SD6014: ANTHOCYANINS

COLORIMETRIC

FOR USE IN WINE SAMPLES

PURPOSE OF THE TEST

Anthocyanins are the main responsible for colour in red wine and contribute especially to the sensation of wine astringency. At the beginning of the ripening process, they form simple complexes with other compounds present in the wine, giving rise to a progressive change in the colour of the wine (turning to bluish tones) but as the maturation progresses, these compounds tend to form other compounds (often polymers) more stable in colour with respect to pH and sulfites. The proportion between the different compounds present (simple and polymeric) is directly related in the final colour obtained and its stability. This determination allows to establish the concentration of non-polymeric anthocyanins (ionizable).

METHOD

In a strongly acidic environment, the ionization of unpolymerized anthocyanins is produced. The concentration is proportional to the absorbance read at 520 nm.

CONTENT

R1	2 x 30 mL	Buffer (pH <1.0), ethanol (<12%), preservatives

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 0.300 OD at 520 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. 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 are not used exactly as stated.

Pipette into a cuvette:

	Blank reaction	Sample Reaction
Reagent 1	800 µL	800 µL
Distilled water	40 µL	--
Sample	--	40 µL

Mix, incubate at 37ºC for 10 minutes and read absorbance at 520 nm (A_1).

Concentration of anthocyanins is calculated as:

$$Anthocyanins = \left((A_1)_{\text{sample}} - (A_1)_{\text{blank}}\right) x\ 420$$

ASSAY PARAMETERS FOR ANALYZERS DIONYSOS®

Dionysos model	**150**	**240**
Name	ANTHOCYANINS	
Method	End Point A	
Direction	Increasing	
Main Wavelength	520	
Sec. Wavelength	--	
Sample	10	
Reagent 1	200	
Reagent 2	--	
Calibration	K Factor = 420	
Blank cycle [150 | 240]	2 - 2	2 - 2
Reading cycle [150 | 240]	18 - 19	28 - 29
Units	mg/L	
Decimals	0.0	
Measure range	0.4 ~ 800	
R1 Lim. Abs	3000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

Procedure is linear up to 800 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: 0.4 mg/L
Limit of linearity: 800 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.

Factor 420 is used to express the result in concentration of total anthocyanins. Use 350 as a factor if you want to express the result in concentration of malvidin-3-glycoside according to the official AOAC method.

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. (1974).
3. Somers, T.C., and Evans, M.E. (1977). J. Sci. Food Agric. 28:279.
4. Rivas-Gonzalo, J.C., Gutierrez, Y., Hebrero, E., and Santos-Buelga, C. (1992). Am. J. Enol. Vitic., 43:210.
5. AOAC Official method 2005.02. J. AOAC Int. 88, 1269(2005).