RESOLUTION OIV-OENO 662H-2022

METHOD FOR 180/160 ISOTOPE RATIO DETERMINATION OF WATER IN GRAPE JUICE

Type IV methods

THE GENERAL ASSEMBLY,

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

AT THE PROPOSAL of the "Methods of Analysis" Sub-Commission,

CONSIDERING that for grape juice the determination could be performed based on the method OIV-MA-AS2-12 of the *Compendium of international methods of wine and must analysis*, with a modification added at the points:

DECIDES to add the following method:

METHOD FOR $^{18}O/^{16}O$ ISOTOPE RATIO DETERMINATION OF WATER IN GRAPE JUICE

1. Scope

The method describes the determination of the isotopic ratio $^{18}O/^{16}O$ of water from grape juice after equilibration with CO_2, using the isotope ratio mass spectrometry.

2. Definitions

Oxygen isotopic ratio $^{18}O/^{16}O$ has been used to identify the water added in the grape juice.

3. Principle

The method is based on the equilibration of water from the grape juice with CO_2, according to the following isotopic exchange reaction:

C16O2 + H218O ◻ C16O18O + H216O

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After equilibration the carbon dioxide in the gaseous phase is used for analysis by means of Isotopic Ratio Mass Spectrometry (IRMS) where the $^{18}O/^{16}O$ isotopic ratio is determined on the CO2 resulting from the equilibration.

4. Reagents and materials

The materials and consumables depend on the method used. The systems generally used are based on the equilibration of water in grape juice with CO_2.

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

4.1. Reference materials

NAME	Issued by	$\delta^{18}O$ related to V-SMOW
VSMOW2	NIST/IAEA	0 ‰
GISP	NIST	-24.78 ‰
SLAP	NIST/IAEA	-55.5 ‰

4.2. Working Standards

4.2.1. Carbon dioxide used for equilibration in the case of continuous flow systems cylinders containing gas mixture helium-carbon dioxide can also be used).

4.2.2. Working Standards with calibrated $\delta^{18}O$ VSMOW values traceable to international reference materials.

4.3. Consumables

- Helium for analysis (CAS 07440-59-7);
- Carbon dioxide for analysis, used as a reference gas (CAS 00124-38-9);

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5. Apparatus

The equipment and interfaces depend on the method and can be used as follow

5.1. Isotope Ratio Mass Spectrometer (IRMS)

Isotope ratio mass spectrometer (IRMS) is able for determining the relative 18O content of CO2 naturally occurring with an internal accuracy of 0.05 ‰ or better expressed as a relative value. The mass spectrometer used to measure isotope ratios is generally equipped with a triple collector to simultaneously measure intensities for m/z = 44, 45 and 46.

5.2. Equipment and Materials

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

5.2.1. Vials with septa appropriate for the used system;

5.2.2. Volumetric pipettes with appropriate tips;

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

5.2.4. Vacuum pump (if needed for the used system);

5.2.5. Autosampler (if needed for the used system);

5.2.6. Syringes for sampling (if needed for the used system);

5.2.7. GC Column to separate CO2 from other elementary gases (if needed for the used system).

5.2.8. Water removal device (e.g. selective permeable membrane)

6. Sampling

Juice samples as well as a reference material are used for analysis without any pre-treatment. Possible fermentation must be avoided adding benzoic acid (or another anti fermentation product) or filtered by membrane with pore diameter of 0.22 µm.

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7. Procedure

The descriptions that follow refer to procedures generally used for the determination of the $^{18}O/^{16}O$ 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 technical systems and the corresponding operation conditions are given as an example

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.

7.1. 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 ($\pm$ 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.

7.2. 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

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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.

7.3. 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 (4.2.1). 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.

7.4. 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.

The temperature and the time for equilibration should be specified according to the manufacturer and/or determined experimentally. The equilibrium is normally reached at a temperature typically of 25 ± 1 °C after a minimum period of 18 hours.

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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.

8. Calculation

The intensities for m/z = 44, 45, 46 are recorded for each sample and reference materials analysed. The 18O/16O isotope ratios are then calculated by the computer and the software of the IRMS instrument. 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 online due to changes in the instrumental conditions. In such a case the □18O of the samples must be corrected according to the difference in the □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).

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

The □^{18}O value normalized versus the V-SMOW/SLAP scale is calculated using the following equation:

$$\delta^{18}O_{V-SMOW/SLAP} = \left[\frac{\delta^{18}O_{échantillon} - \delta^{18}O_{V-SMOW}}{\delta^{18}O_{V-SMOW} - \delta^{18}O_{SLAP}}\right] \times 55{,}5[\%]$$

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9. Characteristics of the method

A validation study was carried out for the purpose of assessing the suitability of the method for the matrices in question, taking into account linearity, limits of detection and quantification and the accuracy of the method. The latter parameter was determined by defining the levels of precision and trueness of the method.

9.1. Precision of the method

The parameters taken into account were repeatability and intralaboratory reproducibility. Table 1 shows the values of these parameters. The repeatability was expressed as ‰ of measurements repeated in the same conditions and in the same day for all the grape juice. And the reproducibility was expressed as the average of a ‰ of measurements of the same grape juice sample, made by two operators.

9.2. Trueness of the method

The recovery percentage was determined using a grape juice sample spiked with 6 concentrations of tap water, ranging from 20% to 99.5%. Each level was analyzed 5 times. The trueness was also expressed in terms of bias using a reference material and calculating the relative error.

Table 1. Characteristics do the method

Correlation coefficient (R2)	Repeatability (n=16) ‰	Reproducibility (n=7) ‰	Recovery (%) ± SD	Trueness (RM) (‰)
0.998	0.20	0.29	101± 2.16	0.17

10. Bibliography

1. OIV. Method for 18O/16O isotope ratio determination of water in wines and must. Method OIV-MA-AS2-12.

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