SD6030: TOTAL ACIDITY (pH 7)

COLORIMETRIC - BTB

PURPOSE OF THE TEST

The total acidity is the sum of all the acid components of the must, especially tartaric, malic and citric, which represent more than 95% of the total acids. The acids act by providing flavour and texture in the mouth, as well as bacteriostatic agents. However, an excessive acidity also has negative effects on the quality of the wine, so it is important to determine its value both at the beginning of the fermentation to correct it if necessary, and at the end of it, since, after fermentation, the acid components have changed significantly and the pH has increased. In the case of the must, a starting acidity between 6.5-7.5 g / L of tartaric, ensures that the decrease in acidity after fermentation does not cause significant changes in the organoleptic characteristics of the wine (especially color). In the case of wine, maintaining high enough acidity is necessary to ensure its stability during ripening and subsequent bottling.

METHOD

The bromothymol blue (BTB) is an indicator that changes from yellow (acid solution) to blue (basic solution) in the pH range between 6.6 and 7.6. When the sample of wine or must is added to a solution of the indicator stabilized at pH 7, a color change is produced that is proportional to the amount of acid added, which can be determined by measuring the variation of absorbance at 620 nm and comparing it with a known concentration pattern in g/L of tartaric acid.

CONTENT

R1	2 x 30 mL	BTB, Ethanol (<20%), Buffer pH 7.0
STD	1 x 3 mL	Tartaric acid 10 g/L

REAGENT PREPARATION

Reagents are 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 lower than 1.500 OD at 620 nm.

SAMPLES

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. 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.

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

Pipette into a cuvette:

	Blank reaction	Sample/Std Reaction
Reagent 1	1000 µL	1000 µL
Distilled water	20 µL	--
Sample/Standard	--	20 µL

Mix, incubate at 37ºC for 3 minutes and read absorbance at 620 nm (A_1).

The total acidity, expressed as tartaric acid, is determined from the variation of absorbance as:

$$\Delta Abs = (A_2 - A_1)_{\text{blank}} - (A_2 - A_1)_{\text{sample/std}}$$

The ΔAbs obtained is compared with the corresponding one of the calibration curve to determine the total acidity expressed in g / L of tartaric acid.

The results can also be expressed as sulfuric acid g/L or mEq/L as:

$$Tartaric\ acid\ \text{g/L} \times 0.65 = Sulfuric\ acid\ \text{g/L}$$

$$Tartaric\ acid\ \text{g/L} \times 13{,}33 = mEq/L$$

ASSAY PARAMETERS FOR ANALYZERS DIONYSOS®

GENERAL	Name	T. ACIDITY
	Method	End Point A
	Direction	Decreasing
	Main wavelenght	620
	Sec. wavelenght	--
	Sample	4
	Reagent 1	200
	Reagent 2	--
	Calibration	Linear
	Blank cycle (D150|D240)	2-2 |2-2
	Read cycle (D150|D240)	8-9 | 10-12
	Units	g/L
	Decimals	0.00
	Meas range	1,0 – 16,0
MONITOR	Ratio Dil. Auto.	5
	Vol. Sample Dil. Auto	40

Procedure is linear up to 16 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): 1,00 g/L
Limit of linearity: 16,00 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, Vol 1&2 (2008).
2. Bermeyer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY.