



## RESOLUTION OIV-OENO 479-2017

### DETERMINATION OF THE $^{13}\text{C}/^{12}\text{C}$ ISOTOPE RATIOS OF GLUCOSE, FRUCTOSE, GLYCEROL AND ETHANOL IN PRODUCTS OF VITIVINICULTURAL ORIGIN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO ISOTOPE RATIO MASS SPECTROMETRY

THE GENERAL ASSEMBLY,

In view of Article 2, paragraph 2 iv of the Agreement establishing the International Organisation of Vine and Wine,

Further to the proposal of the “Methods of Analysis” Sub-Commission,

DECIDES, further to the proposal of Commission II “Oenology”, to introduce the following type II method for glucose, fructose and glycerol and type III method for ethanol into the *Compendium of International Methods of Analysis of Wines and Musts*:

Title	Method type
DETERMINATION OF THE $^{13}\text{C}/^{12}\text{C}$ ISOTOPE RATIOS OF GLUCOSE, FRUCTOSE, GLYCEROL AND ETHANOL IN PRODUCTS OF VITIVINICULTURAL ORIGIN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO ISOTOPE RATIO MASS SPECTROMETRY	<b>II and III</b>

#### 1. Scope of application

This method applies to products of vitivinicultural origin.

This method is:

- type II for glucose, fructose and glycerol,
- type III for ethanol.

#### 2. Principle

The samples are injected into the HPLC instrument after any necessary dilution and filtration. After oxidation in a liquid interface, the  $^{13}\text{C}/^{12}\text{C}$  isotope ratio of the compounds is determined using isotope

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ratio mass spectrometry. This liquid interface, symbolised by the acronym “co”, permits the chemical oxidation of the organic matter into CO<sub>2</sub>. HPLC-co-IRMS coupling can therefore be used to determine the isotope ratio of the following compounds simultaneously: glucose, fructose, glycerol and ethanol.

### **3. Reagents**

3.1 Pure water - resistivity > 18 MΩ cm, HPLC quality

3.2 Ammonium persulfate – analytical purity – [CAS No.: 7727-54-0]

3.3 Orthophosphoric acid (concentration 85%) – analytical purity – [CAS No.: 7664-38-2]

3.4 Analytical-grade helium, used as a carrier gas [CAS No.: 07440-59-7]

3.5 Reference gas: analytical-grade CO<sub>2</sub> (carbon dioxide), used as a secondary reference gas [CAS No.: 00124-38-9]

3.6 International standards

### **4. Equipment**

4.1 Everyday laboratory equipment

4.2 High-performance liquid chromatography instrument

4.3 Liquid interface for the oxidation of eluted compounds

4.4 Isotope ratio mass spectrometer

### **5. Analysis of the samples**

#### **5.1 Preparation of the samples**

Depending on the sugar, glycerol and ethanol contents, the samples should be diluted with the water (3.1) beforehand in order to obtain a concentration which is observable under the experimental

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conditions. Depending on the concentrations of the compounds, two measurements are needed with different dilutions.

## **5.2 Example of analytical conditions**

Total analysis duration: 20 minutes

As an indication, the dilution of grape juices and wines is around 1:200, while that of concentrated musts is approximately 1:500.

### HPLC:

Column: carbohydrate-type column (e.g. 700-CH Carbohydrate column, HyperRez XP Carbohydrate H<sup>+</sup>)

Injection volume: 25 µl

Mobile phase: water (3.1)

Flowrate: 0.4 mL/min

Column T°: 80 °C

### Liquid Interface:

Solution of ammonium persulfate (3.2) (15% in mass) and orthophosphoric acid (2.5% in volume)

Peristaltic pump flow: 0.6 mL/min

Heater temperature: 93 °C

Flow of the helium carrier gas: 15 mL/min

Helium flow for drying: 50 mL/min

### IRMS:

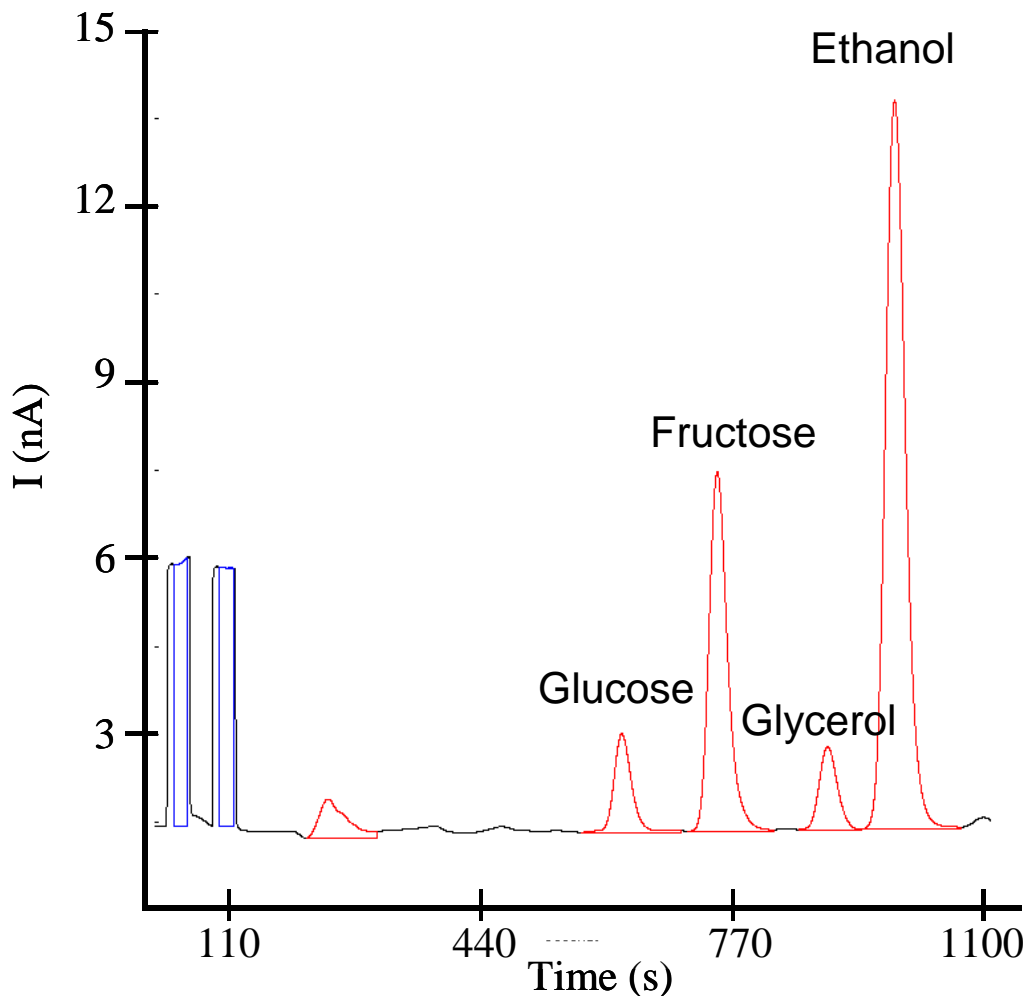
Trap current: 300 µA

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### 5.3 Example chromatogram

Chromatogram of a sweet wine analysed using HPLC-co-IRMS



### 6. Determination of isotope ratios

The reference gas, CO<sub>2</sub>, is calibrated from international commercial standards. The isotope ratios are expressed in δ ‰ in relation to the Pee Dee Belemnite (PDB) and are defined as:

$$\delta^{13}\text{C}_{\text{Sam}} (\text{‰}) = [(R_{\text{Sam}} / R_{\text{St}}) - 1] * 10^3$$

Where: Sam = sample; St = standard; R = <sup>13</sup>C/<sup>12</sup>C isotope ratio

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## 7. Method characteristics

The characteristics of the method for the measurement of the  $\delta^{13}\text{C}$  isotope ratios of glucose, fructose, glycerol and ethanol by HPLC-co-IRMS have been determined from the results obtained from an inter-laboratory analysis of four samples (dry wine, sweet wine, grape juice and rectified concentrated must). The results obtained for each compound analysed and each type of matrix are annexed.

## 8. Bibliography

1. Cabanero, Al.; Recio, JL.; Rupérez, M. (2008) Isotope ratio mass spectrometry coupled to liquid and gas chromatography for wine ethanol characterization. *Rapid Commun. Mass Spectrom.* 22: 3111-3118.
2. Cabanero, Al.; Recio, JL.; Rupérez, M. (2010) Simultaneous stable carbon isotopic analysis of wine glycerol and ethanol by liquid chromatography coupled to isotope ratio mass spectrometry. *J. Agric. Food Chem.* 58: 722-728.
3. Guyon, F.; Gaillard, L.; Salagoity, MH.; Médina, B. (2011) Intrinsic Ratios of Glucose, Fructose, Glycerol and Ethanol  $^{13}\text{C}/^{12}\text{C}$  Isotopic Ratio Determined by HPLC-co-IRMS: Toward Determining Constants for Wine Authentication. *Anal. Bioanal. Chem.* 401:1551-1558

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## Annex

### Statistical treatment of the HPLC-co-IRMS inter-laboratory analysis for the determination of the precision of the method (repeatability and reproducibility)

#### List of laboratories in alphabetical order of country of origin.

Country	Laboratory
Belgium	IRMM
China	CNRIFI
Czech Republic	SZPI
France	SCL-33
Germany	INTERTEK
Germany	UNI DUE
Germany	ELEMENTAR
Germany	QSI
Germany	LVI
Italy	FLORAMO
Japan	AKITA Univ.
Spain	MAGRAMA

#### Responses:

12 laboratories / 14 responses

#### Treatment of the results of inter-laboratory analyses according to ISO 5725-2

#### Samples:

- 1 dry wine (Wine A)
- 1 sweet wine (Wine B)
- 1 rectified concentrated must (RCM)
- 1 grape juice

#### Analytical conditions:

Each sample was analysed in duplicate (repeatability) and double blind (reproducibility)  
Expression of results in ‰ vs. PDB

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**Precision of the glucose measurement**  
**Repeatability and reproducibility**

	Wine B	RCM	Grape juice
Number of laboratories	12	12	12
Number of responses	14	13	14
Number of responses retained (elimination of outliers)	13	13	12
Minimum value	-26.33	-25.04	-25.78
Maximum value	-23.72	-23.74	-24.62
Mean value	-25.10	-24.24	-25.19
Repeatability variance	0.02	0.01	0.01
Repeatability standard deviation ( $S_r$ )	0.14	0.10	0.09
Repeatability limit ( $r$ ‰)	0.40	0.29	0.24
Reproducibility variance	0.39	0.14	0.11
Reproducibility standard deviation ( $S_R$ )	0.62	0.38	0.33
Reproducibility limit ( $R$ ‰)	1.77	1.06	0.94

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**Precision of the fructose measurement**  
**Repeatability and reproducibility**

	Wine B	RCM	Grape juice
Number of laboratories	12	11	12
Number of responses	14	13	14
Number of responses retained (elimination of outliers)	13	13	13
Minimum value	-25.56	-24.19	-25.33
Maximum value	-24.12	-23.19	-23.98
Mean value	-24.87	-23.65	-24.56
Repeatability variance	0.02	0.03	0.02
Repeatability standard deviation ( $S_r$ )	0.14	0.16	0.14
Repeatability limit ( $r$ ‰)	0.40	0.46	0.39
Reproducibility variance	0.15	0.10	0.18
Reproducibility standard deviation ( $S_R$ )	0.39	0.32	0.42
Reproducibility limit ( $R$ ‰)	1.10	0.90	1.19

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**Precision of the glycerol measurement**  
**Repeatability and reproducibility**

	Wine A	Wine B
Number of laboratories	12	12
Number of responses	12	12
Number of responses retained (elimination of outliers)	11	11
Minimum value	-32.91	-30.74
Maximum value	-30.17	-28.27
Mean value	-31.75	-29.54
Repeatability variance	0.13	0.04
Repeatability standard deviation ( $S_r$ )	0.36	0.19
Repeatability limit (r ‰)	1.03	0.55
Reproducibility variance	0.57	0.37
Reproducibility standard deviation ( $S_R$ )	0.76	0.61
Reproducibility limit (R ‰)	2.14	1.72

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**Precision of the ethanol measurement**  
**Repeatability and reproducibility**

	Wine A	Wine B
Number of laboratories	12	12
Number of responses	11	12
Number of responses retained (elimination of outliers)	10	12
Minimum value	-27.85	-27.60
Maximum value	-26.50	-26.06
Mean value	-27.21	-26.82
Repeatability variance	0.03	0.03
Repeatability standard deviation ( $S_r$ )	0.16	0.17
Repeatability limit (r ‰)	0.47	0.47
Reproducibility variance	0.16	0.23
Reproducibility standard deviation ( $S_R$ )	0.40	0.47
Reproducibility limit (R ‰)	1.14	1.34

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