OIV-MA-AS313-04 Organic acids

Type IV method

Wine organic acids may be separated and simultaneously determined by *high performance liquid chromatography* (HPLC).

1. Principle of method

Wine organic acids may be separated using two stationary phases: octyl□bonded silica and ion exchange resin columns. The acids are detected by spectrophotometric absorbance in ultraviolet.

For the determination of malic and tartaric acids, it is advisable to use an octyl□bonded silica column and for citric and lactic acids, an ion exchange resin column. The determination of these acids is performed with reference to an external standard analyzed under similar conditions.

This method is also able to give an evaluation of contents of shikimic, acetic, succinic and fumaric acids.

Note: other types of columns may also give a good separation. The columns and operating conditions given below are given as examples.

2. Apparatus

2.1. *Cellulose membrane filtration apparatus* (*diameter of pores: 0.45* µ*m*)

2.2. *Octadecyl□bonded silica fitted cartridges* (*e.g. Sep Pak - Waters Assoc.*)

2.3. *High Performance Liquid Chromatograph equipped with:*

- a 10 µL loop injector,
- a temperature control apparatus,
- spectrophotometer detector capable of making absorbance measurements at 210 nm,
- a chart recorder, or integrator.

Operating conditions

2.3.1. In the case of citric, lactic and acetic acid separation:

- a column containing a strong cation (H+) exchange resin (300 mm length,

7.8 mm internal diameter, 9 ¤m particle size) (e.g. HPX-87 H BIO-RAD),

- mobile phase: sulfuric acid solution, 0.0125 mol/L,

rate: 0.6 mL/min,

- temperature: 60 - 65°C. (Depending on the type of resin).

2.3.2. In the case of fumaric, succinic, shikimic, lactic, malic and tartaric acid separation.

- Two columns (250 mm length, 4 mm internal diameter) placed in series, fitted with octyl-bonded silica, spherical particles of 5 µm diameter,
- mobile phase: potassium *di*-hydrogen phosphate solution, 70 g/L, ammonium sulfate, 14 g/L, and adjusted to pH 2.1 by adding phosphoric acid,
- flow rate: 0.8 mL/min,
- temperature: 20°C.

3. Reagents

1. Distilled water of HPLC quality
2. Distilled methanol
3. Tartaric acid
4. Malic acid
5. Sodium lactate
6. Shikimic acid
7. Sodium acetate
8. Succinic acid
9. Citric acid
10. Fumaric acid
11. Sulfuric acid (ρ_{20} = 1.84 g/mL)
12. Sulfuric acid solution, 0.0125 mol/L
13. Potassium *di*-hydrogen *ortho*-phosphate, KH_2PO_4

14. Ammonium sulfate, $(NH_4)_2SO_4$

15. O*rtho*-phosphoric acid, 85% (ρ_{20} = 1.71 g/mL)

16. Reference solution made of: tartaric acid, 5 g/L, malic acid, 5 g/L, sodium lactate, 6.22 g/L, shikimic acid, 0.05 g/L, sodium acetate, 6.83 g/L, succinic acid, 5 g/L, fumaric acid, 0.01 g/L and citric acid, 5 g/L.

4. Procedure

1. Preparation of sample

First wash cartridge (2.2) with 10 mL methanol (3.2) then with 10 mL water (3.1).

Remove gas from wine or must sample. Filter through membrane (0.45 µm) (2.1). Put 8 mL of filtered sample into a syringe already rinsed with the sample; pass through the cartridge. Disregard the first 3 mL and collect the following 5 mL (prevent the cartridge from becoming dry).

4.2. *Chromatography*

Inject successively into the chromatograph 10 µL reference solution and 10 µL sample solution prepared according to 4.1. Repeat these injections three times in the same order.

5. Calculation

5.1. Qualitative analysis

Determine the respective times of retention for each of the eluates.

The organic acids of the reference solution are divided in order of elution as follows:

- citric, tartaric, malic, succinic + shikimic, lactic, fumaric and acetic acids in the technique 2.3.1.
- tartaric, malic, shikimic, lactic, acetic, citric, succinic and fumaric acids in the technique 2.3.2.

2. Quantitative analysis

Measure the area of each of the peaks and determine the average of the three answers for the reference and sample solutions to be analyzed. Deduct the sample concentration from the organic acids.

6. Expression of results

The concentrations are expressed as follows:

- grams per liter to one decimal place for the tartaric, malic, lactic and succinic acids
- milligrams per liter for the citric, acetic and fumaric acids.

Bibliography

- TUSSEAU D. et BENOIT C., *F.V., O.I.V.,* 1986, nos 800 et 813; J. Chromatogr., 1987, **395**, 323-333.