OIV-MA-AS313-10 Total malic acid

Type IV method

1. Principle

Malic acid, separated by means of an anion exchange column, is determined colorimetrically in the eluent by measuring the yellow coloration it forms with chromotropic acid in the presence of concentrated sulfuric acid. A correction for interfering substances is made by subtracting the absorbance, obtained using 86% sulfuric and chromotropic acid respectively (malic acid does not react at these acid concentrations), from the absorbance obtained from using 96% strength acids.

2. Apparatus

2.1. Glass column 250 mm approximately in length and 35 mm internal diameter, fitted with drain tap.

2.2. Glass column approximately 300 mm in length and 10 to 11 mm internal diameter, fitted with drain tap.

2.3. Thermostatically controlled water bath at 100°C.

2.4. Spectrophotometer set to measure absorbance at 420 nm using cells of 1 cm optical path.

3. Reagents

1. A strongly basic anion exchanger (e.g. Merck III)
2. Sodium hydroxide, 5% (*m/v*).
3. Acetic acid, 30% (*m/v*).
4. Acetic acid, 0.5% (*m/v*).
5. Sodium sulfate solution, 10% (*m/v*).
6. Concentrated sulfuric acid, 95-97% (*m/m*).
7. Sulfuric acid, 86% (*m/m*).
8. Chromotropic acid, 5% (*m/v*).

Prepare fresh solution before each determination by dissolving 500 mg sodium chromotropate, $C_{10}H_6Na_2O_8S_2 . 2H_2O$, in 10 mL distilled water

3.9. 0.5 g DL□malic acid per liter solution

Dissolve 250 g malic acid ($C_4H_6O_5$) in sodium sulfate solution, 10%, to obtain 500 mL.

4. Procedure

4.1. Preparation of ion exchanger

Place a plug of cotton impregnated with distilled water in a 35 x 250 mm glass column. Pour a suspension of the anion exchange resin into the glass column. The level of the liquid should be 50 mm above the top of the resin. Rinse with 1000 mL of distilled water. Wash the column with sodium hydroxide solution, 5%, allow to drain to approximately 2 to 3 mm of the top of the resin and repeat with two further washings of sodium hydroxide, 5%, and leave for one hour. Wash the column with 1000 mL of distilled water. Refill the column with acetic acid, 30%, allow to drain to approximately 2 to 3 mm above the top of the resin and repeat with two further washings of acetic acid, 30%. Leave for at least 24 hours before use. Keep the ion exchange resin in acetic acid, 30%, for the subsequent analysis.

4.2. Preparation of ion exchange column.

Place a plug of cotton wool at the bottom of the column measuring 11 x 300 mm above the tap. Pour in the ion exchanger prepared as described above in 4.1 to a height of 10 cm. Open the tap and allow the acetic acid solution, 30%, to drain to approximately 2 to 3 mm above the surface of the exchanger. Wash the exchanger with a 50 mL acetic acid solution, 0.5%.

4.3. Separation of DL□Malic acid

Pour onto the column (4.2) 10 mL of wine or must. Allow to drain drop by drop (average rate of one drop per second) and stop the flow 2 to 3 mm from the top of the resin. Wash the column with 50 mL acetic acid, 0.5% (*m/v*), then with 50 mL of distilled water and allow to drain at the same rate as previously, stopping the flow 2 to 3 mm from the top of the resin.

Elute the acids absorbed on the exchange resin with sodium sulfate solution, 10%, at the same rate as in the previous steps (1 drop/sec). Collect the eluate in a 100 mL volumetric flask. The ion exchange column can be regenerated using the procedure described in 4.1

4.4. Determination of malic acid

Take two wide necked 30 mL tubes fitted with ground glass stoppers, A and B. In each tube add 1.0 mL of the eluate and 1.0 mL chromotropic acid solution, 5%. Add to tube A 10.0 mL sulfuric acid, 86% (*m/m*), (reference) and to the tube B 10.0 mL sulfuric acid, 96% (*m/m*), (sample). Stopper and shake to homogenize carefully, without wetting the glass stopper. Immerse the tubes in a boiling water bath for exactly 10 min. Cool the tubes in darkness at 20 C for exactly 90 min. Immediately measure the

absorbance of tube B relative to the sample tube A at 420 nm in 1 cm cells.

4.5 Plotting the calibration curve

Pipette 5, 10, 15 and 20 mL of the DL□malic acid solution (0.5g/L) into separate 50 mL volumetric flasks. Make up to the mark with sodium sulfate solution, 10%.

These solutions correspond to eluates obtained from wines containing 0.5, 1.0, 1.5 and 2.0 g DL□malic acid per liter.

Continue as indicated in 4.4. The graph of the absorbencies of these solutions verses their malic acid concentration should appear as a straight line passing through the origin.

The intensity of the coloration depends to a large extent on the strength of the sulfuric acid used. It is necessary to check the calibration curve to see if the concentration of the sulfuric acid has changed.

5. Expression of results

Plot the absorbance on calibration graph to obtain the content of DL□malic acid in grams per liter. This content is expressed with 1 decimal.

Bibliography

- REINHARD C., KOEDING, G., *Zur Bestimmung der Apfelsäure in Fruchtsäften, Flüssiges Obst.*, 1989, 45, S, 373 ff.