SD6015: CALCIUM

COLORIMETRIC - CPC

FOR USE IN WINE SAMPLES

PURPOSE OF THE TEST

Calcium is a natural element in the must although certain winemaking processes adds calcium salts (calcium carbonate for deacidification) and other calcium-rich substances (casein for clarification). The solubility of calcium decreases as the alcohol graduation increases, with a supersaturation state easily reaching, thus increasing the risk of calcium tartrate precipitates (and in some cases calcium oxalate as well) during aging inside the bottle, since its formation is very slow. This problem is particularly relevant in white wines because of its visibility. The control of calcium levels allows for the precipitation of said crystals, and their subsequent filtering, as part of the elaboration process before bottling.

METHOD

The o-cresophthalein dye forms a specific compound with calcium in an alkaline medium.

The calcium concentration is proportional to the absorbance at 578 nm.

CONTENT

R1	1 x 30 mL	TEA Buffer
R2	1 x 30 mL	o-cresophtalein, 8-hydroxyquinolin, preservatives
STD	1 x 5 mL	Calcium 100 mg/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 higher than 0.500 OD at 578 nm.

SAMPLES

For use with wine 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 reaction	Sample/Std Reaction
Reagent 1	400 µL	400 µL
Distilled water	10 µL	--
Sample/Standard	--	10 µL

Mix, incubate at 37ºC for 1 minutes and read absorbance at 578 nm (A_1). Then add into the cuvette:

	Blank reaction	Sample/Std Reaction
Reagent 2	400 µL	400 µL

Mix, incubate for 2 minutes at 37ºC and read absorbance at 578 nm (A_2).

Concentration of calcium is calculated as:

$$Calcium = \frac{(A_2 - 0.51xA_1)_{\text{sample}} - (A_2 - 0.51xA_1)_{\text{blank}}}{(A_2 - 0.51xA_1)_{\text{standard}} - (A_2 - 0.51xA_1)_{\text{blank}}} \; x \; C \text{ g/L}$$

Factor 0.51 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the label for calcium STD.

ASSAY PARAMETERS FOR ANALYZERS DIONYSOS®

Dionysos model	**150**	**240**
Name	CALCIUM	
Method	End Point A	
Direction	Increasing	
Main Wavelength	578	
Sec. Wavelength	--	
Sample	5	
Reagent 1	200	
Reagent 2	200	
Calibration	Linear	
Blank cycle [150 | 240]	3 – 4	3 - 4
Reading cycle [150 | 240]	20 - 21	31 - 32
Units	mg/L	
Decimals	0	
Measure range	5 ~ 100	
R1 Lim. Abs	5000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

Procedure is linear up to 100 mg/L. Calibrate with a single point using the highest concentration standard or with several points as per your quality procedures.

PERFORMANCE

Limit of quantification: 5 mg/L
Limit of linearity: 100 mg/L

NOTES

The calcium reagent is very sensitive. Use freshly obtained distilled water for the preparation of system liquid, washing solution, blanks and / or dilutions to avoid contamination of the sample from the water.

Using a control sample on a regular basis provides information on the calibration status and possible deterioration of the reagent. In case of deviations greater than 15% on the target value, it is advisable to check the calibration status of the test.

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.