PURPOSE OF THE TEST

Citric acid is not very abundant in the grape compared to other organic acids. At the end of fermentation, it can be added to raise the acidity, which increases the efficiency of the sulphites present, and to prevent iron turbidity, since it forms soluble complexes with iron and copper, although this practice has legal restrictions. Citric acid also brings a feeling of freshness to the wine, but in excessive amounts it is unpleasant.

METHOD

Citric acid is converted to oxaloacetate and oxalate by the action of citrate lyase (CL).

$$Citrate \xrightarrow{CL} Acetate + Oxalacetate$$

The MDH favours this reaction by removing the oxaloacetate from the medium, transforming it to malate with NAD^+ formation.

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

In the case of spontaneous decarboxylation of oxaloacetate to pyruvate (for example in the presence of high concentrations of divalent metals), the presence of LDH makes it possible to count the pyruvate formed by transforming it into lactate, with formation of NAD^+.

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

The decrease in absorbance at 340 nm is directly proportional to the concentration of citrate present in the sample.

CONTENT

R1	1 x 13.5 mL	TRIS buffer 100 mM, LDH (> 5 U/mL), MDH (>5 U/mL)
R2	1 x 1,5 mL	NADH
R3	1 vial	Lyophilized CL
R4	1 x 3 mL	TRIS buffer 100 mM, preservatives
CTRL	1 x 3 mL	Citrate 0,50 g/L (0,43 – 0,58 g/L)

The kit includes one empty vial for Reagent 2 preparation

REAGENT PREPARATION

Reagent 1: Pour the content of R2 into the bottle of R1. Mix gently avoiding foam formation. This mix is stable up to 15 days when stored at 2-8 ºC and avoiding contamination. Do not freeze.

Reagent 2: Resuspend the content of R3 with the content of R4. Be sure that all powder has been properly dissolved. Using a pipette put all the reagent into the empty bottle provided. This mix is stable up to 15 days when stored at 2-8 ºC and avoiding contamination. 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. 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 SD2200) as standard or WINECAL-RTU (code SY2100R).

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	800 µL	800 µL
Distilled water	12 µL	--
Sample/Standard	--	12 µL

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

	Blank reaction	Sample/Std Reaction
Reagent 2	120 µL	120 µL

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

Concentration of citric acid is calculated as:

$$Citric = \frac{(A_2 - 0.87xA_1)_{\text{sample}} - (A_2 - 0.87xA_1)_{\text{blank}}}{(A_2 - 0.87xA_1)_{\text{standard}} - (A_2 - 0.87xA_1)_{\text{blank}}} \; x\; C \text{ g/L}$$

Factor 0.87 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the standard label for citric.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	CITRIC	
Method	End Point A	
Direction	Decreasing	
Main Wavelength	340	
Sec. Wavelength	--	
Sample	3	
Reagent 1	200	
Reagent 2	30	
Calibration	Linear	
Blank cycle [150 | 240]	3 - 4	3 - 4
Reading cycle [150 | 240]	21 - 22	31 - 32
Units	g/L	
Decimals	0.00	
Measure range	0.06 ~ 1.00	
R1 Lim. Abs	10000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

Procedure is linear up to 1.00 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): 0.06 g/L
Limit of linearity: 1.00 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.

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