OIV-MA-AS311-09 Determination of the $^{13}C/^{12}C$ isotope ratios of glucose, fructose, glycerol, ethanol in production of vitivinicultural origin by high-performance liquid chromatography coupled to isotope ratio mass spectrometry

Type II and III method

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 $^{13}C/^{12}C$ isotope ratio of the compounds is determined using isotope ratio mass spectrometry. This liquid interface, symbolised by the acronym "*co*", permits the chemical oxidation of the organic matter into CO2. 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 M□ 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_2 (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 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^+)

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

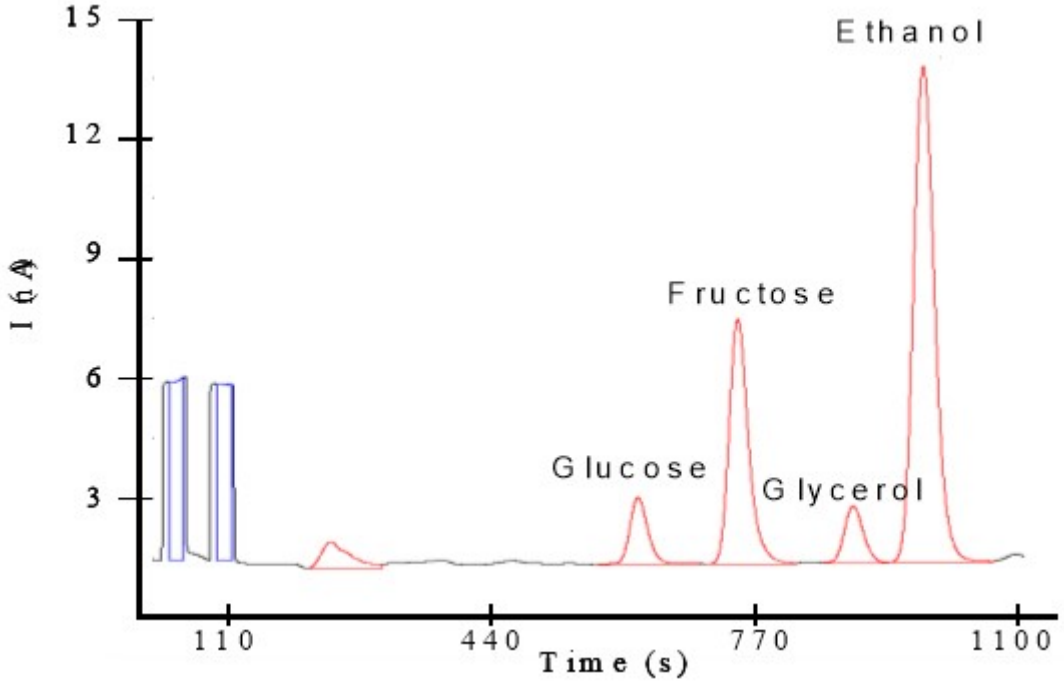
IRMS:

Trap current: 300 µA

5.3. Example chromatogram

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

6. Determination of isotope ratios



The reference gas, CO_2, is calibrated from international commercial standards. The isotope ratios are expressed in □ ‰ in relation to the Pee Dee Belemnite (PDB) and are defined as:

$$\delta^{13}C_{SAM}(\%) = [(R_{SAM}/R_{St}) - 1]*10^3$$

Where: Sam = sample; St = standard; R = $^{13}C/^{12}C$ isotope ratio

7. Method characteristics

The characteristics of the method for the measurement of the $\delta^{13}C$ 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

- Cabanero, AI.; 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.

- Cabanero, AI.; 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.
- Guyon, F.; Gaillard, L.; Salagoïty, MH.; Médina, B. (2011) Intrinsic Ratios of Glucose, Fructose, Glycerol and Ethanol $^{13}C/^{12}C$ Isotopic Ratio Determined by HPLC-*co*-IRMS: Toward Determining Constants for Wine Authentication. Anal. Bioanal. Chem. 401:1551-1558

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	CNRIFFI
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:

Determination of the 13C/12C isotope ratios of glucose, fructose, glycerol, ethanol in production of vitivinicultural origin by high-performance liquid chromatography coupled to isotope ratio mass spectrometry (Type-II-and-III)

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

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

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

Precision of the glycerol measurement

Determination of the 13C/12C isotope ratios of glucose, fructose, glycerol, ethanol in production of vitivinicultural origin by high-performance liquid chromatography coupled to isotope ratio mass spectrometry (Type-II-and-III)

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

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

Determination of the 13C/12C isotope ratios of glucose, fructose, glycerol, ethanol in production of vitivinicultural origin by high-performance liquid chromatography coupled to isotope ratio mass spectrometry (Type-II-and-III)

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