STEP	1	2	3	4	5	6	7	8
DATE		03/2021	10/2021					
		03/2022	10/2022					
		03/2023	10/2023					

PRELIMINARY DRAFT RESOLUTION
OENO-SCMA 21-688 Et3

WINE – DETERMINATION OF RELATIVE RATIO OF NON-EXCHANGEABLE HYDROGEN STABLE ISOTOPES IN ETHANOL OBTAINED FROM THE PRODUCTS DERIVED FROM THE VINE BY MEANS OF INSTRUMENTAL TECHNIQUE EIM - IRMS

Type IV Methods

THE GENERAL ASSEMBLY,

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

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

DECIDES to add the following method to the *Compendium of International Methods of Wine and Must Analysis*:

Determination of relative ratio of non-exchangeable hydrogen stable isotopes in ethanol obtained from the products derived from the vine by means of instrumental technique EIM – IRMS[1]

Type IV Methods

1. Introduction

This resolution establishes a method for determining the relative ratio of non-exchangeable hydrogen stable isotopes in ethanol of products derived from vine.

2. Scope

The method enables measurement of the relative ratio of non-exchangeable hydrogen stable isotopes $(D/H)_n$ in ethanol obtained from products derived from vine.

3. Definitions

On the following documents as a whole or to parts of these documents are the normative reference for this document, and they are necessary for its implementation. For dated references, only cited editions are applied. For undated references, the latest editions of the applicable reference document (including any amendments) are applied.

[1] EIM-IRMS (Ethanol Isotope Measurement – Isotope Ratio Mass Spectrometry) Brevet: République de Serbie, 52615; Europe, EP08793979.9; la Fédération de Russie, 2477855; États Unis: 9,005,979

EN ISO 4797,	Laboratory glassware of glass - flasks for boiling with conical ground joints
EN ISO 1042,	Laboratory glassware of glass - Measuring flasks with one line
OIV-MA-AS311-01A[2]	Reducing substances
OIV-MA-AS312-01A[2]	Alcoholic strength by volume (Pyknometer, using frekvencionog oscillator or hydrostatic weighing)
OIV-MA-AS323-04B[2]	Sulfur Dioxide

Further for the purposes of this resolution, the following terms and definitions are applicable:

3.1 **Relative ratio of non-exchangeable hydrogen hydrogen stable isotopes in the test sample $(D/H)_n$**

relative ratio of non-exchangeable deuterium and hydrogen atoms in the test sample is determined by isotope ratio mass spectrometry (IRMS).

3.2 Ethanol δD_n value

deviation of the relative ratio of non-exchangeable hydrogen stable isotopes in ethanol $(D/H)_n$ in regard to relative ratio of non-exchangeable hydrogen stable isotopes in the **AAWES** - Afusali Authentic Wine Ethanol Standard, expressed in parts per 1000 (‰)

3.3 **Ethanol non-exchangeable hydrogen or deuterium atoms**

deuterium or hydrogen atoms tightly bound to the carbon atoms

3.4 **Ethanol exchangeable hydrogen or deuterium atoms**

deuterium or hydrogen atoms attached to oxygen atoms of the hydroxyl groups

3.5 **AAWES** Afusali Authentic Wine Ethanol Standard

4. Principle

Quantitative extraction of ethanol by distillation of the test sample and determining the content of ethanol in the distillate obtained.

Complete quantitative intramolecular dehydration of ethanol and formation of ethylene as a more stable measuring analyte compared to ethanol.

Ethanol is in constant dynamic equilibrium with its environment due to its hygroscopic nature and possession of exchangeable hydrogen atoms on the hydroxyl group through which hydrogen is building bonds with the water in the surrounding solution. With the addition of water to ethanol, which has a different isotopic profile current profile is distorted due to the exchangeable hydrogen atoms and establishes a new dynamic equilibrium. With the complete quantitative intramolecular dehydration of overheated ethanol vapors over EIM dehydration catalyst at elevated temperature, which is accompanied by kinetic isotope effect, hydroxyl group and hydrogen atom from the methyl site of ethanol are separated to form water and ethylene as reaction products, and deuterium atoms from the ethanol methyl site remains tightly bound the carbon atom inside formed ethylene.

Quantitative reaction of dehydration of ethanol over EIM dehydration catalyst at elevated temperatures is achieved.

Ethylene pyrolysis and extraction of elemental hydrogen gas in a stream of helium, and measuring the relative ratio of hydrogen stable isotopes $(D/H)_n$ by means of mass spectrometer and calculate δD_n values.

5. Reagents and materials

5.1 Reagents

Use only reagents of recognized analytical quality, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

5.1.1 **AAWES**[2] - Afusali Authentic Wine Ethanol Standard with value δD_n = -211.89 ‰.

5.1.2 **Helium** (He), inert gas, CAS number 07440-59-7, analytical quality for continuous flow systems.

5.1.3 **Reference Hydrogen Gas** (H_2), purity 5.0, analytical quality, CAS number 1333-74-0.

5.1.4 **Nitrogen** (N_2), inert gas, purity 4.5, analytical quality.

5.1.5 **EIM (**Ethanol Isotope Measurement) **Dehydration Catalyst**[3]

5.1.6 **Active dry yeast (Saccharomyces Cerevisiae)**[4],

5.1.7 **Nutrients**[5]**:** products available in the market to encourage the fermentation. For dissolving of yeast food use exclusively examined matrix.

5.1.8 **Work reference** Ethanol working standard which δD_n value is calibrated against AAWES (5.1)

5.2 Apparatus

5.2.1 **Fermentation vessel,** made of glass or stainless steel, with capacity of 500 mL, which provides an anaerobic atmosphere and allows alcohol vapor condensation to avoid loss of ethanol during fermentation. Example is presented at Figure 1:

5.2.2 **Boiling flask,** with a round bottom and taper ground neck, with volume of 500 mL in accordance with EN ISO 4797

5.2.3 **Glass flask,** graduated with taper ground neck, 50 mL volume.

5.2.4 **Electromagnetic stirrer**

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[2] Can be obtained from ANA LAB DOO, Dositeja Obradovića 8K, Tamiš Kapija, 26000 Pančevo, Republic of Serbia; www.ana-lab.rs , contact: office@ana-lab.rs

[3] Can be obtained from ANA LAB DOO, Dositeja Obradovića 8K, Tamiš Kapija, 26000 Pančevo, Republic of Serbia; www.ana-lab.rs , contact: office@ana-lab.rs

[4] UVAFERM 43® is an example of a suitable product supplied by Lallemand Wine. www.lalemandwine.com . This information is provided as a convenience to users of this resolution.

[5] GO-FERM® is an example of a suitable product supplied by Lallemand Wine, www.lalemandwine.com . This information is provided as a convenience to users of this resolution.

5.2.5 **Centrifuge,** rotating at a frequency of 3000 rounds/min to 5000 rounds/min and which corresponds to g force (RCF) of 1509 to 2054 for rotor radius of 15 cm.

5.2.6 **Distillation apparatus**

Note: Any method for ethanol extraction can be used as long as the alcohol in the test sample is recovered without isotopic fractionation.
The apparatus for distillation shown in figure 2 is an example of a manual distillation system that allows extracting more than 96 % of the ethanol of a wine without isotopic fractionation and obtaining a distillate with an alcohol grade of from 90 to 94.5 % vol.

Such a system is composed of:

- Electric heater,
- Boiling flask (5.2.2),
- Vigreaux column with length of 900 mm,
- The transition deflegmator ball with inclined core thermometer with a scale from 60 °C to 90 °C with 0,2 °C gradient, or digital thermometer which can measure in the same indicated temperature range
- Liebig condenser,
- Pipe and attachment of the receiving flask.

Commercial automatic distillation systems are also available.[6,7,8]

The performance of the distillation system may be checked periodically for both the yield of extraction as well as for accuracy for the isotopic determination. This control can be done by distillation and measurement of CRM -660.

The following common laboratory equipment and consumables is needed:
- Micropipette with appropriate tips,
- ,
- Precise graduated flasks (50 mL, 100 mL, 250 mL…)
- Flasks equipped with airtight closing systems and inert septa (for storage of aliquots of wines, distillates and residues until measurement)
- Equipment and consumables as specified in the other methods referred to herein.

[6] EIM-PADS® is an example of a suitable product supplied by SGISOTECH DOO Pančevo, Republic of Serbia. https://sgisotech.com/en/product/eim-pads. This information is provided as a convenience to users of this resolution.
[7] The Cadiot Column System is an example of a suitable product supplied by EUROFINS Scientific https://www.eurofins.com/contact-us/ . This information is provided as a convenience to users of this resolution.
[8] The Spinning Band Cadiot Column System is an example of a suitable product supplied by BR Instrument, 9119 Centreville Road, Easton, MD 21601 USA, https://brinstrument.com/blog/fractional-distillation/cadiot-distillation-wine-analysis/ . This information is provided as a convenience to users of this resolution.

The laboratory equipment and consumables indicated in the above lists are examples and may be replaced by other equipment of equivalent performance.

5.2.7 **Online apparatus for dehydration and pyrolysis of ethanol sample**

The apparatus for dehydration and pyrolysis of ethanol sample is an online continuous flow peripheral which consists of dehydration reactor, pyrolysis reactor, gas chromatography column and access to helium as carrier gas. This device[9] is via interface in conjunction with isotope ratio mass spectrometer. It is capable to quantitatively convert ethanol to ethylene with the ability to eliminate the water during ethanol intramolecular dehydration without any isotopic fractioning and with further ethylene pyrolysis to hydrogen gas. This device is via interface coupled to isotope ratio mass spectrometer.

5.2.8 **Isotope Ratio Mass Spectrometer (IRMS)**

Isotope ratio mass spectrometer (IRMS)[10],[11],[12] is an automatic device that basically consists of sources of ionizing radiation, a powerful electromagnet and two detectors, which can simultaneously measure the hydrogen and deuterium ions with masses m/z = 2 and 3. With such a device deuterium content in hydrogen gas can be determined, expressed as the relative value of δD and its natural representation of the internal precision of 0.5 ‰ or greater.

5.2.9 **Laboratory materials**

The following laboratory material is needed when using an apparatus for dehydration and pyrolysis of ethanol sample equipped with a liquid injector:

- Mycrosyringe for liquids
- Vials equipped with sealed closing system and inert septa

6. Preparation of samples for trials

The sample needs to be truly representative and has not changed during transport or storage. Samples for testing are kept in such a way to prevent spoilage and changes in the composition due to uncontrolled aerobic and/or anaerobic fermentation does not start.

6.1 **Preparation of test samples**

6.1.1 **Wine**

Take the test sample of 400 mL of wine.

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[9] EIMPyro® is an example of a suitable product supplied by SGISOTECH DOO Pančevo, Republic of Serbia. https://sgisotech.com/en/product/eimpyro. This information is provided as a convenience to users of this resolution.

[10] Delta V Advantage Isotope Ratio Mass Spectrometer is an example of a suitable product supplied by Thermo Fisher Scientific, www.thermofisher.com. This information is provided as a convenience to the users of this resolution.

[11] Isoprime precision Isotope Ratio Mass Spectrometer is an example of a suitable product supplied by Elementar Analyzen Systeme GmbH, www.elementar.com . This information is provided as a convenience to users of this resolution.

[12] HS2022 Isotope Ratio Mass Spectrometer is an example of a suitable product supplied by Sercon Limited, www.sercon-instruments.com . This information is provided as a convenience to the users of this resolution.

6.1.1.1 **Determination of alcoholic strength by volume in wine**

Determine the alcoholic strength by volume (ASV) in a sample of wine, expressed in % vol, using the method OIV-MA-AS312-01A of the International Organization of Vine and Wine.

6.1.1.2 **Extraction of ethanol distillation**

Transfer the part of the test sample of 250 mL of wine with a measuring cylinder to the boiling flask of 500 mL (5.2.2), which is connected with the rest of the distillation apparatus (5.2.6); receiving graduated flask of 50 mL is used as a receiving vessel for distillate. Turn on the electric heater and start the distillation.
Start the collection of the distillate when a stable temperature of the vapors typical of the ethanol-water azeotrope (78.2 to 78.5 °C) is reached and stop the collection when the temperature increases. The collection of distillate should be continued until the ethanol-water azeotrope is completely recovered.

When using manually a Vigreaux column (Figure 2) the following procedure can be applied:
- Collect the boiling liquid corresponding to the ethanol-water azeotrope,
- When the temperature increases, discontinue collection for five minutes.
- When the temperature returns to 78 °C, recommence collecting the distillate until the temperature of the vapors increases again.
- Repeat this operation until the temperature, after discontinuing collection, does not return to range between 78.2 to 78.5 °C.

The distillate should be stored in the refrigerator in an airtight vial in order to avoid isotopic fractionation and preventing any evaporation, until further use for determination of the alcoholic strength

6.1.2 **Crushed grapes**

Take a sample for testing of up to 1 kg of grapes, separate green parts, then sqeeze the grapes to get the grape pomace.

6.1.2.1 **Determination of the content of fermentable sugars in the crushed grapes**

Determine the content of fermentable sugars (CFS) in the liquid part of the crushed grapes with method OIV-MA-AS311-01A from the International Organisation of Vine and Wine (OIV).

6.1.2.2 **Fermentation of crushed grapes**

Transfer 250 mL prepared crushed grapes to a fermentation vessel (5.2.1) of the corresponding volume, add reactivated yeast (5.1.6) so that its concentration is 1 g/L of pulp and let it fermented in anaerobic atmosphere at a temperature of about 20 ° C until all sugar is fermented. If the fermentation is slow or stopped add nutrients for yeast (5.1.7) according to the manufacturer's instructions.

NOTE: Ensure that the volume of crushed grapes should not take more then 80% of the volume of fermentation vessel.

After completion of the fermentation liquid part is separated from the mash to the receiving vessel of the corresponding capacity, then remove CO_2 with electromagnetic stirrer (5.2.4) for 1 h. Liquid (supernant) which is free of CO_2 is centrifuged in appropriate portions for 10 min using a centrifuge (5.2.5) at 3.000 rounds/min to 3.500 rounds/min.

Supernatant is used for the determination of the actual alcohol content for calculation of fermentation level of fermentable sugars (FLFS) in fermented crushed grapes and ethanol distillation for isolation as defined in 6.1.1.2.

6.1.2.2.1 **Determination of alcoholic strength by volume in fermented crushed grapes**

Determine the alcoholic strength by volume (ASV) in the supernatant obtained in 6.1.2.2, expressed in % vol, using the method OIV-MA-AS312-01A of the International Organisation of Vine and Wine (OIV).

6.1.2.2.2 **Calculation of fermentation level of fermentable sugars in fermented crushed grapes**

Calculate the potential alcohol content (PAC) in grape pomace, expressed in % vol, using the following formula:

$PAC = FSC \times 0{,}059$

where:

FSC is content of fermentable sugars in the grape pomace, determined in accordance with 6.1.2.1, expressed in g/L;

0,059 conversion factor of fermentable sugars into ethanol.

Then calculate the fermentation level of fermentable sugars (FLFS) in fermented crushed grapes, expressed as a percentage, using the following formula:

$FLFS = (ASV/PAC) \times 100$

where:

ASV alcoholic strength by volume in fermented crushed grapes, determined in accordance with 6.1.2.2.1, expressed in % vol.;

PAC potential alcohol content in crushed grapes, expressed in % vol.

Fermentation level of fermentable sugars (FLFS) in fermented crushed grapes must be greater than 98%. If the fermentation level of fermentable sugars in fermented crushed grapes is less than 98% add reactivated yeast (5.1.6) so that its concentration is 1 g/L and let it fermented in anaerobic atmosphere at a temperature of about 20 °C, until all the sugar is fermented into ethanol. If the fermentation is slow or stopped add nutrients for yeast (5.1.7) according to the manufacturer's instructions.

6.1.2.3 **Extraction of ethanol by distillation**

Proceed with the extraction of ethanol by distillation of the supernatant obtained in 6.1.2.2 as it is defined in 6.1.1.2, except that instead of 250 mL of wine, use 250 mL of prepared supernatant.

6.1.3 **grape must**

Take the test sample of at least 500 mL of grape must.

If grape must is sulphured first remove sulphur-dioxide as follows: Put 500 mL of sulphured grape must into the vessel of appropriate volume with feedback condenser. Then transfer the vessel to a water bath heated to a temperature of 70 °C to 80 °C, and then introduce nitrogen (5.4) stream to sulphured grape must for at least 1 hour, or until the sulfur dioxide content is less than 40 mg/L.

The content of sulfur dioxide is determined by the method of the OIV-MA-AS323-04B of the International Organisation for Vine and Wine (OIV).

NOTE: Ensure the effective cooling of the condenser so that grape must would not be concentrated by evaporation of water.

If potassium metabisulfite was used for sulphuring, before you start with SO_2 removal, add 0.25 ml of sulfuric acid (ρ_{20} = 1,84 g / ml) per gram of potassium metabisulphite used per liter of grape must.

6.1.3.1 **Determination of the content of fermentable sugars in fresh grape must**

Determine the content of fermentable sugars (SFS) in the grape must with method OIV-MA-AS311-01A from International Organisation of Vine and Wine (OIV).

6.1.3.2 **grape must fermentation**

Transfer grape must sample to a fermentation vessel (5.2.1) of the appropriate volume and add reactivated yeast (5.1.6) so that its concentration is 1 g/L and allow alcoholic fermentation in anaerobic atmosphere at a temperature of about 20 °C until all the sugar is fermented. If the fermentation is slow or stopped add nutrients for yeast (5.1.7) according to the manufacturer's instructions.

NOTE: Ensure that the volume of grape must does not occupy more than 80% of the volume of fermentation vessel.

After completion of the fermentation liquid part is separated from the lees to the receiving vessel of the corresponding capacity, then remove CO_2 with electromagnetic stirrer (5.2.4) for 1 h. Liquid (supernant) which is free of CO_2 is centrifuged in appropriate portions for 10 min using a centrifuge (5.2.5) at 3.000 rounds/min to 3.500 rounds/min.

Supernatant is used for the determination of the alcoholic strength by volume for calculation of fermentation level of fermentable sugars (FLFS) in fermented grape must (wine) and ethanol distillation for its extraction as defined in 6.1.3.3.

6.1.3.2.1 **Determining the alcoholic strength by volume of the fermented liquid**

Determine the alcoholic strength by volume (ASV) in the supernatant obtained in 6.1.3.2, expressed in % vol, using the method of the OIV-MA-AS312-01A from International Organisation of Vine and Wine (OIV).

6.1.3.2.2 **Calculation of fermentation level of fermentable sugars in the fermented liquid**

Calculate the potential alcohol content (PAC) in grape must, expressed in % vol, using the following formula:

PAC = FSC × 0,059

Where:

FSC fermentable sugar content in grape must, determined in accordance with 6.1.3.1, expressed in g/L;
0,059 a conversion factor of fermentable sugars into ethanol.

Then calculate the fermentation level of fermentable sugars (FLFS) in the fermented liquid, expressed as a percentage, using the following formula:

FLFS = (ASV/PAC) × 100

Where:

ASV alcoholic strength by volume of the fermented liquid, determined in accordance with 6.1.3.2.1, expressed in % vol.;
PAC potential alcohol content in grape must, expressed in % vol.

Fermentation level of fermentable sugars (FLFS) in fermented liquid must be greater than 98%. If the fermentation level of fermentable sugars in fermented liquid is less than 98% add reactivated yeast (5.1.6) so that its concentration is 1 g/L and let it fermented in anaerobic atmosphere at a temperature of about 20 °C, until all the sugar is fermented into ethanol. If the fermentation is slow or stopped add nutrients for yeast (5.1.7) according to the manufacturer's instructions.

6.1.3.3 **Extraction of ethanol by distillation**

Proceed with the extraction of ethanol by distillation of the supernatant obtained in 6.1.3.2 as it is defined in 6.1.1.2, except that instead of 250 mL of wine, use 250 mL of prepared supernatant.

7. Procedure

All preparation steps must be carried out without any significant ethanol loss through evaporation, which would change the isotopic composition of the sample. The description that follows makes reference to the procedure generally used for ethanol sample dehydration and pyrolysis using commercial automatic dehydration and pyrolysis system.

An experimental procedure based on the usage of a dehydration and pyrolysis online peripheral:

a) Placing the vials with ethanol samples in the tray of the auto sampler for liquid samples
- systematically place capsules containing work references at the beginning and the end of the sample series
- regularly insert a check (control) sample in the sample series.

b) Check and adjust the dehydration-pyrolysis online apparatus and mass spectrometer instruments

- adjust the temperature of the dehydration-pyrolysis online apparatus ovens and the helium gas flow for an optimal thermal conversion of the sample;
- check the full system for leaks (for example by checking the ionic current where m/z = 40 corresponding to Ar);
- adjust the mass spectrometer to measure the intensities of ionic current where m/z = 2 and 3;
- check the system using known reference samples before starting to measure the samples.

c) To carry out a series of measurements

The samples that are placed are introduced successively. The hydrogen gas for each sample which is dehydrated and pyrolyzed is eluted towards the mass spectrometer which measures the ionic current. The interface computer records the ionic current intensities and calculates the values δ for each sample (9).

8. Expression of Results

Hydrogen isotopic ratio is calculated on a delta scale per thousand by comparing the results obtained for the sample to be measured to the reference work calibrated before based on the primary reference AAWES (5.1.1). δD_n ethanol values are expressed compared to reference work:

$$\delta D_n\ (‰) = [(R_{sample}/R_{ref}) - 1] \times 1000$$

Where R_{sample} and R_{ref} are respectively the hydrogen isotopic ratio D/H of the sample and the work reference.

δD_n ethanol values are thus expressed using AAWES:

$$\delta D_n \text{ sample / AAWES} = \delta D_n \text{ sample/ref} + \delta D_n \text{ ref/AAWES} + (\delta D_n \text{ sample/ref} \times \delta D_n \text{ ref / AAWES})/1000$$

Where δD_n ref/AAWES is the isotopic deviation determined beforehand for the work reference to AAWES.

Small variations may occur while measuring on line due to changes in the instrumental conditions. In this case the δD_n samples must be corrected according to the difference in the value δD_n from the work reference and the real value, which was calibrated beforehand against AAWES. Between two measurements of the reference work, the variation is the correction applied to the sample results that may be assumed to be linear. The reference work must be measured at the beginning and at the end of all sample series. A correction can be calculated for each sample using linear interpolation between two values (the difference between the assigned value of the reference work and the measurements of obtained values).

9. Precision

The repeatability and reproducibility of the EIM-IRMS method has been studied through collaborative studies as reported in the bibliography here below. These studies considered δD_n ethanol values (‰ vs. AAWES). Data from in-house studies carried out by several laboratories can be considered for establishing the standard deviation of repeatability and the limit of

repeatability as presented in Annex I. The results of testing reported in Annex I provide data that can be used to compute the standard deviation of Reproducibility and the limit of Reproducibility for wines.

The figures can be summarized as follows:

S_R = 1,25 ‰
S_r = 1,14 ‰
r = 3,19 ‰
R = 3,50 ‰

Where:

S_R - Inter-laboratory Reproducibility standard deviation;
S_r - Inter-laboratory Repeatability standard deviation;
r - Repeatability precision limit (2.8 x s*r*);
R - Reproducibility precision limit (2.8 x sR)

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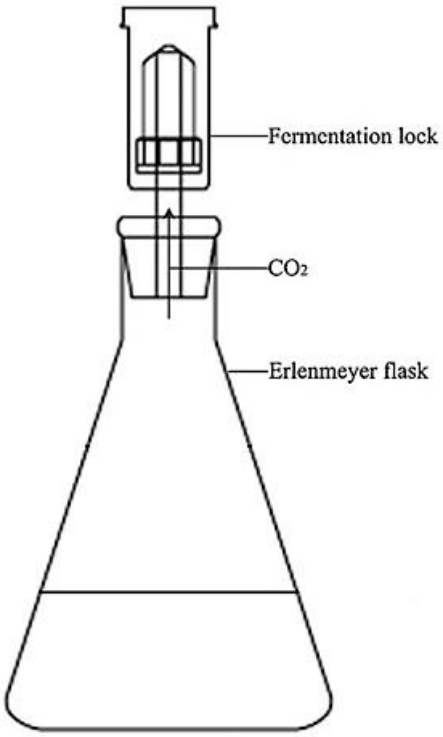


FIGURE 1 - FERMENTATION VESSEL

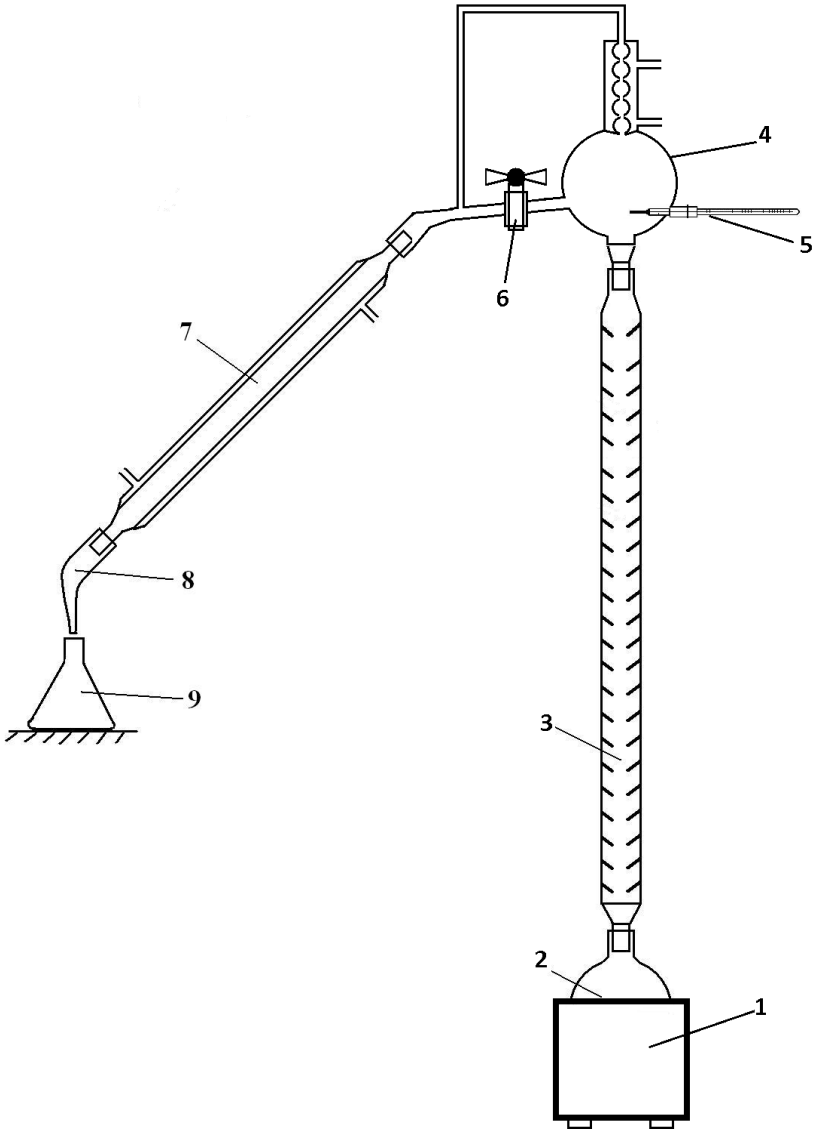

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FIGURE 2 - APPARATUS FOR DISTILLATION

In the picture:
1. Electric heater
2. Boiling flask
3. Vigreaux Column
4. Deflegmator with upper condenser
5. Thermometer
6. Valve
7. Liebig condenser
8. Pipe
9. Receiving flask

ANNEX I - Estimation of the repeatability from in-house repeatability studies

The in-house repeatability studies performed in 3 participating laboratories provide data that allows the estimation of the repeatability of the EIM-IRMS method. These in-house repeatability studies have been performed by measurements of 10 different wine sampleswhich results are shown in Table 1, 2, 3,4,5,6,7,8 and 9 .

Standard deviation for each single sample was calculated using the following formula:

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{mean})^2}{n-1}}$$

where:
s – standard deviation of one sample in one day
n – number of trials of one sample in one day
n-1 – degree of freedom
x_i – *individual value*
x_{mean} – *mean value of the sample*

Intralaboratory Pooled Repeatability Standard Deviation on the multiple days was calculated using the following formula:

$$S_{Pooled} = \sqrt{\frac{(n_1 - 1){S_1}^2 + (n_2 - 1){S_2}^2 + \cdots + (n_k - 1){S_k}^2}{n_1 + n_2 + \cdots + n_k - k}}$$

S_{Pooled} – Pooled Repeatability Standard deviation on multiple days for one sample
n – number of trials of one sample in one day
n-1 – degree of freedom
k – number of samples

The interlaboratory reproducibility standard deviation is computed as

s_R = MAX(S_R,S_r)

with

$$S_R = \sqrt{{S_{\bar{x}}}^2 + {S_r}^2 \frac{n-1}{n}}$$

Where:

$${S_{\bar{x}}}^2 - \text{ standard deviation of the cell averages}$$

$$n - \text{Cell sample size}$$

and S_r denoting the repeatability standard deviation

$$S_r = \sqrt{\sum_{i=1}^{p} {S_i}^2 / p}$$

with p and s_i denoting the number of laboratories and the standard deviation of the i-th laboratory, respectively.

In an interlaboratory study, the reproducibility standard deviation was computed for each material.

Lab 1:

Table 1: Reference material: AFUSALI - Afusali Authentic Wine Ethanol Standard (AAWES) with value -211.89‰

Ethanol δD_n values (‰ vs. AAWES)								
	Thursday		Friday		Saturday		Wednesday	
	13.09.2018.		14.09.2018.		15.09.2018.**		19.09.2018.**	
	Mean	St.Dev.**	Mean	St.Dev.**	Mean	St.Dev.	Mean	St.Dev.
DB73-01	-214,16	1,41	-214,96	0,76	-	-	-213,54	0,48
DB73-02	-220,34	0,95	-221,14	0,52	-223,85	1,34	-224,32	0,90
DB73-03	-211,71*	1,28*	-218,71	1,28	-218,15	2,62	-218,05	1,20
DB73-04	-211,71	0,50	-212,25	3,51	-211,64	1,62	-213,43	0,40
DB73-05	-220,39	2,38	-220,41	0,80	-220,45	1,13	-219,85	1,44
DB73-06	-215,60*	0,59*	-220,74	1,38	-219,78	2,44	-217,85	0,34
DB73-07	-214,79	1,25	-214,37	0,43	-216,06	0,09	-216,21	1,12
DB73-08**	-216,11	0,82	-215,56	1,76	-	-	-216,08	1,06
DB73-09	-220,27	0,55	-220,13	0,83	-221,89	2,55	-221,30	0,51
DB73-10	-222,85	0,34	-223,71	0,75	-223,54	0,98	-224,58	0,87

** Not included in calculation (bad sampling from another vial – wrong number of the vial placement in the software sequence)*

***Repeatability standard deviation of minimum 4 injection runs of the same sample*

Table 2: Intra-laboratory Repeatability and Reproducibility calculation:

Sample Number	Ethanol δD_n mean value (‰ vs. AAWES)	Intra-laboratory Reproducibility standard deviation S_R	Intra-laboratory Repeatability standard deviation S*r*
DB73-01	-214,22	0,71	1,02
DB73-02	-222,41	1,97	1,35
DB73-03	-218,30	0,35	1,93
DB73-04	-212,26	0,83	2,05
DB73-05	-220,27	0,28	1,62
DB73-06	-219,46	1,47	1,73

DB73-07	-215,36	0,92	0,91
DB73-08	-215,92	0,31	1,02
DB73-09	-220,90	0,84	1,45
DB73-10	-223,67	0,71	0,81
Average		0,84	1,39

Lab 2

Table 3: Reference material: AFUSALI - Afusali Authentic Wine Ethanol Standard (AAWES) with value -211.89‰

Ethanol δD_n values (‰ vs. AAWES)												
Date	27.09.2018.		28.09.2018.		29.09.2018.		30.09.2018.		01.10.2018.		02.10.2018.	
Sample number	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*
DB73-01	-214,88	1,63	-214,56	0,81	-214,19	1,78	-214,33	1,45	-213,50	1,76	-214,47	1,29
DB73-02	-221,13	2,25	-221,73	1,28	-220,44	0,89	-220,44	0,77	-218,18	0,29	-218,99	0,43
DB73-03	-218,98	1,87	-217,17	1,25	-217,53	0,85	-218,08	0,20	-218,07	0,81	-216,31	0,81
DB73-04	-213,84	3,86	-214,21	1,01	-212,88	0,17	-213,15	1,42	-214,65	1,09	-214,20	1,11
DB73-05	-219,84	3,79	-220,73	0,94	-219,68	0,59	-220,16	0,63	-220,35	1,76	-220,09	1,32
DB73-06	-219,09	0,60	-214,86	0,77	-216,49	0,93	-217,55	0,53	-216,95	0,61	-216,09	0,29
DB73-07	-220,97	1,25	-215,99	1,12	-216,25	1,47	-215,44	1,00	-215,57	0,94	-214,97	0,44
DB73-08	-216,36	0,07	-214,53	2,13	-218,48	0,88	-216,58	2,41	-216,97	1,26	-216,84	0,27
DB73-09	-224,42	0,42	-221,53	0,28	-220,80	1,07	-220,40	1,04	-220,49	0,64	-219,29	0,30
DB73-10	-224,10	2,03	-219,85	0,88	-220,34	0,48	-220,34	0,48	-220,54	0,46	-219,90	0,91

**Repeatability standard deviation of minimum 4 injection runs of the same sample*

Table 4: Intra-laboratory Repeatability and Reproducibility calculation:

Sample Number	Ethanol δD_n mean value (‰ vs. AAWES)	Intra-laboratory Reproducibility standard deviation S_R	Intra-laboratory Repeatability standard deviation S_r
DB73-01	-214,32	0,47	1,53
DB73-02	-220,15	1,27	1,50
DB73-03	-217,69	0,91	1,12
DB73-04	-213,82	0,68	1,90
DB73-05	-220,14	0,37	1,92
DB73-06	-216,84	1,43	0,67
DB73-07	-216,53	2,22	1,12
DB73-08	-216,63	1,33	1,21
DB73-09	-221,16	1,76	0,73
DB73-10	-220,84	1,62	1,06
Average		1,21	1,28

Lab 3:

Table 5: Reference material: AFUSALI - Afusali Authentic Wine Ethanol Standard (AAWES) with value -211.89‰

Ethanol δD_n values (‰ vs. AAWES)												
Date	21.07.2018.		11.10.2018.		15.10.2018.		17.10.2018.		18.10.2018.		20.10.2018.	
Sample number	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*
DB73-01	-214,50	1,84	-215,00	0,66	-214,07	0,77	-214,68	1,13	-213,32	0,62	-215,78	0,20
DB73-02	-218,50	0,47	-221,64	0,93	-220,64	0,89	-220,89	0,86	-220,69	0,52	-220,27	0,79
DB73-03	-225,60	0,42	-218,87	0,41	-217,16	0,40	-217,28	1,03	-215,64	0,19	-217,81	1,57
DB73-04	-212,40	0,70	-215,13	1,75	-212,07	0,44	-213,26	0,46	-213,11	0,62	-212,09	0,48
DB73-05	-223,20	1,33	-222,49	0,72	-219,67	0,46	-220,38	0,53	-219,70	0,26	-219,36	0,15
DB73-06	-225,30	0,80	-218,36	0,12	-215,68	0,47	-216,12	0,49	-216,37	0,35	-217,06	0,32
DB73-07	-216,40	1,71	-219,03	1,08	-217,51	1,36	-216,05	0,79	-217,80	0,50	-217,83	0,57
DB73-08	-215,40	0,70	-216,60	0,66	-215,51	0,74	-215,37	0,68	-214,77	0,50	-216,57	0,15
DB73-09	-223,10	0,01	-220,87	0,97	-220,81	0,58	-221,07	0,60	-220,62	0,55	-220,71	0,42
DB73-10	-220,20	1,23	-220,81	0,23	-221,08	0,53	-221,12	0,48	-220,62	0,77	-220,48	0,61

**Repeatability standard deviation of minimum 4 injection runs of the same sample*

Ethanol δD_n values (‰ vs. AAWES)		
Date	23.07.2018.	
Sample number	Mean	St.Dev.*
DB73-01	-215,16	0,14
DB73-02	-220,64	0,41
DB73-03	-217,05	0,16
DB73-04	-212,72	0,81
DB73-05	-220,30	0,83
DB73-06	-217,14	0,37
DB73-07	-217,57	0,48
DB73-08	-216,77	0,52
DB73-09	-220,83	0,42
DB73-10	-221,00	1.26

**Repeatability standard deviation of minimum 4 injection runs of the same sample*

Table 6: Intra-laboratory Repeatability and Reproducibility calculation:

Sample Number	Ethanol δD_n mean value (‰ vs. AAWES)	Intra-laboratory Reproducibility standard deviation S_R	Intra-laboratory Repeatability standard deviation S_r
DB73-01	-214,64	0,79	0,96
DB73-02	-220,02	1,23	0,74
DB73-03	-218,49	3,28	0,78
DB73-04	-212,97	1,06	0,89
DB73-05	-220,73	1,50	0,73
DB73-06	-218,01	3,33	0,47
DB73-07	-217,46	0,99	1,05
DB73-08	-216,30	2,08	0,61
DB73-09	-221,14	0,87	0,59
DB73-10	-220,76	0,34	0,83
Average		1,55	0,77

Table 7: Results from all three labs

	Lab 1			**Lab 2**			**Lab 3**		
	Ethanol δD_n mean value (‰ vs. AAWES)	Intra-laboratory Reproducibility standard deviation S_R	Intra-laboratory Repeatability standard deviation S_r	Ethanol δD_n mean value (‰ vs. AAWES)	Intra-laboratory Reproducibility standard deviation S_R	Intra-laboratory Repeatability standard deviation S_r	Ethanol δD_n mean value (‰ vs. AAWES)	Intra-laboratory Reproducibility standard deviation S_R	Intra-laboratory Repeatability standard deviation S_r
DB73-01	-214,22	0,71	1,02	-214,32	0,47	1,53	-214,64	0,79	0,96
DB73-02	-222,41	1,97	1,35	-220,15	1,27	1,50	-220,02	1,23	0,74
DB73-03	-218,30	0,35	1,93	-217,69	0,91	1,12	-218,49	3,28	0,78
DB73-04	-212,26	0,83	2,05	-213,82	0,68	1,90	-212,97	1,06	0,89
DB73-05	-220,27	0,28	1,62	-220,14	0,37	1,92	-220,73	1,50	0,73
DB73-06	-219,46	1,47	1,73	-216,84	1,43	0,67	-218,01	3,33	0,47
DB73-07	-215,36	0,92	0,91	-216,53	2,22	1,12	-217,46	0,99	1,05
DB73-08	-215,92	0,31	1,02	-216,63	1,33	1,21	-216,30	2,08	0,61
DB73-09	-220,90	0,84	1,45	-221,16	1,76	0,73	-221,14	0,87	0,59
DB73-10	-223,67	0,71	0,81	-220,84	1,62	1,06	-220,76	0,34	0,83
		0,84	1,39		1,21	1,28		1,55	0,77

Table 8: INTER-LABORATORY REPEATABILITY AND REPRODUCIBILITY PRECISION LIMITS CALCULATION:

	Sample Number	Ethanol δD_n mean value (‰ vs. AAWES at SGI Scale)	Inter-laboratory Reproducibility standard deviation sR=(STDEV of all Measurements of the same sample)	Inter-laboratory Repeatability standard deviation *sr*= (Average STDEV for the same sample)
	DB73-01	-214.39	0,71	1,17
	DB73-02	-220.86	0,90	1,20
	DB73-03	-218.16	1,22	1,28
	DB73-04	-213.02	1,09	1,61
	DB73-05	-220.38	0,84	1,42
	DB73-06	-218.10	1,80	0,96
	DB73-07	-216.45	1,45	1,02
	DB73-08	-216.28	1,74	0,95
	DB73-09	-221.07	0,92	0,92
	DB73-10	-221.76	1,85	0,90
Average			1,25	1,14
r - Repeatability precision limit (2.8 x S_r)	**3,19**			
R- Reproducibility precision limit (2.8 x S_R)	**3,50**			

Table 9: According to obtained results between three laboratories Z-scores were calculated for all 10 samples:

Sample	**Z - SCORES**		
	Lab 1	Lab 2	Lab 3
DB73-01	0.17	0.07	-0.24
DB73-02	-1.76	0.81	0.95
DB73-03	-0.13	0.43	-0.30
DB73-04	0.61	-0.65	0.04
DB73-05	0.09	0.20	-0.29
DB73-06	-1.67	1.55	0.12
DB73-07	1.22	-0.09	-1.13
DB73-08	0.37	-0.36	-0.02
DB73-09	0.21	-0.12	-0.10
DB73-10	-2.46	1.18	1.28