SD6032: ASCORBIC ACID

COLORIMETRIC - MTT

PURPOSE OF THE TEST

Ascorbic acid (vitamin C) is a powerful antioxidant that is used during the manufacturing process to quickly eliminate any presence of dissolved O_2 that could oxidize phenolic compounds (especially those derived from cinnamic acid) resulting in a darkening of the wine. Ascorbic acid very efficiently converts said oxygen into hydrogen peroxide that is subsequently neutralized by the SO_2 present in the wine (it is recommended that the wine contains between 30 and 50 mg/L of SO_2 before the addition of ascorbic acid to be effective in the elimination of hydrogen peroxide formed). It also prevents oxidation of iron ions as prevention of iron case. An excess of ascorbic can negatively affect the colour of the wine and its subsequent evolution.

METHOD

In acidic medium, ascorbic (and other reducing agents) reacts with the tetrazolium salt MTT (3- (4,5-dimethylthiazoli-2) -2,5-diphenyltetrazolium bromide) producing a coloured complex whose concentration is proportional to the concentration of them. To differentiate between ascorbic and the rest of other complexes, the result (RT) is compared with that obtained from a sample in which ascorbic has been completely removed by the specific action of ascorbate oxidase (RB). The absorbance difference at 578 nm between the two methods corresponds to the concentration of ascorbic acid.

CONTENT

R1	1 x 30 mL	Buffer pH 7.0
R2	1 x 25 mL	Buffer pH 7.0
R3	1 x 5 mL	Ascorbate Oxidase
R4	1 x 8 mL	MTT , PMS
ASC	1 x tube	Ascorbic acid (powder)

REAGENT PREPARATION

RB: Add the content of R3 to R2. This mixture is stable for one month at 2-8 ° C avoiding contamination.

R1 and R4 are ready to use. R4 is very sensitive to light. Keep it in dark and avoid direct sunlight.

ASC: Weigh an exact amount of ascorbic (150 mg) and dissolve it in 100 mL of distilled water. Diluted this solution 1/5 to be used as standard. This solution is stable for 8 hours at 2-8 ° C or one week frozen at -20 ° C. Keep well covered and protected from light. **The use of a calibrator is not necessary if you work with factor calibration mode**.

SAMPLES

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 0.1 g 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

Use distilled water as Blank. Once the calibration factor is established, this factor can be used in subsequent analysis without recalibration. The calibration factor depends on the light path and wavelength used.

Volumes stated below can be adjusted to other analytical procedures. Expected performance can vary if those ratios S:RB/R1:R4 are not used exactly as stated.

Pipette as per the scheme:

	Blank Reaction (RB)		Sample Reaction (RT)	
	Blank	Std	Blank	Sample
Reagent 1/RB	1000 µL	1000 µL	--	--
Reagent 1/R1	--	--	1000 µL	1000 µL
Distilled wáter	50 µL	--	50 µL	--
Sample/Std	--	50 µL	--	50 µL

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

	Blank reaction	Test Reaction
Reagent 2	150 µL	150 µL

Mix, incubate for 10 minutes at 37ºC and read absorbance at 578 nm (A_2).

The ascorbic acid is calculated as:

$$T_{RB} = \frac{(A_2 - 0.87xA_1)_{sample} - (A_2 - 0.87xA_1)_{blank}}{(A_2 - 0.87xA_1)_{standard} - (A_2 - 0.87xA_1)_{blank}} \; x\; C \; mg/L$$

$$T_{RS} = \frac{(A_2 - 0.87xA_1)_{sample} - (A_2 - 0.87xA_1)_{blank}}{(A_2 - 0.87xA_1)_{standard} - (A_2 - 0.87xA_1)_{blank}} \; x\; C \; mg/L$$

$$Ascorbic = (T_{RT} - T_{RB})\; mg/L$$

The factor 0.87 is used to correct the absorbance by dilution after adding R2. C represents the concentration of the standard.

ASSAY PARAMETERS FOR ANALYZERS DIONYSOS®

Dionysos model	**150**	**240**	**150**	**240**
Name	ASCORB-RB		ASCORB-RT	
Method	End Point A		End Point A	
Direction	Increasing		Increasing	
Main Wavelength	578		578	
Sec. Wavelength	--		--	
Sample	10		10	
Reagent 1	200		200	
Reagent 2	30		30	
Calibration	Factor*		Linear/Factor*	
Blank cycle [150 | 240]	3 - 4	3 - 4	3 - 4	3 - 4
Reading cycle [150 | 240]	20 - 21	28 - 29	20 - 21	28 - 29
Units	mg/L		mg/L	
Decimals	0		0	
Measure range	0 ~ 300		2 ~ 300	
R1 Lim. Abs	5000		5000	
Ratio Dil. Auto.	–		–	
Vol. Sample Dil. Auto	–		–	

*If you do not calibrate, use Factor=270 for Dio150 or Factor=300 for Dio240, for both RB and RT. Factor obtained through calibration could be slightly different depending on instrument and lot of reagents.
Use the reagent prepared as RB for the blank reaction (RB) or R1 for the sample reaction (RT) as Reagent 1. Use R4 as Reagent 2.

Use the option of calculated methods for ASCORBIC as:

[ASCORBICO] = [ASCORB-RT]-[ASCORB-RB]
Units: mg/L
Reference range: 2-300
Print experimental test: No

PERFORMANCE

Limit of quantification (LoQ): 0,5 mg/L
Limit of linearity: 300 mg/L

NOTES

O_2 in samples or reagents reacts rapidly with ascorbic, interfering with the analysis. Avoid vigorous agitation of samples and reagents.