OIV-MA-AS313-11 L-Malic acid

Type II method

1. Principle of the method

L-malic acid (L-malate) is oxidized by nicotinamide adenine dinucleotide (NAD) to oxaloacetate in a reaction catalysed by L-malate dehydrogenase (L-MDH).

The equilibrium of the reaction normally lies more strongly in favour of the malate. Removal of the oxaloacetate from the reaction mixture displaces the equilibrium towards the formation of oxaloacetate. In the presence of L-glutamate, the oxaloacetate is transformed into L-aspartate in a reaction catalysed by glutamate oxaloacetate transaminase (GOT):

$$(1) L-malate + NAD^{+} \overset{L-MDH}{\Longleftrightarrow} oxaloacetate + NADH + H^{+}$$

$$(2) Oxaloacdtate + L-glutamate \overset{GOT}{\Longleftrightarrow} L-asparate + \alpha-ketoglutarate$$

The amount of NADH formed, measured by the increase in absorbance at the wavelength of 340 nm, is proportional to the quantity of L-malate originally present.

2. Apparatus

2.1. A spectrophotometer permitting measurement to be made at 340 nm, the wavelength at which absorption by NADH is at a maximum. Failing that, a spectrophotometer, with a discontinuous spectrum source permitting measurements to be made at 334 or 365 nm, may be used.

Since absolute measurements of absorbance are involved (i.e. calibration curves are not used, but standardization is made by consideration of the extinction coefficient of NADH), the wavelength scales and spectral absorbance of the apparatus must be checked.

2.2. Glass cells with optical path lengths of 1 cm or single-use cells.

2.3. Micropipettes for pipetting sample volumes in the range 0,01 to 2 ml.

3. Reagents

Doubly distilled water

3.1. Buffer solution, pH 10

(glycylglycine 0,6 M; L-glutamate 0,1 M):

dissolve 4,75 g of glycylglycine and 0,88 g of L-glutamic acid in approximately 50 ml of

doubly distilled water; adjust the pH to 10 with about 4,6 ml of 10 M sodium hydroxide and make up to 60 ml with doubly distilled water. This solution will remain stable for at least 12 weeks at 4 °C.

3.2. Nicotinamide adenine dinucleotide (NAD) solution, approximately 47 × 10 3 M: dissolve 420 mg of NAD in 12 ml of doubly distilled water. This solution will remain stable for at least four weeks at 4 °C.

3.3. Glutamate oxaloacetate transaminase (GOT) suspension, 2 mg/ml. The suspension remains stable for at least a year at 4 °C.

3.4. L-malate dehydrogenase (L-MDH) solution, 5 mg/ml. This solution remains stable for at least a year at 4 °C.

Note: All the reagents above are available commercially

4. Preparation of the sample

L-malate determination is normally carried out directly on the wine, without prior removal of pigmentation (colouration) and without dilution provided that the L□malic acid concentration is less than 350 mg/l (measured at 365 mg/l). If this is not so, dilute the wine with doubly distilled water until the L-malate concentration lies between 30 and 350 mg/l (i.e. amount of L-malate in the test sample lies between 3 and 35 µg).

If the malate concentration in the wine is less than 30 mg/l, the volume of the test sample may be increased up to 1 ml. In this case, the volume of water to be added is reduced in such a way that the total volumes in the two cells are equal.

5. Procedure

With the spectrophotometer adjusted to a wavelength of 340 nm, determine the absorbance using the cells having optical paths of 1 cm, with air as the zero absorbance (reference) standard (no cell in the optical path) or with water as the standard.

Place the following in the cells having 1 cm optical paths:

	Reference cell (ml)	Sample cell (ml)
Solution 3.1	1,00	1,00
Solution 3.2	0,20	0,20
Doubly distilled water	1,00	0,90

Suspension 3.3	0,01	0,01
Sample to be measured	)	0,10

Mix; after about three minutes, measure the absorbances of the solutions in the reference and sample cells (A1).

Add:

Solution 3.4	0.01 ml	0.01 ml

Mix; wait for the reaction to be completed (about 5 to 10 minutes) and measure the absorbances of the solutions in the reference and sample cells (A_2).

Calculate the differences ($A_2 - A_1$) in the absorbances of the solutions in the reference and sample cells,ΔA_R *and* ΔA_S.

Finally, calculate the difference between those differences: $\Delta A = \Delta A_S - \Delta A_R$

Note: The time needed for the completion of enzyme activity can vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch.

6. Expression of results

L-malic acid concentration is given in grams per litre to one decimal place.

6.1. *Method of calculation*

The general formula for calculating the concentration in g/l is:

$$C = \frac{V \times PM}{\varepsilon \times \delta \times 1000}$$

where:

V = volume of test solution in ml (here 2,22 ml)

V = volume of the sample in ml (here 0,1 ml)

M = molecular mass of the substance to be determined (here, for L-malic acid, M=134,09)

□= optical path in the cell in cm (here, 1 cm)

□= absorption coefficient of NADH, (at 340 nm

□= 6,3 m mol 1 × l × cm 1),

so that for L-malate:

$$C = 0.473 \times \Delta A \; g/l$$

If the sample was diluted during its preparation, multiply the result by the dilution factor.

Note:

Measurement at 334 nm, ε = 6,2 ($mmole^{-1}$ x 1 x cm^2)

$C = 0{,}482 \times \Delta A$

Measurement at 365 nm, ε = 6,2 ($mmole^{-1}$ x 1 x cm^2)

$C = 0{,}876 \times \Delta A$

6.2. *Repeatability (r)*

$r = 0{,}03 + 0{,}034\ x_i$

x_i is the malic acid concentration in the sample in g/l.

6.3. *Reproducibility (R)*

$R = 0{,}05 + 0{,}071\ x_i$

x_i is the malic acid concentration in the sample in g/l.

Bibliography

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