OIV-MA-AS4-02B Detection of preservatives and fermentation inhibitors

Type IV method

1. Detection of the following acids: sorbic, benzoic, *p*-chlorobenzoic, salicylic, *p*-hydroxybenzoic and its esters

1.1. Thin layer chromatography

1.1.1. Principle

The preservatives are extracted with ether from the previously acidified wine. After separation by thin layer chromatography with polyamide powder, they are located and characterized by examining the chromatogram under ultraviolet light.

1.1.2. Apparatus

Chromatography bath.

20 x 20 cm glass plates.

Preparation of the plates - Mix thoroughly 12 g of dry polyamide powder with 0.3 g fluorescent indicator; add, while stirring, 60 mL of methanol; spread on plates to a thickness of 0.3 mm. Dry at normal temperature.

Note: Commercially prepared plates can be used.

1.1.3. Reagents

Diethyl ether

Methanol

Ethanol, 96% (*v/v*).

Sulfuric acid diluted to 20% (*v/v)*

Anhydrous sodium sulfate

Polyamide powder for chromatography (e.g., Macherey-Nagel or Merck).

Fluorescent indicator (F_{254} Merck or equivalent).

Solvent:

n-Pentane :10 vol.

n-Hexane :10 vol.

Glacial acetic acid :3 vol.

Standard solutions:

- Prepare standard solutions containing 0.1 g/100 mL of 96% ethanol (*v/v*) of the

following acids: sorbic, *p*-chlorobenzoic, salicylic, *p*-hydroxybenzoic and its esters.

- Prepare a solution of 0.2 g benzoic acid per 100 mL of 96% ethanol (*v/v*).

4. Procedure

Place 50 mL of wine in a separatory funnel; acidify with dilute 20% sulfuric acid (1.1.3.4), and extract 3 times using 20 mL diethyl ether (1.1.3.1) per extraction. Combine the washed solutions in a separatory funnel and wash with a few milliliters of distilled water. Dry the ether with the anhydrous sodium sulfate (1.1.3.2). Evaporate the ether dry using a 100°C water bath, or a rotary evaporator. If the evaporation is accomplished on a water bath, it is advisable to hasten the evaporation using a mild current of air until 2 or 3 milliliters remain, then finish the evaporation cold.

Dissolve the residue in 1 mL ethanol, deposit 3 to 5 µL of this solution on the polyamide plate, as well as 3 to 5 µL of the various preservative standard alcoholic solutions (1.1.3.9). Place the plate in a chromatography tank, and saturate with solvent vapors. Let the solvent migrate to a height of about 15 cm, which takes from 1.5 to 2.5 hours.

Remove the plate from the tank and allow to dry at normal temperature. Examine in ultraviolet light, at a wavelength of 254 nm. The preservatives appear from the bottom of the plate upward in the following order: *p*-hydroxybenzoic acid, esters of *p*-hydroxybenzoic, salicylic acid, *p*-chlorobenzoic acid, benzoic acid, sorbic acid.

With the exception of salicylic acid, which has a light blue fluorescence, other preservatives give dark spots on a fluorescent yellow-green background.

Sensitivity - This technique allows determination of the following minimum quantities of the miscellaneous preservatives expressed in milligrams per liter:

Salicylic acid 3

Sorbic acid 5

Esters of *p*-hydroxybenzoic acid 5

p-hydroxybenzoic acid 5-10

p-chlorobenzoic acid 5-10

Benzoic acid 20

1.2. High performance liquid chromatography

1.2.1. Procedure

The method is performed directly on the wine, without sample preparation. It is necessary to dilute red wines before injecting them in order to preserve the column.

Using this method, the detection threshold of preservatives in the solution analyzed is about 1 mg/L.

1.2.2. Operating conditions

Conditions which are appropriate are the following:

For the determination of sorbic and benzoic acid

Proceed according to the sorbic, benzoic, salicylic acid assay method in wines by high performance liquid chromatography (AS313-20-SOBESA) provided in the Compendium

B. For the determination of *p*-chlorobenzoic acid, *p*-hydroxybenzoic acid and its esters

Column: see A

Mobile phase:

Solution of ammonium acetate, 0.01 M + methanol (60 : 40)

pH: 4.5 - 4.6

Flow rate: see A

Injected volume: see A

Detector: UV, 254 nm

Temperature: see A

Bibliography

- Junge Ch., *Zeits. Unters. Lebensmit.,* 1967, 133, 319