Method OIV-MA-VI-20 : R2000
Type IV method

Authentification via fine – NMR® and other isotopic methods in vinegars

(OENO 71/2000)

1. Objective and principle

The main objective for this procedure is to detect and measure synthetic acetic acid in vinegars and also detect any other downgrading of vinegars.

Addition of synthetic acetic acid and other downgrading can be traced because the deuterium concentration of the -CH_3 of this acid is thereby altered.

The method includes the following steps:

- measurement of the acetic acid concentration in the vinegar
- extraction of the acetic acid from the vinegar with ether
- measurement of the percentage of residual water in the purified acetic acid
- measurement of the deuterium in the resulting acetic acid, via NMR.

In order to detect the possible addition of alcohol-vinegar coming from plants whose metabolism is C4, one has also to determine other isotopic parameters, such as the acetic acid $^{13}C/^{12}C$ ratio or the water(D/H), using IRMS (*Isotope Ratio Mass Spectrometry*).

Optical isomers of acetic acid and water which can be detected by this method are:

I	CH_2D-COOH
II	CH_3-COOD
III	HOD

2. Reagents

2.1. Reagents for water measurement using the Karl Fischer method

2.2. N,N- tetramethylurea (TMU); use a TMU reference sample with a given and controlled isotopic ratio

2.3. Trifluoracetic anhydride $(CF_3\text{-}CO)_2O$

2.4. Ethylic ether (HPLC quality)

2.5. Nitrogen gas

3. Devices and utensils

Standard laboratory material, plus:

3.1. Equipment for extracting acetic acid, including:

- a liquid-liquid extractor, with an electric flask-heater and a condenser
- a Cadiot column with rotating strip (mobile, in teflon)
- 125 ml conical flasks with "rodage"
- 250 ml double-"rodage" flask

3.2. Karl Fischer device

3.3. NMR equipment:

- NMR spectrometer with:
- a specific "deuterium" probe set at v frequency characteristic of B field (for example, for B = 9.4 T, v = 61.4 MHz),
- a proton (B_2) decoupler, and a field-frequency stabilizer (*lock*) set at the fluorine frequency.
- automatic sample changer (if necessary)
- data processing software
- 10 mm diameter sample tubes

The resolution measured on the spectrum, transformed without exponential multiplication (LB = 0), and expressed by the width at mid-height of the methyl signal of the TMU, must be lower than 0.7 Hz. The sensitiveness, measured with an exponential multiplication factor LB of 2, must be higher than or equal to 150 for the acetic acid methyl signal of the extract, with a titration of 85% m/m, minimum, of acetic acid.

4. Preparation of the sample

4.1. Extraction of acetic acid in vinegar

Put 350 ml of the vinegar sample into the liquid-liquid extractor. Fill 2/3rd of the 250 ml flask of the extractor with ethylic ether; add a few grains of pumice stone. Heat for 5 hours and bring to boiling point. Collect 100 ml of residual water for analysis of $(D/H)_{water}$ (SMRI).

4.2. Separation of ether from acetic acid

Put the organic phase into the 250 ml double-"rodage" flask (with the pumice stone), and place under the Cadiot column. Heat to bring the ether to boiling point. Install a "rodée" 125 ml conical flask to collect the distillate, collect the boiling liquid between

32° C and 40° C. When the temperature goes over 45° C, stop collecting until the temperature comes back down to 40° C. Collect again the distillate up to 45° C. Repeat this procedure until the temperature, after stopping the collection and operating in closed circuit, doesn't come down again anymore.

Heat to 85° C - 90° C. Introduce the needle into the 250 ml flask, to let nitrogen in. Collect the boiling liquid until 95° C, and stop collecting. Repeat this procedure until temperature goes quickly over 95° C.

The whole distillation process lasts about 4 hours. Then let the flask containing the acetic acid and the water cool down. Transfer to a 100 ml flask.

4.3. Measurement of the percentage of water

From a trial sample close to 0.2 ml of acetic acid solution, of p' mass precisely known, the water content is measured by the Karl Fischer method, that is, p in grams.

The acetic acid mass title is given by:

$$t = 100(p_{,} - p)/p'$$

4.4. Preparation of the sample for the NMR analyses

NMR probe of 10 mm in diameter:

- In a flask which has been tared first, place 1.2 ml of the in-house sample solution (TMU) and weigh to an accuracy of 0.1 mg (m_{st}); then add 3.3 ml of the acetic acid from the distillation procedure and weigh to an accuracy of 0.1 mg (M_A). Add 250 µL of trifluoro-acetic anhydride, to be used as field-frequency stabilizer (*lock*). Shake to homogenize the compounded solution.

5. Measurement of isotopic parameters

5.1. Adjustments

Perform the usual homogeneity and sensitivity adjustments according to the NMR manufacturer's instructions.

The standard variation of repetitiveness over an average of 10 trials for each spectrum must be inferior to 0.8 ppm on $(D/H)_{CH3}$.

5.2. Conditions of acquisition of the NMR spectra

Put the acetic acid sample prepared in 4.4, while filtering it, in a 10 mm tube and introduce it into the probe.

The conditions of acquisition of the NMR spectra are as follows:

- temperature of the probe (for example, 302 K) must be constant
- acquisition time: 6.8 s at least for 1200 Hz of spectral width (memory 16 K), that is: about 20 ppm at 61.4 MHz or 27 ppm at 46.1 MHz
- impulse: 90°
- adjust the time frame for the acquisition; this value must be close to the sampling time (*dwell time*)
- detection in quadrature: adjust the *offset* 01 between the TMU OD signal and CH_2D signal
- determine the value of the 0_2 decoupling *offset* from the protonic spectrum measured by the decoupling spool on the same tube. Use the large strip decoupling method.

Perform, for each spectrum, a sufficient number of NS accumulations to reach the signal/noise ratio indicated in the specifications of the NMR equipment. Repeat NE = 10 times this NS accumulations series. The NS values will depend on the types of spectrometer and probe used. The values could be, for example:

Spectrometer	10 mm Probe
7.05 T	NS = 304
9.4 T	NS = 200
11.7 T	NS = 104

6. Expression of the results

For each of the 10 spectra, determine:

$$(D/H)_{CH3} = 2.0661 - T - m_{st}/m_A - (D/H)_{st}/t$$

with:

- T = surface of the acetic acid CH_3 signal / surface of the TMU CH_3 signal
- t = purity of the acetic acid
- m_{st} and m_A = mass of the in-house standard solution and the acetic acid (see 4.4)
- $(D/H)_{st}$ = isotopic ratio of the in-house standard solution (TMU)

For $(D/H)_{CH3}$, calculate the average for the 10 measurements and the confidence interval.
The advice is to use the EUROSPEC software with the EUROLIS module in order to get the best results, under as for precision and repetitiveness.

7. Bibliography

1. EUROFINS, Authentification of vinegars SNIF-NMR by and other isotopic methods, Laboratoires EUROFINS Nantes (1994).
2. OIV, 1990. Recueil des méthodes Internationales d'analyse des vins et des moûts (Compilation of the international methods of analysis of wines and musts) OIV Paris, France.