PURPOSE OF THE TEST

The phenolic compounds of wine (natural phenols and polyphenols) are a broad group of chemical compounds that affect the flavour, colour and mouthfeel of wine contained in skin, pulp and grape seed. The specific distribution of them is what gives the wine its own particular characteristics, identifying the type of grape used and the production process. Its main function is to control the natural oxidation of the wine during the maturation and aging process and to increase the stability of its organoleptic properties.

METHOD

The Folin-Ciocalteu reagent contains molybdenum and tungsten salts capable of oxidizing phenolic groups in basic medium. The intensity of colour generated is measured at 750 nm and is proportional to the concentration of polyphenols.

CONTENT

R1	1 x 30 mL 	Carbonate buffer pH 13.0 *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 30 mL	Folin-Ciocalteu reagent
CTL	1 x 5 mL	Gallic acid anhydrous 2000 mg/L (1700 – 2300 mg/L)
STD	1 x 5 mL	Gallic acid anhydrous 3000 mg/L

REAGENT PREPARATION

Reagents are ready to use and are stable up to expiry date as supplied when stored at 15-25 ºC. Do not freeze.

Discard if absorbance of blank is higher than 0.300 OD at 750 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:R2 are not used exactly as stated.

Pipette into a cuvette:

	Blank reaction	Test Reaction
Reagent 1	400 µL	400 µL
Distilled water	6 µL	--
Sample/Standard	--	6 µL

Mix, incubate at 37ºC for 1 minutes and read absorbance at 750 nm (A_1). Then add into the cuvette:

	Blank reaction	Test Reaction
Reagent 2	400 µL	400 µL

Mix, incubate for 10 minutes at 37ºC and read absorbance at 750 nm (A_2).

Concentration of polyphenols, expressed as Gallic acid, is calculated as:

$$Polyphenols\ (Gallic\ acid) = \frac{(A_2 - 0.50xA_1)_{\text{sample}} - (A_2 - 0.50xA_1)_{\text{blank}}}{(A_2 - 0.50xA_1)_{\text{standard}} - (A_2 - 0.50xA_1)_{\text{blank}}}\ x\ C\ \text{mg/L}$$

Factor 0.50 is used to correct absorbances for dilution after adding reagent 2. C is the value of concentration stated in the standard label for polyphenols.

Results could also be expressed also referred to Tannic acid or Catechins as:

$$Gallic\ acid\ \text{mg/L}\ x\ 1.57 = Tannic\ acid\ \text{mg/L}$$

$$Gallic\ acid\ \text{mg/L}\ x\ 1.24 = Catechins\ \text{mg/L}$$

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	POLYPHENOLS	
Method	End Point A	
Direction	Increasing	
Main Wavelength	750	
Sec. Wavelength	--	
Sample	3	
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	20 ~ 3000	
R1 Lim. Abs	3000	
Ratio Dil. Auto.	–	
Vol. Sample Dil. Auto	–	

Procedure is linear up to 3000 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: 20 mg/L
Limit of linearity: 3000 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 – Method OIV-MA-AS2-10. OIV, Vol 1&2 (2008).
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4. Bermeyer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY. (1974).