
Kis - Izotopska analiza očetne kisline in vode - 3. del: 18O-IRMS-analiza vode v vinskem kisu

Vinegar - Isotopic analysis of acetic acid and water - Part 3: 18O-IRMS analysis of water in wine vinegar

Essig - Isotopenanalyse von Essig und Wasser - Teil 3: 18O-IRMS-Analyse von Wasser in Weinessig

Vinaigre - Analyse isotopique de l'acide acétique et de l'eau - Partie 3: Analyse SMRI-18O de l'eau

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Vinegar - Isotopic analysis of acetic acid and water - Part 3:
¹⁸O-IRMS analysis of water in wine vinegar

Vinaigre - Analyse isotopique de l'acide acétique et de l'eau -
Partie 3: Analyse SMRI-¹⁸O de l'eau dans le vinaigre de vin

Essig - Isotopenanalyse von Essig und Wasser - Teil 3:
¹⁸O-IRMS-Analyse von Wasser in Weinessig

This European Standard was approved by CEN on 3 November 2012.

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Foreword

This document (EN 16466-3:2013) has been based on an international collaborative study of the method published in *Analytica Chimica Acta* 649 (2009) 98-105, and organised under the auspices of the Permanent International Vinegar Committee (CPIV, Brussels).

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 2013, and conflicting national standards shall be withdrawn at the latest by July 2013.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

The European standard, *Vinegar — Isotopic analysis of acetic acid and water*, consists of the following parts:

- *Part 1: ^2H -NMR analysis of acetic acid;*
- *Part 2: ^{13}C -IRMS analysis of acetic acid;*
- *Part 3: ^{18}O -IRMS analysis of water.*

According to the CEN/CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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Introduction

Wine vinegar is defined by the European Regulations 479/2008 and 491/2009 as the product obtained exclusively from the acetous fermentation of wine, which is in turn defined as the product exclusively obtained from the alcoholic fermentation of fresh grapes, whether crushed or not, or of grape must.

In accordance with this, it is clear that the production of wine vinegar by fermenting dried grapes and rehydrating with tap water is not allowed by European Regulations.

The isotopic analysis of water from vinegar by ^{18}O -IRMS enables the distinction of wine vinegar and vinegars from fermented dried grapes which have been rehydrated with water [1], and more generally helps to check the authenticity of wine vinegar.

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1 Scope

This European Standard specifies an isotopic method to control the authenticity of wine vinegar. This method is applicable on wine vinegar in order to characterise the $^{18}\text{O}/^{16}\text{O}$ ratio of water, and allows differentiating wine vinegar from vinegars made from raisins or alcohol vinegar.

NOTE The Oxygen 18 isotopic analysis of water from vinegar is based on a similar method already normalised for wine analysis [2].

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

Not applicable.

3 Principle

The $^{18}\text{O}/^{16}\text{O}$ ratio of water from vinegar is determined on CO_2 gas after equilibration of reference CO_2 gas with vinegar water according to the following isotopic exchange reaction:



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

4 Reagents

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

4.1 Carbon dioxide

For analysis, used as secondary reference gas for the determination of $^{13}\text{C}/^{12}\text{C}$ ratio. Purity 5.2 minimum.

4.2 Carbon dioxide used for equilibration

Depending on the instrument, this gas could be the same as 4.1 or in the case of continuous flow systems cylinders containing gas mixture helium-carbon dioxide can also be used

5 Apparatus

All equipments and materials used shall 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.

5.1 Vials with septa appropriate for the used system.

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- 5.2** Volumetric pipettes with appropriate tips.
- 5.3** Temperature controlled system to carry out the equilibration at constant temperature, typically within ± 1 °C.
- 5.4** Vacuum pump (if needed for the used system).
- 5.5** Autosampler (if needed for the used system).
- 5.6** Syringes for sampling (if needed for the used system).
- 5.7** GC Column to separate CO₂ from other elementary gases (if needed for the used system).
- 5.8** Water removal device (e.g. cryo-trap, selective permeable membranes).

6 Procedure**6.1 General**

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 CO₂ 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 kinds of equilibration devices are available, implying various conditions of operation. Two main technical procedures can be used for introduction of CO₂ 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.

All values given for volumes, temperatures, pressures and time periods are only indicative. Appropriate values shall be obtained from specifications provided by the manufacturer and/or determined experimentally.

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6.2 Manual method

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 CO₂ working standard is allowed to expand into the various flasks. For the equilibration process each manifold is placed in a temperature controlled waterbath typically at 25 °C (± 1 °C) for 12 h (overnight). It is crucial that the temperature of the water-bath is kept constant and homogeneous.

After the equilibration process is completed, the resulting CO₂ 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 CO₂ contained in the sample side and the standard side (CO₂ reference standard gas) of the dual inlet.

This approach is repeated till the last sample of the sequence has been measured.

6.3 Use of an automatic exchange 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 CO₂ working standard is expanded into the vials. Equilibrium is reached at a temperature of typically (22 \pm 1) °C after a minimum period of 5 h 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 CO₂ is transferred from the vials to the sample side below of the dual inlet system. The measurements are performed by comparing several times the ratios of the CO₂ contained in the sample side and the standard side (CO₂ reference standard gas) of the dual inlet.

This approach is repeated till the last sample of the sequence has been measured.

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

A defined volume of sample / standard (e.g. 200 µl) is introduced into a vial using a pipette. The open vials are then placed in a closed chamber filled with the CO₂ used for equilibration (4.2). 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 h at 40 °C. Once the process of equilibration completed, the CO₂ 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 CO₂ contained in the sample side and the standard side (CO₂ reference standard gas) of the dual inlet.

This approach is repeated until the last sample of the sequence has been measured.

6.5 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 CO₂. The CO₂ 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 h. After the equilibration process is completed the resulting CO₂ is transferred by means of the continuous flow system into the ion source of the mass spectrometer. CO₂ 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.

6.6 Calculation and expression of the results

The purpose of the method is to measure the ¹⁸O/¹⁶O ratio of water extracted from vinegar. The ¹⁸O/¹⁶O isotope ratio can be expressed by its deviation from a working reference. The isotopic deviation of oxygen 18 (δ¹⁸O) is then calculated on a delta scale per thousand (‰) by comparing the results obtained for the sample to be measured with those for a working reference previously calibrated on the basis of the primary international reference (V.SMOW2). The δ¹⁸O values (in ‰) are expressed in relation to the working reference as follows:

$$\delta^{18}\text{O} = \frac{R(^{18}\text{O}/^{16}\text{O})_{\text{sample}} - R(^{18}\text{O}/^{16}\text{O})_{\text{standard (V.SMOW2)}}}{R(^{18}\text{O}/^{16}\text{O})_{\text{standard (V.SMOW2)}}} \quad (2)$$

where R_{sample} and R_{standard} are respectively the ¹⁸O/¹⁶O isotope ratios of the sample and of the standard.

Between two measurements of the standard working sample, the variation, and therefore the correction to be applied to the results obtained from the samples, may be assumed to be linear. The standard working sample shall be measured at the beginning and at the end of all sample series. A correction can then be calculated for each sample according to its position in the sequence using linear interpolation.