

**COMMISSION REGULATION (EC) No 822/97**  
**of 6 May 1997**  
**amending Regulation (EEC) No 2676/90 determining Community methods for**  
**the analysis of wines**

THE COMMISSION OF THE EUROPEAN COMMUNITIES,  
Having regard to the Treaty establishing the European Community,

Having regard to Council Regulation (EEC) No 822/87 of 16 March 1987 on the common organization of the market in wine <sup>(1)</sup>, as last amended by Regulation (EC) No 536/97 <sup>(2)</sup>, and in particular Article 74 thereof,

Whereas the Annex to Commission Regulation (EEC) No 2676/90 <sup>(3)</sup>, as last amended by Regulation (EC) No 69/96 <sup>(4)</sup>, contains a description of these analysis methods; whereas a method of analysing the isotope ratio  $^{18}\text{O}/^{16}\text{O}$  of the oxygen in the water of wine has been developed and its validity established in accordance with internationally recognized criteria; whereas the application of this method may constitute a better way of checking the authenticity of wine and other wine products; whereas the description of this new method has been adopted by the International Wine and Wine Office; whereas this method should be added to the said Regulation;

Whereas the measures provided for in this Regulation are in accordance with the opinion of the Management Committee for Wine,

HAS ADOPTED THIS REGULATION:

*Article 1*

Chapter 43 as shown in the Annex hereto is added to the Annex to Regulation (EEC) No 2676/90.

*Article 2*

This Regulation shall enter into force on the seventh day following its publication in the *Official Journal of the European Communities*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 6 May 1997.

*For the Commission*  
Franz FISCHLER  
*Member of the Commission*

<sup>(1)</sup> OJ No L 84, 27. 3. 1987, p. 1.

<sup>(2)</sup> OJ No L 83, 25. 3. 1997, p. 5.

<sup>(3)</sup> OJ No L 272, 3. 10. 1990, p. 1.

<sup>(4)</sup> OJ No L 14, 19. 1. 1996, p. 13.

## ANNEX

43. DETERMINATION OF THE ISOTOPIC RATIO  $^{18}\text{O}/^{16}\text{O}$  OF THE WATER CONTENT IN WINES

## I. DESCRIPTION OF THE METHOD

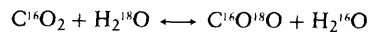
## 1. Method objective

The objective of the present method is to measure the isotopic ratio  $^{18}\text{O}/^{16}\text{O}$  of waters of different origins. The isotopic ratio  $^{18}\text{O}/^{16}\text{O}$  can be expressed in deviation  $\delta$ ‰ in ratio to the value of isotopic ratio of the international reference V.SMOW:

$$\delta_i [\text{‰}] = \left[ \frac{R_i}{R_{\text{SMOW}}} - 1 \right] \times 1\,000$$

## 2. Principle

The isotopic ratio  $^{18}\text{O}/^{16}\text{O}$  is determined by mass spectrometry of isotopic ratios (MSIR) from ionic currents  $m/z$  46 ( $^{12}\text{C}^{16}\text{O}^{18}\text{O}$ ) and  $m/z$  44 ( $^{12}\text{C}^{18}\text{O}_2$ ) produced by carbon dioxide obtained after an exchange with the water in wine according to the reaction:



The carbon dioxide in the gaseous phase is used for analysis.

## 3. Reagents

- Carbon dioxide for analysis
- SMOW (Standard Mean Ocean Water)
- GISP (Greenland Ice Sheet Precipitation)
- SLAP (Standard Light Arctic Precipitation)
- Reference water specific to the laboratory carefully standardized in relation to the reference sample of the International Agency of Atomic Energy in Vienna (IAEA).

## 4. Laboratory equipment

- mass spectrometer of isotopic ratios with an internal repeatability of 0,05 ‰
- triple collector for simultaneous recording of ions  $m/z$  44, 45 and 46 or, by default, a double collector for measuring ions  $m/z$  44 and 46
- thermostated system ( $\pm 0,5^\circ\text{C}$ ) to carry out the equilibration between  $\text{CO}_2$  and the water content in wine
- vacuum pump able to reach an internal pressure of 0,13 Pa
- phials for samples having 15 ml volume and a capillary annex tube with an interior diameter of about 0,015 mm
- Eppendorf pipette with plastic throw-away cone.

## 5. Experimental determinations

## 5.1. Manual method

## Operational mode of the equilibration method

## Introduction of the sample

- Take the Eppendorf pipette at the fixed volume of 1,5 ml, adapt a cone and pump the liquid to be analysed in order to insert it in a balloon flask. Then, place silicon grease around the neck of the balloon flask and attach the balloon flask to the valve while verifying that it is tightly shut,
- Repeat the operation for each balloon flask on the work ramp while introducing the laboratory's reference water into one of the balloons.

## Degasing of the ramp

The two ramps are cooled down with liquid nitrogen, then the whole system is purged up to 0,1 mm Hg by opening the valves.

Then, shut the valves off and let it all heat up. The degasing cycle is repeated until there is no more pressure variation.

### Equilibration of the water and the CO<sub>2</sub>

Cool the work ramps to  $-70\text{ }^{\circ}\text{C}$  (Liquid nitrogen and alcohol mix) to freeze the water and put it all in a vacuum. After stabilization of the vacuum, isolate the ramp by actioning the valve and purge the CO<sub>2</sub> introduction system. Insert the gaseous CO<sub>2</sub> into the work ramp and, after having isolated it from the rest of the system, introduce the ramp in a thermostated bath at  $25\text{ }^{\circ}\text{C}$  ( $\pm 0,5\text{ }^{\circ}\text{C}$ ) for 12 hours (one night). To optimize the necessary time for equilibration, it is advisable to prepare the samples at the end of the day and let the balance settle overnight.

### Transfer of the CO<sub>2</sub> exchanged in the measuring cells

A sample holder which supports as many measuring cells as balloon flasks containing exchanged CO<sub>2</sub> is adapted on the empty line next to the work ramp. The empty cells are carefully purged and the exchanged gases contained in the balloons are transferred one after the other, into the measuring cells which have been cooled by liquid nitrogen. Then the measuring cells are allowed to heat up at room temperature.

#### 5.2. Use of an automatic exchange apparatus

In order to carry out the equilibration, sample phials are filled with either 2 ml of wine or 2 ml of water (laboratory work reference) and cooled down to  $-18\text{ }^{\circ}\text{C}$ . The sample slides containing the frozen products are adapted to the equilibration system, and after having created a vacuum in the system, carbon dioxide is introduced at a pressure of 800 hPa.

The balance is reached at a temperature of  $22 \pm 0,5\text{ }^{\circ}\text{C}$  after a minimum period of five hours and with moderate agitation. Since the equilibration duration depends on the phial's geometry, the optimum duration should be determined first for the system used.

Carbon dioxide contained in the phials is then transferred to the introduction chamber of the mass spectrometer by a capillary tube and the measurement is carried out according to a specific protocol for each kind of equipment.

#### 6. Calculation and expression of the results

The relative difference  $\delta'$  of the ratio intensities of ions  $m/z$  46 and 44 ( $I_{46}/I_{44}$ ) between the sample and the reference is expressed in ‰ by means of the following equation:

$$\delta' \text{ sample} = \left[ \frac{(I_{46}/I_{44}) \text{ sample}}{(I_{46}/I_{44}) \text{ reference}} - 1 \right] \times 1\,000$$

The <sup>18</sup>O content of the sample compared to the reference V.SMOW on the V.SMOW/SLAP scale, is given by the relation:

$$\delta' ^{18}\text{O} = \left[ \frac{\delta' \text{ sample} - \delta' \text{ SMOW}}{\delta' \text{ SMOW} - \delta' \text{ SLAP}} \right] \times 55,5$$

The value accepted for SLAP is equal to  $-55,5\text{ }‰$  compared to V.SMOW. The isotopic ratio of reference must be determined after each series of 10 measurements on unknown samples.

#### 7. Fidelity

- the repeatability (r) is equal to  $0,24\text{ }‰$
- the reproductibility (R) is equal to  $0,50\text{ }‰$ .