PURPOSE OF THE TEST

Acetic acid is the main indicator of wine deterioration although in small quantities (less than 300 mg / L) contribute to endow it with organoleptic characteristics through the formation of esters and other compounds. It is produced, mainly from the oxidation of ethanol, by certain bacteria (especially of the genus *Acetobacter*). The assessment of acetic acid allows monitoring possible deterioration situations along the elaboration process.

METHOD

Acetate kinase (AK) phosphorylates acetic acid in the presence of ATP producing ADP. Phosphoenolpyruvate (PEP) transfers the phosphate group to ADP through the action of pyruvate kinase (PK), regenerating the ATP consumed in the previous reaction and producing pyruvate.

$$Acetate + ATP \xrightarrow{AK} Acetyl\text{-}P + ADP$$
$$PEP + ADP \xrightarrow{PK} Pyruvate + ATP$$

Pyruvate is reduced to lactate by consuming NADH due to the action of lactate dehydrogenase (LDH)

$$Pyruvate + NADH^{+} \xrightarrow{LDH} Lactate + NAD^{+}$$

The decrease in absorbance at 340 nm associated with the consumption of NADH is directly proportional to the acetic concentration in the sample.

CONTENT

R1	2 x 30 mL	MOPS buffer 100 mM, pH 7.5, $NADH^{+}$ 0,56 mM, PEP 1,25 mM, ATP 5 mM
R2	1 x 15 mL	MOPS buffer 100 mM, pH 7.5, AK (>50 UI/mL), PK (>100 UI/mL), LDH(>40 UI/mL)
CTRL	1 x 3 mL	Acetic 0,50 g/L (0,42 – 0,57 g/L)

REAGENT PREPARATION

Reagents are ready to use. Stable up to expire date when stored at 2-8 ºC. Do not freeze.

Discard if absorbance of blank is lower than 1.000 OD at 340 nm.

SAMPLES

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. As acetic acid is volatile, keep samples in closed tubes until time of analysis. 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 SD2200) or WINECALRTU (code SY2100RT) 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 wáter	9 µL	--
Sample/Standard	--	9 µL

Mix, incubate at 37ºC for 1 minutes. Then add into the cuvette:

	Blank reaction	Test Reaction
Reagent 2	180 µL	180 µL

Mix, incubate for 10 minutes at 37ºC and read absorbance at 340 nm immediately after adding R2 (A_1) and after 10 minutes (A_2).

Concentration of acetic acid is calculated as:

$$Acetic = \frac{(A_2 - A_1)_{\text{sample}} - (A_2 - A_1)_{\text{blank}}}{(A_2 - A_1)_{\text{standard}} - (A_2 - A_1)_{\text{blank}}} \; x \; C \text{ g/L}$$

C is the value of concentration stated in the standard label for acetic.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	ACETIC	
Method	End Point C	
Direction	Decreasing	
Main Wavelength	340	
Sec. Wavelength	--	
Sample	3	
Reagent 1	240	
Reagent 2	60	
Calibration	Linear	
Blank cycle [150 | 240]	0 - 0	0 - 0
Reading cycle [150 | 240]	5 - 15	5 - 46
Units	g/L	
Decimals	0.00	
Measure range	0,03 ~ 1,20	
R1 Lim. Abs	10000	
Ratio Dil. Auto.	-	
Vol. Sample Dil. Auto	-	

[#] Procedure is linear up to 1.20 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): 0.03 g/L
Limit of linearity: 1.20 g/L

NOTES

It is recommended to use wine controls to verify quality of calibration. Each laboratory should establish its own quality criteria for acceptance, as well as proper corrective action procedures in case of rejection.

REFERENCES

1. Compendium of International methods of analysis – OIV, Vol1&2 (2008). RESOLUTION OIV-OENO 621-2019
2. Bermeyer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY.