Method OIV-MA-BS-23 : R2009

Type I method

Determination of the distribution of deuterium in ethanol in spirit drinks of vitivinicultural origin by application of nuclear magnetic resonance of deuterium (SNIF-NMR/RMN-FINS[1])

(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.

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	$(D/H)_I$	$(D/H)_{II}$	$(D/H)^{O}_{W}$	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:

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

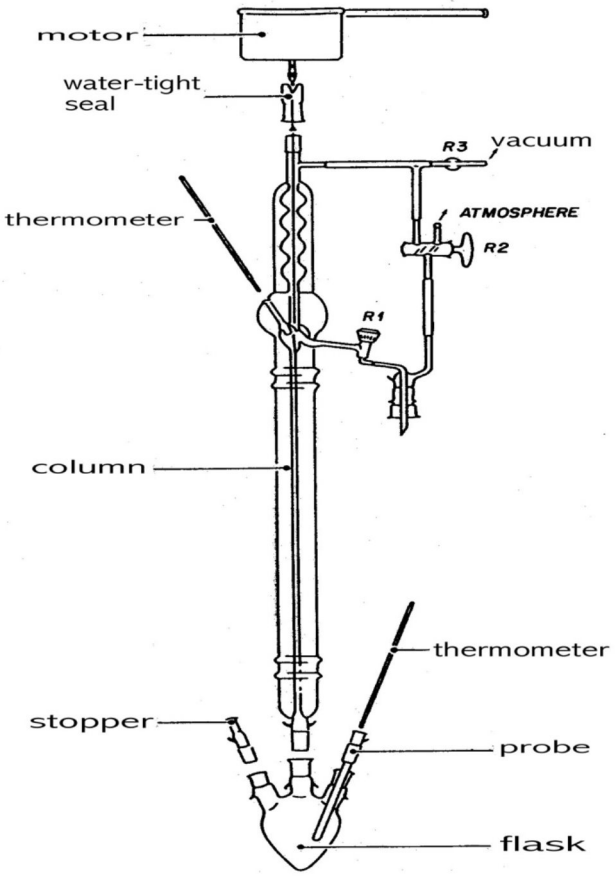


Figure 1 - Apparatus for extracting ethanol

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 *v*o, characteristic of channel Bo (e.g. Bo = 7.05 T, *v*o = 46.05 MHz and for Bo = 9.4 T, *v*o = 61.4 MHz) having a decoupling channel (B2) and a field-frequency stabilization channel (lock) at the fluorine frequency

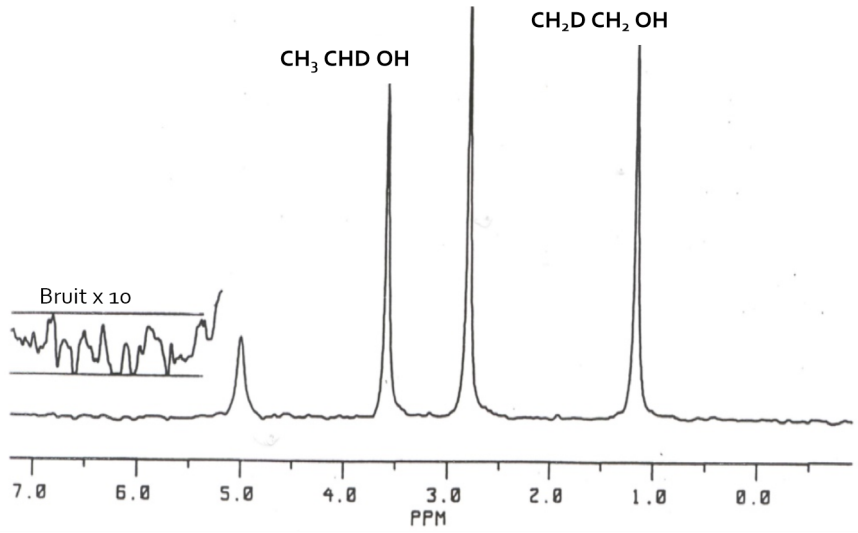


Figure 2a
2H NMR spectrum of an ethanol from wine with an internal standard (TMU: N, N□tetramethylurea)

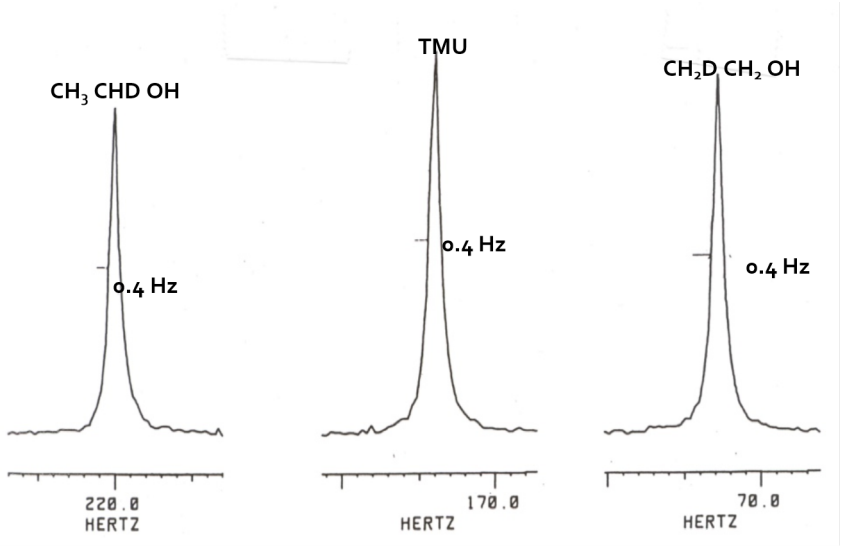


Figure 2b
2H spectrum of ethanol taken under the same conditions as those of Figure 2a, but without exponential multiplication (LB = 0)

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 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;
- 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 CH3- and CH2- 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 9,4 T	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:

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

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

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

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

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

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

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:

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

6. Bibliography

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