2. Mixed indicator solution:

Weigh 0.1 g of indigo carmine and 0.1 g of phenol red.

Dissolve in 40 mL water and make up to 100 mL with ethanol.

6. Apparatus and Equipment

Standard laboratory apparatus, "A" grade volumetric glassware and, in particular, the following:

6.1. Equipment for applying vacuum (water pump, vacuum flask, etc.), or other system for eliminating carbon dioxide (bubbling or other).

6.2. Flat-bottomed stainless-steel cylindric capsule, of sufficient dimensions to avoid loss of liquid when evaporating.

6.3. Equipment for potentiometric titration (optional).

7. Sampling and samples

Samples are stored at room temperature prior to analysis.

8. Procedure

8.1. Total acidity

8.1.1. Preparation of sample

If necessary, the spirit is stirred for at least two minutes under vacuum to remove carbon dioxide, or the latter is eliminated by any other convenient method.

8.1.2. Titration

Pipette 25 mL of the spirit into a 500 mL conical flask.

Add about 200 mL of cooled boiled distilled water (freshly prepared) and 2-6 drops of the mixed indicator solution (5.2).

Titrate with the 0.05 M sodium hydroxide solution (5.1) until the yellow-green colour changes to violet in the case of colourless spirit drinks, or the yellow-brown colour to red-brown in the case of brown-coloured spirit drinks.

The titration may also be carried out by potentiometry, to pH 7.5.

Let n_1 mL be the volume of the 0.05 M sodium hydroxide solution added.

8.1.3. Calculation

- The total acidity (TA) expressed in milliequivalents per L of spirit drink is equal to 2 x n_1.
- The total acidity (TA') expressed in mg of acetic acid per L of spirit drink is equal to 120 x n_1.
- The total acidity (TA') expressed in g of acetic acid per hL of pure 100 % vol alcohol is equal to 120 x n_1 x 10/A, where A is the alcoholic strength by volume of the spirit drink.

8.2. Fixed acidity

8.2.1. Preparation of sample

Pipette 25 mL (or a larger volume if the fixed acidity is very low) of the spirit drink into a flat-bottomed cylindrical evaporating dish (6.2). 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.

If necessary, complete the drying by placing the evaporating dish in a drying oven at 105 °C for two hours. Allow the evaporating dish to cool in a desiccator.

8.2.2. Titration

Take up the residue left after evaporating with cooled boiled distilled water (freshly prepared), make up to a volume of about 100 mL and add 2-6 drops of the mixed indicator solution (5.2).

Titrate with the 0.05 M sodium hydroxide solution (5.1) until the yellow-green colour changes to violet if the solution is colourless, or the yellow-brown colour to red-brown if the solution is brown-coloured.

The titration may also be carried out by potentiometry, to pH 7.5.

Let n_2 mL be the volume of the 0.05 M sodium hydroxide solution added, and V mL the volume of sample evaporated.

8.2.3. Calculation

- The fixed acidity (FA) expressed in milliequivalents per L of spirit drink is equal to 2 x n_2 x 25/V.
- The fixed acidity (FA') expressed in mg of acetic acid per L of spirit drink is equal to 120 x n_2 x 25/V.
- The fixed acidity (FA') expressed in g of acetic acid per hL of pure 100% vol alcohol is equal to 120 x n_2 x 25/V x 10/A, where A is the alcoholic strength by volume of the spirit drink.

8.3. Calculation of volatile acidity

8.3.1. Expression in milliequivalents per L :

Let:

- TA = total acidity in milliequivalents per L
- FA = fixed acidity in milliequivalents per L
- Volatile acidity,VA, in milliequivalents per L is equal to :
- TA - FA

8.3.2. Expression in mg of acetic acid per L:

Let:

- TA' = total acidity in mg of acetic acid per L
- FA' = fixed acidity in mg of acetic acid per L
- Volatile acidity,VA, in mg of acetic acid per L is equal to :

- TA‘ - FA’

8.3.3. Expression in g of acetic acid per hL of pure 100 % vol alcohol is equal to :

$$\frac{TA' - FA'}{A} \times 10$$

- where A is the alcoholic strength by volume of the spirit drink.

9. Method performance characteristics (Precision)

The following data were obtained in 2000 from an international method-performance study on a variety of spirit drinks, carried out following internationally-agreed procedures.

Key to the tables below:

nLT	Number of laboratories (2 results per laboratory)
nL	Number of laboratories to calculate precision values
r	repeatability limit
Sr	repeatability standard deviation
RSDr	repeatability standard deviation expressed in % of the level
R	reproducibility limit
SR	reproducibility standard deviation
RSDR	reproducibility standard deviation expressed in % of the level
PRSDR	RSDR predicted with the Horwitz formula (%)

HoR	HorRat value = RSDR / PRSDR
SH240	Aqueous-alcoholic solution: acetic acid (240 mg/L), tartaric acid (200 mg/L), sucrose (10 g/L)

All the acidities are expressed as mg of acetic acid per L of spirit drink.

9.1. Total acidity

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Rum 1	18	18	53	8	2,7	5,1	34	12	23	8,8	2,6
Slibowitz	18	17	55	10	3,7	6,7	19	6,6	12	8,8	1,4
Brandy	20	18	193	16	5,7	2,9	43	15	7,9	7,2	1,1
Brandy	18	18	194	16	5,8	3,0	38	13	6,9	7,2	1,0
Calvados	18	17	282	21	7,5	2,7	34	12	4,3	6,8	0,6
SH240	20	17	400	14	4,9	1,2	18	6,2	1,6	6,5	0,2
Marc	18	18	547	16	5,8	1,1	42	15	2,7	6,2	0,4

Armagnac	20	19	580	27	9,4	1,6	53	19	3,2	6,1	0,5
Rum 2	18	18	641	41	14,3	2,2	66	23	3,7	6,0	0,6

9.2. Fixed acidity

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Slibowitz	18	16	9,5	5,1	1,8	19	14	4,9	52	11	4,6
Rum 1	18	18	22	6,1	2,2	9,7	28	10	45	10	4,5
Calvados	18	16	25	7,7	2,7	10,8	24	8,4	34	9,9	3,4
Rum 2	18	18	25	5,7	2,0	7,9	28	9,9	39	9,8	4,0
Marc	18	17	51	25	8,8	17	60	21	42	8,8	4,7
Brandy	18	18	87	17	6,0	6,9	47	17	19	8,2	2,3
Brandy	20	19	89	12	4,2	4,7	33	12	13	8,1	1,6
Armagnac	20	19	159	13	4,7	2,9	80	28	18	7,5	2,4
SH240	20	17	162	12	4,1	2,5	32	11	7,1	7,4	1,0

9.3. Volatile acidity

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Rum 1	18	18	30	10	3,5	12	24	8,4	28	9,6	2,9
Slibowitz	18	14	46	10	3,7	8,1	13	4,6	10	9,0	1,1
Brandy	20	18	107	23	8,0	7,5	44	16	15	7,9	1,8
Brandy	18	18	107	19	6,6	6,2	38	13	13	7,9	1,6
SH240	20	17	242	21	7,2	3,0	48	17	6,9	7,0	1,0
Calvados	18	16	257	23	8,0	3,1	24	8,5	3,3	6,9	0,5
Armagnac	20	17	418	22	7,8	1,9	62	22	5,2	6,5	0,8
Marc	18	18	492	24	8,5	1,7	69	24	5,0	6,3	0,8
Rum 2	18	18	616	42	15	2,4	71	25	4,1	6,1	0,7

10. Bibliography

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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
3. FV 1322 (2009), Measurement of acidities in spirits - estimation of precision

Method OIV-MA-BS-13: R2009

Type IV method

Determination of pH in spirit drinks of viti-vinicultural origin

(OENO 6/94;

OIV/OENO 382A/2009)

1. Presentation

The pH is closely related to the concentration of hydrogen ions (H^+) present in alcoholic beverages (the pH characteristics of alcoholic beverages depend on various parameters, such as the quality of the reducing water, the duration of maturation in casks, the nature of the aromatic raw materials, and of any additives).

Due to the presence of ethyl alcohol in alcoholic beverages, the pH should be measured according to specific procedures.

2. Purpose

The purpose of the present document is to describe the measurement of pH in alcoholic beverages by the potentiometric method, using a glass electrode.

On the one hand it specifies the essential characteristics required of the appliance in order to obtain comparable results, and on the other, standardises the operating instructions for the glass electrode and the techniques to follow in order to obtain satisfactory results.

3. Scope

The method is applicable in the case of measurements on beverages with an alcoholic strength by volume ranging between 15 and 50% vol.

4. Principle of the method

4.1. Definition of pH

The pH of a solution is the decimal cologarithm of the solution activity in hydrogen ions and is measured in pH units.

4.2. General principle

The potential difference between a glass electrode and reference electrode immersed in the same solution is a linear function of its pH. According to the laws of Nernst, the electrode potential is related to the activity of the H+ ions present as indicated by the relationship

$$H = k + RT \text{ Loa } (H+l F$$

where

- R is the gas constant, in joules / degrees
- T is the absolute temperature (°K)
- F is the Faraday symbol (96,500 coulombs)
- (H+) is the activity of the H+ ions,
- k is a constant depending on the nature of the glass electrode and the measuring device.

Determining the pH is therefore equivalent to the accurate measurement of potential difference. This measurement is made using a potentiometric apparatus with amplifiers. The assembly is required because of the high resistance of the electrical circuit and, in particular, that of the glass electrode.

Given the influence of temperature on the measurement result, the electrometer used must be equipped with an apparatus for temperature compensation and the exact conversion of the electromotive force in pH units, at a temperature of the sample.

The asymmetry potential of the glass membrane and the junction and diffusion potentials between the reference electrode and the liquid being tested will be eliminated by calibrating the system using standard solutions of known pH. One these standard solutions will be considered as the primary standard.

4.3. Principle applied to alcoholic beverages

4.3.1. The measurement of pH in organic media

The traditional pH range extending from 0 to 14 is determined by the dissociation of water. If the water content of a solution is gradually reduced or the water is replaced by another solvent, it is the dissociation equilibrium, i.e. the latter's ionic product which is taken into account instead of that of the water. This results in totally different concentration ranges for the "free" H^+ ions (i.e. which are not chemically bound).

In non-aqueous media, it is not possible to carry out absolute measurements of pH. Only relative measurements can be made. In addition, partially aqueous media are often low-ion.

However, from a water content of at least 5%, the classic definition of pH can be used, i.e. expressed in terms of absolute values and not just relative values.

Under these operating conditions, at the interface between the electrolyte and solution to be measured a phase separation is often formed which makes the signal unstable. There is also a risk of precipitation at the membrane level. The same problem is also encountered when using concentrated solutions of KCl as the reference electrolyte.

4.3.2. Specific measurement conditions

To avoid the problems described above, the basic requirement is that the electrolyte solution to measure and form a homogeneous solution without phase separation or precipitation. This condition can be met using lithium chloride (LiCl) in an ethanol medium.

A second condition is the use of an electrode with cylindrical membrane and a ground-in diaphragm, to ensure optimum contact between the reference electrolyte and the solution to be measured.

5. Apparatus

5.1. pHmeter

pH meter calibrated in pH units, enabling measurements to a minimum accuracy of: ± 0.01 pH i.e. ± 1 mV.

The instrument is preferably to be equipped with an electronic device for the automatic compensation of the temperature to a minimum accuracy of ± 0.5°C.

The pH meter should be used in a place sheltered from pollutants, acid or alkaline vapours in particular, hydrogen sulphide (H2S) and ammonia (NH3).

5.2. Electrodes

5.2.1. Combined electrode

The electrodes marketed for this specific purpose are generally of the type: combined electrode.

The useful part of the electrode consists of a cylindrical membrane and a ground-in diaphragm made of Teflon.

The reference electrolyte is an ethanol solution at 95% vol. of lithium chloride (LiCl) to 1 mol/L. Its alcoholic strength should be close to that of the alcoholic beverage to be analysed.

5.2.2. Immerse the electrode tip when not used continuously, in an ethanol solution of lithium chloride to 1 mol/1, unless otherwise specified by the manufacturer of the electrode.

5.3. Small laboratory equipment

5.3.1. Common glassware: beakers, crystallizers, etc.

5.3.2. Stirring device: magnetic stirrer and bar, for example.

5.3.3. Cleaning supplies: Joseph paper, etc.

6. Reagents

6.1. Deionised or distilled water.

Free from carbon dioxide and metal ions, with a maximum conductivity of 20°C 200 µS/m.

6.2. Standard buffer solution.

With reference to standard NFT 01012 "pH measurement - standard solutions for calibration of a pH meter"

6.2.1. pH buffer solution: 3.57 at 20°C

Saturated solution of potassium acid tartrate. Solution containing at least 5.7 g/1 of potassium acid tartrate (HOOC $C_2H_4O_2$ COOK) at 20°C. This solution can be kept for two months in the presence of 0.1 g of thymol per 200 ml.

pH	3.57 at 20°C
	3.56 at 25°C
	3.55 at 30°C

6.2.2. pH buffer solution: 4.00 at 20°C

- 0.05 M solution of potassium hydrogen phthalate. Solution containing 10.211 g/1 of potassium hydrogen phthalate (HOOC C_2 H_4 COOK) at 20°C (maximum storage time: 2 months).

pH	3.999 at 15°C
	4.003 at 20°C
	4.008 at 25°C
	4.015 at 30°C

6.2.3. pH buffer solution: 6.88 at 20°C

- Solution containing
- Dihydrogen phosphate, KH_2PO_4: 3.402 g
- Dipotassium phosphate, K_2HPO_4: 4.354 g
- Water q.s.p 1 L
- (Shelf life: 2 months)

pH	6.90 at 15°C
	6.88 at 20°C
	6.86 at 25°C

	6.85 at 30°C

6.2.4. pH buffer solution: 9.22 at 20°C Solution containing

- Decahydrated Borax, $B_4O_7Na_2.10\ H_2O$...: 3.810 g
- Water q.s.p: 1 L

(Basic buffer solutions are quickly altered by the carbon dioxide in the surrounding air, and it is therefore necessary to renew the solution for each calibration).

pH: 9.22 at 20°C

Note: market-available reference buffer solutions can also be used (according to the DIN 19266 standard and NBS, for example).

7. Procedure

7.1. Calibration of the measurement chain

7.1.1. Two standard solutions are needed to calibrate the pH meter. Their pH should, if possible, be located on either side of the presumed pH value of the test solution; if this is not possible, one of them must not differ by more than one unit pH from the presumed value.

7.1.2. Zero setting the measurement chain (pH)

Operate in accordance with the instructions provided with the apparatus used.

Rinse the electrodes with the first standard buffer solution by pouring the liquid along them.

Introducing a sufficient volume of the same standard solution into the measuring vessel, which should be clean and dry, and immerse the electrodes.

Adjust the indication of the pH meter on the pH value of the standard solution taking into account its temperature (if necessary).

Remove the electrodes and discard the standard solution contained in the measuring vessel.

7.1.3. Setting the slope of the electrode

Rinse the electrodes with distilled or deionised water and then with the second standard buffer solution

Introduce a sufficient volume of the same standard buffer solution and immerse the electrodes.

If the result matches the known value of the pH of the standard solution, the unit is in working condition and is properly calibrated.

7.1.4. Calibration Check

Use a buffer solution with an intermediate pH value in relation those used for calibration.

7.2. pH measurements

Once the device has been calibrated, rinse the electrodes and the measuring vessel, first with deionised or distilled water, then with the test solution by proceeding as above. Homogenize the test solution, introduce a sufficient volume in the measuring vessel and immerse the electrodes.

Lightly stir the test solution.

Verify that the indication given by the pH meter is stable and record it.

8. Expression of results

In the operating conditions described above, the accuracy of the determination is ± 0.02 pH units

The results are expressed in units of pH, at a temperature of: 20°C, in the form

pH at 20 °C = xx, xx

9. Test report

Indicate in the test report:

- The method used,
- The specific details and any unforeseen events recorded during the measurement,
- Operations that were not scheduled or were optional.

10. Bibliography

1. Method written with reference to the standards
2. NFTO1-013 Mesure électrométrique du pH au moyen d'une électrode de verre - Vocabulaire et méthode de mesure –
3. NFTO1-012 pH-métrie - Solutions étalons pour l'étalonnage d'un pH-mètre

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4. Norme NF T 90-008 - Essais des Eaux. Mesure électrométrique du pH avec l'électrode de verre.
5. Utilisation et entretien des instruments de mesure - Cahier technique - Fondation de l'eau - Ministère de l'Environnement –
6. Théorie der Glaselektrode - K. Schwabe et H.D. Suschke-Angeur - Chem.76 (1964) 39-49

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Method OIV-MA-BS-14 : R2009

Type II method

Determination of the principal volatile substances of spirit drinks of viti-vinicultural origin

(OIV/OENO 379/2009)

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. Définition

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.

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 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 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.

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 μm film thickness (stabilised polyethylene glycol) followed by a Carbowax 400 column 50 m x 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 °C 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 x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 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 °C to 110 °C at 5 °C/min., 110 °C 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 x 2 mm i.d.
	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.
	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, 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 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.

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

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

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 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).

$$(1)\ \text{Response factor} = \frac{\text{Peak area of IS}}{\text{Peak area of congener}} * \frac{\text{Conc.congener (µg/g))}}{\text{conc.IS (µg/g)}}$$

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).

9.2. Sample analysis

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

$$(2)\ \text{Congener concentrations, (µg/g)} = \frac{\text{Peak area of congener}}{\text{Peak area of IS}} \times \frac{\text{M}_{\text{IS}}\text{(g)}}{\text{M}_{\text{SAMPLE}}\text{ (g)}} \times \text{Conc. IS (µg/g)} \times \text{RF}$$

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.

9.3. 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= $\frac{\text{concentration of analyte in QC standard}}{\text{concentration of analyte in solution D}} \times 100$

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

9.4. 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 (μg/g) × ρ × 10/(strength(% vol.) × 1000)

- where ρ = 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

Principal volatile substances

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 13.8*	28.6 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 ($\bar{X}$) $\mu g/g$	96.8	1046	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 ($\bar{X}$) $\mu g/g$	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

Principal volatile substances

Number of outliers (Laboratories)	1	5	2	3	2
Number of accepted results	46	38	44	42	44
Mean value ($\bar{X}$) $\mu g/g$	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

Principal volatile substances

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*

Principal volatile substances

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 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 ($\bar{X}$) $\mu g/g$	86.4	3541	159.1	272.1 229.3*	177.1 222.1*
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	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
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 ($\bar{X}$) $\mu g/g$	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

Principal volatile substances

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	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

Principal volatile substances

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	142.2 120.4*	212.3 245.6*
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*

12. 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

Method OIV-MA-BS-15 : R2009

Type II method

Anethole. Gas chromatography determination of trans-anethole in spirit drinks of viti-vinicultural origin

(OIV/OENO 379/2009)

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 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.

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

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

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

$$RF_i = (C_i/area_i) \times (area_{is}/C_{is})$$

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:

$$C_i = C_{is} \times (area_i/area_{is}) \times RF_i$$

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)

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.

12.6. Calculation of results

Use the following formula to calculate the results

$$C_i = (m_{is}/V) \times (area_i/area_{is}) \times RF_i$$

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 obtained 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 outlirs (laboratories)	1	1	1	3	-	-
Number of accepted results	30	30	30	26	16	16
Mean value g/l	1,477	1,955	1,940	1,833	1,741	1,754

Repeatability 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, 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	0,778 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
Reproducibility 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

Method OIV-MA-BS-16: R2009

Type II method

Determination of the principal compounds extracted from wood during ageing of spirit drinks of viti-vinicultural origin

(OIV/OENO 382A/2009)

1. Purpose and applicability

The present method pertains to the determination of furfural, 5-hydroxymethylfurfural,5-methylfurfural, vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, gallic, ellagic , vanillic, and syringic acids, and scopoletin, by high-performance liquid chromatography.

2. Principle

Determination by high-performance liquid chromatography (HPLC), with detection by ultraviolet spectrophotometry at several wavelengths, and by spectrofluorimetry.

3. Reagents

The reagents must be of analytical quality. The water used must be distilled water or water of at least equivalent purity. It is preferable to use microfiltered water with a resistivity of 18.2 M Ω.

3.1. 96% vol. alcohol.

3.2. HPLC-quality methanol (Solvent B).

3.3. Acetic acid diluted to 0.5% vol. (Solvent A).

3.4. Mobile phases: (given only an example).

Solvent A (0.5% acetic acid) and solvent B (pure methanol). Filter through a membrane (porosity 0.45 µm). Degas in an ultrasonic bath, if necessary.

3.5. Reference standards of 99% minimum purity: furfural, 5-hydroxymethyl furfural, 5-methylfurfural, vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, gallic, ellagic, vanillic, and syringic acids, and scopoletin.

3.6. Reference solution: the standard substances are dissolved in a 50% vol. aqueous-alcoholic solution. The final concentrations in the reference solution should be of the order of:

- furfural: 5 mg/L
- 5-hydroxymethyl furfural: 10 mg/L
- 5-methylfurfural 2 mg/L
- vanillin: 5 mg/L
- syringaldehyde: 10 mg/L
- coniferaldéhyde: 5 mg/L
- sinapaldehyde: 5 mg/L

- gallic acid: 10 mg/L
- ellagic acid: 10 mg/L
- vanillic acid: 5 mg/L
- syringic acid: 5 mg/L
- scopoletin: 0.5 mg/L.

4. Apparatus

Standard laboratory apparatus

4.1. A high-performance liquid chromatograph capable of functioning in binary gradient mode and equipped with:

4.1.1. A spectrophotometric detector capable of measuring at wavelengths from 280 to 313 nm. It is however preferable to work with a multiple wavelength detector with a diode array or similar, in order to confirm the purity of the peaks.

4.1.2. A spectrofluorimetric detector – excitation wavelength: 354 nm, emission wavelength: 446 nm (for the trace determination of scopoletin; which is also detectable at 313 nm by spectrophotometry).

4.1.3. 4.1.3 An injection device capable of introducing 10 or 20 µL (for example) of the test sample.

4.1.4. A high-performance liquid chromatography column, RP C18 type, 5 µm maximum particle size.

4.2. Syringes for HPLC.

4.3. Device for membrane-filtration of small volumes.

4.4. Integrator-computer or recorder with performance compatible with the entire apparatus, and in particular, it must have several acquisition channels.

5. Procedure

5.1. Preparation of the injection

The reference solution and the spirit drink are filtered if necessary through a membrane with a maximum pore diameter of 0.45 μm.

5.2. Chromatographic operating conditions: Carry out the analysis at ambient temperature under the conditions defined in 4.1 using the mobile phases (3.4) with a flow of approximately 0.6 ml per minute following the gradient below (given as an example only)

Time	0 min	50 min	70 min	90 min
solvent A (water acid)	100%	60%	100%	100%
solvent B (methanol)	0%	40%	0%	0%

Note that in certain cases this gradient should be modified to avoid co-elutions.

5.3. Determination

5.3.1. Inject the reference standards separately, then mixed.

Adapt the operating conditions so that the resolution factors of the peaks of all the compounds are equal to at least 1.

5.3.2. Inject the sample as prepared in 5.1, after filtering it through a membrane.

5.3.3. Measure the area of the peaks in the reference solution and the spirit drink and calculate the concentrations.

6. Expression of results

Express the concentration of each constituent in mg/l.

7. Performance characteristics of the method (precision)

The following data were obtained in 2009 from an international method-performance study on a variety of spirit drinks, carried out following internationally-agreed procedures.

Key to the tables below:

nLT	Number of participating laboratories
nL	Number of laboratories used to calculate precision data
r	repeatability limit
Sr	repeatability standard deviation

RSDr	repeatability standard deviation expressed as % of the mean
R	reproducibility limit
SR	reproducibility standard deviation
RSDR	reproducibility standard deviation expressed as % of the mean
PRSDR	RSDR predicted with the Horwitz formula (%)
HoR	HorRat value = RSDR / PRSDR

7.1. Gallic acid

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	16	15	1.2	0.2	0.07	6.1	1.2	0.43	36	16	2.3
Brandy	15	14	0.4	0.1	0.04	8.1	0.6	0.20	47	18	2.6
Rum	16	16	2.0	0.2	0.06	2.9	1.7	0.62	31	14	2.1
Cognac 1	16	16	6.1	0.5	0.18	3.0	9.1	3.3	53	12	4.4
Bourbon	16	16	7.3	0.5	0.18	2.4	6.2	2.2	30	12	2.6

Cognac 2	16	16	21.8	1.7	0.60	2.8	21.7	7.7	35	10	3.5

7.2. 5-Hydroxymethylfurfural

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	16	14	5.0	0.2	0.09	1.7	1.1	0.39	8	13	0.6
Brandy	16	14	11.1	0.3	0.09	0.8	2.8	1.01	9	11	0.8
Rum	16	14	9.4	0.3	0.09	1.0	1.4	0.50	5	11	0.5
Cognac 1	16	14	33.7	1.2	0.42	1.3	12.5	4.5	13	9	1.4
Bourbon	16	14	5.8	0.2	0.07	1.2	1.1	0.4	7	12	0.6
Cognac 2	16	14	17.5	0.4	0.13	0.8	4.6	1.6	9	10	0.9

7.3. Furfural

	nLT	nL	Mean	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR

			(mg/L)								
Whisky	15	14	2.9	0.1	0.04	1.4	0.7	0.24	8	14	0.6
Brandy	15	12	1.2	0.2	0.05	4.5	0.5	0.18	15	16	0.9
Rum	15	13	1.7	0.1	0.04	2.3	0.3	0.09	5	15	0.4
Cognac 1	15	14	10.6	0.5	0.18	1.7	3.8	1.4	13	11	1.1
Bourbon	15	13	15.3	0.6	0.23	1.5	1.4	0.49	3	11	0.3
Cognac 2	15	13	13.9	0.6	0.20	1.5	1.9	0.69	5	11	0.5

7.4. Vanillic acid

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	15	12	0.2	0.1	0.03	14.2	0.2	0.06	28	20	1.4
Brandy	15	11	0.2	0.1	0.04	16.5	0.1	0.05	20	20	1.0
Rum	15	14	1.5	0.1	0.03	2.3	1.4	0.51	35	15	2.3

Cognac 1	15	14	0.8	0.3	0.10	12.6	0.7	0.2	31	17	1.9
Bourbon	15	15	2.4	0.4	0.13	5.3	3.4	1.22	51	14	3.6
Cognac 2	15	14	2.7	0.6	0.21	7.7	2.0	0.70	26	14	1.9

7.5. 5-Methylfurfural

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	11	11	0.1	0.0	0.01	10.7	0.1	0.03	35	24	1.5
Brandy	11	11	0.2	0.0	0.01	6.1	0.1	0.04	18	20	0.9
Rum	11	8	0.1	0.1	0.02	13.6	0.1	0.03	22	22	1.0
Cognac 1	11	11	0.5	0.1	0.02	4.7	0.5	0.18	39	18	2.2
Bourbon	11	10	1.7	0.1	0.03	2.0	0.6	0.20	12	15	0.8
Cognac 2	11	11	0.8	0.2	0.07	10.0	0.7	0.26	35	17	2.1

7.6. Syringic acid

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	16	16	0.4	0.1	0.03	6.7	0.2	0.08	19	18	1.0
Brandy	15	15	0.2	0.1	0.02	12.6	0.1	0.05	29	21	1.4
Rum	16	15	2.5	0.2	0.06	2.3	0.8	0.29	11	14	0.8
Cognac 1	16	15	1.4	0.4	0.13	9.0	0.7	0.26	18	15	1.2
Bourbon	16	16	3.4	0.2	0.08	2.3	1.2	0.43	13	13	0.9
Cognac 2	16	15	4.8	0.3	0.11	2.3	1.9	0.67	14	13	1.1

7.7. Vanillin

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	16	16	0.5	0.1	0.03	6.8	0.3	0.09	19	18	1.1

Brandy	15	15	0.2	0.1	0.02	9.6	0.2	0.06	25	20	1.2
Rum	16	16	1.2	0.2	0.06	4.6	0.5	0.18	15	16	1.0
Cognac 1	16	16	1.2	0.3	0.11	8.9	0.8	0.27	22	16	1.4
Bourbon	16	16	3.2	0.3	0.11	3.5	1.2	0.41	13	13	0.9
Cognac 2	16	16	3.9	0.3	0.09	2.3	1.7	0.62	16	13	1.2

7.8. Syringaldehyde

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	16	13	1.0	0.1	0.03	2.6	0.2	0.08	8	16	0.5
Brandy	15	13	0.2	0.1	0.02	8.1	0.2	0.07	33	20	1.6
Rum	16	13	4.8	0.1	0.04	0.8	0.7	0.23	5	13	0.4
Cognac 1	16	12	3.2	0.2	0.08	2.6	0.5	0.19	6	14	0.4

Bourbon	16	14	10.5	0.3	0.10	0.9	1.1	0.39	4	11	0.3
Cognac 2	16	13	9.7	0.3	0.09	0.9	1.2	0.43	4	11	0.4

7.9. Scopoletin

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	10	9	0.09	0.007	0.0024	2.6	0.04	0.01	15	23	0.6
Brandy	10	8	0.04	0.002	0.0008	2.2	0.02	0.01	16	26	0.6
Rum	10	9	0.11	0.005	0.0018	1.6	0.07	0.03	23	22	1.0
Cognac 1	10	8	0.04	0.004	0.0014	3.3	0.02	0.01	17	26	0.7
Bourbon	10	8	0.65	0.015	0.0054	0.8	0.26	0.09	15	17	0.8
Cognac 2	10	8	0.15	0.011	0.0040	2.7	0.06	0.02	15	21	0.7

7.10. Coniferaldéhyde

	nLT	nL	Mean	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR

			(mg/L)								
Whisky	13	12	0.2	0.04	0.02	9.2	0.1	0.04	23	21	1.1
Brandy	12	12	0.2	0.04	0.02	9.8	0.1	0.04	27	21	1.3
Rum	13	13	0.6	0.07	0.03	4.6	0.3	0.11	21	18	1.2
Cognac 1	12	12	0.8	0.09	0.03	4.3	0.5	0.18	23	17	1.4
Bourbon	13	13	4.6	0.24	0.09	1.9	1.1	0.38	8	13	0.6
Cognac 2	13	13	1.3	0.16	0.06	4.5	0.7	0.25	19	15	1.2

7.11. Sinapaldehyde

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	14	14	0.3	0.06	0.02	7.5	0.2	0.09	31	19	1.6
Brandy	14	13	0.2	0.03	0.01	4.6	0.2	0.05	27	20	1.3
Rum	14	12	0.2	0.06	0.02	11.2	0.2	0.08	46	21	2.2

Cognac 1	14	13	1.6	0.17	0.06	3.7	0.6	0.20	13	15	0.8
Bourbon	15	13	8.3	0.38	0.14	1.6	2.3	0.81	10	12	0.8
Cognac 2	14	12	0.3	0.08	0.03	11.4	0.5	0.18	73	20	3.7

7.12. Ellagic acid

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	PRSDR (%)	HoR
Whisky	7	7	3.2	0.6	0.20	6.3	4.0	1.41	44	13	3.2
Brandy	7	7	1.0	0.4	0.16	16	1.2	0.42	43	16	2.7
Rum	7	7	9.5	0.9	0.30	3.2	11	4.0	42	11	3.7
Cognac 1	7	7	13	1.1	0.41	3.2	14	5.0	39	11	3.6
Bourbon	7	7	13	2.7	0.95	7.4	14	4.9	39	11	3.5
Cognac 2	7	6	36	1.0	0.34	1.0	40	14	40	9	4.3

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Method OIV-MA-BS-17 : R2010

Type IV method

Analysis of α-dicarbonyl compounds in spirituous beverages of viti-vinicultural origin by gas chromatography after derivation by 1,2-diaminobenzene

(OIV/OENO 382D/2010)

1. Introduction

The principal α-dicarbonyl compounds found in wine spirits (Figure 1) are: glyoxal, methylglyoxal, diacetyl and pentane-2,3-dione.

Glyoxal	OCH–CHO (ethanedial
Methylglyoxal	CH_3–CO–CHO (2-oxopropanal)
Diacetyl	CH_3–CO–CO–CH (butane-2,3-dione)
Pentane-2,3-dione	CH_3–CH_2–CO–CO–CH_3
Hexane-2,3-dione	CH_3–CH_2–CH_2–CO–CO–CH_3
Figure 1. The principal α-dicarbonyl compounds of wine (hexane-2,3-dione is not naturally present in wine but it is used as internal standard).	

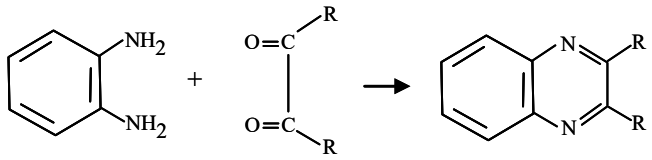
Dicarbonyl compounds are important because of their sensory impact

2. Applicability

This method applies to spirituous beverages of vitivinicultural origin, for a content of carbonyl compounds included between 0.05 mg/L and 20 mg/L.

3. Principle

The method is based on the formation of quinoxaline derivatives from α-dicarbonyl compounds with 1,2-diaminobenzene (figure 2).



1,2-Diaminobenzène *Dicarbonyle* *Quinoxaline*

Figure 2 Formation of derivatives.

The reaction takes place in the spirituous beverage diluted four-fold, pH 8 and after a reaction time of 3 hrs at 60° C. The analysis of the derivatives is then carried out after extraction of the derivatives by dichloromethane and analysis by gas chromatography with detection by mass spectrometry (GC-MS) or using a specific detector of nitrogenous compounds.

4. Reagents and products

4.1. Dicarbonyl compounds

4.1.1. Glyoxal (CAS N° 107-22-3) in a 40% solution

4.1.2. Methylglyoxal (CAS N° 78-98-8) in a 40% solution

4.1.3. Diacetyl (CAS N° 431-03-8) > 99 % pure

4.1.4. Pentane-2,3-dione (CAS N° 600-14-6) > 97 % pure

4.1.5. Hexane-2,3-dione (CAS N° 3848-24-6) > 90 % pure

4.2. 1,2-Diaminobenzene (CAS N° 95-54-5) in the forme of powder, > 97 % pure

4.3. Water for HPLC (according to standard EN ISO 3696)

4.4. Ethanol (CAS N° 64-17-5) pure for HPLC

4.5. Sodium Hydroxide (CAS N° 1310-73-2) in a 0,1M solution

4.6. Acetic acid (CAS N° 7664-93-9) pure cristallisable

4.7. Dichloromethane (CAS N° 75-09-2)

4.8. Anhydrous sodium sulphate (n°cas 7757-82-6).

4.9. 50% vol. hydroalcoholic solution.

Mix 50 ml of pure ethanol for HPLC (4.4) with 50 ml of water (4.3)

4.10. Solution of internal standard hexane-2,3-dione at 2.0 g/l

Place 40 mg of hexane-2,3-dione (4.2) in a 30 ml flask, dilute in 20 ml of 50% vol. hydroalcoholic solution. (4.9), stir until complete dissolution.

5. Apparatus

5.1. Gas chromatography with detection by mass spectrometry (GC-MS) or using a special nitrogenised compound detector.

5.1.1. Moderately polar, polyethylene glycol capillary column (such as the Carbowax 20M, BP21) with the following characteristics (as an example) 50 m X 0.32 mm X 0.25 µm.

5.1.2. Data acquisition system

5.2. pH measuring apparatus

5.3. Magnetic stirrer

5.4. Mg analytical balance

5.5. Oven which can be set to 60°C

5.6. Standard laboratory glassware including pipettes, 30-ml screw-cap flasks, and microsyringes

6. Preparation of the sample

Dilute the spirituous beverage four-fold in water (4.3)

7. Procedure

Place 10 ml of spirituous beverage diluted four-fold (6) in a 30 ml flask

Bring to pH 8 while stirring, with sodium hydroxide 0.1 M (4.5)

Add 5 mg of 1,2-diaminobenzene (4.2)

Add 10 µl of hexane-2,3-dione (internal standard) at 2.0 g/1 (4.10)

Close the flask using a screw-cap fitted with a Teflon-faced seal

Stir until the reagent has completely disappeared (5.3)

Place in the oven at 60°C for 3 hrs (5.5)

Cool.

7.1. Analysis

7.1.1. Extraction of quinoxalines

- The reactional medium prepared at 7, is brought to pH 2 using H_2SO_4 2M (4.6);
- Extract 2 times using 5 ml of dichloromethane (4.7) by magnetic stirring for 5 minutes;
- Elutriate the lower phase each time;
- Mix the two solvent phases;
- Dry on approximately 1 g of anhydrous sodium sulphate (4.8);
- Elutriate.

7.1.2. Chromatographic analysis (given as an example)

- *Detection.* For GC-MS analysis, a Hewlett Packard HP 5890 gas-phase chromatograph was coupled with a chemstation and an HP 5970 mass spectrometer (electron impact 70eV, 2.7 kV),

- *Note*: A specific detector of the nitrogenous compounds can be used.
- *Column.* The column is a BP21 (SGE, 50 m x 0.32 mm x 0.25 µm).
- *Temperatures.* The temperatures of the injector and the detector are 250°C and 280□C, respectively; the oven is kept at 60°C for 1 min., then programmed to increase at a rate of 2□C/min to 220°C, and the final isotherm lasting 20 min.
- *Injection.* The volume injected is 2 µl and the closing time of the injector valves (splitless) is 30s.

7.1.3. Analysis of quinoxalines formed

- *Separation.* The chromatogram of the derivatives by 1,2-diaminobenzene of a wine according to the ion selection method (SIM) is shown in Figure 3.
- *Identification of the peaks.* GC-MS was used to identify the dicarbonyl compounds derived from the wine spirit based on the total ionic current method (scan) which is used to obtain the mass spectra of quinoxaline derivatives and to compare them with those memorised in the spectra library; in addition, the retention times were compared with those for pure compounds treated in the same way. Table 1 shows the principal ions of the mass spectra for the obtained dicarbonyl compound derivates.
- *Proportioning.* The quantitative determination of the dicarbonyl compounds is carried out with the SIM method, by selecting ions M/Z = 76, 77, 103, 117, 130, 144, 158 and 171. The ions M/Z = 76 and 77 are used for the quantification and the others as qualifiers, i.e. glyoxal: ions M/Z = 103 and 130, methylglyoxal: ions M/Z = 117 and 144, diacetyl: ions M/Z = 117

and 158, pentan-2,3-dione: ions M/Z = 171 and hexane-2,3-dione: ions M/Z = 158 and 171.

7.1.4. Characteristics of the method

- Some internal validation were defined but these are not a formal validation as per the protocol governing the planning, the implementing and the interpreting of the performance studies relating to the analysis methods (OIV 6/2000)
- Repeatability. The repeatability of the GC-MS-SIM method displays variation coefficients ranging between 2 and 5% for the four dicarbonyl compounds;
- Recovery rate. The quantities added to a wine were recovered with with a below 6% deviation from expected results;
- Linearity. Linear correlations were obtained in concentration domains ranging between 0.05 to 20 mg/l.
- Detection limit. The detection limit of most of the derived dicarbonylated products is 0.05 mg/l.

Dicarbonylated compound	Derivative	Mass spectrum (principal ions and abundance)
Glyoxal	Quinoxaline	130 (100), 103 (56.2), 76 (46.8), 50 (20.2)
Methylglyoxal	2-Methylquinoxaline	144 (100), 117 (77.8), 76 (40.5), 77 (23.3), 50 (21.9), 75 (11.3), 145 (10.3)

α-dicarbonyl compounds by gas chromatography after derivation by 1,2-diaminobenzene

Diacetyl	2,3-Diméthylquinoxaline	117 (100), 158 (75.6), 76 (32.3), 77 (23.1), 50 (18.3), 75 (10.4)
Pentane-2,3-dione	2-Ethyl-3-methylquinoxaline	171 (100), 172 (98), 130 (34.1), 75 (33.3), 77(21) 50 (19.4), 144 (19), 143 (14.1), 103 (14)
Hexane-2,3-dione	2,3- Diethylquinoxaline	158 (100), 171 (20.1), 76 (13.7), 77 (12.8), 159 (11.4), 157 (10.8), 50 (8.1)

Table 1. Mass spectra (ion m/Z (intensity of the molecular ion in relation to that of the basic peak) of derivatives of dicarbonyl compounds by 2,3-diaminobenzene

α-dicarbonyl compounds by gas chromatography after derivation by 1,2-diaminobenzene

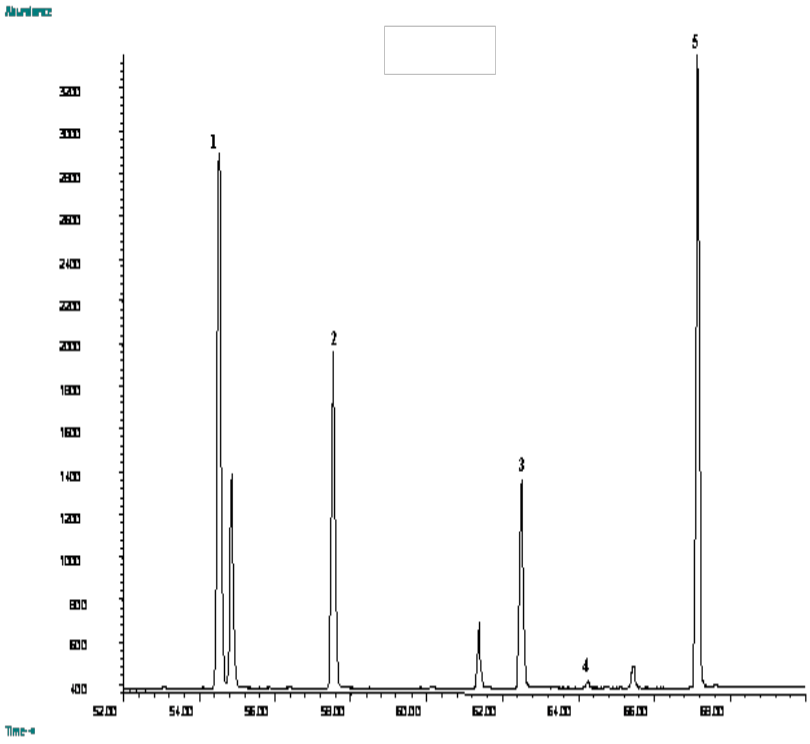


Figure 3. High-performance liquid phase chromatogram of dicarbonyl compounds derived by 1,2-diaminobenzene from wine spirit, detected through mass spectrometry by selecting the ions m/z = 76, 77, 103, 117, 130, 131, 144, 158, 160 and 171. BP21 column, 50m _ 0.32mm 0.25 µm oven Temperature 60 ° C for 1 min, then programmed to increase 2 ° C/min up to 220 ° C. injector Temperature: 250° C.

1. *Glyoxal*
2. *Methylglyoxal*
3. *Diacetyl*
4. *Pentane-2,3-dione*
5. *Hexane-2,3-dione (internal standard)*
6. *Phenylglyoxal (not determined for this method)*

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Method OIV-MA-BS-18 : R2010

Type IV method

Analysis of α -dicarbonyl compounds in spiritous beverages of viti-vinicultural origin by HPLC after derivation by 1,2 diaminobenzene

(OIV/OENO 382C/2010)

1. Introduction

The principal α-dicarbonyl compounds found in wine-based spirits (Figure 1) are: glyoxal, methylglyoxal, diacetyl and pentane-2,3-dione.

Glyoxal	OCH–CHO (ethanedial)
Methylglyoxal	CH_3–CO–CHO (2-oxopropanal)
Diacetyl	CH_3–CO–CO–CH_3(butane-2,3-dione)
Pentane-2,3-dione	CH_3–CH_2–CO–CO–CH_3
Hexane-2,3-dione	CH_3–CH_2–CH_2–CO–CO–CH_3
Figure 1. The principal α-dicarbonyl compounds of wine-based spirits (hexane-2,3-dione is not naturally present in wine but it is used as internal standard).	

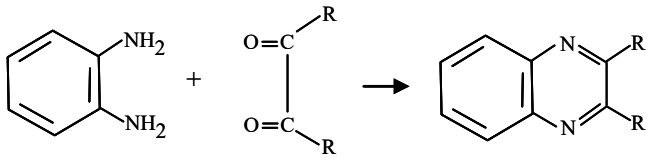
Dicarbonyl compounds are important because of their sensory impact,

2. Applicability

This method applies to spirituous beverages of vitivinicultural origin for dicarbonyl compounds with a content ranging between 0.05 mg/L and 20 mg/L;

3. Principle

The method is based on the formation of quinoxaline derivatives from α - dicarbonyl compounds with 1,2-diaminobenzene (figure 2).



1,2-Diaminobenzene Dicarbonyl Quinoxaline

Figure 2 Formation of derivatives.

The reaction takes place in the spirituous beverage diluted four-fold, pH 8 and after a reaction time of 3 hrs at 60° C. The analysis of the derivatives is then carried out either directly by chromatography in the high-performance liquid phase (HPLC) and detection by UV absorptiometry at 313 Nm,.

4. Reagents and products

4.1. Dicarbonyl compounds

4.1.1. Glyoxal (CAS N° 107-22-3) in a 40% solution

4.1.2. Methylglyoxal (CAS N° 78-98-8) in a 40% solution

4.1.3. Diacetyl (CAS N° 431-03-8) > 99 % pure

4.1.4. Pentane-2,3-dione (CAS N° 600-14-6) > 97 % pure

4.1.5. Hexane-2,3-dione (CAS N° 3848-24-6) > 90 % pure

4.2. 1,2-Diaminobenzene (CAS N° 95-54-5) in the form of powder, > 97 % pure

4.3. Water for HPLC (according to standard EN ISO 3696)

4.4. Ethanol (CAS N° 64-17-5) pure for HPLC

4.5. Sodium Hydroxide (CAS N° 1310-73-2) in 0.1M solution

4.6. Acetic acid (CAS N° 64-19-7) pure crystallisable

4.7. Solvent A for the analysis by HPLC

In 1 water L for HPLC (4.3), add 0.5 ml of acetic acid (4.8), mix, degas (by ultrasound, for example)

4.8. Solvent B for HPLC

Pure HPLC methanol (CAS N° 67-56-1)

4.9. 50% vol. hydroalcoholic solution.

Mix 50 ml of pure ethanol for HPLC (4.4) with 50 ml of water (4.3)

4.10. Solution of internal standard hexane-2,3-dione at 2.0 g/L

Place 40 mg of hexane-2,3-dione (4.2) in a 30 ml flask, dilute in 20 ml of 50% vol. hydroalcoholic solution. (4.11), stir until complete dissolution.

5. Apparatus

5.1. High-performance liquid phase chromatography with detection by UV absorption (313 nm);

5.1.1. Analytical column filled with silica grafted by octadecyl radicals of 5 µm with dimensions of 250 mm x 4.6 mm, for example.

5.1.2. Data acquisition system.

5.2. pH measuring apparatus

5.3. Magnetic stirrer

5.4. Mg analytical balance

5.5. Solvent degasification system for HPLC (an ultrasound apparatus, for example)

5.6. Oven which can be set to 60°C

5.7. Standard laboratory glassware including pipettes, 30-ml (5.7) screw-cap flasks, and microsyringes.

6. Preparation of the sample

Dilute the spirituous beverage four-fold in water (4.3)

7. Procedure

Place 10 ml of spirituous beverage diluted four-fold (6) in a 30 ml flask

Bring to pH 8 while stirring, with sodium hydroxide 0.1 M (4.5)

Add 5 mg of 2,3-diaminobenzene (4.2)

Add 10 µl of hexane-2,3-dione (internal standard) at 2.0 g/1 (4.10)

Close the flask using a screw-cap fitted with a Teflon-faced seal

Stir until the reagent has completely disappeared (5.3)

Place in the oven at 60°C for 3 hrs (5.6)

Cool.

7.1. Analysis

Injection. After cooling, the reactional medium containing the quinoxalines is directly injected into the HPLC system at an amount of 20 µl.

- *Elution programme.* For separation, an example of an elution schedule is displayed in Table 1

Table 1. Example of HPLC analysis elution schedule

Time in minutes	Solvent A	Solvent B
0	80	20
8	50	50

26	25	75
30	0	100
32	0	100

The flow rate being 0.6 ml/min

- *Separation.* The chromatogram obtained by HPLC is shown in Figure 3
- *Detection.* The maximum absorption was studied for all the dicarbonyl compound derivatives and set at 313 Nm as being optimal.
- *Identification of the derivatives.* The identification of the derivatives was carried out by comparing the retention times with standard reference solutions. The chromatographic conditions enable a good separation of the peaks in all the wines.

7.1.1. Characteristics of the method

Some internal validation elements were determined but these are not a formal validation according the protocol for the planning, the implementing and the interpretation of the performance studies pertaining to analysis methods (OIV 6/2010)

- *Linearity.* The linearity of the method was tested using standard solutions (the hydroalcoholic solution at 12% vol. was used as a matrix) (Table 2). The quantitative analysis of the additions of dicarbonyl compounds showed that the method is linear for the four compounds with recovery rate varying between 92 and 117%.

Table 2. Study of the linearity and recovery tests with standard solutions (12% v/v water-ethanol) correlation coefficients			
Glyoxal	Methylglyoxal	Diacetyl	Pentane-2,3-dione
value[a] surface[b]	value[a] surface[b]	value[a] surface[b]	value[a] surface[b]
R=0,992	R=0,997	R=0,999	R=0,999

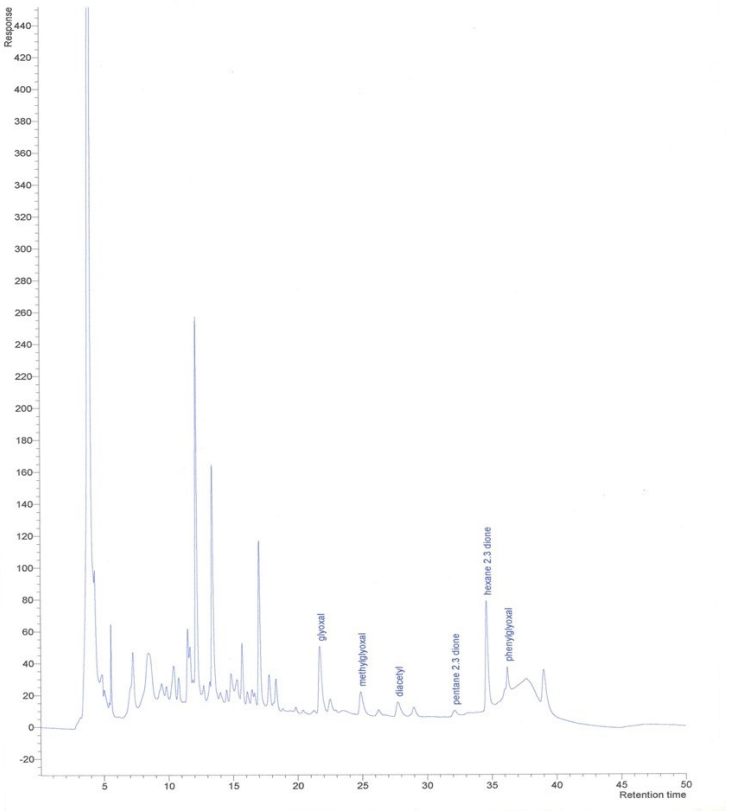
a: mg/l, b: arbitrary units, c: response factor in relation to the internal standard.

- *The quantification limit* of the dicarbonyl compounds is very low, the best results being obtained with diacetyl, the detection limit of which is 10 times weaker than that of the other compounds (table 3).

Tableau 3. Performance of the HPLC method for the quantification of dicarbonyl compounds			
Limits	detection[a]	determination[a]	quantification[a]
Glyoxal	0,015	0,020	0,028
Methylglyoxal	0,015	0,020	0,027
Diacetyl	0,002	0,002	0,003
Pentane-2,3-dione	0,003	0,004	0,006

a: results in mg/L, hydroalcoholic solution (10% vol.).

Figure 3. High-performance liquid phase chromatogram of dicarbonyl compounds derivatized by 1,2-diaminobenzene, detected by UV at 313 nm. Spherisorb ODS Column 250 mm x 4.6 mm x 5 µm.



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Method OIV-MA-BS-19 : R2010

Type IV method

Overall determination of phenolic compounds in spirituous beverages of viti-viniculture origin without added caramel

(OIV/OENO 382B/2010)

1. Definition

Folin-Ciocalteu assay measures the total quantities of phenolic compounds originating from wood present in barrel-aged spirits that haven't received any added caramel.

This assay is not specific to phenolic compounds (cf. principle). Caramel also reacts to the Folin-Ciocalteu reagent. However, in the case of wood-aged spirituous beverages, the vast majority of results are related to the presence of phenolic compounds derived from the oak wood (VIDAL and Al, 1991).

Folin-Ciocalteu phenolic compound content corresponds to the response to the result described below. This result is expressed in mg of gallic acid/l by calibration.

2. Principle

All the phenolic compounds are oxidized by the Folin-Ciocalteu reagent. This reagent consists of a mixture of phosphotungstic acid and phosphomolybdic acid which is reduced, during the oxidation of the

phenolic substances, into a mixture of blue molybdenum and tungsten oxides.

The blue colouring produced has a maximum absorption of about 750-760 nm. It is proportional to the quantity of oxidized phenolic compounds.

3. Apparatus

3.1. Standard laboratory apparatus, and in particular:

- Temperature-controlled bath (70° C), spectrophotometer.

4. Reagents

4.1. Folin-Ciocalteu reagent

This reagent is available for purchase ready to use. It can be prepared in the following manner:

- 100 g of sodium tungstate (No. CAS : 13472-45-2),
- 25 g of sodium molybdate (No. CAS : 7631-95-0), are dissolved in 700 ml of distilled water (No. CAS : 7732-18-5).

Add:

- 50 ml of 85% phosphoric acid (No. CAS : 7664-38-2) (ρ20=1.71 g/mL),
- 100 ml of concentrated chlorhydric acid (No. CAS : 7647-01-0) (ρ20=1.19 g/mL).

Bring to a boil under reflux for 10 hours, then add:

- 150 G of lithium sulphate (No. CAS : 10377-48-7),
- a few bromine drops (No. CAS : 7726-95-6),

- and bring to a boil for another 15 minutes. Cool and add 1 litre of distilled water.

4.2. Anhydrous sodium carbonate

- Prepare a 4.25% solution (m/v) in distilled water.

4.3. Anhydrous gallic acid (No. CAS : 149-91-7),

5. Procedure

5.1. Calibration in gallic acid

Produce a hydroalcoolic gallic acid stock solution by weighed quantity, then some surrogate solutions by dilution (at least 2). The calibration range also includes a blank (hydroalcoolic solution). As an example, the range can include the following levels: 0.200 and 400 mg/L. Check the linearity of the calibration.

5.2. Preparation of the samples

The sample must be perfectly limpid and free of beeswing.

5.3. Reaction

In a test tube, introduce:

- 0.2 ml of sample (or of calibration solution)
- 1 ml of Folin-Ciocalteu reagent,
- 18.8 ml of sodium carbonate solution.

After stirring, bring to approximately 70°C for 20 minutes in the temperature-controlled bath, then cool under running cold water.

5.4. Absorptance measurement at 760 Nm

Absorbance at 760 nm is measured under a 1 cm optical path.

6. Expression of results

Express the result in mg of gallic acid/L (linear calibration), accounting for the possible dilution of the sample. If the absorptance is greater than 1, a new measurement is carried out after dilution of the sample, if linearity is guaranteed.

7. References

1. BLOUIN J., LLORCA L., MONTREAU F.R., DUFOUR J.H., 1972. Etude des conditions optimales pour la détermination des composés phénoliques totaux par le réactif de Folin-Ciocalteu. Connaissance de la vigne et du vin, 6, 405-413.
2. VIDAL J.-P., CANTAGREL R., FAURE A., BOULESTEIX J.M., 1991. Comparaison de trois méthodes de dosage des composés phénoliques totaux dans les spiritueux. FV OIV n°904.

Method OIV-MA-BS-20 : R2009

Type IV method

Propanol-2-ol Determination by gas chromatography in spirit drinks of viti-viniculture origin

(OENO 6/94;

OIV/OENO 382A/2009)

Introduction

This assay is not part of the official determinations provided by the international regulations, but is quite often requested since propan-2-ol is not a natural constituent of fermented beverages of vinous origin. It may be added to the alcohol during its denaturation. The presence (or more accurately lack thereof) of this compound must be verifiable. In addition it may be present in various alcoholic beverages.

1. Purpose

Determination of propan-2-ol in alcoholic beverages of vitivinicultural origin

2. Principle, apparatus

2.1. The separation of propan-2-ol from ethanol is carried out by means of gas chromatography.

2.2. Apparatus

- Gas chromatograph equipped with a flame ionization detector.

- Classic stainless steel column 6 m long and with an internal diameter of 2 mm.
- Stationary phase: for example, coated with diglycerol at 5% on Chromosorb P 60-80 mesh (0.22 to 0.32 mm).

It is also possible to use a mixture of phases known as the ESD: Erytritol, sorbitol, diglycerol respectively at 1%, 2.5%, and 5% weight of the support (it can be used in other phases: porapak, poraplot, etc.)

- Nitrogen R * carrier gas (Air Liquide standard). - Isothermal temperature 80°C.

The settings of the various gas flows must be performed to obtain proper performance of the chromatograph.

3. Sample preparation

For a qualitative test, the sample of the alcoholic beverage can be injected directly into the gas chromatograph (1 to 2 µl).

For accurate dosing is possible to use an internal standard separated from the other alcohols such as pentan-1-ol.

Its content must be the same order of magnitude as that of the propan-2-ol.

4. Assay

Depending on whether the purpose is to detect the presence of the propan-2-ol or measure it, a reference solution of propan-2-ol must be injected into the pure alcohol, its content depending on the required dose (in principle several grams per litre).

For accurate dosing the internal calibration method will be applied using pentan-1-ol.

5. Expression of results

The concentrations of propan-2-ol will be calculated using the traditional method in gas chromatography with the use of an internal standard (c.f. volatile substances) and expressed in g/hl of alcohol at 100% vol.

Method OIV-MA-BS-21 : R2009

Type IV method

UV absorption of rectified alcohols of vitiviniculture origin

(OENO 6/94;

OIV/OENO 382A/2009)

1. Purpose and scope

This method can be used to determine the optical permeability of neutral alcohol liable to enter into the composition of certain alcoholic beverages.

2. Principle

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

3. Apparatus

3.1. UV-visible spectrophotometer.

3.2. Quartz cells 10 mm thick, with identical spectral transmission.

4. Reagents

Hexane for spectroscopy.

5. Procedure

- Rinse the tanks clean beforehand with a sample solution and then fill with the sample, dry the tanks outside,
- treat the reference cell (n) with hexane in the same way and fill it,
- determine the absorbance value and build the graph.

6. Expression of results

The absorbance values recorded at 270, 240, 230 and 220 nm should not exceed the following values: 0.02, 0.08, 0.18 and 0.3. The absorbance curve must be smooth and regular.

7. Bibliography

1. Methods of analysis of neutral alcohol applicable in the wine sector. EEC Regulation No. 1238/92 (May 8, 1992), EEC Regulation No. 2009/92 (July 20, 1992).

Method OIV-MA-BS-22 : R2009

Type II method

Determination by isotope ratio mass spectrometry of the $^{13}C/^{12}C$ ratio of wine ethanol of spirit drinks of viti-vinicultural origin

(OIV/OENO 381/2009)

1. Field of application

The method enables the measuring of the $^{13}C/^{12}C$ isotope ratio of the ethanol of spirit drinks of vitivinicultural origin.

2. Reference standards

ISO 5725 :1994 «Accuracy (trueness and precision) of measurement methods and results: Basic method for the determination of repeatability and reproducibility of a standard measurement method»

V-PDB : Vienna-Pee-Dee Belemnite (R_{PDB} = 0.0112372).

Method OIV «Detection of enriching musts, concentrated musts, grape sugar and wine by application of nuclear magnetic deuterium resonance (SNIF-NMR)»

3. Terms and definitions

$^{13}C/^{12}C$: Carbon 13 and carbon 12 isotope ratio for a given sample

$\delta^{13}C$: Carbon 13 contents (^{13}C) expressed in parts per 1000 (‰)

SNIF-NMR: Site-specific natural isotope fractionation studied by nuclear magnetic resonance

V-PDB : Vienna-Pee-Dee Belemnite. or PDB, is the main reference for measuring natural variations of carbon 13 isotopic contents. Calcium carbonate comes from a Cretaceous belemnite from the Pee Dee formation in South Carolina (USA). Its isotopic ratio $^{13}C/^{12}C$ or R_{PDB} is R_{PDB} = 0.0112372. PDB reserves have been exhausted for a long time, but it has remained the primary reference to express natural variations of Carbon 13 isotopic contents. Reference material is calibrated based on this content and is available at the International Agency of Atomic Energy (IAEA) in Vienna (Austria). The isotopic indications of naturally occurring carbon 13 are expressed by V-PDB, as is the custom.

m/z:Mass to charge ratio

4. Principle

During photosynthesis, the assimilation of carbon dioxide by plants occurs according to 2 principle types of metabolism that are: metabolism C_3 (Calvin cycle) and C_4 (Hatch and Slack). These two means of photosynthesis present a different type of isotope fractionation. Products, such as sugars and alcohol, derived from C_4 plants and fermentation, have higher levels of Carbon 13 than from C_3 plants. Most plants, such as vines and sugar beet belong to the C_3 group. Sugar cane and corn belong to the C_4 group. Measuring the carbon 13 content enables the detection and the quantification of C_4 (sugar cane or corn isoglucose) origin sugars which are added to products derived from grapes (grape musts, wines). The combined information on carbon 13 content and information obtained from SNIF-NMR enable the quantification of mixed sugars added or alcohol of plant origin C_3 and C_4.

The carbon 13 content is determined on carbon dioxide resulting from the complete combustion of the sample. The abundance of the main mass isotopomers 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$ et $^{12}C^{17}O^{16}O$) and 46 ($^{12}C^{16}O^{18}O$), resulting from different possible combinations of isotopes ^{18}O, ^{17}O, ^{16}O, ^{13}C et ^{12}C, are determined from ion currents measured by three different collectors of isotopic mass spectrometers. The contributions of isotopomers $^{13}C^{17}O^{16}O$ et $^{12}C^{17}O_2$ can be neglected because of their low abundance. The ion current for $m/z = 45$ is corrected for the contribution of $^{12}C^{17}O^{16}O$ which is calculated according to the current intensity measured for $m/z = 46$ while considering the relative abundance of ^{18}O and ^{17}O (Craig adjustment). The comparison with the calibrated reference and the international reference V-PDB enable the calculation of carbon 13 content on a relative scale of $\delta^{13}C$.

5. Reagents

The material and the consumables depend on the apparatus (6) used by the laboratory. The systems generally used are based on elementary analysers. These systems can be equipped to introduce the samples placed in sealed metal capsules or for the injection of liquid samples through a septum using a syringe.

Depending on the type of instrument used, the reference material, reagents, and consumables can be used:

- Reference material available from the IAEA:

Name	Material	$\delta^{13}C$ versus V-PDB (9)
-IAEA-CH-6	saccharose	-10,4 ‰
-IAEA-CH-7	polyethylene	-31,8 ‰

- NBS22	oil	-29,7 ‰
- USGS24	graphite	-16,1 ‰

- Available from the IRMM in Geel (B) (Institut des Matériaux et Mesures de Référence) :

Name	Material	$\delta^{13}C$ versus V-PDB (9)
- CRM 656	Wine alcohol	-26,93 ‰
- CRM 657	glucose	-10,75 ‰
- CRM 660	Aqueous alcoholic solution (ABV 12%)	-26,72 ‰

Standard working standard with a known $^{13}C/^{12}C$ ratio calibrated with international reference materials.

A standard list of consumables established for continuous flow systems follows below:

- Helium for analysis (CAS 07440-59-7)
- Oxygen for analysis (CAS 07782-44-7)
- Carbon dioxide for analysis, used as a secondary reference gas for the content of carbon13 (CAS 00124-38-9)
- Oxidation reagent for the oven and the combustion system as follows: copper oxide (II) for elementary analysis (CAS 1317-38-0)
- Drying agent to eliminate water produced by combustion. For example: anhydrone for elementary analysis (magnesium perchlorate) (CAS 10034-81-8).

This is not necessary for apparatus equipped with a water elimination system by cryo-trapping or through selective permeable capillaries.

6. Apparatus and materials

6.1. Isotope ratio mass spectrometry (IRMS*)*

Isotope ratio mass spectrometry (IRMS) enables the determination of the relative contents of ^{13}C of naturally occurring CO_2 gas with an internal accuracy of 0.05‰ or expressed in relative value (9). Internal accuracy here is defined as the difference between 2 measurements of the same sample of CO_2. The mass spectrometer used to measure isotope ratios is generally equipped with a triple collector to simultaneously measure m/z = 44, 45 and 46 intensities. The isotope ratio mass spectrometry must either be equipped with a double introduction system to alternately measure the unknown sample and a reference sample, or use an integrated system that carries out quantitative combustion on samples and separates the carbon dioxide from the other combustion products before measuring the mass spectrum.

6.2. Combustion apparatus

Combustion apparatus able to quantitatively convert ethanol into carbon dioxide and capable of eliminating all other combustion products including water, without any isotopic fractionation. The apparatus can be either an integrated continuous flow system integrated with mass spectrometry (6.2.1), of an autonomous combustion system (6.2.2). The apparatus must be as precise as indicated in (11).

6.2.1. Continuous flow systems

These consist of either an elemental analyser, or a gas chromatograph equipped with an online combustion system.

The following laboratory material is used for systems equipped for the introduction of samples contained in metallic capsules :

- volumetric micropipette with appropriate cones
- balance with 1 μg accuracy or better
- tool for capsule sealing
- tin capsules for liquid samples
- tin capsules for solid samples

The following laboratory material is needed when using an elemental analyser equipped with a liquid injector or in the case of a preparation system for combustion chromatography:

- syringe for liquids
- flasks equipped with sealed closing system and inert septa

The laboratory materials indicated in the lists are examples that are susceptible of being replaced by other equivalent performance material depending on the type of combustion apparatus and of mass spectrometry used by the laboratory.

6.2.2. Separate preparation systems

In this case the samples of carbon dioxide resulting from the combustion of samples to be analyzed and the reference sample are collected in ampoules which are then put in a double entry spectrometer system to carry out isotopic analyses. Several types of combustion apparatus described in the literature can be used:

- Closed combustion system filled with circulating oxygen gas

- Elemental analyser with helium and oxygen flows
- Sealed glass ampoule filled with copper oxide (II) used as an oxidation agent

7. Preparation of samples for tests

Ethanol must be extracted from the spirit drink before isotopic testing. This is carried out by distilling the beverage as described in §3.1 of the SNIF-NMR method for the determination by NMR of the deuterium distribution in the ethanol of spirit drinks of vitivinicultural origin.

8. Procedure

All preparation steps must be carried out without any significant ethanol loss through evaporation, which would change the isotopic composition of the sample.

The description that follows makes reference to the procedure generally used for ethanol sample combustion using commercial automatic combustion systems. All other methods, ensuring that ethanol samples are converted quantitatively into carbon dioxide without the evaporation of ethanol, can use the preparation of carbon dioxide for isotopic analyses. An experimental procedure based on the use of an elemental analyser:

a. Placing the samples in capsules
   - use capsules, tweezers and a clean preparation tray
   - take an appropriate sized capsule using tweezers
   - introduce an appropriate amount of liquid into the capsule using a micropipette

Note: 3.84 mg of absolute ethanol or 4.17 mg of distillate with an alcohol content of 92 % m/m are necessary to obtain 2 mg of carbon. The appropriate quantity of distillate must be calculated in the same way according to the quantity of carbon necessary based on the mass spectrometer's sensitivity.

- close the capsule with the sealing tool.
- each capsule must be completely sealed. If not, it must be discarded and a new capsule must be prepared.
- two capsules must be prepared for every sample
- place the capsules in an appropriate place on the elemental analyser sample tray. Every capsule must be carefully identified in order by number.
- systematically place capsules containing working references at the beginning and the end of the sample series
- regularly insert a check sample in the sample series.

b. check and adjust the elemental analysis and mass spectometry instrumentation

- adjust the temperature of the elemental analyzer ovens and the helium and oxygen gas flow for an optimal combustion of the sample;
- check the elemental analysis system and the mass spectometry system for leaks (for example by checking the ion current where m/z = 28 corresponds to N_2);
- adjust the mass spectrometer to measure the ion current intensities for m/z = 44, 45 and 46;
- check the system using known reference samples before starting to measure the samples.

c. To carry out a series of measurements

The samples that are placed on the autosampler of the elemental analyser or chromatographare introduced successively. The carbon dioxide for each sample combustion is eluted into the mass spectrometer which measures the ion current. The data system records the ion current intensities and calculates the δ values for each sample (9).

9. Calculation

The objective of the method is to measure the $^{13}C/^{12}C$ isotopic ratio of the ethanol extracted from spirit drinks. The $^{13}C/^{12}C$ isotopic ratio can be expressed by its deviation compared to the reference work. The carbon 13 ($\delta\ ^{13}C$) isotopic ratio is calculated on a delta scale per thousand by comparing the results obtained for the sample to be measured to the working reference calibrated previously based on the primary international reference (V-PDB). The $\delta\ ^{13}C$ values are expressed compared to the working reference:

$$\delta^{13}C_{ech/ref}\% = 1000 \times (R_{ech} - R_{ref})/R_{ref}$$

where R_{ech} and R_{ref} are respectively the isotopic ratio $^{13}C/^{12}C$ of the sample and the working reference.

The $\delta\ ^{13}C$ values are thus expressed using V-PDB:

$$\delta^{13}C_{ech/_{V-PDB}}\% = \delta^{13}C_{ech/ref} + \delta^{13}C_{ref/_{V-PDB}} + \left(\delta^{13}C_{\frac{ech}{ref}} \times \delta^{13}C_{ref/_{V-PDB}}\right)/1000$$

where $\delta^{13}C_{ref/V\text{-}PDB}$ is the isotopic deviation determined beforehand for the working reference to V-PDB.

Small variations may occur while measuring on line due to changes in the instrumental conditions. In this case the $\delta\ ^{13}C$ samples must be corrected according to the difference in the $\delta^{13}C$ value from the working reference

and the real value, which was calibrated beforehand against V-PDB by comparison with one of the international reference materials. Between two measurements of the working reference, the variation and therefore the correction applied to the sample results may be assumed to be linear. The working reference must be measured at the beginning and at the end of all sample series. A correction can then be calculated for each sample using linear interpolation between the two values (the difference between the assigned value of the working reference and the measurement values obtained).

10. Quality assurance and control

Check that the ^{13}C value for the working reference does not differ by more than 0.5‰ of the accepted value. If not, the spectrometer must be checked and possibly readjusted.

For each sample, verify that the difference in the results between the 2 capsules measured successively is under 0.3 ‰. The final result for a given sample is the average value of the 2 capsules. If the deviation is higher than 0.3 ‰ the measurement should be repeated.

Measurement condition monitoring can be based on the ion current intensity for $m/z = 44$ and is proportional to the quantity of carbon injected in the elemental analyzer. Under standard conditions, the ion current intensity should be almost constant for the samples analysed. A significant deviation could be indicative of ethanol evaporation (an imperfect seal on a capsule), an instability of the elemental analyser, or the mass spectrometer.

11. Method performance characteristics (precision)

One collaborative analysis (11.1) was carried out on distillates containing alcohol of vinous origin, and cane and beet alcohol, in addition to

different mixtures of these three origins. This study did not take into account the distillation step, and further information from other joint laboratory studies on wine (11.2) and in particular proficiency testing (11.3) for isotopic measurements have also been considered. The results show that different distillation systems under satisfactory conditions, and in particular those used for SNIF-NMR measurements, do not have significant variability for determining the $\delta^{13}C$ of ethanol in wine. It is reasonable to suppose that this would likewise be true for the ethanol of spirit drinks. The precision parameters observed for wine are almost identical to those obtained in the joint study on distillates (11.1).

11.1. Joint study on distillates

Year of inter laboratory study	1996
Number of laboratories	20
Number of samples	6 samples in double-blind comparison
Analysis	ethanol $\delta^{13}C$

Sample code	Vinous origin alcohol	Beet alcohol	Sugar cane alcohol
A & G	80%	10%	10%
B & C	90%	10%	0%
D & F	0%	100%	0%
E & I	90%	0%	10%

H & K	100%	0%	0%
J & L	0%	0%	100%

Samples	A / G	B / C	D / F	E / I	H / K	J / L
Number of laboratories retained after eliminating anomalous results	19	18	17	19	19	19
Number of results accepted	38	36	34	38	38	38
Average value (δ ^{13}C) ‰	-25.32	-26.75	-27.79	-25.26	-26.63	-12.54
Sr^2	0.0064	0.0077	0.0031	0.0127	0.0069	0.0041
Repeatability standard deviation (Sr) ‰	0.08	0.09	0.06	0.11	0.08	0.06
Repeatability limit r ($2.8 \times S_r$) ‰	0.22	0.25	0.16	0.32	0.23	0.18
S_R^2	0.0389	0.0309	0.0382	0.0459	0.0316	0.0584
Reproducibility standard deviation (S_R) ‰	0.20	0.18	0.20	0.21	0.18	0.24

Reproducibility limit R (2,8× S_R) ‰	0.55	0.9	0.55	0.60	0.50	0.68

11.2. Inter laboratory study on two wines and one alcohol

Year of inter laboratory trial:	1996
Number of laboratories	14 for distillation of wine and 7 for also measuring the $\delta\ ^{13}C$ of ethanol in wine 8 for measuring the $\delta\ ^{13}C$ in the alcohol sample
Number of samples	3 (White wine ABV 9.3 % vol., white wine ABV 9.6 % and alcohol of strength 93% m/m)
Analysis	ethanol $\delta^{13}C$

Samples	Red wine	White wine	Alcohol
Number of laboratories	7	7	8

Number of accepted results	7	7	8
Average value ($\delta\ ^{13}C$) ‰	-26.20	-26.20	-25.08
Reproducibility variance S_R^2	0.0525	0.0740	0.0962
Reproducibility standard deviation (S_R) ‰	0.23	0.27	0.31
Reproducibility limit R (2.8× S_R) ‰	0.64	0.76	0.87

Different distillation systems were used by the participating laboratories. The $\delta\ ^{13}C$ isotopic determinations carried out in one laboratory on all of the distillates

returned by the participants, did not reveal any anomalous values or significantly distinct average values. The variation in results ($S^2 = 0.0059$) is comparable to repeatability variances Sr^2 from the collaborative study on distillates (11.1).

11.3. Results from proficiency-testing studies

Since December 1994 international proficiency testing to determine the isotopic measurements for wine and alcohol (ABV of distillates 96 % vol.) have been organized regularly. The results enable participating laboratories to check the quality of their analyses. Statistical results enable an appreciation of the variability of derterminations under reproducibility conditions. This enables an estimation of the variance parameters and the reproducibility limit. The results obtained for the wine and ethanol distillate $\delta\ ^{13}C$ determinations are summarized in the table below:

Wines	Distillates

Date	N	SR	S2R	R	N	SR	S2R	R
Dec. 1994	6	0,210	0,044	0,59	6	0,151	0,023	0,42
June 1995	8	0,133	0,018	0,37	8	0,147	0,021	0,41
Dec. 1995	7	0,075	0,006	0,21	8	0,115	0,013	0,32
March 1996	9	0,249	0,062	0,70	11	0,278	0,077	0,78
June 1996	8	0,127	0,016	0,36	8	0,189	0,036	0,53
Sept. 1996	10	0,147	0,022	0,41	11	0,224	0,050	0,63
Dec. 1996	10	0,330	0,109	0,92	9	0,057	0,003	0,16
March 1997	10	0,069	0,005	0,19	8	0,059	0,003	0,16
June 1997	11	0,280	0,079	0,78	11	0,175	0,031	0,49
Sept 1997	12	0,237	0,056	0,66	11	0,203	0,041	0,57
Dec. 1997	11	0,127	0,016	0,36	12	0,156	0,024	0,44
March 1998	12	0,285	0,081	0,80	13	0,245	0,060	0,69
June 1998	12	0,182	0,033	0,51	12	0,263	0,069	0,74
Sept 1998	11	0,264	0,070	0,74	12	0,327	0,107	0,91
Weighted average		0,215	0,046	0,60		0,209	0,044	0,59

N: number of participating laboratorie

12. Bibliography

1. Determination by NMR of the distribution of deuterium in ethanol of spirit drinks of vitivinicultural origin
2. OIV Compendium of international methods of analysis of spirit drinks of vitivinicultural origin.

3. Determination by isotopic mass spectrometry of the $^{13}C/\ ^{12}C$ isotopic ratio of wine ethanol or ethanol obtained from fermentation of musts, concentrated musts or grape sugar
4. OIV Compendium of International Methods of Analysis of Wine and Musts. Detecting enrichment of musts, concentrated musts, grape and wine sugars by application of nuclear magnetic resonance of deuterium (RMN-FINS/SNIF-NMR)
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9. Stable carbon isotope content in ethanol of EC data bank wines from Italy, France and Germany. A Rossmann ; H-L Schmidt ; F. Reniero ; G. Versini ; I. Moussa ; M.- H. Merle. Z. Lebensm. Unters. Forsch., 1996, 203, PP. 293-301.

Method OIV-MA-BS-23 : R2009

Type I method

Determination of the distribution of deuterium in ethanol of spirit drinks of vitivinicultural origin by application of nuclear magnetic resonance of deuterium (SNIF-NMR/RMN-FINS1)

(OIV/OENO 381/2009)

1. Definition

The deuterium contained in the sugars and the water in grape must will be redistributed after fermentation in molecules I, II, III and IV of the wine:

$CH_2D\ CH_2OH$	$CH_3CHD\ OH$	CH_3CH_2OD	HOD
I	II	III	IV

The addition of exogenous sugar (chaptalisation) before the must ferments will have an effect on the distribution of the deuterium.

[1] Specific Natural Isotope Fractionation studied by Nuclear Magnetic Resonance). Patent: France, 8122710; Europe, 824022099; USA, 854550082; Japan 57123249

As compared with the figures for parameters for a natural control wine from the same region, the enrichment of an exogenous sugar will lead to the following variations:

Parameter	$(D/H)_I$	$(D/H)_{II}$	$(D/H)^{Q}_{W}$	R
Vin natural	→	→	→	→
Vin enriched				
Beet sugar	↘	↗	↗	↗
Cane sugar	↗	↗	↗	↘
Maize sugar	↗	↗	↗	↘

- $(D/H)_I$: isotope ratio associated with molecule I
- $(D/H)_{II}$: isotope ratio associated with molecule II
- $(D/H)\frac{Q}{W}$: isotope ratio of the water in the wine.
- $R = 2\ (D/H)_{II}/(D/H)_I$, expresses the relative distribution of deuterium in molecules I and II: R is measured directly from the *h* intensities of the signals and then $R = 3h_{II}/h_I$.
- $(D/H)_I$ mainly characterizes the vegetable species which synthesized the sugar and to a lesser extent the geographical location of the place of harvest (type of water used during photosynthesis).
- $(D/H)_{II}$ represents the climatology of the place of production of the grapes (type of rain-water and weather conditions) and to a lesser extent the sugar concentration of the original must.

- (D/H) $\frac{Q}{W}$represents the climatology of the place of production and the sugar content of the original must. Henceforth, this parameter shall no longer be considered, since it is not characteristic of water of a spirituous beverage.

2. Principle

The parameters defined above (R, (D/H)I and (D/H)II) are determined by nuclear magnetic resonance of the deuterium in the ethanol extracted from a spirituous beverage; they may be supplemented by determining the ratio 13C/12C in the ethanol.

3. Preparation of the sample for analysis

Note: Any method for ethanol extraction can be used as long as 98 to 98.5% of the total alcohol in the wine is recovered in a distillate which contains 92 to 93% (*m/m*) (95% vol.).

3.1. Extraction of ethanol

3.1.1. Apparatus and reagents

Apparatus for extracting ethanol (Figure 1) comprising:

- Electric heating mantle with voltage regulator,
- One-liter round-bottom flask with ground glass neck joint,
- Cadiot column with rotating band (moving part in Teflon),
- 125 mL conical flasks with ground glass neck joints,
- 125 and 60 mL bottles with plastic stoppers.

Reagents for the determination of water by the Karl Fischer method.

3.1.2. Procedure

3.1.2.1. Extraction of the ethanol

Introduce a homogeneous sample of 50 to 300 mL of spirit drink (depending on its alcoholic strength) into the flask of the distillation apparatus with a constant reflux ratio of about 0.9. Place a 125 mL ground conical flask, calibrated beforehand, to receive the distillate. Collect the boiling liquid between 78.0 and 78.2°C, i.e. approximately 40 to 60 mL. When the temperature exceeds 78.5°C, discontinue collection for five minutes.

When the temperature returns to 78°C, recommence collecting the distillate until 78.5°C and repeat this operation until the temperature, after discontinuing collection and operating within a closed system, remains constant.

Complete distillation lasts approximately five hours. This procedure enables between 98 and 98.5% of the total alcohol in the wine to be recovered in a distillate with a strength of between 92 and 93% mass (95% vol.), a strength for which the NMR conditions described in section 4 have been established. The collected ethanol is weighed.

3.1.2.2. Determination of the alcoholic strength of the alcohol extracted.

The water content (p' g) is determined by the Karl Fischer method using a sample of about 0.5 mL of alcohol of exactly known mass p'.

The strength by mass of the alcohol is given by:

$$t\frac{D}{m} = \frac{\mathrm{p} - \mathrm{p}'}{\mathrm{p}} \times 100$$

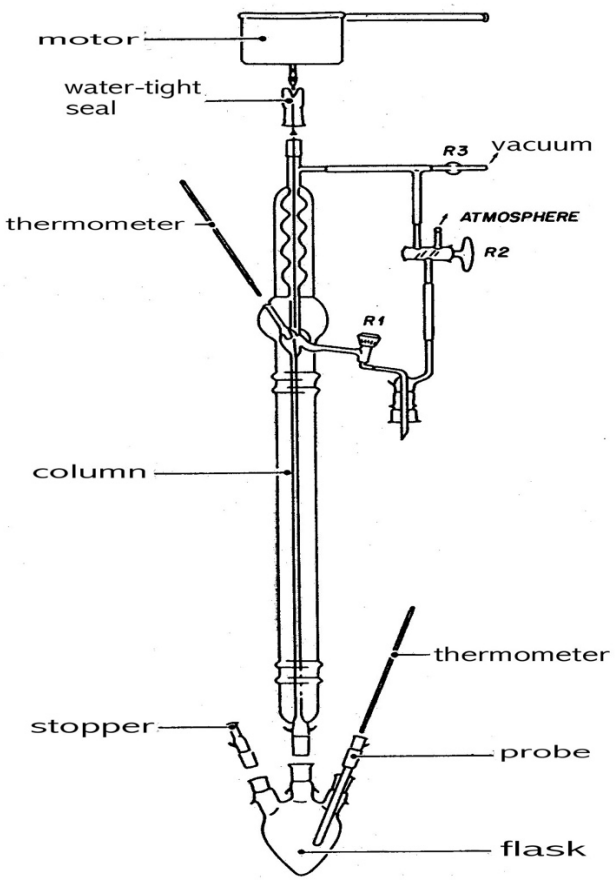

Figure 1 – Apparatus for extracting ethanol

3.2. Preparation of alcohol sample for NMR measurement

3.2.1. Reagents

N,N-tetramethyl urea (TMU); use a sample of standard TMU with a given, monitored isotope ratio D/H. Such samples may be supplied by Community Bureau of Reference, Brussels, or other authoritative body.

3.2.2. Procedure

- 15 mm diameter NMR probe:

In a previously weighed bottle, collect 7 mL alcohol as in 3.1.2 and weigh it to the nearest 0.1 mg (m_A); then take a 3 mL sample of the internal

standard (TMU) and weigh to the nearest 0.1 mg (m_{ST}). Homogenize by shaking.

- 10 mm diameter NMR probe:

.

3.2 mL of alcohol and 1.3 mL TMU are sufficient

Depending on the type of spectrometer and probe used (cf.. section 4), add a sufficient quantity of hexafluorobenzene as a field-frequency stabilization substance (lock).

Spectrometer	Probe	
	10mm	15 mm
7,05 T	150 µl	200 µl
9,4 T	35 µl	50 µl

4. Recording of ^2H NMR spectra for the alcohol.

Determination of isotope parameters.

4.1. Apparatus

- NMR spectrometer fitted with a specific "deuterium" probe tuned to a frequency ν_0, characteristic of channel B_0 (e.g. B_0 = 7.05 T, ν_0 = 46.05 MHz and for B_0 = 9.4 T, ν_0 = 61.4 MHz) having a decoupling channel (B_2) and a field-frequency stabilization channel (lock) at the fluorine frequency

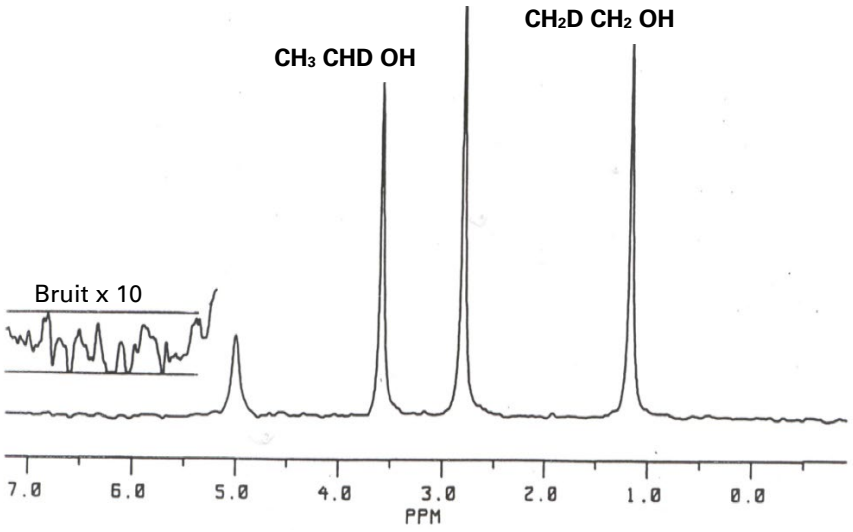

Figure 2a

2H NMR spectrum of an ethanol from wine with an internal standard (TMU: N, N-tetramethylurea)

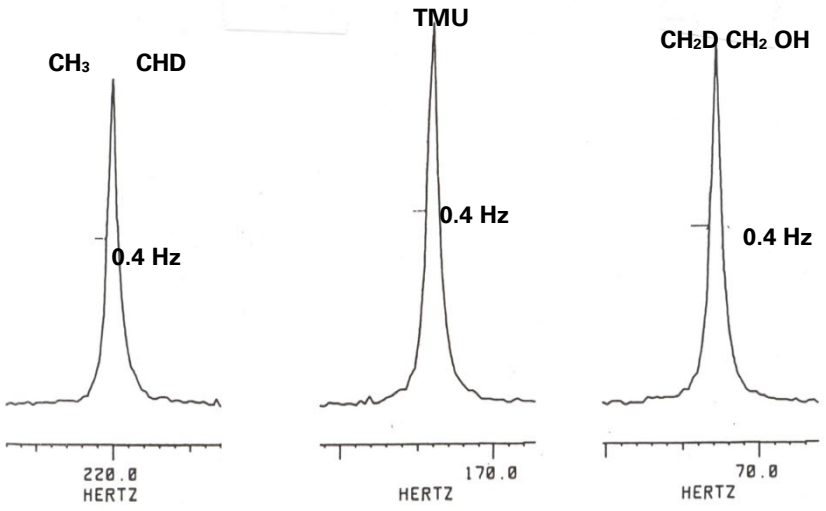

Figure 2b

2H spectrum of ethanol taken under the same conditions as those of

Figure 2a, but without exponential multiplication (LB = 0)

The resolution measured on the spectrum, transformed without exponential multiplication (i.e. LB = 0) (Figure 2b) and expressed by the (half-height) of the methyl and methylene signals of ethanol and the methyl signal of TMU, must be less than 0.5 Hz. The sensitivity, measured with an exponential multiplying factor LB equal to 2 (Figure 2a) must be

greater than or equal to 150 for the methyl signal of ethanol of alcoholic strength 95% vol. (93.5% mass**).**

Under these conditions, the confidence interval for the measurement of the signal height, calculated for a 97.5% probability (one-sided test) and 10 repetitions of the spectrum, is 0.35%.

Automatic sample changer (optional).

Data-processing software.

15 mm or 10 mm sample tubes according to spectrometer performance.

4.2. Standardization of spectrometer and checks

4.2.1. Standardization

Carry out customary standardization for homogeneity and sensitivity according to the manufacturer's specifications.

4.2.2. Checking the validity of the standardization

Use standard ethanol solutions designated by the letters:

C: alcohol from cane sugar or maize,

V: wine spirit,

B: alcohol from beet sugar.

These samples are supplied by the Community Bureau of Reference or other authoritative body.

Following the procedure described in 4.3, determine the isotope values of these alcohols, denoting them C_{mes}, V_{mes}, B_{mes} (see 5.3).

Compare them with the given corresponding standard values, denoted by a superscript C_{st}, B_{st}, V_{st} (see 5.3).

The standard deviation for repeatability obtained on an average of 10 repetitions of each spectrum must be less than 0.01 for the ratio R and less than 0.3 ppm for $(D/H)_I$ and $(D/H)_{II}$.

The average values obtained for the various isotopic parameters (R, $(D/H)_I$, $(D/H)_{II}$) must be within the corresponding standard deviation of repeatability given for those parameters for the three standard alcohols by the Community Bureau of References or other authoritative bodies. If they are not, carry out the checks again.

4.3. Conditions for obtaining NMR spectra

Place a sample of alcohol prepared as in 3.2 in a 15 mm or 10 mm tube and introduce it into the probe.

The conditions for obtaining NMR spectra are as follows:

- a constant probe temperature (e.g. 302 K);
- acquisition time of at least 6.8 s for 1200 Hz spectral width (16K memory) (i.e. about 20 ppm at 61.4 MHz or 27 ppm at 46.1 MHz);
- 90° pulse;
- adjustment of acquisition time: its value must be of the same order as the dwell time;
- parabolic detection: fix the offset O1 between the OD and CHD reference signals for ethanol and between the HOD and TMU reference signals for water;
- determine the value of the decoupling offset O2 from the proton spectrum measured by the decoupling coil on the same tube. Good decoupling is obtained when O2 is located in the middle of the frequency interval existing between the CH_3- and CH_2- groups. Use the wide band-decoupling mode.

For each spectrum, carry out a number of accumulations NS sufficient to obtain the signal-to-noise ratio given in 4.1 and repeat this set of NS accumulations NE = 10 times. The values of NS depend on the types of spectrometer and probe used (cf. section 4). Examples of the possible choices are:

Spectrometer	Probe	
	10 mm	15 mm
7,05 T	NS = 304	NS = 200
9,4 T	NS = 200	NS = 128

5. Expression of results

5.1. For each of the 10 spectra (see NMR spectrum for ethanol, Figure 2a), determine:

$$R = 3 \times \frac{\text{height of signal II } (h_{II})(CH_3\ CHD\ OH)}{\text{height of signal I } (h_I)(CH_2D\ CH_2OH)}$$

$$\left(D/H\right)_I = 1.5866 \cdot T_I \cdot \frac{m_{st}}{m_A} \cdot \frac{\left(D/H\right)_{st}}{t_m^D}$$

$$\left(D/H\right)_{II} = 2.3799 \cdot T_{II} \cdot \frac{m_{st}}{m_A} \cdot \frac{\left(D/H\right)_{st}}{t_m^D}$$

With

$$T_I = \frac{\text{height of signal I } (CH_2D\ CH_2OH)}{\text{height of signal of internal standard (TMU)}}$$

$$T_{II} = \frac{\text{height of signal II } (CH_3CHD\ OH}{\text{height of signal of internal standard (TMU)}}$$

- m_{st} and m_A, see 3.2.2.
- t_m^D, see 3.1.2.2.
- $(D/H)_{st}$ = isotope ratio of internal standard (TMU) indicated on the bottle supplied by the Community Bureau of Reference or other appropriate body.

The use of peak heights instead of peak area, which is less precise, supposes that peak width at half height is uniform and is a reasonable approximation if applicable (Figure 2b).

5.2. For each of the isotope parameters, calculate the average of 10 determinations and the confidence interval.

Optional software (e.g. SNIF-NMR) suitable for the spectrometer computer enables such calculations to be carried out on-line.

Note: If, after standardization of the spectrometer, there is a systematic difference between the average values obtained for the characteristic isotopes of the standard alcohols (4.2.2) and the values indicated by the Community Bureau of Reference or other authoritative body, to within the standard deviation, the following corrections may be applied to obtain the true value for any sample X.

The interpolation will be made by taking the values for the standard sample which straddle that of the sample X.

Let $(D/H)_I^{X\,mes}$ be the measured value and $(D/H)_I^{X\,corr}$ be the corrected value. This will give:

$$(D/H)_I^{Xcorr} = (D/H)_I^{Bst} + \alpha (D/H)_I^{Xmes} - (D/H)_I^{Bmes}$$

$$\alpha = \frac{(D/H)_I^{Vst} - (D/H)_I^{Bst}}{(D/H)_I^{Vmes} - (D/H)_I^{Bmes}}$$

Example:

Standard samples supplied and standardized by the Community Bureau of Reference or other authoritative body:

$(D/H)_I^{Vst}$ = 102,0 ppm $(D/H)_I^{Bst}$ = 91,95ppm

Standard samples measured by the laboratory:

$(D/H)_I^{Vmes}$ = 102,8 ppm $(D/H)_I^{Bmes}$ = 93,0 ppm

Reviewed non corrected sample:

$(D/H)_I^{Xmes}$ = 100,2 ppm

Calculation:

α = 1,0255 $(D/H)_I^{Xcorr}$ = 99,3 ppm

6. Bibliography

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2. MARTIN G.J., MARTIN M.L., *J. Chim. Phys.*, 1983, **80,** 294-297.
3. MARTIN G.J., GUILLOU C., NAULET N., BRUN S., Tep Y., Cabanis J.C.,
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5. MARTIN G.J., ZHANG B.L., NAULET N. and MARTIN M.L., *J. Amer. Chem. Soc.*, 1986, **108,** 5116-5122.
6. MARTIN G.J., GUILLOU C., MARTIN M.L., CABANIS M.T., Tep Y. et Aerny J., *J. Agric. Food Chem.*, 1988, **36,** 316.

Method OIV-MA-BS-24 : R2009

Type IV method

Determination of ^{14}C content by liquid scintillation spectrometry in spirit drinks of viti-vinicultural origin

OENO 6/94;

OIV/OENO 382A/2009

Introduction

Since alcohol can be used for the development of certain alcoholic beverages it is necessary to verify its agricultural or fossil origin.

1. Procedure for determining the type of alcohol

The determination of ^{14}C content in ethanol can be used to distinguish between alcohol made with fossil raw materials (referred to as synthetic alcohol) and alcohol made with existing raw materials (referred to as fermentation alcohol).

2. Definition

The expression ^{14}C content in ethanol means the ^{14}C content determined by the specified method. The natural content in the atmosphere of ^{14}C (reference value) which is absorbed by living plants is not a constant value. Accordingly, the reference value is determined on the basis of ethanol taken each time from raw materials grown in the most recent vegetation period. This annual reference value is determined each year by collaborative analyses organised by the Community Bureau of References and the Joint Research Centre, Ispra.

3. Principle

^{14}C content is determined directly by liquid scintillation counting in samples containing alcohol with at least 85% (by mass) of ethanol.

4. Reagents

4.1. Toluene scintillator.

5.0 g 2,5-diphenyloxazole (PPO).

0.5 g p-p-bis [4-methyl-5-phenyloxazole (2)]-benzene (dimethyl-POPOP) in one litre of pure toluene.

Toluene scintillators of this composition, commercially available, ready to use, can also be used.

4.2. Standard ^{14}C.

^{14}C n-hexadecane with an activity of about 1 x 106 dpm/g (approximately 1.67. 106 cBq/g) and a guaranteed accuracy of the determined activity of $\pm$ 2% rel.

4.3. Ethanol free of ^{14}C. Synthetic alcohol made from raw materials of fossil origin with at least 85 wt% ethanol to determine the zero effect.

4.4. The reference solution i.e. alcohol made with current raw materials from the most recent vegetation period with at least 85 wt% ethanol.

5. Apparatus

5.1. Multichannel liquid scintillation spectrometer with a computer and automatic external standardization and indication of the conditions of the external standard channel (usual design: three meter channels and two external standard channels).

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

5.3. Volumetric pipettes, 10 ml.

5.4. Automatic dosing device, 10 ml.

5.5. 250 ml round-bottom flask.

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

5.7. Microlitre syringe 50 µl.

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

5.9. Thermostat at a constant temperature ± 0.01°C.

5.10. Alcoholometric tables to international standards.

6. Procedure

6.1. Adjusting the apparatus.

The apparatus should be adjusted according to the manufacturer's instructions.

Measuring conditions are optimal when the value E2/B, the quality index, is at its maximum.

- E = efficiency
- B = background

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

6.2. Selection of counter tubes.

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

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

When setting the channel provided for in point 6.10, at the same time, when calculating the efficiency coefficient using the corresponding computer program, the external standard channel ratio (CEGN) is also determined. The external standard used is 137 caesium, which is already built-in by the manufacturer..

6.4. Preparation of sample

Samples having an ethanol content of at least 85 % mass and free from impurities, which absorb at wavelengths below 450 nm can be measured. The low residue of esters and aldehydes has no disruptive effect. After the first few ml have been discarded, the sample is distilled direct into the pycnometer and the alcohol content of the sample is determined by pycnometry. The values to be determined are taken from the Official Alcohol Tables.

7. Measurement of samples using an external standard

7.1. The low absorbance samples as described in section 6.4 with an ESCR value of approximately 1.8 can be measured through the external standard channel ratio, which provides a measure of the efficiency ratio.

7.2. Procedure

Using a pipette, introduce into a counter tube checked (selected) for background noise 10 ml of the sample prepared in accordance with section 6.40 and then each time add 10 ml of toluene scintillator to using an automatic dosing device. The samples are homogenized in the calibration bottle by rotation, taking care to ensure that the liquid does not wet the polyethylene insert in the screw-top. A tube containing ^{14}C-free fossil ethanol is prepared in the same way to measure the background noise. To check the relevant annual ^{14}C value a duplicate of recent ethanol from the latest vegetation period is prepared, mixing a counter tube with an internal standard, in accordance with paragraph 8.

The control and background samples are placed at the beginning of the measurement series, which should contain no more than 10 samples for analysis. Total measuring time per sample is at least 2 × 100 minutes, with the individual samples being measured in partial stages of 100 minutes in order to detect any equipment drift or other defect. (One cycle therefore corresponds to a measuring interval of 100 minutes per sample). Background and control samples should be freshly prepared every four weeks. This method requires little time and material and is particularly suitable for experienced laboratories processing large numbers of samples.

For samples with low absorbance (external standard channel ratio of about 1.8) the efficiency is only slightly affected by the change in this value. When this change does not exceed ± 5% rel., the calculation can be done with the same efficiency. For samples with higher absorbance such as for denatured alcohols, the efficiency can be established via the extinction correction graph. If an appropriate computer program is not available the internal standard must be used, and this gives an unambiguous result.

8. Measuring samples using internal standard hecadecane ^{14}C

8.1. Procedure

Control and background samples (recent and fossil ethanol) and the unknown material are each measured as duplicates. One sample of the duplicate is prepared in a non-selected tube and an accurately dosed quantity (30 µl) of hexadecane 14C is added as an internal standard (added activity around 26,269 dpm/gC, approximately 43,782 cBq/gC). For the sample preparation and measuring time of the other samples see 7.20, but the measuring time for the samples with the internal standard can be reduced to about five minutes by presetting at 105

pulses. One duplicate each of background and control samples is used per measuring series; these are placed at the beginning of the measuring series.

8.2. Use of internal standard and counter tubes

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

9. Expression of results

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

The indication of specific radio-activity is expressed as Becquerels relative to one gram carbon = Bq/gC.

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

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

9.2. Expression of results with an external standard

- (cpmpr - cpmNg) . 1,918. 100 cBq/g C = V. F. Z. 60

9.3. Expression of results with an internal standard

- (cpmpr - cpmNg) . dpmis . 1,918. 100 cBq/g C = (cpmis - cpmpr) . V. F. 60

9.4. Abbreviations

- cpmpr = the mean sample count rate over the total measuring time.
- cpmNg = the mean background pulse rate calculated in the same way.
- cpmis = count rate of samples, with an internal standard.
- dpmls = the quantity of internal standard added (calibration radioactivity dpm).
- V = the volume of the samples used in ml.
- F = the content in grams of pure alcohol per ml corresponding to its concentration.
- Z = the efficiency corresponding to the ESCR value.
- 1.918 = the number of grams of alcohol per gram of carbon.

10. Repeatability of the method

10.1. Repeatability (r)

- r = 0.632 cBq/g C ; S(r) = ± 0.223 cBq/gC

10.2. Reproducibility (R)

- R = 0.821 cBq/g C ; S(R) = ± 0.290 cBq/gC.

11. Bibliography

1. Methods of analysis of neutral alcohol applicable in the wine sector. EEC Regulation No. 1238/92 (8 May 1992), EEC Regulation No. 2009/92 (20 July 1992).

Method OIV-MA-BS-25 : R2017

Type IV method

Determination of ethyl carbamate in spirit drinks of viti-vinicultural origin

OENO 6/94;

OIV-OENO 590-2017

1. Principle

The assay is performed by direct injection of the drink into a chromatograph coupled to a mass spectrometer operating under the principle of electron impact, in "Selected Ion Monitoring (SIM)" acquisition mode.

2. Chromatography

Capillary column of the Carbowax 20 M (50 mx 0.22 mm) type, film thickness 0.2 μm.

- Temperature programming from 60 to 200°C, 3°C per minute.
- Data acquisition method of the mass spectrometer: Selected Ion Monitoring (SIM), MZ = 62, 74, 84.

The chromatograms are re-processed with the single ion M/Z = 62. The other ions are used to confirm peak purity by taking into account the ratio of their respective intensities.

Note: Certain NP or Hall sensors can be used.

3. Sample preparation

3.1. Internal standard

The internal standard is propyl carbamate (reference ICN, K & K Laboratories 217188) at 100 mg/L in a 50% vol. hydroalcoholic solution. (Check that the alcohol used is free of ethyl carbamate).

3.2. Addition of the internal standard

At 5 ml of the alcoholic beverage, add 50 µL of the solution of propyl carbamate at 100 mg/L which results in 1 mg/1 in the sample.

Note: this final quantity of the internal standard in the sample can be modulated according to the ethyl carbamate content in the medium to be analyzed.

3.3. In the case of sweet alcoholic beverages (over 10 g/L),

after adding the internal standard it is preferable to extract the ethyl carbamate, for example, method (1) can be used, which consists in extracting the ethyl carbamate with ether after saturating the medium with excess sodium sulphate to fix the water, or by method (2) which involves fixing the carbamates (ethyl carbamate or the internal standard) on a porous polymer (of Extrelut type) followed by elution with dichloromethane.

4. Preparation of the reference solution

- According to the alcoholic beverage to be analyzed, prepare a solution of ethyl carbamate at 50 µg/L or 400 µg/L or more if necessary.

- 5 ml of the reference solution are added by 50 µL of the internal standard solution (propyl carbamate at 100 mg/L).
- The solution is injected using the Splitless mode (valve closure for 20 to 30 seconds) by 2 µL of the prepared solution into the chromatograph after being properly adjusted.

5. Expression of results

The ethyl carbamate is expressed in µg/L of the spirit.

6. Bibliography

1. Dosage du Carbamate d'éthyle dans les vins et eaux de vie, 1988, BERTRAND A. et BARROS P.; connaissance Vigne Vin 22 (1) 39-47.
2. Method for the analysis of ethyl carbamate in alcoholic beverages by capillary gas chromatography, 1986, DENNIS M.J. HOWARTH N. MASSEY R.C. PARKER I. SCOTTER M. and STARTIN J.R. J.AOAC, 369 193.<0}

Method OIV-MA-BS-26 : R2009

Type IV method

Measurement of colour intensity in spirit drinks of viti-vinicultural origin

OENO 6/94

OIV/OENO 382A/2009

1. Principle

Colour intensity is determined by measuring the absorbance at 445 nm for an optical length of 1 cm thick (for traditional alcoholic beverages).

2. Apparatus

- A spectrophotometer enabling measurements at different wavelengths.
- Glass tanks with an optical path length of 1 cm and 0.2 cm.

3. Procedure

3.1. Alcoholic beverage of a natural "golden yellow" colour. Measure the absorbance at the wavelength 445 nm of the alcoholic beverage placed in a glass tank with an optical path length of 1 cm by setting the zero of the absorbance scale compared with distilled water.

Remarks.

- It is possible to measure the absorbance at any wavelength for alcoholic beverages naturally aged in wood and/or supplemented by caramel and/or supplemented by "woody" brandies because in all cases the absorption curves are continuous, without any maximum, or even a significant change in slope.
- Taking into account the maximum perceived by human vision it would be preferable to perform the measurement at 530 nm.
- The hue or hue gamut between two alcoholic beverages can be expressed, in certain cases, by measuring absorbance at 620 nm.
- Theoretically the sample should not be filtered if it is a product intended for direct consumption, but care should be taken to ensure that the sample is free of particles that are not a priori contained in the alcoholic beverage, especially those resulting from corking.

3.2. Alcoholic beverage containing synthetic dyes. First, the absorption maximum should be measured, and then the wavelength corresponding to the selected maximum, if necessary using a tank with an optical path length of 0.2 cm.

4. Expression of results

Express the colour intensity by the absorbance measured under the conditions specified above, indicating the size of the colorimeter tank, and the chosen wavelength.

5. Bibliography

1. Compendium of International Methods of Analysis of Spirituous Beverages of Vitivinicultural origin, 1990, BERTRAND A., F.V. O.J.V. n° 867.

Method OIV-MA-BS-27 : R2009

Type IV method

Determination of chromatic characteristics in spirit drinks of viti-vinicultural origin

OENO 6/94

OIV/OENO 382A/2009

1. Definitions

The chromatic characteristics of an alcoholic beverage are its luminosity and its chromaticity. Luminosity corresponds to the transmittance that varies inversely with colour intensity. Chromaticity corresponds to the dominant wavelength (hue) and purity.

Theoretical monochromatic components referred to as X, Y, Z, were defined by the International Commission on Illumination in 1931 (CIE). Each colour is therefore defined by its coordinates X, Y, Z in a system of three axes (also referred to as tristimulus values) forming between them an equal angle.

The trichromatic coordinates of any colour stimulus are obtained from the ratio of one component to the sum of all three, such that $x = X / (X + Y + Z)$, just as $y = Y / (X + Y + Z)$, etc.

It is therefore possible to reduce the spatial configuration of the colour in a flat presentation.

To fully define the colour, the notion of intensity is lacking, although the value Y is directly proportional to the visual intensity perceived by the human eye.

The colour of a solution is therefore fully defined by

- x, y, and Y (CIE 1931)

However, this space is not homogeneous and it is very difficult to correlate a chromatic gamut and a visual gamut.

Since this technique is not ultimately used, mathematicians have tried to define a new space that is more consistent referred to as L*,

L (psychometric clarity) is defined from Y; u and v being derived from X, Y and Z as well as from Xn, Yn, Zn the chosen surface colour as the nominal white stimulus under the illuminant used for the measurement. (CIE 1976).

The work of Adams-Nickerson enables the definition of a new space referred to as L*, a*, b* based on the same tristimulus values X, X, Y, etc.

It is represented by a sphere (Fig. 2) materialised by three axes L which vary from 0 for the white to 100 for the black, a* (-a = green, +a = red) and b* (-b = blue, +b = yellow).

- Clarity is defined by the value of L,
- Purity or chroma or saturation is the value C = (a2 + b2)1/2
- The hue angle, h = tg-1 (b/a).

Fig.2 Representation of colour in space L*, a*, b*.

The difference between two colours is measured by the relationship (total chromatic gamut)

AE= [(AL)2 + (Aa)2 + (Ab)2] 1/2 = [(AL)2 + (AC)2 + (Ah)2] 1/2

- The "chroma" gamut AC = [(C1)2 +(C2)2]1/2
- The clarity gamut AL = [(L1)2 +(L2)2]1/2
- The difference in hue Ah = [(AE)2 - (AL)2 - (AC)2] 1/2

The illuminants. As a result in particular of the observation of surface colours (introduced in the measurement of Xn, Yn and Zn) it was necessary to know the spectral distribution of the illumination used. At

present, the illuminant the most commonly used is the illuminant D 65 (Daylight colour close to 6504°K). Spectral distributions neighbouring the theoretical illuminant are obtained with tungsten or xenon arc lamps.

Transmission measurements. It is possible to work with an illumination angle and a viewing angle, but in fact, it is preferable to work in conditions that can be most easily normalised, namely illumination at 0° and observation at 0 ° (or 180°), observation being from the side opposite to that which is illuminated and in the extension of the axis of the illuminating beam. (less than 5° in deviation) this type of measurement is called "0/0".

2. Measurement of colour

2.1. Choice of an illuminant At present, it is recommended to choose the illuminant D65 although the illuminant C leads to very similar results.

2.2. Calculation of tristimulus values X, Y, Z. These tristimulus values of a colour stimulus can be determined from the summation of values calculated for a wavelength ranging from 380 to 770 nm (minimum) and with a measurement at least every 5 nm (in some cases , a measurement every 20 nm may be acceptable).

Refer to the Compendium of International Methods of Analysis of the OIV: Determination of chromatic characteristics according CIELab. *Resolution* OENO 1/2006

2.3. Carry out the calibration of the appliance using a vessel suitable to the spectrophotometer or colorimeter used. The size of the tank depends on the colour intensity of the alcoholic beverage (in principle 10 mm, exceptionally 1 mm or on the contrary 20 mm). The calculations are performed based on the transmittance values for an optical path length of 10 mm, when other optical path lengths are used the absorbance must be measured and rounded off to an optical path length of 10 mm and then the transmittance calculated.

2.4. Perform the measurement on the alcoholic beverage

Theoretically the sample should not be filtered if it is a product intended for direct consumption, because a certain degree of opacity and can be sought and expressed directly by L; care, however, should be taken to ensure that the sample is free of particles that are not a priori contained in the alcoholic beverage, especially those resulting from corking. Perform the measurement. Record the results.

2.5. Make colour comparisons

If appropriate, if the appliance used allows comparisons of colour, for example in relation to a chosen reference standard and thus directly determine

- the purity gamut AC
- the clarity gamut AL
- the difference in hue Ah

3. Expression of results

- The reference of illuminant A, C, or D65
- The optical path length under which the measurement is made, - the luminosity L*.
- The values of a* and b*,
- The purity or saturation C, - the hue angle h.

For comparative measurements, note

- The purity gamut AC
- The clarity gamut AL
- The difference in hue Ah

4. Bibliography

1. DORET Y. La colorimétrie, principes et applications Eyrolles ed. - Minolta CT-210 doc. Minolta.
2. TROUVÉ A. La mesure de la couleur Principes, technique et produits du marché AFNOR CETIM ed.
3. Détermination des caractéristiques chromatiques des boissons spiritueuses, 1984, SAUCADO A., ESCOLAR D., HARO Ma R., GOMEZ J. et ALVAREZ J. A., F.V. 0.1.V. 981.
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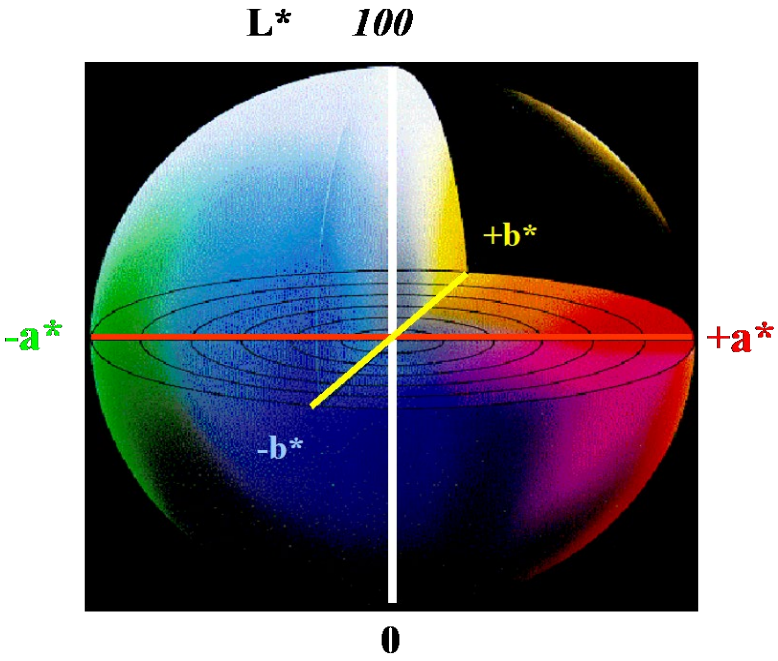
5. The Compendium of International Methods of Analysis of the OIV: Determination of chromatic characteristics according to CIELab. *Resolution* Oeno 1/2006.

Figure 1 Representation of the different parameters of the colour


L*
100
+b*
-a*
+a*
-b*
0

Method OIV-MA-BS-28 : R2009

Type IV method

Measurement of turbidity by nephelometry in spirit drinks of viti-vinicultural origin

OENO 6/94

OIV/OENO 382A/2009

1. Purpose

The purpose of the present document is to describe an optical method for determining the turbidity index (or diffusion index) of an alcohol or alcoholic beverage.

2. Scope

The method described is applicable to alcohol and alcoholic beverages with a high optical purity and containing low quantities of suspensoid material.

Its application is of less interest in liquids containing suspended solids (very turbid).

3. Principle

Turbidity is an optical effect.

The diffusion index is an intrinsic property of liquids used to characterize their optical appearance.

This optical effect is caused by the presence of very fine particles distributed in a liquid dispersion medium; the refractive index of the particles differs from that of the dispersion medium.

If optically clean water contained in a known volume is illuminated and if we measure the luminous flux diffused by the incident beam, the value read for this diffused flux characterizes the molecular diffusion of water.

If the value obtained with the water analyzed is greater than that corresponding to the molecular diffusion, which is constant for a given wavelength, for the same incident flux from the same angle of measurement, for the same tank geometry and a given temperature, the difference is attributed to the light diffused by the solid, liquid or gaseous particles suspended in the water.

The measurement of diffused luminous flux, performed as described, is a nephelometric measurement.

4. Definition

4.1. Unit of expression of the turbidity index

The turbidity unit used is the N T U - NEPHELOMETRIC TURBIDITY UNIT which corresponds to the measurement of light diffused by a standard suspension of Formazine, with a value of 1 NTU, at an angle of 90° with respect to the direction of the incident beam.

4.2. Preparation of the standard Formazine suspension.

4.2.1. Water for the preparation of control solutions.

Soak a filter membrane with pore size of 0.1 microns (the type used in bacteriology) for 1 h in 100 ml of distilled water. Filter 250 ml of distilled

water twice through the membrane and retain the water for the preparation of the standard solutions.

4.2.2. Formazine ($C_2H_4N_2$) solutions.

The combination referred to as Formazine, the formula for which is $C_2H_4N_2$, is not commercially available. It is obtained by means of the following solutions:

- Solution A: Dissolve 10.0 g of hexamethylenetetramine (formula $CH_2)_6N_4$ in distilled water prepared according to 4.2.1. Then fill to 100 ml with distilled water prepared according to 4.2.1
- Solution B: Dissolve 1.0 g of hydrazinium sulphate $N_2H_6SO_4$ in distilled water prepared according to 4.2.1. Then fill to 100 ml with distilled water prepared according to 4.2.1.

Warning: Hydrazinium sulphate is poisonous and may be carcinogenic.

4.3. Procedure

Mix 5 ml of solution A and 5 ml of solution B. After 24 h at 25°C ± 3°C, dilute the solution to 100 ml with water. (4.2.1).

The turbidity of this standard solution is: 400 NTU

In the dark, the standard suspension can be kept at room temperature for approximately 4 weeks.

By dilution to 1/400 with distilled water according to 4.2.1, we obtain a turbidity of 1 NTU.

This solution is only stable for one week.

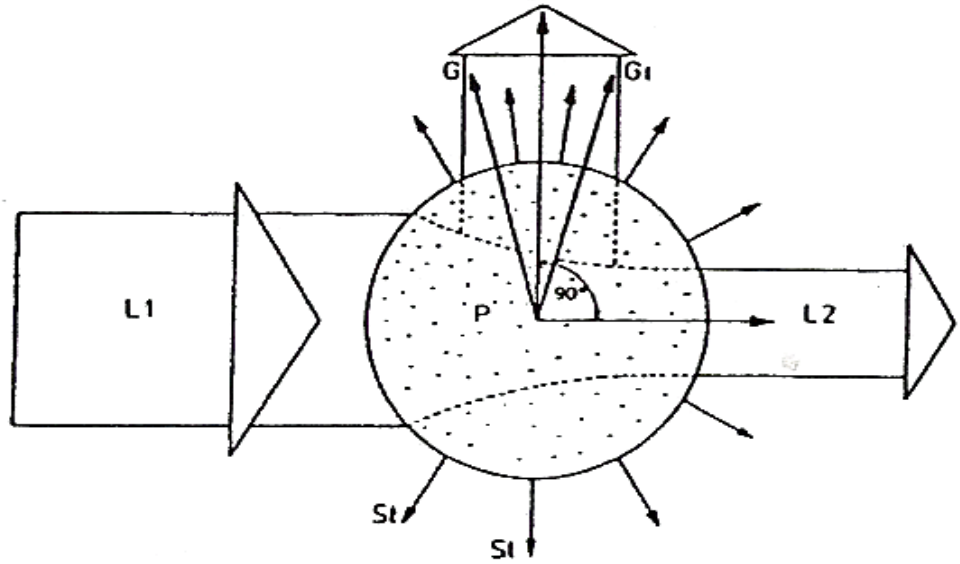
NB: Formazine standards have been compared to standards based on polymers.

The discrepancies observed may be considered to be negligible. Polymer-based standards have the following disadvantages, however:

their cost is very high and their service life is limited. They must be handled carefully to avoid breaking the polymer particles, which changes the turbidity index. This possibility is offered as an alternative to Formazine.

4.4. Principle of optical measurement

Measuring principle



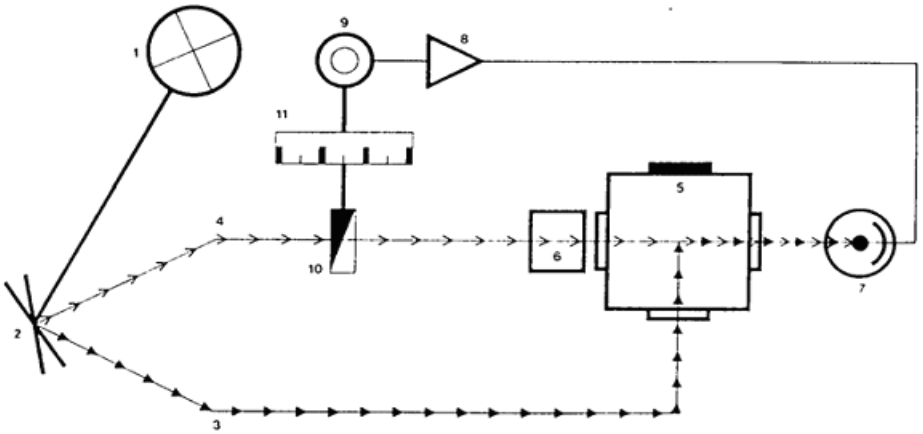
- L1 =Incident light beam
- L2 = beam having passed through the sample
- P = Sample
- St = diffused light
- G/G1 = limit rays of the diffused light beam, used for the measurement

- G/GL: Limit rays of the diffused beam of light for measurement.
- Observation of the diffused light should be at 90 degrees in relation to the direction of the incident beam.

5. Apparatus

This method refers to the use of apparatus making the measurement by optical compensation with two light beams.

5.1. Optical principle



A light source (1), powered by the mains, sends a beam of light onto the oscillating mirror (2) which reflects alternatively a measuring beam (3) and a comparison beam (4) about 600 times per second.

The measuring beam (3) is propagated in the fluid to be measured (5), while the comparison beam (4) is propagated in an optically stable turbidity comparison standard (6).

The diffused light produced in the fluid (5) by the particles which generate turbidity and the light diffused by the comparison standard (6) are alternately received by a photoelectric cell (7).

This cell thus receives, at the same frequency, on the one hand a measuring beam (3), and on the other hand, a comparison beam (4) whose luminous intensities are different.

The photoelectric cell (7) transforms these unequal luminous intensities into photoelectric currents which are then amplified (8) and fed to a synchronous motor (9) acting as a servomotor.

By means of a mechanical measuring diaphragm (10), the servomotor varies the intensity of the comparison beam until the two rays reach the photo-electric cell with the same luminous intensity.

This state of equilibrium can be used to measure the solid content of the measured fluid.

The absolute value of the measurement depends on the dimensions of the comparison standard and the position of the diaphragm.

5.2. Range of measurement

The apparatus must enable measurement in the range of 0 to 50 NTU

5.3. Measuring ranges

The measurements ranging from 0 to 5 NTU must be performed by placing the test sample in a glass measuring cell of optical quality with the following dimensions: 60 x 60 mm, i.e. a minimum sample volume of 140 ml.

The measurements ranging beyond 5 NTU must be performed by placing the sample in a glass measuring cell of optical quality with the following dimensions: 35 x 35 mm, i.e. a minimum sample volume of 60 ml.

Measuring cells of different shape but with identical characteristics in terms of optical path length and volumes may be used.

5.4. Limit of detection of stray light

Part of the light beam is diffused at the entry, exit and other areas of the measuring cell, including when measuring clear, clean water.

Due to the apparatus, this diffused light also reaches the photoelectric cell. This light, referred to as: stray light, creates a slight error in each measured value.

The apparatus must not induce an error due to stray light greater than: 0.01 NTU on the measuring range of: 0 to 0.1 N**Effect of colouring of the test sample**

The apparatus must compensate for the colour of the product to be measured without affecting the turbidity measurement up to the following absorbance values:

NTU Measuring ranges	Total admissible absorbance
0- 0.1	0.5
0 - 0.2	0.5
0 - 0.5	0.7
0 - 1.0	0.8
0 - 2.0	0.9
0- 5.0	1.1

0-10.0	1.2
0-20.0	1.3
0-50.0	1.5

5.6. Temperature conditions

The measurement should be made at a temperature of between 15 and 25°C

6. Procedure for measurement

6.1. Verification of the apparatus

Before any measurement or series of measurements, check the electrical and mechanical operation of the apparatus in accordance with the recommendations of the manufacturer.

6.2. Verification of calibration of the measurement scale

Before any measurement or series of measurements, using a previously calibrated apparatus, check the calibration of its measurement scale in accordance with its principle of construction.

6.3. Cleaning the measuring cell

Clean the measuring cell with great care before any determination. Take all the precautions required to avoid the introduction of dust into the apparatus and even more so in the measuring cell before and during the determination of the turbidity index.

6.4. Measurement

- Operate at a temperature as close as possible to 20°C. Prior to the measurement, thoroughly mix and without any sudden movements the flask containing the product to be measured,
- Thoroughly rinse the measuring cell twice using a small volume of the product to be measured,
- Introduce the product to be measured with care into the measuring cell, avoiding any turbulent flow which might lead to the formation of air bubbles and carry out the test measurement,
- Wait one minute if the index value is stable
- Note the turbidity index obtained.

7. Expression of results

The turbidity index of the product examined is recorded and expressed in NTU

8. Test report

The test report must state the results obtained, the references and identification of the sample, and all the test conditions, the type of apparatus used and all the operating details, whether optional or not provided in the method, and any incidents which may have influenced the results.

Appliances using the optical compensation method of measurement include solid turbidity standards in which the measuring light is diffused by a standard suspension of Formazine.

The solid standards are to have been checked beforehand by an optical laboratory.

It is recommended to periodically check the calibration using three standards distributed in the measurement range habitually used.

These three standard solutions are obtained by dilution of the standard suspension of Formazine described in section 4.10 of the method.

The standard liquids must be packaged in bottles made of glass or another inert material of low capacity (e.g. 200 ml) to prevent multiple successive manipulations from altering their purity. Keep the bottles in a cool, dark place. Any opened bottle should be carefully and quickly recapped after sampling the volume required for measurement. The sample must never be used for a second determination.

9. Bibliography

1. Norme NF T 90 - 033 - Essai des eaux. Mesure de l'indice de diffusion, dite mesure de la turbidité.
2. Standard Methods for the examination of water and wastewater (13th Edition - 1971) ALPHA - American Public Health Association
3. Methods for the Chemical Examination of Water and Wastes (1973) EPA - Environmental Protection Agency.
4. "Stray light in turbidity measurements" Dr. Ing. W. SIGRIST - Gas, Wasser, Abwasser - Ed No. May 1976 published by the Association of Swiss Water and Gas.
5. Notices techniques turbidimeters - COMPANY SIGRIST PHOTOMETER - Horfurlistrasse 1 - CH 6373 - Ennetbürgen - Switzerland.
6. Informations techniques sur la mesure de turbidité - SOCIETE HACH COMPANY - P.O. BOX 389 - Loveland Colorado - 80539 USA.
7. German Standard for water analysis - DIN 38404.
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9. Projet de norme sur la mesure de l'indice de turbidité dans les boissons spiritueuses, 1991, BOULANGER P., F.V. O1.V. no. 903.

Method OIV-MA-BS-29 : R2009

Type IV method

Calcium- Determination by atomic absorption in spirit drinks of viti-vinicultural origin

OENO 6/94

OIV/OENO 382A/2009

1. Principle

Calcium is determined by atomic absorption spectrophotometry with a reductive air acetylene flame using a calcium hollow-cathode lamp, wavelength of 422.7 nm, on the dealcoholised alcoholic beverage, concentrated 2 times. The measurement is performed in the presence of lanthanum chloride referred to as the "matrix modifier".

2. Appartus

2.1. Glassware

2.1.1. 25, 50, 100, 1000 ml volumetric flasks (class A).

2.1.2. 1, 2, 3, 4, 10, 50 ml volumetric pipettes (class A)

2.1.3. 100 ml test tube

2.1.4. 250 ml beaker (class A).

2.1.5. 20 ml tablet bottle

2.2. Spectrophotometer (sample setting for Varian 575 model)

2.2.1. Reducing air-acetylene flame, flow rates:

- air: : 7.5 Vmin
- C2 H2: 4.0 Vmin

2.2.2. Calcium hollow-cathode lamp with calcium; Wavelength: 422.7 nm, slit (slit): 0.2 nm, lamp intensity: 5 mA.

3. Reagents

3.1. Ultrapure demineralised water resistivity 18.2 M4.

3.2. Stock solution 2.3 to 1 g/l of Calcium: (e.g. Titrisol Merck).

3.3. Solution of 3.3 to 100 mg/1 of calcium

Place 10 ml of stock solution (3.2) in a 100 ml flask (2.1.1), fill to volume with demineralised water (3.1).

3.4. Hydrochloric acid d = 1.18 (35% minimum)

3.5. Lanthanum Chloride Solution, 25 g/1

Weigh 63.6 g of lanthanum chloride ($LaC1_3.6H_20$) in a 1000 ml flask

(2.1.1), add approximately 500 ml of demineralised water (3.1) then to the test tube (2.1.3) 50 ml of hydrochloric acid (3.4). After solubilisation, allow to cool and fill to volume with demineralised water (3.1).

3.6. Calibration range: 2, 4, 6, 8 mg/l of calcium.

Place successively 1.0, 2.0, 3.0, 4.0 ml of the solution at 100 mg/1Calcium (3.3) in four 50 ml vials (2.1.1), add 10 ml of the solution of lanthanum chloride (3.5), and fill to volume with demineralised water (3.1). Perform a blank test without calcium in the same conditions.

4. Sample preparation

The calcium content in alcoholic beverages is often very low, it is therefore necessary to concentrate the sample by evaporating the alcohol.

Pipette (2.1.2) 50 ml of the alcoholic beverage into a 250 ml beaker (2.1.4). Evaporate the alcohol in a water bath to about one volume of 10 ml. Leave to cool, then pour the concentrate into a vial of 25 ml (2.1.1), rinse the beaker and fill to volume with demineralised water (3.1).

Place 4 ml of this solution to be determined prepared in a clean, dry tablet bottle (2.1.5) with 1 ml of lanthanum chloride solution (3.5); cork, stir.

5. Determinations

Successively present the calibration solutions, the blank solution (3.6), and the samples (4.); ; note the corresponding absorbances.

Establish the calibration curve absorbance = f (concentration in mg/1 calcium) by the least squares method.

Deduce the concentration of calcium in mg/l taking into account the concentration factor.

6. Bibliography

1. Compendium of International methods of wine and must analysis, 1990, O.I.V. ed.

Method OIV-MA-BS-30 : R2009

Type IV method

Copper- Determination by atomic absorption in spirit drinks of viti-vinicultural origin

OENO 6/94

OIV/OENO 382/2009

1. Principle

Copper content is determined by atomic absorption spectrophotometry by means of an oxidizing acetylene air flame using a copper hollow-cathode lamp, at wavelength 324.7 nm, on the dealcoholised alcoholic beverage, according the so-called "standard addition" method.

2. Apparatus

2.1. Glassware.

2.1.1. 50 ml, 200 ml volumetric flasks (class A).

2.1.2. 5 ml, 10 ml, 50 ml volumetric pipettes (class A).

2.1.3. 20 ml

2.1.4. 200 µl automatic micropipette.

2.1.5. 250 ml beaker

2.2. Spectrophotometer (sample setting for Varian 575 model

2.2.1. Oxidising air-acetylene flame

- Flow rates air: 7.5 1/min
- C2 H2: 1.8 l/min

2.2.2. Copper hollow-cathode lamp; Wavelength:

- 324.7 nm, slit (slit): 0.5 nm, lamp intensity: 3.5 mA.

3. Reagents

3.1. Ultrapure demineralised water resistivity 18.2 MΩ.m (e.g. Milli Q).

3.2. Stock solution to 1 g/l of copper: (eg. Titrisol Merck).

3.3. Solution 10 mg/1 of copper.

Place 2 ml of stock solution (3.2) in a 200 ml flask (2.1.1); fill to volume with demineralised water (3.1).

4. Sample preparation standard addition method

4.1. Evaporation of alcohol

Pipette (2.1.2) 50 ml of the alcoholic beverage in a 250 ml beaker (2.1.5). Evaporate the alcohol in a water bath to about one volume of 10 ml. Leave to cool, then pour the concentrate into a vial of 50 ml (2.1.1), rinse the beaker and fill to volume with demineralised water (3.1).

4.2. Add 0.2 mg/l of copper

Place 5 ml of the test sample (3.1) in a tablet bottle (1.1.3), add using the micropipette (1.1.4) 100 µl of the solution to 10 mg/l of copper (2.3).

4.3. Add 0.2 mg/l of copper

Place 5 ml of the test sample (4.1) in a tablet bottle (2.1.3), add using the micropipette (2.1.4) 200 µl of the solution to 10 mg/l of copper (3.3).

5. Determination

Successively present the test sample (4.1), the addition solutions (4.2), (4.3);

note the corresponding absorbances.

Establish the calibration curve for the additions: absorbance = f (concentration in mg/1 of copper) by the least squares method.

The concentration of copper is given by the intersection of the calibration curve for the additions absorbance = f (concentration mg/l of copper) with the x-axis.

6. Bibliography

1. Compendium of International methods of wine and must analysis, O.I.V. ed.

Method OIV-MA-BS-31 : R2009

Type IV method

Iron- Determination by atomic absorption in spirit drinks of viti-vinicultural origin

OENO 6/94

OIV/OENO 382A/2009

1. Principle

Iron is determined by atomic absorption spectrophotometry by means of an oxidising air-acetylene flame, using an iron hollow-cathode lamp, at a wavelength of 248.3 nm on the alcoholised alcoholic beverage.

2. Apparatus

2.1. Glassware.

2.1.1. 50 ml, 100 ml volumetric flasks (class A).

2.1.2. 1, 2, 3, 4, 10, 50 ml volumetric pipettes (class A)

2.1.3. 250 ml beaker (class A).

2.2. Spectrophotometer (sample setting for Varian 575 model)

2.2.1. Oxidising air-acetylene flame

- Flow rates: air:: 7.5 1/min
- C2 H2: 3.5 1/min

2.2.2. Iron hollow-cathode lamp; Wavelength: 248.3 nm, slit: 0.5 nm, lamp intensity: 5 mA.

3. Reagents

3.1. Ultrapure demineralised water resistivity 18.2 MΩ.m (e.g. Milli-Q).

3.2. Stock solution to 1 g/l of iron: (e.g. Titrisol Merck).

3.3. Stock solution to 1 g/l of iron.

Place 10 ml of stock solution (3.2) in a 100 ml flask (2.1.1), fill to volume with demineralised water (3.1).

3.4. Calibration range: 2, 4, 6, 8 mg/1 of iron.

Place successively 1.0, 2.0, 3.0, 4.0 ml of the solution at 100 mg/1 of iron (3.30) in four 50 ml vials (2.1.1), fill to volume with demineralised water (3.1).

4. Sample preparation

4.1. Evaporation of alcohol.

Pipette (2.1.2) 50 ml of the alcoholic beverage in a 250 ml beaker (2.1.3). Evaporate the alcohol in a water bath to about one volume of 10 ml. Leave

to cool, then pour the concentrate into a 50 ml vial (2.1.1), rinse the beaker and fill to volume with demineralised water (3.1).

Dilution in demineralised water (3.1) is only required if the concentration of iron is greater than 8 mg/l.

5. Determinations

Successively present the calibration solutions (3.4), and samples (4.1.);

note the corresponding absorbances.

Establish the calibration curve absorbance = f (concentration in mg/1 calcium) by the least squares method.

Deduce the concentration of iron (mg/1) taking into account any dilution.

6. Bibliography

1. Compendium of International Methods of wine and must analysis, 1990, O.I.V. ed.

Method OIV-MA-BS-32 : R2009

Type IV method

Lead- Determination by atomic absorption in spirit drinks of viti-vinicultural origin

OENO 6/94

OIV/OENO 382A/2009

1. Principle of the method

Lead is determined directly in the alcoholic beverage, using a lead hollow-cathode lamp by flameless atomic absorption spectrometry, using a matrix modifier.

2. Apparatus

All the glassware must be washed prior to use with hot concentrated nitric acid (70-80°C) and rinsed in double-distilled water.

2.1. Atomic absorption spectrophotometer equipped with a graphite oven, a non-selective absorption corrector and a multipotentiometric recorder.

2.2. Lead hollow-cathode lamp.

2.3. 5 l micropipettes with special tips for atomic absorption measurements

3. Reagents

All the reagents must be of analytical purity and, in particular must be lead-free. The water used must be double-distilled in a borosilicate glass apparatus or with water of equivalent purity.

3.1. Phosphoric acid to 85 p. 100 (p20 = 1.71 g/ml)

3.2. Phosphoric acid solution obtained by dilution of 6 ml of phosphoric acid to 100 ml with water.

3.3. Nitric acid (ρ20 = 1.38 g/ml)

3.4. Lead solution to 1 g per litre.

Use a standard commercial solution. This solution can be obtained by dissolving 1.600 g of lead nitrate II, Pb $(NO_3)_2$ in nitric acid diluted to 1% (v/v) and adjusting the volume to 1 litre.

Keep the solution in a borosilicate glass bottle with a ground glass stopper.Nitric acid solution diluted to 1% (v/v).

3.6. The solution is obtained by diluting the phosphoric acid solution at 6% at 1/2 with the nitric acid solution at 1%.

4. Procedure

4.1. Sample preparation

Add to the test sample of the alcoholic beverage an equal volume of the solution (3.6) of phosphoric and nitric acids. Determine its absorbance

If it is greater than 0.6, dilute the alcoholic beverage (a dilution of 1/5 is sufficient in most cases).

Prepare the test solution by adding to the test sample of the diluted alcoholic beverage an equal volume of the solution of phosphoric and nitric acids.

4.2. Preparation of the solutions in the calibration range

Using the control solution of lead, prepare dilutions in which 50% of the final volume is the solution (3.6) of phosphoric and nitric acids The concentration scale of the range depends on the sensitivity of the apparatus. For example, prepare solutions containing 10 - 20 - 30 micrograms of lead per litre.

4.3. Determination

4.3.1. Oven program.

Step	Temperature (°C)	Time (s)	Nitrogen (L/min.)	Reading
1	75	2	3	
2	95	20	3	
3	140	15	3	
4	300	8	3	
5	450	7	3	
6	480	10	3	
7	900	20	3	
8	900	1	0	
9	2 250	0,7	0	L

10	2 250	1	0	L
11	2 250	2	3	

4.3.2. Measurements

Select wavelength 283.3 nm. Set to zero the absorbance scale with double-distilled water Using a micropipette or an automatic sampler, inject into the programmed oven 3 times 5 tl of each solution in the calibration range and of the solution of the sample to be analysed.

Record the measured absorbances. Calculate the mean absorbance value based on the results for the three injections. The absorbances are measured in height of peaks.

5. Expression of results

5.1. Calculation

Plot the changes in absorbance versus the concentrations of lead in solutions of the calibration range. The relationship is linear. Record the mean value of the absorbance of the sample solution on the calibration curve and determine the concentration C of lead.

The lead concentration in micrograms per litre of alcoholic beverage is equal to:

$$C \times F$$

- F = dilution factor.

6. Bibliography

1. Application de la spectrométrie d'absorption atomique sans flamme au dosage de quelques métaux dans les vins, 1978, MEDINA B.,Thèse Doc. en Oenologie, Université deBordeaux II.

2. MEDINA B. et SUDRAUD P., 1979, Fcuillet. Vcrt. O.I.V., n° 695.
3. Recueil des méthodes internationales d'analyse des vins et des moûts, 1990, plomb 233-234, O.I.V. ed.
4. Comparaison de deux méthodes de minéralisation en vue du dosage du plomb par spectrométrie d'absorption atomique électrothermique.
5. Application à des échantillons de sols, feuilles de vigne, raisins, moûts et lies, 1993, Analusis, TEISSEDRE P.L., CABANIS M.T., CABANIS J.C.
6. Dosage du Plomb dans les vins, 1993, TEISSEDRE P.L., BRUN S., MEDINA B.,
7. Feuillet. Vert. OIV

Method OIV-MA-BS-33 : R2013

Type IV method

Method of determination of phthalates in spirituous beverages by gas chromatography/ mass spectrometry

OIV-OENO 521-2013

1. Scope of application

This method applies to the detection and assay of some phthalates in spirit drinks.

2. Principle

The sample is extracted using a non-polar solvent. The extract is then analysed by gas chromatography/mass spectrometry (GC/MS) with an internal standard.

Analysis may also be carried out directly but with higher detection limits. In this case, the internal standard is added directly before injection in GC/MS.

3. Reagents and products

Unless otherwise specified, all the reagents used are of recognised analytical quality:

3.1. DBP (Dibutyl phthalate) [CAS N°: 84-74-2];

3.2. DEHP (Di-(2-ethylhexyl) phthalate) [CAS N°: 117-81-7];

3.3. BBP (Butyl benzyl phthalate) [CAS N°: 85-68-7];

3.4. DIBP (Diisobutyl phthalate) [CAS N°: 84-69-5];

3.5. other phthalates if necessary (note: diisodecyl and diisononyl phthalate are each a mixture of compounds, some of which are common to both);

3.6. internal standard (for example: dipentyl phthalate [CAS N° 131-18-0]);

3.7. absolute ethanol;

3.8. Milli-Q water;

3.9. non-polar extraction solvent, free from phthalates, such as toluene.

Standard solutions

The concentrations provided in this method are for indicative purposes:

3.10. Internal standard stock solution

- 500 mg/L in ethanol,

3.11. Internal standard working solution

- 50 mg/L in ethanol,

3.12. Phthalates stock solution

- 500 mg/L of different phthalates in ethanol:
- Diisobutyl phthalate,
- Dibutyl phthalate,
- Diethylhexyl phthalate,
- Butylbenzyl phthalate,

Others if required

3.13. Phthalates working solution

The solution is prepared using a 1:5 dilution of the stock solution in the ethanol.

3.14. Calibration range

Prepare a 40% vol. aqueous-alcoholic solution: pour 80 mL of ethanol into a 200 mL flask then make up to volume with water. A multi-point range is prepared according to Table 1:

Table 1: Preparation of standards

40% vol. aqueous-alcoholic solution (mL)	Concentration level (mg/L)	Volume of working solution (µL) [3.13.]	Concentration of working solution (mg/L)
25	0 (blank)	0	100
25	0.2	50	100
25	0.4	100	100

25	0.8	200	100
25	1.2	300	100
25	1.6	400	100
25	2.0	500	100

4. Equipment

- Glassware and volumetric laboratory equipment
- Analytical balance
- GC-MS system

5. Procedure

5.1. Precautions

Due to the presence of phthalates in the environment, precautions must be taken throughout the analysis of these compounds:

- avoid any contact with plastic equipment,
- test the solvents used,
- use glassware rinsed with an appropriate solvent,
- avoid contamination from the septum of the injection vials.

5.2. Preparing the samples

Samples with an ABV of 40% vol. or which have been adjusted to 40% vol. (+/- 5% vol.) are extracted.

Extraction: in a glass test tube

- 25 mL of sample or calibration solution,
- 50 µL of internal standard solution,
- 2 mL of non-polar solvent.

Extract with a Vortex mixer for 3 minutes.

Recover the organic phase in the automatic injector vials.

Prepare a blank in the same way (with a 40% vol. aqueous-alcoholic solution).

5.3. Chromatography conditions (as an example)

- non-polar type column (DB5 MS: 30 m x 0.25 mm x 0.25 µm),
- injector at 250°C,
- splitless mode of injection,
- in the case of direct analysis, choose the split mode with a ratio of 1:10,
- programming of oven temperature (example): 80°C (0.7 min) at 20°C/min up to 110°C then 6°C/min up to 245°C (30 min isothermal),
- Helium: 1mL/min

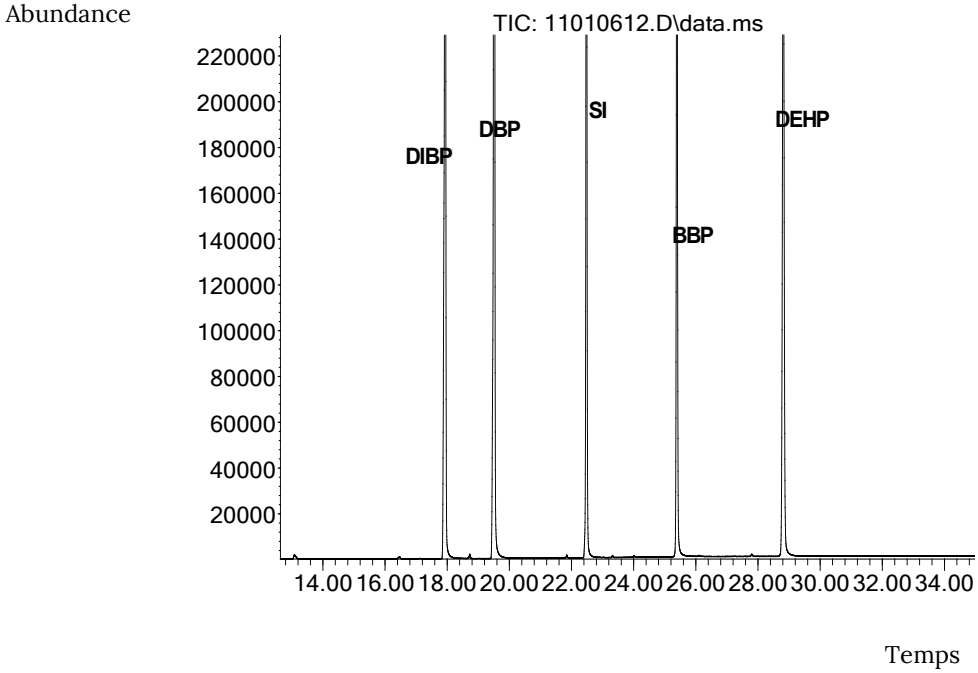
Injection volume: 1 µL

MS conditions:

Ionisation in EI Mode

SIM mode on ion m/z 149 or SIM/SCAN mode

Figure 1 – GC/MS Chromatogram of a phthalates solution and the internal standard



5.4. Injection sequence

Start the sequence by analysing the "blank", then inject the calibration solutions and the samples. Regular injection of the blanks is recommended.

5.5. Expressing the results

Identification is carried out using the retention time.

The results are expressed in µg/L or in mg/L.

Use the multiplication factor corresponding to the dilution performed to adjust the sample to 40% vol.

The results of the blanks (average and dispersion) should be considered, with or without correction of the blank, to evaluate:

the limits of quantification and of detection,

the uncertainty of the measurement.

The average of the blanks and their dispersion should be estimated based on the repetitions carried out with different calibrations. The consistency of the results of the blanks with these references should be verified.

6. Method characteristics

The method characteristics are presented in relation to the described procedure with liquid/liquid extraction.

6.1. Linearity

The linearity (Table 2) was evaluated at 7 levels (see Table 1). For the higher concentration levels, a curve was observed.

Table 2: Linearity

Compound	Working Range (mg/L)	Linear R^2
DIBP	0.0-2.5	1.000
DBP	0.0-2.5	1.000
BBP	0.0-2.5	1.000
DEHP	0.0-3.5	1.000

6.2. Recoveries

Table 3 shows the observed recoveries.

Table 3: Recoveries					
		DIBP	DBP	BBP	DEHP
	Average recovery (%)	91%	93%	101%	98%
	Min. recovery (%)	86%	85%	96%	91%
	Max. recovery (%)	95%	101%	104%	101%

6.3. Repeatability and intermediate precision (intralaboratory)

The analyses to determine the repeatability and intermediate precision have been carried out on a spiked sample (Tables 4 and 5). The intermediate precision corresponds to 2 months of results based on this sample. used as an internal control.

Table 4: Repeatability (mg/L)				
	N° of degrees of freedom	Level	Standard deviation	CV (%)
DIBP	9	0.3135	0.0026	0.8%
DBP	9	0.3290	0.0024	0.7%
BBP	9	0.5141	0.0034	0.7%
DEHP	9	1.4887	0.0159	1.1%

Table 5: Intermediate precision (mg/L)				
	N° of degrees of freedom	Level	Standard deviation	CV (%)
DIBP	28	0.3075	0.0088	2.8%
DBP	28	0.3230	0.0076	2.3%
BBP	28	0.5211	0.0111	2.1%
DEHP	28	1.5213	0.0663	4.4%

6.4. Limits of detection and of quantification

Table 6 shows the evaluated limits of detection and of quantification.

Table 6: limits of detection and of quantification (mg/L)			
	Direction injection Calculated detection limit *	Extraction Calculated detection limit*	Extraction. Quantification limit **
DIBP	0.019	<0.001	0.010
DBP	0.059	<0.001	0.010

BBP	0.100	<0.001	0.010
DEHP	0.033	<0.001	0.050

* according to the calculation based on the signal: noise (S:N) ratio

** limit of quantification taking the blanks into consideration

7. References

1. WENZL T.. 2009. Methods for the determination of phthalates in food. Outcome of a survey conducted among European food control laboratories. EUR 23682 EN – 2009.

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