OIV-OENO 645-2020 Method of determination of the mean molecular mass of potassium polyaspartate

THE GENERAL ASSEMBLY,

IN VIEW OF THE ARTICLE 2, paragraph 2 b) ii of the Agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Specifications of Oenological Products" Expert Group,

CONSIDERING the already adopted Resolution OIV-OENO 543-2016, "Treatment with potassium polyaspartate in wine",

CONSIDERING the already adopted Resolution OIV-OENO 572-2017, "Monograph on potassium polyaspartate",

DECIDES to add the following Annex to the resolution OIV-OENO 572-2017 in the *International Oenological Codex*:

Method of determination of the mean molecular mass of potassium polyaspartate

1. Introduction

The effectiveness of potassium polyaspartate used in tartaric stabilisation depends on the parameter of mean molecular mass, therefore it is necessary to have a method to determine the latter.

2. Objective

Parameter to be determined: mean molecular mass, expressed in g/mol.

3. Definitions

GPC/SEC: gel permeation chromatography.

4. Principle

The determination of the mean molecular mass requires the use of gel permeation chromatography (GPC/SEC). This is a type of molecular exclusion chromatography used to separate molecules according to their size.

5. Reagents and equipment

1. Double-distilled water with resistivity of > 10 MΩ.cm at 25°C
2. Anhydrous sodium sulphate (Na_2SO_4) with a purity of $\geq$ 99% (CAS No. 7757-82-6)
3. Anhydrous monobasic potassium phosphate (KH_2PO_4) with a purity of $\geq$ 99% (CAS

Beglaubigte Ausführung Ensenada, 4. November 2022
Der Generaldirektor der OIV
Sekretär der Generalversammlung
Pau Roca

No. 7787-77-0)

4. Sodium azide with a purity of $\geq$ 99% (CAS No. 26628-22-8). In using sodium azide, preventative measures should be taken to mitigate the risks of toxicity and instability (explosion)
5. Sodium polyacrylate salts with molecular masses of between 1000 and 1250 g/mol (CAS No. 9003-04-7)
6. L-aspartic acid with a purity of $\geq$ 98% (CAS No. 56-84-8)
7. Potassium polyaspartate with a purity of $\geq$ 98% (CAS No. 64723-18-8)

6. Apparatus

6.1. Filtration system with porosity of 0.22 µm

6.2. GPC column adapted to molecular mass intervals of between 500 and 10,000 g/mol

6.3. UV detector

7. Preparation of the sample

Prepare a volume of around 15 mL of 0.1% potassium polyaspartate solution (5.7) in the mobile phase (8.1) and filter it on a 0.22 µm filter (6.1). The polyaspartate solution in the buffer becomes unstable after 3 hours. Fresh solutions should therefore be prepared before each injection.

7.1. Calibration

Prepare 0.1% solutions for each standard (5.5 and 5.6) in the mobile phase (8.1).

Inject the standards to be used in order of decreasing molecular mass.

The calibration curve is obtained by representing the retention time (variable x) on a graph as a function of the logarithm of the mean molecular mass of the standards (5.5) (variable y) ($r^2 \geq 0.99$).

8. Procedure

8.1. Preparation of the mobile phase

The mobile phase is a buffer solution composed of 0.1 M Na_2SO_4 (14.2 g/L) (5.2), 0.01 M KH_2PO_4 (1.36 g/L) (5.3) and 20 mg/L sodium azide (5.4) in double-distilled water (5.1) filtered using a 0.22- µm filter. The buffer solution should be used within 4 days of preparation.

Beglaubigte Ausführung Ensenada, 4. November 2022
Der Generaldirektor der OIV
Sekretär der Generalversammlung
Pau Roca

8.2. Chromatography conditions

- Flow rate: 0.7 mL/min
- Column: Ultrahydrogel™ Linear or similar, dimensions: 7.8 x 300 mm, filled with particles of an average diameter of 6 µm
- Column temperature: 50 °C
- Run time: 40 min
- Injection volume: 200 µL
- UV detector: wavelength of 220 nm

9. Calculations

Compare the chromatographic profile corresponding to the sample with that of the standards (5.5). Calculate the molecular mass of polyaspartate, expressed in g/mol, according to the retention time of the sample and the calibration curve.