RESOLUTION OIV/OENO 353/2009

METHOD FOR 18O/16O ISOTOPE RATIO DETERMINATION OF WATER IN WINE AND MUST

THE GENERAL ASSEMBLY,

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

CONSIDERING the resolution OENO 2/96 related to the determination method of the isotopic ratio 18O/16O of the water content in wines and the collaborative inter-laboratory study,

UPON the proposal of the "Methods of Analysis" Sub-commission,

DECIDES to replace the existing type II method for isotopic ratio 18O/16O determination of water content in wines in Annex A of the Compendium of International Methods of Analysis of Wine and Must with the following method:

Title	Type of the method
Method for 18O/16O isotope ratio determination of water in wine and must	II

1. SCOPE

The method describes the determination of the 18O/16O isotope ratio of water from wine and must after equilibration with CO_2, using the isotope ratio mass spectrometry (IRMS).

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-SMOW : Vienna-Standard Mean Ocean Water (18O/16O = RV-SMOW = 0.0020052)

GISP : Greenland Ice Sheet Precipitation

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

SLAP : Standard Light Antarctic Precipitation

3. DEFINITIONS

18O/16O: Isotope ratio of oxygen 18 to oxygen 16 for a given sample

$\delta 18O_{V-SMOW}$:Relative scale for the expression of the isotope ratio of oxygen 18 to oxygen 16 for a given sample. δ18OV-SMOW is calculated using the following equation:

$$\delta 18O_{V-SMOW} = \left[\frac{\left(\frac{18O}{16O}\right) - \left(\frac{18O}{16O}\right)_{standard}}{\left(\frac{18O}{16O}\right)_{standard}} \right] \times 1000[\%]$$

using the V-SMOW as standard and as reference point for the relative δ scale.

BCR: Community Bureau of Reference

IAEA: International Atomic Energy Agency (Vienna, Austria)

IRMM: Institute for Reference Materials and Measurements

IRMS: Isotope Ratio Mass Spectrometry

m/z: mass to charge ratio

NIST: National Institute of Standards & Technology

RM: Reference Material

4. PRINCIPLE

The technique described thereafter is based on the isotopic equilibration of water in samples of wine or must with a CO_2 standard gas according to the following isotopic exchange reaction:

$$C^{16}O_2 + H_2{}^{18}O \leftrightarrow C^{16}O^{18}O + H_2{}^{16}O$$

After equilibration the carbon dioxide in the gaseous phase is used for analysis by means of Isotopic Ratio Mass Spectrometry (IRMS) where the 18O/16O isotopic ratio is determined on the CO_2 resulting from the equilibration.

5. REAGENTS AND MATERIALS

The materials and consumables depend on the method used (see chapter 6). The systems generally used are based on the equilibration of water in wine or must with CO_2.

The following reference materials, working standards and consumables can be used:

5.1. Reference materials

Name	Issued by	$\delta 18O$ versus $V - SMOW$
V-SMOW, RM 8535	IAEA/ NIST	0 %
BCR-659	IRMM	-7.18%
GISP, RM 8536	IAEA/ NIST	-24.78%
SLAP, RM 8537	IAEA/ NIST	-55.5%

5.2. Working Standards

5.2.1. Carbon dioxide as a secondary reference gas for measurement (CAS 00124-38-9).

5.2.2. Carbon dioxide used for equilibration (depending on the instrument this gas could be the same as 5.2.1 or in the case of continuous flow systems cylinders containing gas mixture helium-carbon dioxide can also be used)

5.2.3 Working Standards with calibrated □18OV-SMOW values traceable to international reference materials.

5.3. Consumables

Helium for analysis (CAS 07440-59-7)

6. APPARATUS

6.1. Isotope ratio mass spectrometry (IRMS)

The Isotope ratio mass spectrometer (IRMS) enables the determination of the relative contents of 13C of CO_2 gas naturally occurring with an internal accuracy of 0.05‰.

Internal accuracy here is defined as the difference between 2 measurements of the same sample of CO2.

The mass spectrometer used for the determination of the isotopic composition of CO_2 gas is generally equipped with a triple collector to simultaneously measure the following ion currents:

- m/z = 44 (12C16O16O)
- m/z = 45 (13C16O16O and 12C17O16O)
- m/z = 46 (12C16O18O, 12C17O17O and 13C17O16O)

By measuring the corresponding intensities, the 18O/16O isotopic ratio is determined from the ratio of intensities of m/z = 46 and m/z = 44 after corrections for isobaric species (12C17O17O and 13C17O16O) whose contributions can be calculated from the actual intensity observed for m/z= 45 and the usual isotopic abundances for 13C and 17O in Nature.

The isotope ratio mass spectrometry must either be equipped with:

- a double introduction system (dual inlet system) to alternately measure the unknown sample and a reference standard.
- or a continuous flow system that transfers quantitatively the CO_2 from the sample vials after equilibration but also the CO_2 standard gas into the mass spectrometer.

6.2. Equipment and Materials

All equipments and materials used must meet stated requirements of the used method / apparatus (as specified by the manufacturer). However, all equipments and materials can be replaced by items with similar performance.

6.2.1 Vials with septa appropriate for the used system

6.2.2 Volumetric pipettes with appropriate tips

6.2.3 Temperature controlled system to carry out the equilibration at constant temperature, typically within ± 1 °C

6.2.4 Vacuum pump (if needed for the used system)

6.2.5 Autosampler (if needed for the used system)

6.2.6 Syringes for sampling (if needed for the used system)

6.2.7 GC Column to separate CO_2 from other elementary gases (if needed for the used system)

6.2.8 Water removal device (e.g. cryo-trap, selective permeable membranes)

7. SAMPLING

Wine and must samples as well as reference materials are used for analysis without any pre-treatment. In the case of the possible fermentation of the sample, benzoic acid (or another anti-fermentation product) should be added or filtered with a with a 0,22 µm pore diameter filter.

Preferably, the reference materials used for calibration and drift-correction should be placed at the beginning and at the end of the sequence and inserted after every ten samples.

8. PROCEDURE

The descriptions that follow refer to procedures generally used for the determination of the 18O/16O isotopic ratios by means of equilibration of water with a CO_2 working standard and the subsequent measurement by IRMS. These procedures can be altered according to changes of equipment and instrumentation provided by the manufacturers as various kind of equilibration devices are available, implying various conditions of operation. Two main technical procedures can be used for introduction of CO_2 into the IRMS either through a dual inlet system or using a continuous flow system. The description of all these technical systems and of the corresponding conditions of operation is not possible. Note: all values given for volumes, temperatures, pressures and time periods are only indicative. Appropriate values must be obtained from specifications provided by the manufacturer and/or determined experimentally.

8.1. Manual equilibration

A defined volume of the sample/standard is transferred into a flask using a pipette. The flask is then attached tightly to the manifold.

Each manifold is cooled down to below – 80 °C to deep-freeze the samples (manifold equipped with capillary opening tubes do not require this freezing step). Subsequently, the whole system is evacuated. After reaching a stable vacuum the gaseous CO_2 working standard is allowed to expand into the various flasks. For the equilibration process each manifold is placed in a temperature controlled water-bath typically at 25°C (± 1 °C) for 12 hours (overnight). It is crucial that the temperature of the water-bath is kept constant and homogeneous.

After the equilibration process is completed, the resulting CO_2 is transferred from the flasks to the sample side bellow of the dual inlet system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet. This approach is repeated till the last sample of the sequence has been measured.

8.2. Use of an automatic equilibration apparatus

A defined volume of the sample/standard is transferred into a vial using a pipette. The sample vials are attached to the equilibration system and cooled down to below – 80 °C to deep-freeze the samples (systems equipped with capillary opening tubes do not require this freezing step). Subsequently, the whole system is evacuated.

After reaching a stable vacuum the gaseous CO_2 working standard is expanded into the vials. Equilibrium is reached at a temperature of typically 22 ± 1 °C after a minimum period of 5 hours and with moderate agitation (if available). Since the equilibration duration depends on various parameters (e.g. the vial geometry, temperature, applied agitation ...), the minimum equilibrium time should be determined experimentally.

After the equilibration process is completed, the resulting CO_2 is transferred from the vials to the sample side bellow of the dual inlet system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet. This approach is repeated till the last sample of the sequence has been measured.

8.3. Manual preparation manual and automatic equilibration and analysis with a dual inlet IRMS

A defined volume of sample / standard (eg. 200 μL) is introduced into a vial using a pipette. The open vials are then placed in a closed chamber filled with the CO_2 used for equilibration (5.2.2). After several purges to eliminate any trace of air, the vials are closed and then placed on the thermostated plate of the sample changer. The equilibration is reached after at least 8 hours at 40 °C. Once the process of equilibration completed, the CO_2 obtained is dried and then transferred into the sample side of the dual inlet introduction system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet. This approach is repeated till the last sample of the sequence has been measured.

8.4. Use of an automatic equilibration apparatus coupled to a continuous flow system

A defined volume of the sample/standard is transferred into a vial using a pipette. The sample vials are placed into a temperature controlled tray.

Using a gas syringe the vials are flushed with mixture of He and CO_2 . The CO_2 remains in the headspace of the vials for equilibration.

Equilibrium is reached at a temperature typically of 30 ± 1 °C after a minimum period of 18 hours.

After the equilibration process is completed the resulting CO_2 is transferred by means of the continuous flow system into the ion source of the mass spectrometer. CO_2 reference gas is also introduced into the IRMS by means of the continuous flow system. The measurement is carried out according to a specific protocol for each kind of equipment.

9. CALCULATION

The intensities for m/z = 44, 45, 46 are recorded for each sample and reference materials analysed in a batch of measurements. The 18O/16O isotope ratios are then calculated by the computer and the software of the IRMS instrument according to the principles explained in section 6.1. In practice the 18O/16O isotope ratios are measured against a working standard previously calibrated against the V-SMOW. Small variations may occur while measuring on line due to changes in the instrumental conditions. In such a case the δ 18O of the samples must be corrected according to the difference in the δ 18O value from the working standard and its assigned value, which was calibrated beforehand against V-SMOW. Between two measurements of the working standard, the variation is the correction applied to the sample results that may be assumed to be linear. Indeed, the working standard must be measured at the beginning and at the end of all sample series. Therefore a correction can be calculated for each sample using linear interpolation between two values (the difference between the assigned value of the working standard and the measurements of the obtained values).

The final results are presented as relative δ18OV-SMOW values expressed in ‰.

$\delta 18O_{V-SMOW}$ values are calculated using the following equation:

$$\delta 18O_{V-SMOW} = \left[\frac{(\frac{18O}{16O})_{sample} - (\frac{18O}{16O})_{V-SMOW}}{(\frac{18O}{16O})_{V-SMOW}}\right] \times 1000\ [\%]$$

The $\delta 18O$ value normalized versus the V-SMOW/SLAP scale is calculated using the following equation:

$$\delta 18O_{V-SMOW/SLAP} = \left[\frac{\delta 18O_{sample} - \delta 18O_{V-SMOW}}{\delta 18O_{V-SMOW} - \delta 18O_{SLAP}}\right] \times 55.5\ [\%]$$

The $\delta 18O_{V-SMOW}$ value accepted for SLAP is -55.5‰ (see also 5.1).

10. PRECISION

The repeatability (r) is equal to 0.24 ‰.

The reproducibility (R) is equal to 0.50 ‰.

Summary of statistical results

	General average (‰)		Standard deviation of repeatability (‰) sr		Repeatability (‰) r		Standard deviation of reproducibility (‰) sR		Reproducibility (‰) R
Water									
Sample 1	-8.20		0.068		0.19		0.171		0.48
Sample 2	-8.22		0.096		0.27		0.136		0.38
Wine N° 1									
Sample 5	6.87		0.098		0.27		0.220		0.62
Sample 8	6.02		0.074		0.21		0.167		0.47

Sample 9	5.19		0.094		0.26		0.194		0.54
Sample 4	3.59		0.106		0.30		0.205		0.57
Wine N° 2									
Sample 3	-1.54		0.065		0.18		0.165		0.46
Sample 6	-1.79		0.078		0.22		0.141		0.40
Sample 7	-2.04		0.089		0.25		0.173		0.49
Sample 10	-2.61		0.103		0.29		0.200		0.56

11. INTER-LABORATORIES STUDIES

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