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

Type IV method

1. Examination of dehydroacetic acid

1.1. Principle

Wine acidified with sulfuric acid is extracted with a mixture of equal parts of diethyl ether and petroleum ether. After evaporation of the solvent, the extract, recovered with a small quantity of 96% ethanol (*v/v*) is deposited on a thin layer of polyamide and silica gel with fluorescent indicator and subjected to the action of the mobile solvent (benzene-acetone-acetic acid). The dehydroacetic acid is identified and characterized by ultraviolet examination of the chromatogram.

1.2. Apparatus

1.2.1. Equipment for thin layer chromatography

1.2.2. Oven

1.2.3. Rotary evaporator

1.2.4. UV lamp 254 nm.

1.3. *Reagents*

1.3.1. Diethyl ether

1.3.2. Petroleum ether (boiling point □ 40 °C)

1.3.3. Methanol

1.3.4. Sulfuric acid, 20% (*v/v*)

1.3.5. Anhydrous sodium sulfate.

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

1.3.7. Chromatographic separation layer: 10 g polyamide powder with fluorescent indicator(e.g. polyamide DC II UV_{254} from Macherey-Nagel) mixed vigorously with 60 mL methanol. Add while stirring, 10 ml of water and 10ml of silica gel (with fluorescent indicator), e.g. Kiesselgel GF_{254} Merck. Spread this mixture on 5 plates (200 x 200 mm) to a thickness of 0.25 mm. Dry the plates at room temperature for 30 minutes, then place in a 70°C oven for 10 min.

1.3.8. Migration solvent:

Crystallizable benzene :60 vol.

Acetone :3 vol.

Crystallizable acetic acid :1 vol.

1.3.9. Reference solutions:

Dehydroacetic acid and benzoic acid, 0.2%, in alcoholic solution.

Sorbic acid, *p*-chlorobenzoic acid, salicylic acid, *p*-hydroxybenzoic acid and its propyl, methyl and ethyl esters, 0.1 % (*m/v*), in alcoholic solution.

1.4. *Procedure*

Acidify 100 mL of wine using 10 mL of 20% sulfuric acid (1.3.3), then proceed to extract 3 times using 50 mL of a (50:50) diethyl ether-petroleum ether mixture for each extraction. Remove the clear aqueous phase leaving an aqueous emulsion and the ether phase. Mix again the remaining liquid in the separation flask composed of an emulsion and the ether phase. The remaining aqueous phase usually separates clearly from the ether phase. If there is any residual emulsion, it should be eliminated by the addition of a few drops of ethanol.

The diethyl ether-petroleum ether phases recovered are washed with 50 mL water, dried using sodium sulfate, then evaporated by rotary evaporator, at 30 - 35 °C. The residue is recovered with 1 mL of ethanol.

Deposit 20 µL of this solution on the starting line in a 2 cm wide band, or 10 µL in a circular spot. For a comparison standard, deposit 5 µL of each of the reference solutions described above. After the chromatography (ascending height of migration 15 cm, duration 1 hour 15 min. to 1 hour 45 min., at normal saturation of the chamber), the plate is dried at room temperature. Any dehydroacetic acid and other preservatives present show up under a UV lamp at 254 nm.

When the examination of the chromatogram has revealed the presence of *para*-chlorobenzoic acid, the propyl or methyl esters of *para*-hydroxybenzoic acid which are only partly separated by this method may be identified consequently on the extract above, following the method described in *Examination of Sorbic, Benzoic, Parachlorobenzoic Acids*, 2.1. *Thin layer chromatography.*

Bibliography

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