RESOLUTION OIV/OENO 381/2009

UPDATE OF COMPEMDIUM OF INTERNATIONAL METHODS OF ANAYSIS OF SPIRIT DRINKS OF VITIVINICULTURE ORIGIN– PART 3

THE GENERAL ASSEMBLY

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

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

CONSIDERING the works of the Sub-commission of Methods of Analysis and

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

IN VIEW OF the compendium of international methods of analysis of wine and musts

CONSIDERING that the methods described below are likewise described in the compendium of international methods of analysis of wine and musts with interlaboratory validation parameters.

CONSIDERING that the principle of these methods lies in measuring the parameters concerned with the ethanol fraction so obtained;

DECIDES to incorporate these methods in the new edition of the Compendium of International Methods of Analysis of Spirit Drinks of Vitivinicultural Origin

DETERMINATION BY ISOTOPE RATIO MASS SPECTOMETRY OF THE $^{13}C/^{12}C$ RATIO OF WINE ETHANOL OF SPIRIT DRINKS OF VITIVINICULTURAL ORIGIN.

Type II method

Year: 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.

Certified in conformity Zagreb, 3rd July 2009
The Director General of the OIV
Secretary of the General Assembly
Frederico CASTELLUCCI

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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

□^{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

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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 ‰

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- USGS24	Graphite	-16.1 ‰

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

Name	Material	□^{13}C versus V-PDB (9)
- CRM 656	Wine alcohol	-26.93 ‰
- CRM 657	glucose	-10.75 ‰
- CRM 660	aqueous-alcoholic solution (ABV 12% vol.)	-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 carbon 13 (CAS 00124-38-9)
- Oxidation reagent for the oven and the combustion system as follows: copper oxide (□□) 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

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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 □g 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 (□□) 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

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- 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

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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:

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

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:

- $\delta^{13}C_{ech/V\text{-}PDB}\text{‰} = \delta^{13}C_{ech/ref} + \delta^{13}C_{ref/V\text{-}PDB} + (\delta^{13}C_{ech/ref} \times \delta^{13}C_{ref/V\text{-}PDB})/1000$

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

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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 CHARCTERISTICS (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 δ^{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

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Analysis: ethanol □ ^{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 (□ ^{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

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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 δ^{13}C of ethanol in wine

8 for measuring the δ^{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 δ^{13}C

Samples	Red wine	White wine	Alcohol
Number of laboratories	7	7	8
Number of accepted results	7	7	8
Average value (δ^{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 δ^{13}C

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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 □ ^{13}C determinations are summarized in the table below:

	Wine				Distillates			
Date	N	S_R	S^2_R	R	N	S_R	S^2_R	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

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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 laboratories

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)
5. E.C. Regulation. Community analytical methods which can be applied in the wine sector, N°.2676/90. Detecting enrichment of grape musts, concentrated grape musts, rectified concentrated grape musts and wines by application of nuclear magnetic resonance of deuterium (SNIF-NMR)
6. *Official Journal of the European Communities*, NoL 272, Vol 33, 64-73, 3 October 1990.
7. Inter-laboratory study about the determination of □^{13}C in wine ethanol OIV FV No 1051

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8. Fidelité de la determination du rapport isotopique $^{13}C/^{12}C$ de l'éthanol du vin OIV FV No 1116.
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.

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□FINS ([1]))

Type I method

Year: 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.

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	$(D/H)_I$	$(D/H)_{II}$	$(D/H)_W^Q$	R
Wine – Natural	□	□	□	□

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Wine – Enriched: beet sugard	□	□	□	□
Cane sugar Maize sugar	□	□	□	□

$(D/H)_I$: isotope ratio associated with molecule I

$(D/H)_{II}$: isotope ratio associated with molecule II

$(D/H)_W^Q$: 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.

$(D/H)_W^Q$ 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 $^{13}C/^{12}C$ 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

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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.

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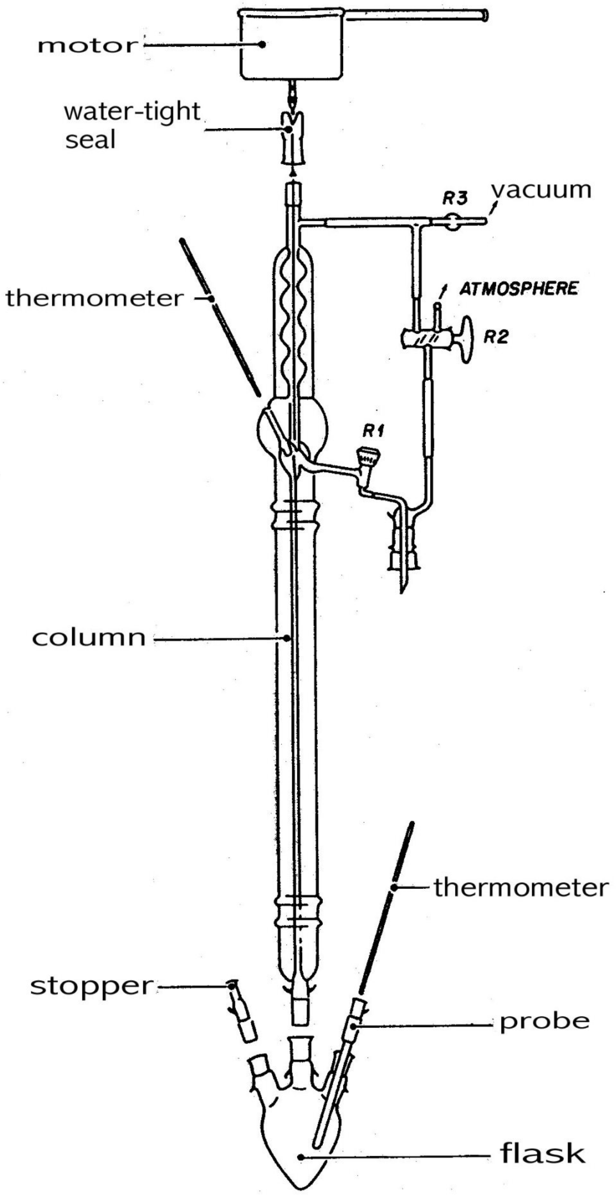

Figure 1 - Apparatus for extracting ethanol

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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:

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

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

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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.

A. 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 10 mm 15 mm
7.05T 9.4T	150 µL 200 µL 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 νo, characteristic of channel Bo (e.g. Bo = 7.05 T, νo = 46.05 MHz and for Bo = 9.4 T, νo = 61.4 MHz) having a decoupling channel (B2) and a field-frequency stabilization channel (lock) at the fluorine frequency.

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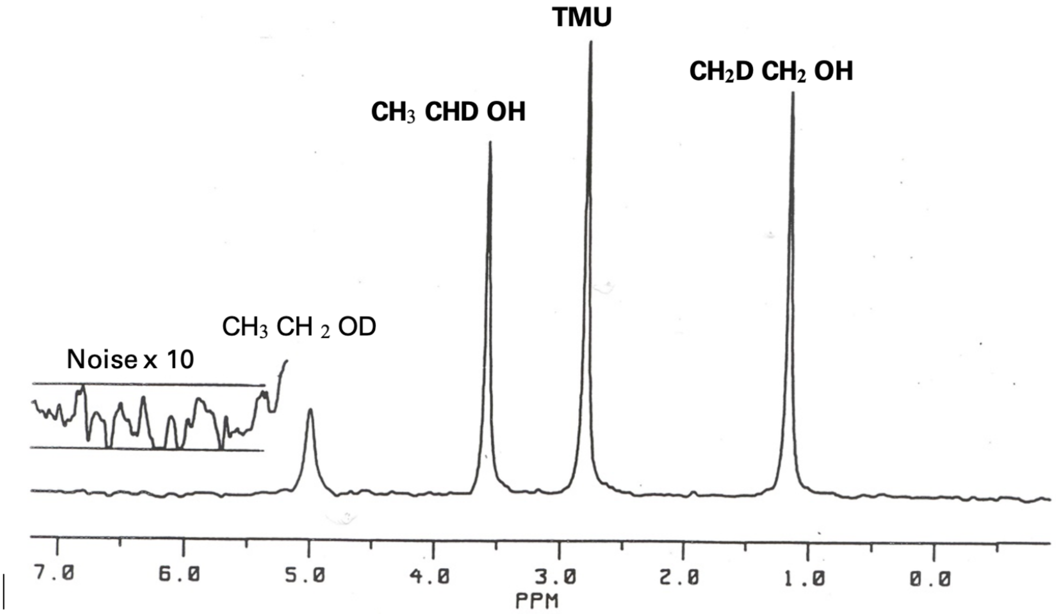

Figure 2a
^{2}H NMR spectrum of an ethanol from wine with an internal standard (TMU: N, N-tetramethylurea)

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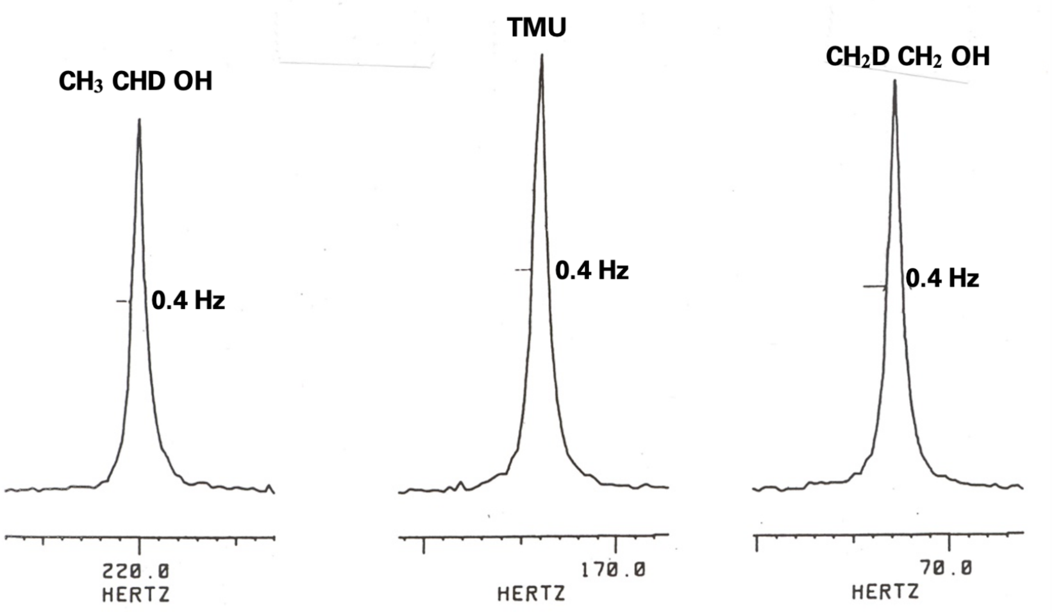

Figure 2b
^{2}H spectrum of ethanol taken under the same conditions as those of Figure 2a, but without exponential multiplication (LB = 0)

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

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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 Cmes, Vmes, Bmes (see 5.3).

Compare them with the given corresponding standard values, denoted by a superscript Cst, Bst, Vst (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;

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- 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 O_2 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
7.05T 9.4T	NS = 304 NS = 200 NS = 200 NS = 128

5. Expression of results

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

$$R = 3\frac{h_{II}}{h_I} = 3\frac{height\ of\ signal\ II\ (CH_3CHD\ OH}{height\ of\ signal\ I\ (CH_2D\ CH_2OH)}$$

$$(D/H)_I = 1{,}5866.T_I.\frac{m_{st}}{m_A}.\frac{(D/H)_{st}}{t_m^D}$$

$$(D/H)_{II} = 2{,}3799.T_{II}.\frac{m_{st}}{m_A}.\frac{(D/H)_{st}}{t_m^D}$$

With

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

$$T_I = \frac{height\ of\ signal\ II\ (CH_3CHDOH)}{height\ of\ signal\ of\ internal\ standard\ (TMU)}$$

- mst 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 $(D/H)_I^{Xmes}$ be the measured value and $(D/H)_I^{Xcorr}$ be the corrected value. This will give:

$$(D/H)_I^{Xcorr} = (D/H)_I^{Bst} + \alpha\left[(D/H)_I^{Xmes} - (D/H)_I^{Bmes}\ (D/H)_I^{Vmes}\right]$$

Where

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

Example:

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

$(D/H)_I^{Vst}$ = 102.0 ppm $(D/H)_I^{Bst}$ = 91.95 ppm

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 and $(D/H)_I^{Xcorr}$= = 99.3 ppm

BIBLIOGRAPHY

1. Martin G.J., Martin M.L., MABON F., *Anal. Chem.,* 1982, **54,** 2380-2382.
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.,
4. Cabanis M.T., Sudraud P., *Sci. Alim.,* 1986, **6,** 385-405.
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.

([1]) **Fractionnement Isotopique Naturel Spécifique étudié par Résonance Magnétique Nucléaire (Site□Specific Natural Isotope Fractionation studied by Nuclear Magnetic Resonance). □ Brevet: France, 81□22710; Europe, 82□402□209□9; USA, 85□4□550□082; Japan 57□123□249.**

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