

Linea Polyquid

L-Malic Acid

UV-METHOD standardized with liquid reagent for the determination of L-Malic Acid in wine samples

Test-Combination for 50 determinations

CONF 1x100mL

REF PM4100

Contents:

Reagent 1: <i>(Buffer – Substrate)</i>	1 bottle with 90mL
Reagent 2: <i>(Coenzyme - NAD)</i>	1 bottle with 5,0mL
Reagent 3: <i>(Enzyme)</i>	1 bottle with 5,0mL
Calibrator: <i>(L-Malic Acid 1,0g/L)</i>	1 bottle with 3,0mL

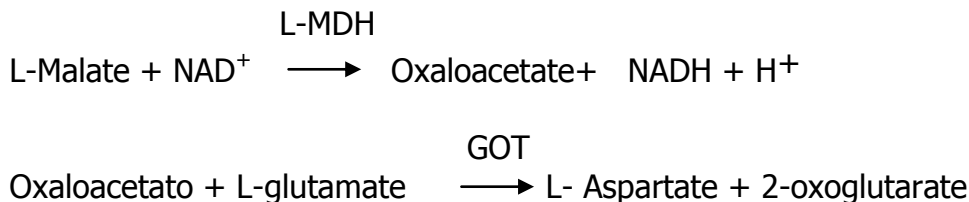


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1. PRINCIPLE OF THE METHOD

L-Malic Acid is oxidized to oxaloacetate by nicotinamide-adenine dinucleotide (NAD) in the presence of L-malate dehydrogenase (L-MDH); from this reaction is obtained the NADH proportional to the concentration of L-Malate in the sample; the NADH solution present an increase of absorbance at 340nm, so the NADH is measured by the increase in absorbance at 340nm.

The equilibrium of the reaction system is shifted toward the formation of NADH until exhaustion of the Malic Acid present in the sample, obtaining that oxaloacetate is removed from the reaction system, as indicated in details below. In this reaction system oxaloacetate is converted to 2-oxoglutarate, by the intervention of the enzyme glutamate-oxaloacetate Transaminase (GOT).



2. REAGENT

The concentration are referred to the final reactions solution

1. One bottle contains 90 mL of reagent solution, consisting of :

Tris- buffer , pH = 9,8 a 37°C

L-Glutamate	240	mmol/L
Sodium Azide	0,9	gr/L
Inert stabilizer		

2. One bottle contains 5 mL of reagent solution, consisting in:

NAD	0,18	mM
Sodium Azide	0,9	g/l
Inert stabilizer		

3. One bottle contains 5 mL of reagent solution, consisting in:

Tris-Buffer, pH = 7,8 a 37°C	80	mmol/L
GOT (Glutamate-oxaloacetate transaminase)	> 0,5	U/mL
MDH (Malate Dehydrogenase)	> 1,0	U/mL
Sodium Azide	0,9	g/L
Inert stabilizer		

4. One bottle contains 3 mL of reagent solution ready to use:

L-Malic Acid	1,0g/L
Inert stabilizer	

3 PREPARATION OF SOLUTIONS

The reagent are ready to use

4. STORAGE AND STABILITY OF REAGENTS

The single reagent are stable until the date of expiration indicated in the label.

The reagent (1+2) is stable for 15 days at 2-8°C

The mixed working reagent ready to use is stable for 7 days at 2-8 °C.

Store the package at 2-8 °C away from direct light.

5. INSTRUCTION FOR PERFORMANCE OF ASSAY

REQUIRED MATERIAL NEED BUT NOT PROVIDED WIT THE ASSAY

- Photometer with optical path length of 1,0cm and filter for readings at a wavelength of 340nm;
- Disposable cuvettes with optical path 1,0cm;
- Micropipettes

SAMPLE CHARACTERISTICS

The sample is red or white wine, directly or after dilution with distilled water or after treatment with activated charcoal to decolorize most structured wines.

5.1. APPLICATION

CONDITION OF REACTION

Wavelength	340nm
Temperature	Room temperature or 37°C
Optical path	1 cm
Final Volume	2,050mL

Pipette into cuvette:

	Reagent Blank	Calibrator (c)	Sample(s)
Distilled Water	0,050mL		
Calibrator 1,0g/L		0,050mL	
Sample			0,050mL
Reagent 1	1,800mL	1,800mL	1,800mL
Reagent 2	0,100mL	0,100mL	0,100mL
Mix the components and read the first time (A1) at 340nm, readings are zeroing against its reagent blank and add to each cuvette:			
Reagent 3	0,100mL	0,100mL	0,100mL
Incubate the vials for 10 minutes at 37°C. For readings at room temperature, add in the best way the reaction time. Proceed to the readings (A2)			

6. CALCULATION

$$\text{Concentration of L-Malic Acid sample solution} = \frac{A2s - A1s}{A2c - A1c} \times \text{conc. Calibrator (1,0g/L)}$$

7. PERFORMANCE AND FEATURES OF THE METHOD

The reagent has a linear response up to a maximum of about 1,2g/L of L-Malic Acid. Repeated analysis in the same serie and between the series showed a coefficient of variation between 2% and 3%. The specificity of the method of determination is proven in literature: it's not possible to have cross-reactions with similar compounds.

8. INTERNAL QUALITY CONTROL

It's should be better to process an internal quality control on the quality of the outcomes, using an assay control solution suitable on the market, or a pool of home control. Repeat such monitoring for each test provide with a confidence data of the test, that in case of out of range

result respect to the sheet of the manufacturer, invite to reevaluate the analytical result obtained in the session.

9. SAMPLE PRETREATMENT

As it's not knowing if the malolactic fermentation is completed or not, is recommended at the outset, to dilute the sample 1:1 with distilled water and then multiply the result by 2.

Generally a sample pretreatment is not needed. However, it may be useful to bleach highly structured red wines with activated charcoal.

10. WARNINGS

IN VITRO DIAGNOSTIC USE ONLY

Guidelines for personal safety

Always operate according to "Good Laboratory Practice".

The reagent contains irritants, thus avoid ingestion and contact with mucous membranes. It's present the Sodium Azide which may form explosive mixtures with copper and lead: delete using large quantities of water.

Never pipet by mouth, use gloves and appropriate personal protective equipment.

Analytical warnings

- Before use take the reagents and sample at room temperature (15-25°C). After use immediately return reagents to the storage temperature.
- Do not use reagents after the expiration date. Avoid microbial contamination of reagents, as reduce the stability of the product and can lead to erroneous results.
- Strictly follow the procedure and don't substitute the reagent with some other manufacturer reagents. .
- Avoid cross-contamination between reagents.

11. LITERATURE

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