PURPOSE OF THE TEST

Malic acid, both in free and esterified form, is usually present in ripening fruit and is responsible for the astringent taste of the unripe fruit. Its concentration is reduced as it matures. In the process of winemaking, more than 30% of the malic acid is transformed by a process of fermentation in lactic acid (malolactic fermentation) that helps to reduce the initial acidity of the must. Control of the malic acid level is necessary to maintain the desired taste, acidity and astringency characteristics of wine.

METHOD

Malate dehydrogenase (MDH) catalyzes the oxidation of malic acid to oxaloacetate with the formation of NADH.

$$L\text{-}Malate + NAD^{+} \xrightarrow{MDH} Oxalacetate + NADH + H^{+}$$

Oxalacetate is removed from the medium by its transformation to aspartate by the action of aspartate amino transferase (AST / GOT).

$$Oxalacetate + L\text{-}Glutamate \xrightarrow{GOT} L\text{-}Aspartate + \alpha\text{-}Ketoglutarate$$

The increase in absorbance at 340 nm associated with the formation of NADH is directly proportional to the concentration of malic acid in the sample.

CONTENT

R1A	2 x 24 mL	Glycylglycine buffer 200 mM, pH 9.0, Glutamate 50 mM, MDH (>3 U/mL)
R1B	2 x 8 mL	MDH (> 3 U/mL), AST (> 2 U/m)
R2	1 x 16 mL	NAD^{+} 20 mM, GOT (>2 U/mL)
CTRL	1 x 3 mL	L-Malic Acid control 2,50 g/L (2,12 – 2,88 g/L)

REAGENT PREPARATION

Reagent 1: Pour the content of a vial of R1B into a vial of R1A. Mix gently avoiding foam formation. A lower volume can be prepared using a ratio of 3 parts of R1A and 1 part of R1B. Mixture is stable up to 4 months if stored at 2-8 ºC and contamination is avoided.

Reagent 2 is ready to use and are stable up to expiry date as supplied when stored at 2-8 ºC. Do not freeze.

Discard if absorbance of blank is higher than 0.500 OD at 340 nm.

SAMPLES

For use with wine and other beverages.

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. The presence of CO_2 introduces instability in the measure. Samples containing CO_2 must be degassed beforehand. In samples with very high colour intensity, the pigment may interfere with the measurement. Treat with polyvinylpolypyrrolidone (PVPP 0.1g for each 10 mL) to reduce the level of colour. Samples with concentration higher than the measurement range must be diluted accordingly with distilled water. Multiply the final result by the dilution factor.

PROCEDURE OVERVIEW

Treat standard, controls and samples as Sample. Use distilled water as Blank.

Use WINECONTROL (code SD2100) or WINECAL-RTU (code SY2100RTU) as standard.

Volumes stated below can be adjusted to other analytical procedures. Expected performance can vary if those ratios S:R1:R2 are not used exactly as stated.

Pipette into a cuvette:

	Blank reaction	Sample/Std Reaction
Reagent 1	720 µL	720 µL
Distilled water	9 µL	--
Sample/Standard	--	9 µL

Mix, incubate at 37ºC for 1 minutes and read absorbance at 340 nm (A_1). Then add into the cuvette:

	Blank reac.	Sample/Std Reac.
Reagent 2	180 µL	180 µL

Mix, incubate for 10 minutes at 37ºC and read absorbance at 340 nm (A_2).

Concentration of L-Malic acid is calculated as:

$$L-Malic = \frac{(A_2 - 0.80xA_1)_{\text{sample}} - (A_2 - 0.80xA_1)_{\text{blank}}}{(A_2 - 0.80xA_1)_{\text{standard}} - (A_2 - 0.80xA_1)_{\text{blank}}} \; x\; C \text{ g/L}$$

Factor 0.80 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the standard label for L-Malic.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	L-MALIC	
Method	End Point A	
Direction	Increasing	
Main Wavelength	340	
Sec. Wavelength	--	
Sample	3	
Reagent 1	240	
Reagent 2	60	
Calibration	Linear	
Blank cycle [150 | 240]	3- 4	3 - 4
Reading cycle [150 | 240]	20 - 21	31 - 32
Units	g/L	
Decimals	0.00	
Measure range	0,05 ~ 5,20	
R1 Lim. Abs	5000	
Ratio Dil. Auto.	-	
Vol. Sample Dil. Auto	-	

Procedure is linear up to 5.20 g/L. Calibrate with a single point using the highest concentration standard or with several points as per your quality procedures.

PERFORMANCE

Limit of Quantification (LoQ): 0.05 g/L
Limit of linearity: 5.20 g/L

NOTES

Using a control sample on a regular basis provides information on the calibration status and possible deterioration of the reagent. In case of deviations greater than 15% on the target value, it is advisable to check the calibration status of the test.

REFERENCES

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