COMMISSION REGULATION (EC) No 440/2003

of 10 March 2003

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 (EC) No 1493/1999 of 17 May 1999 on the common organisation of the market in wine [1], as last amended by Regulation (EC) No 2585/2001 [2], and in particular the first subparagraph of Article 46(3) thereof,

Whereas:

(1) The Annex to Commission Regulation (EEC) No 2676/90 [3], as last amended by Regulation (EC) No 1622/2000 [4], describes analysis methods.

(2) An analysis method for D-malic acid suitable for measuring the low levels found in wines has been developed and validated according to recognised international criteria. The International Vine and Wine Office adopted the description of this new method at its General Assembly in June 2002.

(3) A new method for determining the carbon isotope ratio of wine ethanol or of ethanol obtained through the fermentation of grape musts, concentrated grape musts or rectified concentrated grape musts has been developed and validated according to recognised international criteria. The International Vine and Wine Office adopted the description of this new method at its General Assembly in 2001.

(4) The use of these methods can ensure better control of wine quality and authenticity and prevent disputes caused by the application of less accurate testing methods, particularly as regards enrichment with mixtures of sugars of various origins and the acidification of wines using malic acid.

(5) The existing method for the dosage of D-malic acid described in the Annex to Regulation (EEC) No 2676/90 should be supplemented by the description of the procedure for determining low levels and the description of the new isotopic method for carbon in ethanol should be added.

(6) 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

The Annex to Regulation (EEC) No 2676/90 is hereby amended as follows:

1. In Chapter 20, 'D-malic acid', point 8 is replaced by Annex I hereto.

2. Chapter 45 in Annex II hereto is added.

Article 2

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

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

Done at Brussels, 10 March 2003.

For the Commission

Franz FISCHLER

Member of the Commission

[1] OJ L 179, 14.7.1999, p. 1.
[2] OJ L 345, 29.12.2001, p. 10.
[3] OJ L 272, 3.10.1990, p. 1.
[4] OJ L 194, 31.7.2000, p. 1.

ANNEX I

'8. DOSAGE OF D-MALIC ACID (D(+)-MALIC ACID) IN WINES WITH LOW LEVELS

8.1. **FIELD OF APPLICATION**

The method described is applied to the dosage, by enzymatic means, of D-malic acid of wines with levels under 50 mg/l.

8.2. **PRINCIPLE**

The principle of the method is described in point 1. The formation of NADH after the introduction into the cuvette of 50 mg/l of D-malic acid is proportional to the quantity of D-malate present and is measured on the basis of the increase in absorbance at a wavelength of 340 nm.

8.3. **REAGENTS**

A 0,199 g/l D-malic acid solution plus the reagents indicated in point 2.

8.4. **APPARATUS**

Apparatus indicated in point 3.

8.5. **PREPARATION OF THE SAMPLE**

As indicated in point 4.

8.6. **PROCEDURE**

The procedure is as described in point 5, but with the introduction into the measuring cuvette of 50 mg/l of D-malic acid. (Introduction of 0,025 ml of 0,199 g/l D-malic acid solution, displacing the equivalent volume of water); the values obtained are decreased by 50 mg/l.

8.7. **INTERNAL VALIDATION**

The table below summarises the internal validation file on the method for determining the dosage of D(+)-malic acid after the addition of 50 mg/l of the isomer.

Working range	0 mg to 70 mg of D-malic acid per litre. Within these limits, the method is linear with a correlation coefficient of between 0,990 and 0,994
Limit of quantification	24,4 mg/l
Limit of detection	8,3 mg/l
Sensitivity	0,0015 abs/mg/l
Recovery ratio	87,5 to 115,0 % for white wines and 75 to 105 % for red wines
Repeatability	= 12,4 mg/l for white wines (according to the OIV method = 12,5 mg/l) = 12,6 mg/l for red wines (according to OIV method = 12,7 mg/l)
Coefficient of variation	4,2 % to 7,6 % (white wines and red wines)
Intralaboratory variability	CV=7,4 % (s = 4,4 mg/l; mean = 59,3 mg/l)'

ANNEX II

'45. DETERMINATION BY ISOTOPE MASS SPECTROMETRY OF THE $^{13}C/^{12}C$ RATIO IN WINE ETHANOL OR ETHANOL OBTAINED BY THE FERMENTATION OF MUSTS, CONCENTRATED MUSTS OR RECTIFIED CONCENTRATED MUSTS

1. FIELD OF APPLICATION

The method enables measurement of the $^{13}C/^{12}C$ isotope ratio in wine ethanol and ethanol obtained by fermentation of products of the vine (musts, concentrated musts, rectified concentrated musts).

2. REFERENCE STANDARDS

ISO:	5725:1994 "Accuracy (trueness and precision) of measurement methods and results: Basic method for the determination of repeatability and reproducibility of a standard measurement method".
V-PDB:	Vienna-Pee-Dee Belemnite (R_{PDB} = 0,0112372).
Method 8 of the Annex to this Regulation:	"Detecting enrichment of grape musts, concentrated grape musts, rectified concentrated grape musts and wines by application of nuclear magnetic resonance of deuterium (SNIF-NMR)."

3. TERMS AND DEFINITIONS

$^{13}C/^{12}C$:	ratio of carbon 13 (^{13}C) to carbon 12 (^{12}C) isotopes for a given sample.
$\delta^{13}C$:	carbon 13 content (^{13}C) expressed in parts per 1 000 (‰).
SNIF-NMR:	fractionating the particular natural isotope under study by nuclear magnetic resonance.
V-PDB:	Vienna-Pee-Dee Belemnite. PDB is the primary reference material for measuring natural variations of carbon 13 isotope content, consisting of calcium carbonate from a Cretaceous belemnite guard from the Pee Dee Formation in South Carolina (USA). Its $^{13}C/^{12}C$ isotope ratio or R_{PDB} is 0,0112372. PDB reserves have been exhausted for a long time, but it has remained the primary reference for expressing natural variations of carbon 13 isotope content and against which the reference material available at the International Atomic Energy Agency (IAEA) in Vienna (Austria) is calibrated. Isotopic indications of naturally occurring carbon 13 are conventionally expressed in relation to V-PDB.
m/z:	mass-to-charge ratio.

4. PRINCIPLE

During photosynthesis, the assimilation of carbon dioxide by plants occurs via two principle forms of metabolism, the C_3 metabolism (Calvin cycle) and the C_4 metabolism (Hatch and Slack). These two photosynthesis mechanisms present a different type of isotope fractionation. Products of C_4 plants, such as sugars and alcohol derived from fermentation, have higher levels of carbon 13 than similar products of C_3 plants. Most plants, including vines and sugar beets, belong to the C_3 group. Sugar cane and maize belong to the C_4 group. Measuring the carbon 13 content enables the detection and evaluation of sugars of C_4 origin (sugar cane or maize isoglucose) added to grape products (grape musts, wines, etc.). The information on carbon 13 content combined with that obtained from SNIF-NMR enables the added quantities of mixtures of sugars or alcohols derived from C_3 and C_4 plants to be determined.

The carbon 13 content is determined on carbon dioxide produced during the complete combustion of the sample. The abundance of the principle isotopomers of masses 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$) and 46 ($^{12}C^{16}O^{18}O$), resulting from the different possible combinations of isotopes ^{18}O, ^{17}O, ^{16}O, ^{13}C and ^{12}C, are determined from the ionic currents measured by three different collectors of a mass isotopic spectrometer. The contributions of isotopomers $^{13}C^{17}O^{16}O$ and $^{12}C^{17}O_2$ may be disregarded given their low levels. The ionic current for m/z = 45 is corrected for the contribution of $^{12}C^{17}O^{16}O$, which is calculated according to the current intensity measured for m/z = 46, while taking the relative abundance of ^{18}O and ^{17}O into account (Craig correction). Comparison with a reference calibrated against the international reference V-PDB permits calculation of carbon 13 content on the $\delta^{13}C$ relative scale.

5. REAGENTS

The material and the consumables depend on the apparatus (point 6) used by the laboratory. The systems generally used are based on elemental analysers. These systems can be equipped to allow the introduction of samples placed in sealed metal capsules or the injection of liquid samples through a septum using a syringe.

Depending on the type of instrument used, the following reference materials, reagents, and consumables may be used:

— reference materials

— available from the IAEA:

Name	Materiel	$\delta^{13}C$ relative to V-PDB (9)
— IAEA-CH-6	Sucrose	- 10,4 ‰
— IAEA-CH-7	Polyethylene	- 31,8 ‰
— NBS22	Oil	- 29,7 ‰
— USGS24	Graphite	- 16,1 ‰

— available from the IRMM in Geel (B) (Institute for Reference Materials and Measurements):

Name	Material	$\delta^{13}C$ relative to V-PDB (9)
— CRM/BCR 656	Wine alcohol	- 26,93 ‰
— CRM/BCR 657	Glucose	- 10,75 ‰
— CRM/BCR 660	Hydroalcoholic solution (ASV 12 %)	- 26,72 ‰

— a standard working sample with a known $^{13}C/^{12}C$ ratio calibrated against international reference materials,

— the following is an indicative list of consumables for continuous-flow systems:
  — helium for analysis (CAS 07440-59-7),
  — oxygen for analysis (CAS 07782-44-7),
  — carbon dioxide for analysis, used as a secondary reference gas for the carbon 13 content (CAS 00124-38-9),
  — oxidation reagent for the furnace of the combustion system, for example copper (II) oxide for elemental analysis (CAS 1317-38-0),
  — a desiccant to eliminate water produced in combustion, for example anhydrone for elemental analysis (magnesium perchlorate) (CAS 10034-81-8) (This is not necessary for apparatuses equipped with a water elimination system using cryogenic traps or selectively permeable capillaries).

6. APPARATUS AND EQUIPMENT

6.1. **Isotope ratio mass spectrometer (IRMS)**

Isotope ratio mass spectrometer (IRMS) capable of determining the relative ^{13}C content of naturally occurring CO_2 gas with an internal accuracy of 0,05 ‰ or better expressed as a relative value (point 9). Internal accuracy here is defined as the difference between two measurements of the same sample of CO_2. 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. The isotope ratio mass spectrometer must either be equipped with a dual inlet, to alternately measure the unknown sample and a reference sample, or use an integrated system that carries out the quantitative combustion of samples and separates the carbon dioxide from the other combustion products before measurement in the mass spectrometer.

6.2. **Combustion apparatus**

Combustion apparatus able to quantitatively convert ethanol into carbon dioxide and eliminate all other combustion products including water, without any isotopic fractionation. The apparatus may be either a continuous-flow system integrated with the mass spectrometry apparatus (point 6.2.1) or a separate combustion system (point 6.2.2). The apparatus must permit an accuracy of at least that indicated in (point 11).

6.2.1. *Continuous-flow systems*

These comprise either an elemental analyser or a gas chromatograph with an online combustion system.