RESOLUTION OIV-OENO 511-2013

METHOD FOR 18O/16O ISOTOPE RATIO DETERMINATION OF WATER IN WINE VINEGAR USING ISOTOPIC MASS SPECTROMETRY

THE GENERAL ASSEMBLY,

CONSIDERING Article 2 paragraph 2 iv of the agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine;

CONSIDERING resolution Oeno 2/96 related to the determination of the 18O/16O isotope ratio of the water content in wine and the collaborative inter-laboratory study;

CONSIDERING resolution OIV/OENO 353/2009, “method for 18O/16O isotope ratio determination of water in wines and must”;

On the proposal of the “Methods of Analysis” Sub-commission;

DECIDES to complete the “Compendium of International Methods of Analysis of wine vinegars” with the following method:

1. Title

Method for $^{18}O/^{16}O$ isotope ratio determination of water in wine vinegar using isotopic mass spectrometry

Method type: II

2. Object/Scope of application

The method enables the determination of the $^{18}O/^{16}O$ isotope ratio of water in wine vinegar after equilibration with CO_2 , using isotope ratio mass spectrometry (IRMS).

3. Definitions

$^{18}O/^{16}O$:Isotope ratio of oxygen 18 to oxygen 16 in a given sample.

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

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

using the V-SMOW standard as a reference for the relative □ 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: U.S. National Institute of Standards & Technology
RM: Reference Material

V-SMOW: Vienna Standard Mean Ocean Water ($^{18}O/^{16}O = R_{V-SMOW} = 0.0020052$)
GISP : Greenland Ice Sheet Precipitation
SLAP: Standard Light Antarctic Precipitation

4. Principle

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

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

After equilibration, the carbon dioxide in the gaseous phase is used to analyse the $^{18}O/^{16}O$ isotopic ratio by means of Isotopic Ratio Mass Spectrometry (IRMS).

5. Reagents and materials

The materials and consumables depend on the method used (see point 6).
The following reference materials, working solutions and consumables can be used:

5.1. Reference materials

Name	Issuing organisation	□^{18}O according to 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 solutions

5.2.1. Carbon dioxide (or carbonic anhydride) as a secondary reference gas for measurement (CAS N° 00124-38-9).

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

5.2.3.Working solutions with $\delta^{18}O_{V-SMOW}$ values calibrated according to international reference materials.

5.3. Consumables

Helium for analysis (CAS N° 07440-59-7)

6. Apparatus

6.1. Isotope ratio mass spectrometer

The Isotope ratio mass spectrometer (IRMS) enables the determination of the relative contents of ^{18}O and ^{16}O in CO_2 gas with an internal precision of 0.05‰. Internal precision is defined here as the difference between two measurements of the same sample of CO_2.

The mass spectrometer designed for the determination of the isotopic composition of CO2 gas is generally equipped with a triple collector capable of simultaneously measuring the following ion currents:

- m/z = 44 ($^{12}C^{16}O^{16}O$)
- m/z = 45 ($^{13}C^{16}O^{16}O$ and $^{12}C^{17}O^{16}O$)
- m/z = 46 ($^{12}C^{16}O^{18}O$, $^{12}C^{17}O^{17}O$ and $^{13}C^{17}O^{16}O$)

When measuring the corresponding intensities, the $^{18}O/^{16}O$ isotopic ratio is determined from the m/z = 46 and m/z = 44 intensity ratios after conducting a series of corrections for $^{12}C^{17}O^{17}O$ and $^{13}C^{17}O^{16}O$ isobaric species, whose contributions can be calculated from the actual intensity observed for m/z= 45 and from the usual isotopic abundances for ^{13}C and ^{17}O in nature.

The isotope ratio mass spectrometer must either be equipped with:

- a double entry system (dual inlet system) to alternately measure the sample and a reference sample,
- or a continuous flow system that transfers quantitatively the CO2 from the sample vials after equilibration and the CO_2 standard gas into the mass spectrometer.

6.2. Equipment and materials

All equipment and materials used must satisfy the stated requirements of the method or of the apparatus used (as specified by the manufacturer). However, other equipment and materials of equivalent performance can also be used.

6.2.1. Vials with septa appropriate for the system used

6.2.2. Volumetric pipettes with appropriate tips

6.2.3. Temperature control system to carry out the isotopic exchange reaction at a constant temperature (typically within ±1°C)

6.2.4. Vacuum pump (if needed for the system used)

6.2.5. Autosampler (if needed for the system used)

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

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

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

7. Preparation of the samples

Wine vinegar samples as well as reference materials are used for analysis without any pre-treatment. If the sample contains impurities, it should be filtered with a 0.22 µm pore diameter filter.

Preferably, the reference materials used for calibration and data correction should be placed at the beginning and at the end of each series and inserted after every ten samples.

8. Procedure

The procedure generally used for the determination of the $^{18}O/^{16}O$ isotope ratio by means of equilibration of water with a CO_2 working solution and subsequent analysis by IRMS is presented below. These procedures can be altered according to the apparatus and equipment used, given that various kinds of equilibration devices with different functions are available. The two main technical procedures for introduction of CO_2 into the IRMS provide a dual inlet system and a continuous flow system respectively.

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 or determined experimentally.

8.1. Manual equilibration

A defined volume of the sample/working solution 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 (manifolds equipped with capillary opening tubes do not require this freezing phase). Subsequently, the whole system is evacuated.

After reaching a stable vacuum, the CO_2 resulting from the working solution in the round bottom flasks is distributed. For the equilibration process, each manifold is placed in a water-bath 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 complete, 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 the isotope ratios of the CO_2 contained in the sample side with those of the CO2 contained in the working solution side (gas reference standard:

CO_2) of the dual inlet. This approach is repeated till the last sample of the sequence has been measured.

8.2. Use of automatic equilibration apparatus

A defined volume of the sample/working solution is transferred into a round bottom flask using a pipette. The flasks containing the sample are attached to the equilibration apparatus 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 CO_2 resulting from the working solution in the round bottom flasks is distributed. Equilibrium is reached at a temperature of 22 ± 1°C after a minimum period of 5 hours with moderate stirring (if possible). Since the equilibration duration depends on various parameters (e.g. the flask geometry, temperature, amount of stirring), the minimum equilibrium time required should be determined experimentally.

After the equilibration process is complete, the resulting CO_2 is transferred from the flasks to the sample -side bellows of the dual inlet system. Analysis is carried out by comparing the isotope ratios of the CO_2 contained in the sample side with those of the CO_2 contained in the working-solution side of the dual inlet. This approach is repeated till the last sample of the sequence has been measured.

8.2.1. Manual preparation and automatic equilibration and analysis using a dual inlet system coupled to a continuous flow IRMS

A defined volume of sample/working solution (e.g. 200 µL) is transferred to 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 thermal tray of the autosampler. The equilibration is reached after at least 8 hours at 40°C. Once the process of equilibration is complete, the CO_2 obtained is dried and then transferred into the sample side of the dual inlet system. The measurements are performed by comparing the isotope ratios of the CO_2 contained in the sample side and the working solution side (CO_2 reference standard gas) of the dual inlet several times. This process is repeated until the last sample of the sequence has been measured.

8.3. Use of automatic equilibration apparatus by means of a continuous flow system

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

A mixture of He and CO_2 is added to the vials using a gas syringe. The CO_2 remains in the top of the vials for equilibration.

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

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

9. Calculations

The intensities for m/z = 44, 45, 46 are recorded for each sample and reference material analysed in a series of measurements. The $^{18}O/^{16}O$ isotope ratios are then calculated by a computer, using the IRMS apparatus' software, according to the principles explained in point 6.1. In practice, the $^{18}O/^{16}O$ isotope ratios are measured against a working solution previously calibrated against the V-SMOW. Changes in the instrumental conditions while measuring on line may entail small variations. If this occurs, the $\delta^{18}O$ of the samples must be corrected according to the difference in the $\delta^{18}O$ value from the working solution and its assigned value, which was calibrated beforehand against V-SMOW. The correction that is applied to the samples varies in a linear fashion with respect to the values of the two working solutions that precede and follow the samples. Indeed, the working solution must be measured at the beginning and at the end of all sample series. A correction can therefore be calculated for each sample using linear interpolation between two values (the difference between the assigned value and the measured value of the working solution).

The final results are presented as $\delta^{18}O_{V-SMOW}$ values expressed in ‰.

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

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

The $\delta^{18}O$ value normalized with respect to the V-SMOW/SLAP scale is calculated according to the following equation:

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

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

10. Validation of the method

An international collaborative study was carried out (Thomas and Jamin, 2009) for validation of the method.

The repeatability limit (r) is, on average, 0.15‰.

The reproducibility limit (R) is, on average, 0.54‰.

The data and results of the international collaborative study are presented in Annex A.

11. Bibliography

1. OIV-MA-AS1-08: Reliability of analytical results (Resolution 5/99)
2. OIV-MA-AS1-09: Protocol for the design, conducts and interpretation of collaborative studies (Resolution 6/2000)
3. OIV-MA-AS2-12: Method for 18O/16O isotope ratio determination of water in wines and must
4. IUPAC Protocol in W. Horwitz, Pure Appl. Chem. 67 (2) (1995) 331
5. Thomas, F., Jamin, E. (2009). (2)H NMR and (13)C-IRMS analyses of acetic acid from vinegar, (18)O-IRMS analysis of water in vinegar: International collaborative study report. Analytica Chimica Acta, 649, 98-105

ANNEX A

RESULTS OF THE INTERNATIONAL COLLABORATIVE STUDY

This document sets out the results of the study for the validation of the method of

analysis of the $^{18}O/^{16}O$ ratio of water in wine vinegar (Thomas and Jamin 2009).
The study was carried out in accordance with documents OIV-MA-AS1-08 and OIV-MA-AS1-09.

1. Participating laboratories

14 laboratories participated in the study:

Bundesinstitut für Risikobewertung, Berlin	Germany
Central Science Laboratory, York	United Kingdom
Custom Technical Laboratory, Prague	Czech Republic
Chemical Institute of the Hungarian Customs and Finance Guard, Budapest	Hungary
Eurofins, Nantes	France
FEM-IASMA, San Michele all'Adige, Trento	Italy
Joint Research Center, Ispra	Italy
Arbitral Agroalimentario Del MAPA, Madrid	Spain
Landesuntersuchungsamt, Speyer	Germany
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Wurzburg	Germany
Service Commun des Laboratoires, Bordeaux	France
Service Commun des Laboratoires, Montpellier	France
Service Commun des Laboratoires, Paris	France
Unione Italiana Vini, Verona	Italy

2. Samples

The study was carried out on 5 double-blind samples: three wine vinegar samples (B, C, D), an alcohol vinegar sample (A) and a wine vinegar sample with 20% of A (Thomas and Jamin, 2009).

For each sample, the $\delta^{18}C$ of the water was analysed. The results are indicated in the following table.

3. Statistical evaluation

Statistical calculations were performed according to the OIV-MA-AS1-09 protocol, which is based on Horwitz' IUPAC protocol. Outliers were removed in the following way: Cochran tests for removal of the laboratories with the highest variance; single- and pair-value Grubbs tests for individual or paired individual outliers; then more Cochran tests, etc., keeping a proportion of outliers <2/9. The standard deviations of repeatability (sr) and of reproducibility (sR) were then calculated for each material from valid results pairs from the blind duplicates.

For more information, refer to the publication by Thomas and Jamin, 2009.

Description of the sample	N° of valid results	N° of replicates	□^{13}C average (‰)	s$_r$ (‰)	Repeatability limit r (2.8×S$_r$) ‰	s$_R$ (‰)	Reproducibility limit R (2.8× SR) ‰
A	8	2	-6.96	0.06	0.17	0.22	0.62
B	8	2	-0.86	0.08	0.22	0.21	0.59
C	8	2	-1.38	0.02	0.06	0.2	0.56
B+20%A	8	2	-1.79	0.05	0.14	0.2	0.56
D	5	2	-1.23	0.05	0.14	0.14	0.39

The average repeatability limit (r) was 0.15‰, and the average reproducibility limit (R) was 0.54‰, comparable to the values observed for wine alcohol (OIV-MA-AS2-12).