Method OIV-MA-BS-11 : R2009

Type II method

Determination of sugars in spirit drinks of viti-vinicultural origin

(OIV/OENO 380/2009)

Introduction

Spirit drinks of viti-vinicultural origin may be sweetened by various compounds, and in certain legislations the concentrations of sweetener are subject to minimum or maximum levels.

1. Scope

This method is suitable for the determination of the glucose, fructose, and sucrose contents of spirit drinks of viti-vinicultural origin. It is not suitable for spirit drinks containing dairy products or eggs.

2. Normative References

ISO 3696:1897 Waters for analytical use - Specifications and test methods.

3. Principle

High performance liquid chromatography (HPLC) to determine the glucose, fructose, and sucrose concentrations.

This method is described as an example. It uses an alkylamine stationary phase and differential refractometry detection. Other columns/detectors may be used, for example anion exchange resins as the stationary phase.

4. Reagents and Materials

4.1. Glucose (CAS 50-99-7), at least 99 % pure.

4.2. Fructose (CAS 57-48-7), at least 99 % pure.

4.3. Sucrose (CAS 57-50-1), at least 99 % pure.

4.4. Pure acetonitrile (CAS 75-05-8) for HPLC analysis.

Acetonitrile is a highly flammable liquid. It is toxic by inhalation, in contact with skin and if swallowed. It is irritating to eyes.

4.5. Distilled or demineralised water, preferably micro-filtered.

4.6. Solvents (example)

The elution solvent is prepared beforehand by mixing:

- 75 parts by volume of acetonitrile (4.4),
- 25 parts by volume of distilled or demineralised water (4.5).

Pass helium through at a slow rate for 5 - 10 minutes prior to use to degas.
If the water being used has not been micro-filtered, it is advisable to pass the solvent through a filter for organic solvents with a pore size less than or equal to 0.45 μm.

4.7. Ethanol, absolute (CAS 64-17-5).

4.8. Ethanol solution (5 %, v/v).

4.9. Preparation of stock standard solution (20 g/L)

Weigh 2 g each of the sugars to be analysed (4.1 to 4.3), transfer them without loss to a 100 mL volumetric flask. Adjust to 100 mL with a 5 % vol. alcohol solution (4.8), shake and store at around +4 °C. Prepare a new stock solution once a week if necessary.

4.10. Preparation of working standard solutions (2.5, 5.0, 7.5, 10.0 and 20.0 g/L)

Dilute the stock solution, 20 g/L, (4.9) appropriately with a 5% vol. alcohol solution (4.8) to give five working standards of 2.5, 5.0, 7.5, 10.0 and 20.0 g/L. Filter with a filter of a pore size less than or equal to 0.45 µm (5.3.).

5. Apparatus and Equipment (as an example - other systems that provide equivalent performance can be used)

Standard laboratory apparatus, "A" grade volumetric glassware and, in particular, the following:

5.1. HPLC system capable of achieving baseline resolution of all of the sugars.

5.1.1. High-performance liquid chromatograph with a six-way injection valve fitted with a 10 µL loop or any other device, whether automatic or manual, for the reliable injection of micro-volumes.

5.1.2. Pumping system enabling one to achieve and maintain a constant or programmed rate of flow with great precision.

5.1.3. Differential refractometer.

5.1.4. Computational integrator or recorder, the performance of which is compatible with the rest of the set-up.

5.1.5. Pre-column:

It is recommended that a suitable pre-column is attached to the analytical column.

5.1.6. Column (example):

Material: stainless steel or glass

Internal diameter: 2-5 mm

Length: 100-250 mm (depending on the packing particle size), for example 250 mm if

the particles are 5 µm in diameter

Stationary phase: cross-linked silica with radicals containing the alkylamine functional group, maximum particle size 5 µm.

5.1.7. Chromatography conditions (example):

Elution solvent (4.6), flow rate: 1 mL/minute

Detection: Differential refractometry

To make certain that the detector is perfectly stable, it may be advisable to switch it on a few hours before use. The reference cell must be filled with the elution solvent.

5.2. Analytical balance accurate to 0.1 mg.

5.3. Filtration equipment for small volumes using a 0.45 µm membrane.

6. Sample storage

On receipt, samples are to be stored at room temperature prior to analysis.

7. Procedure

7.1. PART A: Sample Preparation

7.1.1. Shake the sample.

7.1.2. Filter the sample through a filter with a pore size less than or equal to 0.45 µm (5.3).

7.2. PART B: HPLC

7.2.1. Determination

Inject 10 µL of the standard solutions (4.10) and samples (7.1.2.). Perform the analysis

under suitable chromatography conditions, for example those described above.

7.2.2. Should any peak of a sample have a greater area (or height) than the corresponding peak in the most concentrated standard, then the sample should be diluted with distilled or demineralised water and re-analysed.

8. Calculation

Compare the two chromatograms obtained for the standard solution and spirit. Identify the peaks by their retention times. Measure their areas (or heights) to calculate the concentrations by the external standard method. Take into account any dilutions made to the sample.

The final result by convention is the sum of sucrose, glucose, and fructose, in g/L.

9. Method performance characteristics (Precision)

The following data were obtained in 2000 from an international method- performance study carried out on a variety of spirit drinks, following internationally-agreed procedures.

Key to the tables below:

nLT	Number of laboratories (2 results per laboratory)
nL	Number of laboratories to calculate precision values
r	repeatability limit
Sr	repeatability standard deviation
RSDr	repeatability standard deviation expressed in % of the level
R	reproducibility limit
SR	reproducibility standard deviation

RSDR	reproducibility standard deviation expressed in % of the level
PRSDR	RSDR predicted with the Horwitz formula (%)
HoR	HorRat value = RSDR / PRSDR

9.1. Glucose

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	24	92,4	5,4	1,9	2,1	13	4,8	5,2	1,8
Liqueur 2	24	23	93,2	9,7	3,5	3,7	28	10	11	3,8

9.2. Fructose

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	22	87	3,2	1,2	1,3	8,5	3,0	3,5	1,2
Liqueur 2	24	21	93	6,6	2,3	2,5	22	7,7	8,3	2,9

9.3. Saccharose

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR

Liqueur 1	26	24	174	12	4,2	2,4	24	8,7	5,0	1,9
Liqueur 2	24	18	320	12	4,3	1,3	45	16	5,0	2,1
Liqueur 3	24	18	349	22	8,0	2,3	30	11	3,1	1,3
Pastis	24	19	11	0,2	0,1	0,8	2,2	0,8	7,3	1,9
Ouzo	24	19	24	2,1	0,8	3,1	2,6	0,9	3,8	1,1
Kirsch	24	20	103	6,1	2,2	2,1	12	4,2	4,0	1,4

9.4. Sucres totaux

	nLT	nL	Mean (mg/L)	r (mg/L)	Sr (mg/L)	RSDr (%)	R (mg/L)	SR (mg/L)	RSDR (%)	HoR
Liqueur 1	26	21	353	8,7	3,1	0,9	41	15	4,2	1,8
Liqueur 2	24	18	510	16	5,6	1,1	41	15	2,9	1,3
Liqueur 3	24	18	349	22	8,0	2,3	30	11	3,1	1,3
Pastis	24	20	11	0,4	0,1	1,2	2,2	0,8	7,3	1,8
Ouzo	24	19	24	2,1	0,8	3,1	2,6	0,9	3,8	1,1
Kirsch	24	20	103	6,1	2,2	2,1	12	4,2	4,0	1,4

10. Bibliography

1. R. Wittkowski, A. Bertrand, P. Brereton, C. Guillou, 2000. PROJECT SMT4-CT96-2119, Validation of analytical methods of analysis for spirit drinks. REPORT NO. 02/09 - WORKSTREAM 10.
2. P. Brereton, S. Hasnip, A. Bertrand, R. Wittkowski, C. Guillou, Analytical methods for the determination of spirit drinks, Trends in Analytical Chemistry, Vol. 22, No. 1, 19-25, 2003.