OIV-MA-AS311-03 Dosage of sugars in wine by HPLC

Type II method

1. Scope of application

This method is applicable to the direct quantification of sugars in musts and wines up to 20 g/L and, after dilution, beyond.

Glycerol (between 0.5 and 15 g/L) and sucrose (between 1 and 40 g/L) may also be quantified in the same way.

2. Principle

Sugars and glycerol are separated by HPLC using an alkylamine column and detected by refractometer.

3. Reagents

3.1. Demineralised Type I water (ISO 3696) or equivalent (HPLC grade);
3.2. acetonitrile [75-05-8] (minimal transmission at 200 nm - purity $\geq$ 99%);
3.3. fructose [57-48-7] (purity $\geq$ 99%);
3.4. glucose [492-62-6] (purity $\geq$ 99%);
3.5. sucrose [57-50-1] (purity $\geq$ 99%);
3.6. glycerol [56-81-5] (purity $\geq$ 99%).

Preparation of reagent solutions

3.7. Demineralised water (3.1): filtered through a 0.45 µm cellulose membrane;
3.8. eluent: acetonitrile (3.2)/water (3.9) with a respective ratio of 80/20.

Note 2: the water/acetonitrile ratio may be adapted according to the objectives.

4. Apparatus

4.1. 0.45 µm Cellulose filtration membrane;
4.2. silica-based, octadecyl-bonded filter cartridge (e.g. Sep-Pak C_{18});
4.3. common apparatus for high-performance liquid chromatography;
4.4. alkylamine column (5 µm, 250 x 4.6 mm);

Note 3: columns of different lengths, internal diameter and particle size may be used but the type II method refers to the dimensions provided.

4.5. refractometric index detector (RID);
4.6. common laboratory apparatus.

5. Sampling

The samples are degassed beforehand if necessary (e.g. with nitrogen or helium, or in an ultrasonic bath).

6. Procedure

6.1. Preparation of the sample

6.1.1. Dilution

Wines containing less than 20 g/L of (glucose + fructose) are analysed undiluted. Musts and wines containing more than 20 g/L have to be diluted to be within the range of calibration.

6.1.2. Filtration

The samples must be filtered using a 0.45 µm membrane (4.1) before analysis.

6.1.3. Elimination of phenolic compounds (if necessary)

For a must or wine, pass over a C_{18} cartridge (4.2).

6.2. Analyses

6.2.1. Analytical conditions

Note 4: The following instructions are mandatory for the type II method.

Note 5: Conditions may be adapted by the laboratory with the loss of the type II reference.

HPLC system (4.3) equipped with column (4.4) and RID (4.5).

Mobile phase: isocratic acetonitrile/water eluent (3.10).

Flow: 1 mL/min.

Injected volume: between 10 and 50 µL, to be adapted according to the material used.

Examples of chromatograms are shown in Annex B (Figures 1 and 2).

The fructose-glucose resolution is recommended to be $\geq$ 2.

6.2.2. External calibration

The calibration solution that applies to all compounds described in this procedure may contain the following:

- 10 g/L glycerol (3.6) $\pm$ 0.01 g/L,
- 10 g/L fructose (3.3) $\pm$ 0.01 g/L,
- 10 g/L glucose (3.4) $\pm$ 0.01 g/L,
- 10 g/L sucrose (3.5) $\pm$ 0.01 g/L.

Note 6: if quantifying only one of these compounds, a solution that contains only the one required can be prepared.

6.3. Calculation of response factors for external calibration used in routine analyses

$RF_i = area_i / C_i$

where

- *area i* = peak area of the product in the calibration solution
- and C_i = quantity of product present in the calibration solution.

It is also possible to use a calibration curve.

7. Expression of results

1. Calculation of concentrations

$C_e = area_e / RF_i$

Where:

$area_e$ = peak area of product present in the sample.

The results are expressed in g/L.

Note 7: the results are indicated to a maximum of one decimal place.

8. Quality assurance and control

Traceable to the international references through mass, volume and temperature.

Synthetic mixtures or samples coming, for instance, from proficiency ring test are used as internal quality control. A control chart may be used

9. Performance of the method

No known compound co-elutes with fructose, glucose or sucrose.

Robustness: the analysis is sensitive to slight variations in temperature. Columns should be protected from temperature variations.

10. Precision

(See Annex B.3)

10.1. Glucose (content $\geq$ 3 g/L)

- Repeatability limit $\cong$ reproducibility limit = 13%

2. Fructose (content $\geq$ 2 g/L)

- Repeatability limit = 7%
- Reproducibility limit = 10%

3. Glucose + fructose (content $\geq$ 5 g/L)

- Repeatability limit $\cong$ Reproducibility limit = 10%

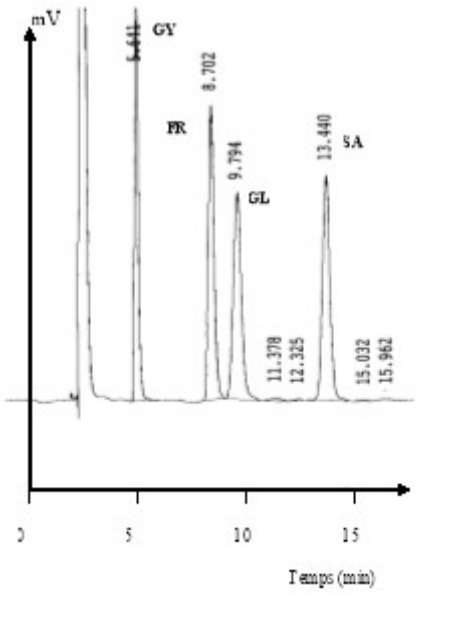


Figure 1 Chromatogram of a calibration solution (sugars and glycerol at 10 g/l

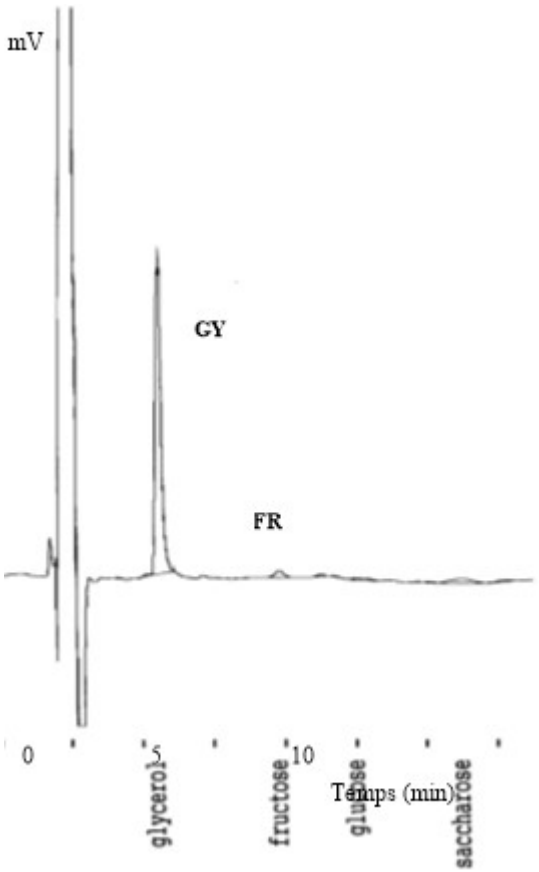
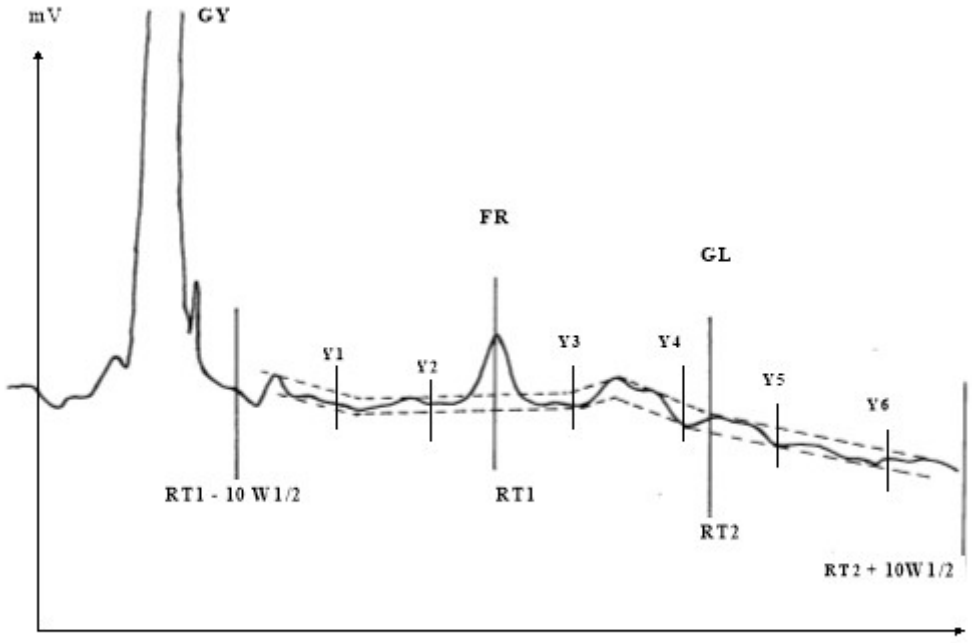


Figure 2 Chromatogram of a rosé wine

Glycerol (GY), fructose (FR), glucose (GL), saccharose (SA)



fructose (FR), glucose (GL), saccharose (SA) Glycerol (GY),

Figure 3 - Measure of pitches of noise after enlargement of chromatogram

RT1: retention time of fructose; RT2: retention time of glucose

W1/2: width of peak at mid-height; Yi: pitch of noise at point i

Annex B

(informative)

Precision data

B.1 - Samples in the interlaboratory test trial

This study was carried out by the Interregional Laboratory of the Répression de Fraudes in Bordeaux. The test trial involved 6 samples in blind duplicates (12 samples in total), identified as A to J (4 white wines and 4 red wines; 2 white Port wines and 2 red Port wines), containing glucose and fructose and whose content of each sugar was between 2 and 65 g/L. The wines from the region of Bordeaux were supplemented with glucose and fructose and stabilised with 100 mg/L of SO_2 (TRICARD and

MEDINA, 2003).

B.2 - Chromatographic conditions

Considering the response factors of these two sugars and the scales of the chromatograms, the noise corresponds to a concentration of 0.04 g/L for fructose and of 0.06 g/L for glucose (see Figure A3).

The limits of detection (3 times the noise) and of quantification (10 times the noise) are then obtained:

- $LD_{fructose} = 0.12\ g/L$
- $LD_{glucose} = 0.18\ g/L$
- $LQ_{fructose} = 0.4\ g/L$
- $LD_{glucose} = 0.6\ g/L$

These results are compliant with those determined by TUSSEAU and BOUNIOL (1986) and are repeatable on other chromatograms.

B.3 - Precision

Nine laboratories participated in the interlaboratory study:

Istituto Sperimentale per l'Enologia, Asti, Italy;

Laboratoire de la DGCCRF de Montpellier, France;

Laboratoire LARA, Toulouse, France;

Instituto do vinho do Porto, Porto, Portugal;

Instituto da Vinha e do Vinho, Unhos, Portugal;

Estación de Viticultura y Enología, Vilafranca del Penedés, Spain;

Comité Interprofessionnel du vin de Champagne, Epernay, France;

Station fédérale de Changins, Switzerland;

Laboratoire de la DGCCRF de Talence, France.

The analyses of 3 points of the set of calibration solutions and the 12 samples were carried out successively by applying the method of analysis given.

The results were analysed according to the OIV protocol (Validation protocol of methods of analysis – Resolution OENO 6/1999).

This protocol does not require the analyses to be repeated, whereas 4 laboratories gave results of analyses repeated 3 times. A single series was chosen (the first one) for the analysis of the results, in compliance with the OIV protocol.

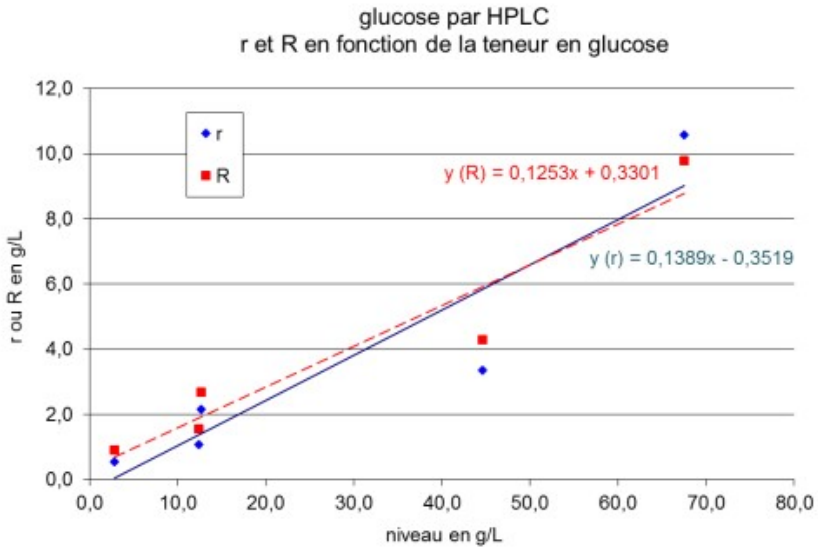
The calculations of repeatability according to Youden, reproducibility and Cochran and Grubbs tests were performed.

Data on the repetitions made it possible to work out the standard deviations of repeatability in another way (according to ISO 5725).

B.3.1 – GLUCOSE

Glucose by HPLC (g/L)						
Number of laboratories	9	9	9	9	9	9
Number of samples	2	2	2	2	2	2
Average value	2.9	2.9	12.6	12.4	44.6	67.5
Repeatability standard deviation	0.44	0.17	0.67	0.34	1.05	3.31
Repeatability limit	1.42	0.55	2.15	1.07	3.35	10.58
Reproducibility standard deviation	0.78	0.30	0.90	0.52	1.43	3.28
Reproducibility limit	2.32	0.90	2.68	1.55	4.28	9.78
Horrat value	5.7*	2.1	1.84	1.08	1.01	1.62

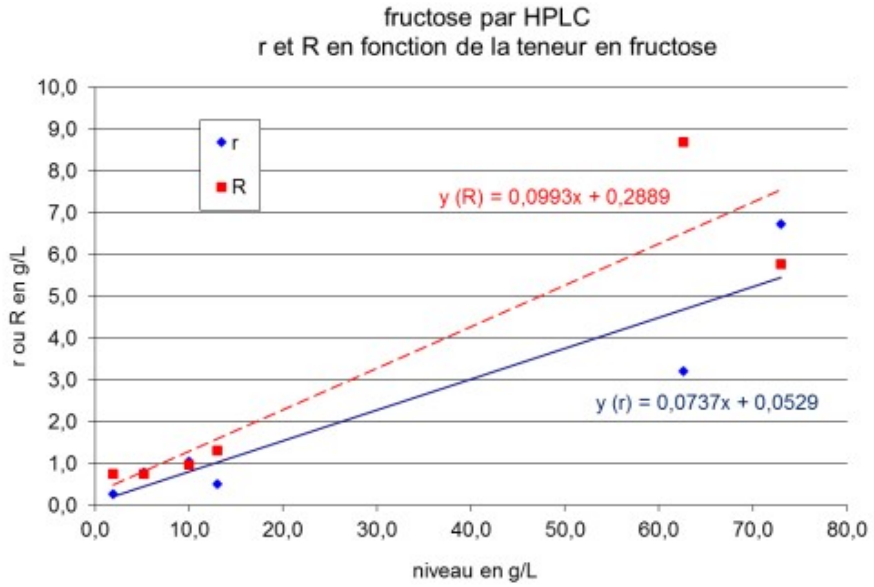
* not taken into account for the expression of precision



Correlation between r and R and the concentration for glucose (ISO 5725)

B.3.2 – FRUCTOSE

Fructose by HPLC (g/L)						
Number of laboratories	9	9	9	9	9	9
Number of samples	2	2	2	2	2	2
Average value	1.9	5.2	10.0	13.0	62.6	73.0
Repeatability standard deviation	0.09	0.24	0.32	0.16	3.20	2.10
Repeatability limit	0.27	0.79	1.03	0.51	3.20	6.72
Reproducibility standard deviation	0.25	0.25	0.32	0.43	2.91	1.93
Reproducibility limit	0.75	0.75	0.96	1.30	8.68	5.77
Horrat value	2.54	1.09	0.81	0.87	1.53	0.89

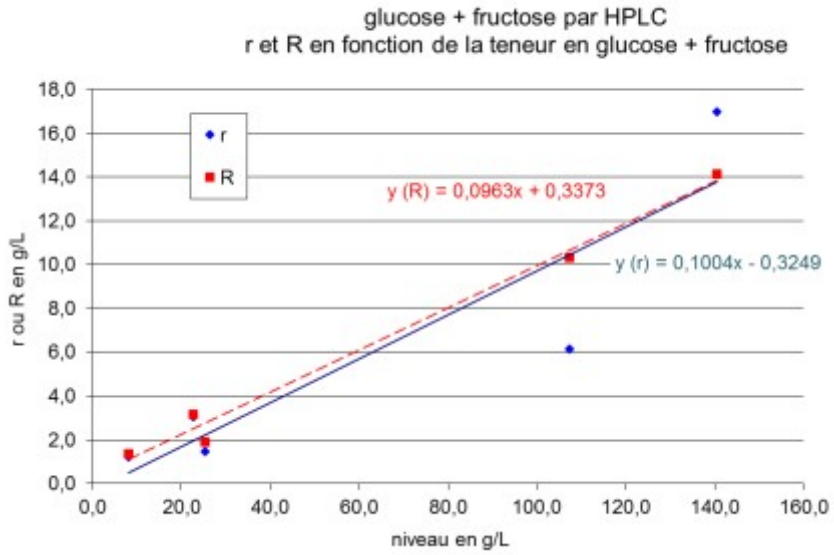


Correlation between r and R and the concentration for fructose (ISO 5725)

B.3.3 – GLUCOSE + FRUCTOSE

Glucose + fructose by HPLC (g/L)						
Number of laboratories	9	9	9	9	9	9
Number of samples	2	2	2	2	2	2
Average value	4.7	8.1	22.6	25.4	107.3	140.5
Repeatability standard deviation	0.48	0.38	1.06	0.46	1.92	5.30
Repeatability limit	1.52	1.21	3.07	1.48	6.13	17.0
Reproducibility standard deviation	0.89	0.46	1.06	0.64	3.47	4.74
Reproducibility limit	2.64	1.38	3.17	1.90	10.34	14.15
Horrat value	4.17*	1.39	1.33	0.72	1.15	1.26

* not taken into account for the expression of precision



Correlation between r and R and the concentration for glucose + fructose (ISO 5725)

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