SD6009: FREE SULFITES

COLORIMETRIC – FUCHSIN

FOR USE IN WINE SAMPLES

PURPOSE OF THE TEST

Sulfites are produced naturally during fermentation, but they are also added to the wine in order to stop the fermentation process (for example, the delay of malolactic fermentation in the presence of sulfites is well-known). A good part of sulfite is reversibly linked to other compounds such as sugars or polyphenols. The free sulfites (either in the form of SO_2, HSO_3^- or H_2SO_3), on the other hand, acts as a preservative to prevent deterioration both due to its antioxidant capacity and its antimicrobial action. Free sulfites have been associated with different allergic reactions, so its specific determination is a legal requirement in many countries.

METHOD

The method is based on the specific reaction of Fuchsin and formaldehyde with the bisulfite ions present in an acid medium to form a coloured complex. Other substances able to react with the fuchsin are discounted with a parallel reaction in which sulfites has been previously removed by an oxidant agent. Difference in the absorbance measured at 578 nm for both reactions is proportional to the concentration of free sulfites in the sample.

CONTENT

R1T	1 x 30 mL 	Buffer (pH < 1.5) *WARNING: H290: May be corrosive to metals. H317: May cause an allergic skin reaction. P262: Do not get in eyes, on skin, or on clothing.*
R1B	1 x 27 mL 	Buffer (pH <1.5) *WARNING: H290: May be corrosive to metals. H317: May cause an allergic skin reaction. P262: Do not get in eyes, on skin, or on clothing.*
R2	1 x 3 mL	Hydrogen peroxide
R3	2 x 6 mL	Fuchsin solution (< 0.2 mM), Formaldehyde (< 0.05%)
STD	1 x 3 mL	Sodium Metabisulfite 100 mg/L

REAGENT PREPARATION

Reagent (RT): Pour one vial of R3 in the R1T bottle. Mix gently avoiding foam formation. Wait at least 20 minutes before use. This mixture is stable for up to 15 days at room temperature (20-25 ºC) and preserved from light.

Blank Reagent (RB): Pour R2 vial and one R3 vial into R1B. Mix gently avoiding foam formation. Wait at least 20 minutes before use. This mixture is stable for up to 15 days at room temperature (20-25 ºC) and preserved from light.

Discard if the blank absorbance is greater than 0.500 OD at 578 nm.

SAMPLES

Use FREE SULFITE CAL (Code SD6009) for calibration. Stable 6 months at 15-25 ºC once opened. Sulfites are volatile: keep it tightly closed when not in use.

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. Use the standard included for both tests.

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

Pipette into separate cuvettes:

	Reaction Blank (RB)		Reaction Sulfite (RT)	
	Blank	Sample/Std	Blank	Sample/Std
Distilled water	20 µL	--	20 µL	--
Sample/Standard	--	20 µL	--	20 µL
Reagent 1	800 µL	800 µL	--	--
Reagent 2	--	--	800 µL	800 µL

Mix, incubate at 37ºC for 10 minutes and read absorbance at 578 nm for both reaction blank (RB) and reaction sulfite (RS).

Concentration of free sulphite is calculated as:

$$T_{RB} = \frac{(A_{sample} - A_{blank})}{(A_{std} - A_{blank})} \; x \; C \; mg/L \qquad T_{RT} = \frac{(A_{sample} - A_{blank})}{(A_{std} - A_{blank})} \; x \; C \; mg/L$$

$$Free\ Sulfite = (T_{RT} - T_{RB})\ mg/L$$

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**	**150**	**240**
Name	SO2-RB		SO2-RT	
Method	End Point A		End Point A	
Direction	Increasing		Increasing	
Main Wavelength	578		578	
Sec. Wavelength	--		--	
Sample	5		5	
Reagent 1	200		200	
Reagent 2	--		--	
Calibration	*Factor		Lineal	
Blank cycle [150 | 240]	2 - 2	2 - 2	2 - 2	2 - 2
Reading cycle [150 | 240]	20 - 21	31 - 32	20 - 21	31 - 32
Units	mg/L		mg/L	
Decimals	0		0	
Measure range	2 ~ 160		2 ~ 160	
R1 Lim. Abs	5000		5000	
Ratio Dil. Auto.	--		--	
Vol. Sample Dil. Auto	--		--	

*Use 10000/K value of calibration for SO2-RT, as K-factor for SO2-RB
Use Reagent RB for SO2-BLANK procedure, and Reagent RT for SO2-TEST procedure.
Use calculated methods for FREE SULFITE as:
[FREE SULFITE]
[SO2-RT]-[SO2-RB]
Units: mg/L
Reference range: 2-160
Print experimental test: No

Procedure is linear up to 160 mg/L. Calibrate with a single point using the standard provided or with multiple points as per your quality procedures.

PERFORMANCE

Limit of quantification (LoQ): 2 mg/L
Limit of linearity: 160 mg/L

NOTES

It is recommended to use control wines to verify the quality of the calibration. Each laboratory must establish its own acceptance criteria, as well as the necessary corrective actions in case of rejection.

REFERENCES

1. Compendium of International methods of analysis – OIV, Vol 1&2 (2008).