AK0001_IFU_EN_V2302

L-Malic acid, UV method

Catalogue number	Presentation
AK00011	58 tests (manual) / 580 tests (microplate)

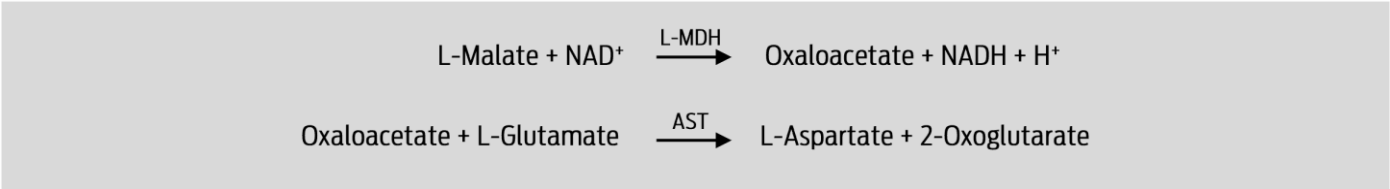
Application

This rapid and simple stereo-specific enzymatic method is used for the determination of L-malic acid (L-malate) in foodstuffs such as wine, beer, bread, fruit and vegetable products, fruit juice, as well as in cosmetics, pharmaceuticals and biological samples.

Introduction

L-Malic acid is a relevant component of the citric acid cycle that is found in animals, plants and microorganisms. It is one of the most important fruit acids found in nature and it is the acid present in highest concentrations in wine. L-Malic acid may be used in food production because it is a stronger acid than citric acid. Microbial decomposition of L-malic acid leads to the formation of L-lactate; this can be a desirable reaction in the wine industry, where the level of L-malic acid is monitored, along with L-lactic acid, during malolactic fermentation. L-Malic acid may be used as a food preservative (E296) or flavour enhancing additive.

Principle



The amount of NADH formed through the action of L-malate dehydrogenase (L-MDH) and Aspartate aminotransferase (AST), measured at 340 nm, is stoichiometric with the amount of L-malic acid in sample volume.

Specificity

The method is specific for L-malic acid. D-Malic acid, as well as D- and L-lactic acid, L-aspartic acid and fumaric acid do not react. L-Malic acid esters also do not react.

Sensitivity and detection limit

The smallest differentiating absorbance for the assay is 0.005 AU. This corresponds to 0.12 mg/L of sample solution at the maximum sample volume of 2.00 mL. The detection limit is 0.25 mg/L, which is derived from an absorbance difference of 0.010 and the maximum sample volume of 2.00 mL.

Linearity and precision

The assay is linear over the range of 0.5 to 30 µg of L-malic acid per assay. In duplicate determinations using one sample solution, an absorbance difference of 0.005 to 0.010 may occur. With a sample volume of 0.10 mL, this corresponds to a L-malic acid concentration of approx. 2.49 to 4.98 mg/L of sample solution. If in sample preparation, the sample is weighed, e.g. 10 g/L, a difference of 0.02 to 0.05 g/100 g can be expected.

In a double determination using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. The relative standard deviation, or coefficient of variation, is approx. 1 to 2% in the measuring range.

Kit composition

Solution 1. Glycylglycine buffer (6 mL, 1 M, pH 10.0) plus L-glutamate (1 M) and sodium azide (0.02% w/v) as a preservative. Store at 2 °C to 8°C.

Solution 2. NAD^+ (380 mg) plus Polyvinylpyrrolidone (PVP; 60 mg). Store at 2 °C to 8°C (Long term storage: -30 °C to -15 °C)

Dissolve in 6 mL of distilled water, divide into appropriately sized aliquots and store in polypropylene tubes at -30 °C to -15 °C between use and on ice during use.

Suspension 3. Aspartate aminotransferase (AST) / GOT (EC 2.6.1.1) suspension (1.25 mL). Store at 2 °C to 8°C. Swirl bottle before use.

Suspension 4. L-Malate dehydrogenase (EC 1.1.1.37) suspension (1.25 mL). Store at 2 °C to 8°C. Swirl bottle before use.

Solution 5. L-Malic acid standard solution (5 mL, 0.15 mg/mL). This standard solution can be used when there is some doubt about the method accuracy. Store at 2 °C to 8°C.

Protocol (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~25 °C

Final volume: 2.34 mL

Sample solution: 0.5-30 µg of L-malic acid per cuvette (in 0.10-2.0 mL sample volume)

Read against air (without a cuvette in the light path) or against water

PIPETTE TO CUVETTES (mL)	**BLANK**	**SAMPLE**
Distilled water	2.10	2.00
Sample	-	0.10
Solution 1 (glycylglycine buffer)	0.10	0.10
Solution 2 (NAD^+)	0.10	0.10
Suspension 3 (AST)	0.02	0.02
Mix*, measure the absorbances of the above solutions (A1) after approx. 3 min and start the reactions by addition of		
Suspension 4 (L-MDH)	0.02	0.02
Mix*, measure the absorbances of the above solutions (A2) at the end of the reaction (approx. 3 min)		

*Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

Calculation

Determine the absorbance difference (A2-A1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{\text{L-malic acid}}$. The concentration of L-malic acid (g/L), based on the ε of NADH at 340 nm (6300 $L \times mol^{-1} \times cm^{-1}$), is calculated as follows:

$$C\ (\text{L-Malic acid}) = 0.4980 \times \Delta A_{\text{L-Malic acid}} \quad [g/L]$$

If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor.

Alternative procedures (micro-volumes)

Although this kit has been developed to work in cuvettes, it can be easily adapted for use in 96-well microplates or in auto-analysers. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than 1 cm, which is the standard cuvettes pathlength. Thus, to perform the calculation of the amount of analyte in the samples follow one of the three possible strategies described in the “Alternative Procedures”, available on the NZYtech website.

Interferences

An internal standard should be included during sample analysis if the presence of interfering substances is suspected. A quantitative recovery of this standard should be expected. Identification of losses in sample handling and extraction may be identified by performing recovery experiments, i.e., by adding L-malic acid to the sample in the initial extraction steps. PVP has been incorporated into the assay system to prevent inhibition from polyphenolics (tannins) in the sample.

General information on sample preparation

The amount of L-malic acid present in the cuvette should range between 0.5 and 30 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield a L-malic acid concentration between 5 and 300 mg/L. However, the sample volume can vary from 0.10 to 2.00 mL, by replacing water (analyte range from 0.25 to 300 mg/L).

To implement this assay use clear, colorless or slightly colored and practically neutral liquid samples directly, or after dilution; filter turbid solutions; degas samples containing carbon dioxide (e.g. by filtration); adjust acid samples, which are used undiluted for the assay, to pH 8-10 by adding sodium or potassium hydroxide solution; adjust acid and weakly colored samples to pH 8-10 and incubate for approx. 30 min; measure "colored" samples (if necessary adjusted to pH 8-10) against a sample blank; treat "strongly colored" samples that are used undiluted or with a higher sample volume with PVP; crush or homogenize solid or semi-solid samples, extract with water or dissolve.

Examples of sample preparation

Determination of L-malic acid in wine

Determination of free L-malic acid concentration [F] of white and red wine can be usually performed without any sample treatment, except dilution. Typically, a dilution of 1:10 and a sample volume of 0.1 mL are satisfactory. Free and esterified L-malic acid [F + E] concentration in white and red wine may be determined as follows: to 20 mL of wine add 6 mL of 2 M NaOH and heat under reflux for 30 min with stirring. When sample is cooled, carefully adjust the pH to 10 with 1 M H_2SO_4 and adjust the volume to 50 mL with distilled water. Proceed with the analysis according to the general procedure. The concentration obtained is the sum of the free and esterified L-malic acid [F + E], and thus the esterified L-malic acid concentration alone [E] can be calculated as follows: [E] = [F + E] – [F].

Determination of L-malic acid in beer

Usually, the sample does not need to be diluted and a volume of 0.1 mL is satisfactory. Previous removal of carbon dioxide is required and may be achieved by stirring with a glass rod.

Determination of L-malic acid in fruit juice, concentrates and related beverages

L-malic acid concentration in the above mentioned liquids can generally be determined without any sample treatment, except dilution. If the liquid is turbid, sample filtration is however required. Coloured solutions are usually appropriate for analysis, following dilution to an appropriate L-malic acid concentration. Usually, a dilution of 1:50 and sample volume of 0.1 mL are satisfactory.

Determination of L-malic acid in solid foodstuffs

With solid foodstuffs, homogenize approximately 10 g of sample using a mortar and pestle or an electric blender. Extract approximately 2 g of representative material in 40 mL of distilled water for 30 min, with heating at 60 °C if necessary. Transfer the extract to a 50 mL volumetric flask and adjust to volume with distilled water. If necessary filter the turbid solution and dilute.

References

Mollering, H. (1985). L-Malate. In: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol.VII, pp. 39-47, VCH Publishers (UK), Cambridge, UK.

AOAC Official Methods of Analysis (2002). Method 993.05 “L-Malic acid/total malic acid ratio in apple juice”. 17th ed., Chapter 37, p. 15.

Recommendations

This method is recommended/approved by the:

– European Community regulations (analysis of wine);

– European, French, Dutch, German and Russian standards (EN, NF, NEN, DIN, GOST);

– German, Swiss and Italian food laws;

– International Wine Office (OIV), International Federation of Fruit Juice Producers (IFU), Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (A.I.J.N.), and Mitteleuropäische Brautechnische Analysenkommission (MEBAK) (Central European Commission for Brewing Technology);

– Association of Official Analytical Chemists (AOAC) (analysis of apple juice).

Please enquire info@nzytech.com to obtain any additional information about this kit, including additional specific applications