RESOLUTION OIV-OENO 665-2022

DETERMINATION OF SWEETENERS IN WHITE WINE AND WHITE WINE-BASED BEVERAGES BY HIGH PERFORMANCE LIQUID CHROMATOGEAPHY COUPLED WITH A DIODE ARRAY DETECTOR AND A CHARGED AEROSOL DETECTOR

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL OF the "Methods of Analysis" Sub-Commission,

DECIDES to add the following method to Annex A of the Compendium of International Methods of Wine and Must Analysis:

Determination of sweeteners in white wine and white wine-based beverages by high performance liquid chromatography coupled with a diode array detector and a charged aerosol detector

Type IV method

1. Scope of application

This method makes it possible to determine five artificial sweeteners (acesulfame-K, aspartame, saccharine, sodium cyclamate and sucralose) in white wine (and white-wine-based beverages), within concentration ranges of up to 50 mg/L for saccharine, 125 mg/L for acesulfame-K and 250 mg/L for sucralose, sodium cyclamate and aspartame.

For greater concentrations, dilution of the sample is necessary.

Note: The presence of anthocyanins interferes with the determination of these sweeteners in rosé wine and red wines.

2. Principle

The five sweeteners are analysed by high performance liquid chromatography through separation on a C18 reverse-phase column coupled with a diode array detector and charged aerosol detector connected in series (HPLC/UV-CAD).

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3. Reagents and solutions

3.1. Reagents:

3.1.1. Water compliant with EN ISO 3696 or equivalent

3.1.2. Acesulfame-K (purity ≥ 99%) (CAS No. 55589-62-3)

3.1.3. Aspartame (purity ≥ 98%) (CAS No. 22839-47-0)

3.1.4. Sodium cyclamate (purity ≥ 98%) (CAS No. 139-05-9)

3.1.5. Saccharine sodium salt dihydrate (purity ≥ 98%) (CAS No. 6155-57-3)

3.1.6. Sucralose (purity ≥ 98%) (CAS No. 56038-13-2)

3.1.7. Formic acid (purity ≥ 98%) (CAS No. 64-18-6)

3.1.8. Ammonium bicarbonate (purity ≥ 98%) (CAS No. 1066-33-7)

3.1.9. HPLC-grade methanol (purity ≥ 99.9%) (CAS No. 67-56-1)

3.1.10. HPLC-grade acetone (purity ≥ 99.8%) (CAS No. 67-64-1)

3.2. Preparation of buffer solution

Prepare an ammonium bicarbonate (3.1.8) buffer solution at 0.4 g/L in water (3.1.1) and adjust the pH to 4.6 with formic acid (3.1.7).

This solution can be kept for 1 month at room temperature.

4. Apparatus

4.1. Everyday laboratory equipment

4.2. pH meter

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4.3. Stirrer

4.4. Ultrasound bath

4.5. Analytical balance with precision of ± 0.01 mg

4.6. Class A volumetric flasks

4.7. 0.45 µm regenerated cellulose syringe filters (for example)

4.8. C18 HPLC column (15 cm in length, 4.6 mm in internal diameter, 5 µm)

4.9. Chromatography system composed of:

- pump system with a minimum of three channels,
- thermostatically-controlled sampler,
- column oven,
- diode array or UV-VIS detector,
- charged aerosol detector (CAD),
- system of data acquisition, integration and calculation.

5. Procedure

5.1. Preparation of samples

- Filter the sample with syringe filters before placing it in a vial,
- if the sample is too concentrated, dilute with the buffer solution to bring the concentration within the above-specified range.

5.2. Preparation of standard solutions

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5.2.1. Preparation of the stock solution, L3 (given by way of example)

In a 100-mL flask, add the following approximate amounts (weighed and recorded accurately):

- 12.5 mg acesulfame-K (3.1.2),
- 25 mg aspartame (3.1.3),
- 25 mg sodium cyclamate (3.1.4),
- 5 mg saccharine (3.1.5),
- 25 mg sucralose (3.1.6).

Dissolve in approx. 20 mL water/ethanol (1:1), then make up to the mark with the buffer solution. Use ultrasonic processing if necessary.

This solution, L3, can be stored for 6 months at between 2 °C and 8 °C.

5.2.2. Preparation of working solutions, L2 and L1

Prepare two other concentration levels from solution L3:

L2: solution L3 diluted to 1/2 its initial concentration. For example, add 10 mL L3 solution to a 20 mL flask and make up to volume with the buffer solution.

L1: solution L3 diluted to 2/25 its initial concentration. For example, add 2 mL L3 solution to a 25 mL flask and make up to volume with the buffer solution.

Filter the standard solutions with syringe filters and place the vials in the sampler.

Summary table of standard solutions (given by way of example):

Sweeteners	Stock solution (Level 3)			Working solution (L2)			Working solution (L1)		
	Weight (mg)	Flask volume	Concentration in mg/L	Sampled volume of L3	Flask volume	Concentration in mg/L	Sampled volume of L3	Flask volume	Concentration in mg/L
Acesulfame-K	12.5	100 mL	125	10 mL	20 mL	62.5	2 mL	25 mL	10
Aspartame	25		250			125			20
Na cyclamate	25		250			125			20
Saccharine	5		50			25			4
Sucralose	25		250			125			20

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5.3. Chromatography conditions

By way of example, the conditions used to achieve the performance described in the annex are as follows:

- Column oven temperature: 30 °C
- Sampler temperature: 20 °C
- Composition of the mobile phase (HPLC-quality reagents):
    - Phase A: 72% methanol / 25% buffer / 3% acetone
    - Phase B: 12% methanol / 88% buffer
- UV detection wavelength: 210 nm
- Flow rate: 1 mL/min
- Injection volume: 10 µL
- CAD parameters:
    - Filter: 3.6
    - Data collection rate: 10 Hz
    - Temperature: high (50 °C)
    - Power function: 0-13 min: 1.50; 13-40 min: 1.48
- Elution gradient to be applied:

Temps (min)	Percentage of A	Percentage of B
0	0%	100%
4	0%	100%
11	53%	47%
18.5	82%	18%

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25	82%	18%
27	100%	0%
31	100%	0%
32	0%	100%
40	0%	100%

Stabilise the column for as long as necessary with mobile phase B, in addition to the CAD and DAD.

6. Calculations

The results are calculated by external calibration according to the peak area of each sweetener and expressed in mg/L, according to the calculation formula:

$$Concentratoion\ of\ sample\left(\frac{mg}{L}\right) = \frac{A_S - Int}{P} \times Dilution$$

Where A_S is the peak area of the sample, *Int* is the intercept of the calibration curve and *P* is the slope of the calibration curve.

7. Expression of results

For concentrations of $<$ 10 mg/L, the results can be expressed in mg/L to one significant figure after the decimal point.

For concentrations of $\geq$ 10 mg/L, the results can be expressed in mg/L to the nearest whole number.

8. Bibliography

1. International Organisation of Vine and Wine (OIV), *Compendium of International Methods of Analysis of Wines and Musts*, Vol. 1 and 2.
2. NF EN 15911: Simultaneous determination of nine sweeteners by high performance

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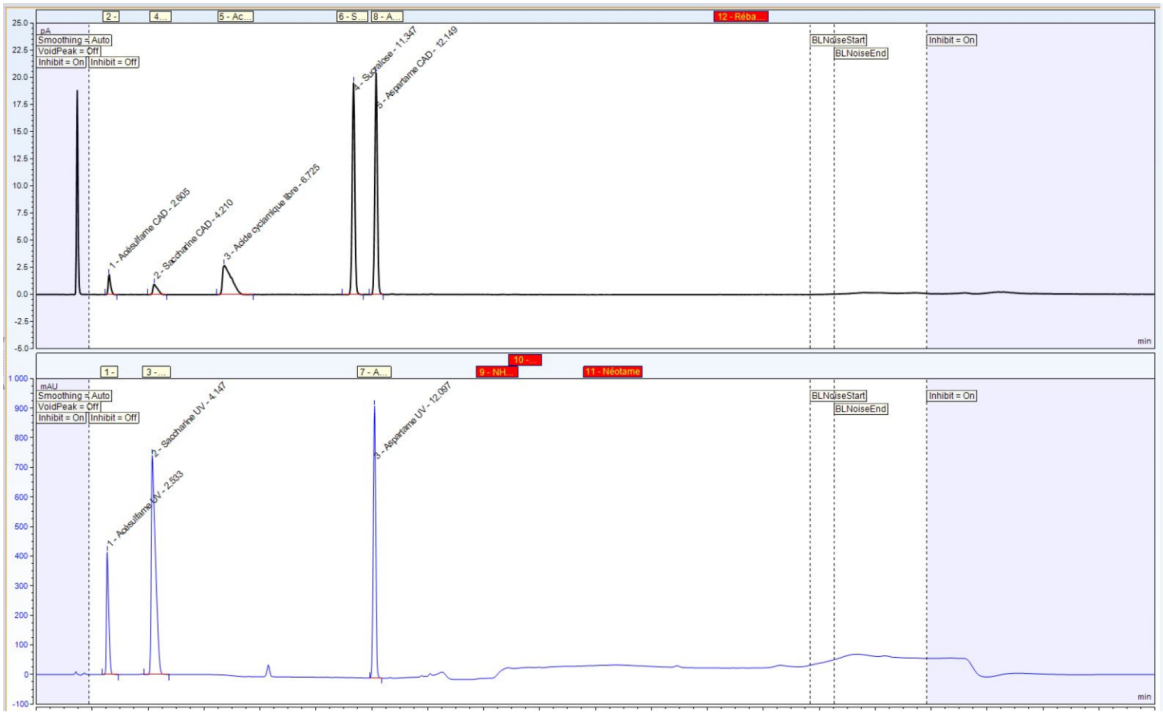
liquid chromatography and evaporative light scattering detection in beverages and canned fruits.

3. ISO 3696: Water for analytical laboratory use — Specification and test methods.
4. ISO 11352: Water quality — Estimation of measurement uncertainty based on validation and quality control data.

Annex 1: Example chromatogram for a level 3 standard solution



Annex 2: Example internal validation

The method has been the object of a performance evaluation and an internal validation study has been carried out for the white wine matrix and the white-wine-based beverage.

The method characteristics obtained as a result of this work are summarised in the following table:

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Sweetener	Repeatability in % (r%)	Interlaboratory reproducibility in % (R%)	Limit of Detection applied in mg/L	Limit of Quantification applied in mg/L	Linearity range in mg/L
Acesulfame-K	2.5	8.5	2	5	5-125
Saccharine	1.7	6.8	1	2	2-50
Aspartame	1.9	8.7	4	10	10-250
Na Cyclamate	5.5	12.8	4	10	10-250
Sucralose	6.6	12.9	4	10	10-250

The Limit of Quantification applied corresponds to level 1; this is the first point within the calibration range.

The Limit of Detection applied corresponds to 1/3 of the Limit of Quantification.

The linearity has been verified by analysis of 5 concentration levels for each sweetener from 5 repetitions per level on different days.

The repeatability and reproductivity were obtained by analysis of each sweetener in each type of matrix on 5 different days with 2 repetitions each time, being on 3 concentration levels chosen in the linearity range of the method.

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