SD6018: COPPER

COLORIMETRIC -3,5-DIBr-PAESA

PURPOSE OF THE TEST

Presence of copper in wine is common due to the phytosanitary treatments carried out on the grape as well as part of the elaboration process through the controlled addition of copper salts. Most of the copper is precipitated in the form of sulfites and filtered later. However, a high residual concentration of copper is toxic and can severely affect the alcoholic fermentation process, accelerate phenolic oxidation, cause turbidity and produce precipitates in reducing media. The control of copper concentration is essential to ensure both the stability during the maturation process and ensure safe consumption.

METHOD

Copper reacts specifically with the chromogen 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylaniline (3,5-diBr-PAESA) in the presence of sodium dodecyl sulfate (SDS).

The absorbance increase at 578 nm is directly proportional to the copper concentration.

CONTENT

R1	1 x 30 mL 	Acetate buffer (pH 4.5), SDS *WARNING: H319: Causes serious eye irritation. P262: Do not get in eyes, on skin, or on clothing. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.*
R2	1 x 10 mL	Acetate buffer (pH 4.5), 3,5-DiBr-PAESA
CTRL	1 x 1 mL	Copper 1,05 mg/L (0,89 – 1,21 mg/L)
STD	1 x 1 mL	Copper 1,90 mg/L

REAGENT PREPARATION

Reagents are ready to use. Keep them closed at 2-8 ºC when not in use and protected from light.

Discard if absorbance of blank is higher than 0.500 at 578 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.

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	Sample/Std
Distilled water	50 µL	--
Sample/Standard	--	50 µL
Reagent 1	750 µL	750 µL

Mix, incubate at 37ºC for 1 minute and read absorbance at 578 nm (A_1).

Then add

Reagent 2	250 µL	250 µL

Incubate at 37ºC for 3 minutes and read absorbance at 578 nm (A_2).

Concentration of copper is calculated as:

$$Copper = \frac{(A_2 - 0.77xA_1)_{\text{sample}} - (A_2 - 0.77xA_1)_{\text{blank}}}{(A_2 - 0.77xA_1)_{\text{standard}} - (A_2 - 0.77xA_1)_{\text{blank}}} \; x \; C \; \text{g/L}$$

Factor 0.77 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the standard label for copper.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	COPPER	
Method	Endpoint A	
Direction	Increasing	
Main Wavelength	578	
Sec. Wavelength	--	
Sample	10	
Reagent 1	150	
Reagent 2	50	
Calibration	Lineal	
Blank cycle [150 | 240]	3 - 4	3 - 4
Reading cycle [150 | 240]	10 - 11	8 - 9
Units	mg/L	
Decimals	2	
Measure range	0 ~ 5.0	
R1 Lim. Abs	5000	
Ratio Dil. Auto.	5	
Vol. Sample Dil. Auto	40	

Procedure is linear up to 5 mg/L. Calibrate with a single point using the highest concentration standard or with several points as per your quality procedures. Reagent blank must be requested at each session.

PERFORMANCE

Limit of quantification (LoQ): 0.15 mg/L
Limit of linearity: 5 mg/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. Abe A, Yamashita S, Noma A. Clin. Chem 1989; 35: 552-554.
3. Ribéreau-Gayon J, Peynaud E, Sudraud P, Ribéreau-Gayon P. Tratado de Enología. Ciencias y técnicas del vino. Vol 1, pp 259-261 (2008).