
OIV-MA-AS313-14C Sorbic acid**Type IV method****1. Principle of Methods***Identification of traces by thin-layer chromatography*

Sorbic acid extracted in ethyl ether is separated by thin layer chromatography and its concentration is evaluated semi-quantitatively.

2. Identification of traces of sorbic acid by thin layer chromatography**2.1. Apparatus**

- 2.1.1. Precoated 20 x 20 cm plates for thin layer chromatography coated with polyamide gel (0.15 mm thick) with the addition of a fluorescence indicator
- 2.1.2. Chamber for thin layer chromatography
- 2.1.3. Micropipette or microsyringe for delivering volumes of 5 µL to within ± 0.1 µL
- 2.1.4. Ultraviolet lamp (254 nm)

2.2. Reagents

- 2.2.1. Diethyl ether, (C₂H₅)₂O
- 2.2.2. Aqueous sulfuric acid solution: sulfuric acid ($\rho_{20} = 1.84$ g/mL), diluted 1/3 (v/v)
- 2.2.3. Standard solution of sorbic acid, approximately 20 mg/L, in a 10% (v/v) ethanol/water mixture.

Mobile phase: hexane + pentane + acetic acid (20:20:3).

2.3. Procedure**2.3.1. Preparation of sample to be analyzed**

Into a glass test tube of approximately 25 mL capacity and fitted with a ground glass stopper, place 10 mL of wine; add 1 mL of dilute sulfuric acid (2.2.2) and 5 mL of diethyl ether (2.2.1). Mix by repeatedly inverting the tube. Allow to settle.

2.3.2. Preparation of dilute standard solutions

Prepare five dilute standard solutions from the solution in 2.2.3. containing 2, 4, 6, 8 and 10 mg sorbic acid per liter.

2.3.3. Chromatography

Sorbic Acid (TLC) (Type-IV)

Using a microsyringe or micropipette, deposit 5 μL of the ether-extracted phase obtained in 2.3.1 and 5 μL each of the dilute standard solutions (2.3.2) at points 2 cm from the lower edge of the plate and 2 cm apart from each other.

Place the mobile phase in the chromatograph tank to a height of about 0.5 cm and allow the atmosphere in the tank to become saturated with solvent vapor. Place the plate in the tank. Allow the chromatogram to develop over 12 to 15 cm (development time approximately 30 minutes). Dry the plate in a current of cool air. Examine the chromatogram under a 254 nm ultraviolet lamp.

The spots indicating the presence of sorbic acid will appear dark violet against the yellow fluorescent background of the plate.

2.4. Expression of the results

A comparison of the intensities of the spots produced by the test sample and by the standard solutions will enable a semi-quantitative assessment of a sorbic acid concentration between 2 and 10 mg/L. A concentration equal to 1 mg/L may be determined by using a 10 μL sample size.

Concentrations above 10 mg/L may be determined using a sample volume of less than 5 μL (measured out using a microsyringe).

Bibliography

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