

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 560 nm is directly proportional to the copper concentration.

CONTENT

| | | |
|------|-----------|---|
| R1 | 1 x 45 mL | Acetate buffer (pH 4.5), SDS  <i>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.</i> |
| R2 | 1 x 15 mL | Acetate buffer (pH 4.5), 3,5-DiBr-PAESA |
| CTRL | 1 x 1 mL | Copper 1,05 mg/L (0,90 – 1,2 mg/L) |
| STD | 1 x 1 mL | Copper 2 mg/L |

REAGENT PREPARATION

Reagents are ready to use. Keep at 2-8 °C closed and preserved from light.

Discard if absorbance of blank is higher than 0.500 at 560 nm.

SAMPLES

For use with wine samples.

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. The presence of CO₂ introduces instability in the measure. Samples containing CO₂ must be degassed beforehand. In samples with very high colour intensity, the pigment may interfere with the measurement. Treat with polyvinylpyrrolidone (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 or S:R2 are not used exactly as stated.

Pipette into a cuvette:

| | Reaction Blank (RB) | |
|-----------------|---------------------|------------|
| | Blank | Sample/Std |
| Distilled water | 50 µL | -- |
| Sample/Standard | -- | 50 µL |
| Reagent 1 | 750 µL | 750 µL |

Mix, incubate at 37°C for 1 minute and read absorbance at 560 nm (A₁). Then add:

| | | |
|-----------|--------|--------|
| Reagent 2 | 250 µL | 250 µL |
|-----------|--------|--------|

Incubate at 37°C for 3 minutes and read absorbance at 560 nm (A₂).

Concentration of copper is calculated as:

$$Copper = \frac{(A_2 - 0.77 \times A_1)_{sample} - (A_2 - 0.77 \times A_1)_{blank}}{(A_2 - 0.77 \times A_1)_{standard} - (A_2 - 0.77 \times A_1)_{blank}} \times C \text{ g/L}$$

Factor 0.77 is used to correct absorbance after adding R2. C is the concentration value stated in the label of the standard.

ASSAY PARAMETERS FOR ANALYZER Y15/Y25®

| GENERAL | |
|------------------------|----------------|
| Name | COPPER |
| Analysis mode | Dif. Bireagent |
| Type of sample | ST1 |
| Units | mg/L |
| Direction | Increasing |
| Decimals | 0 |
| PROCEDURE | |
| Reading | Monochromatic |
| Sample | 12 |
| Reagent 1 | 180 |
| Reagent 2 | 60 |
| Washing | 1,2 |
| Predilution factor | |
| Main wavelenght | 560 |
| Sec. Wavelengt | -- |
| Reading 1 | 24s |
| Reading 2 | 192s |
| Reagent 2 | 48s |
| CALIBRATION | |
| Type of calibration | specific |
| Calibration curve | Linear |
| OPTIONAL | |
| Blank Limit absorbance | 0,5000 |
| Kinetic blank limit | -- |
| Linearity limit | 5 |

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).

