PURPOSE OF THE TEST

The iron is present in the wine coming from the grapes as well as from the remains of the earth and from contact with the tools used during the elaboration process. Iron is capable of forming complex-coloured salts and is therefore a critical element when it comes to bringing tonality to wine. An excess of iron, in addition to providing a bluish hue, can cause the appearance of precipitates of ferric phosphate (white) and ferric tannate (blue) in oxidation conditions.

METHOD

The 3-(2-pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5',5"disodium disulfonate (Ferene S) is able to complex iron (Fe^{2+}) in medium acid and in the presence of a reducing agent.

The concentration of iron present is the sample is proportional to the absorbance at 578 nm.

CONTENT

R1	2 x 30 mL 	Acetate buffer pH 4.5, Thiourea 50 mM *WARNING: H317: May cause an allergic skin reaction. P262: Do not get in eyes, on skin, or on clothing.*
R2	1 x 15 mL	Ferene S, Ascorbic acid 1 mM, preservatives
STD	1 x 5 mL	Iron solution 20 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 0.300 OD at 578 nm.

SAMPLES

It is advised to pre-dilute red wine 1:5 at sample request.

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	720 µL	720 µL
Distilled water	120 µL	--
Sample/Standard	--	120 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	180 µL	180 µL

Mix, incubate for 3 minutes at 37ºC and read absorbance at 578 nm (A_2).

Concentration of iron is calculated as:

$$Iron = \frac{(A_2 - 0.82xA_1)_{\text{sample}} - (A_2 - 0.82xA_1)_{\text{blank}}}{(A_2 - 0.82xA_1)_{\text{standard}} - (A_2 - 0.82xA_1)_{\text{blank}}} \; x\; C \text{ mg/L}$$

Factor 0.83 is used to correct absorbances for dilution after adding reagent 2. C is the value of concentration stated in the standard label for iron.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	IRON	
Method	End Point A	
Direction	Increasing	
Main Wavelength	578	
Sec. Wavelength	--	
Sample	24	
Reagent 1	240	
Reagent 2	60	
Calibration	Linear	
Blank cycle [150 | 240]	3 - 4	3 - 4
Reading cycle [150 | 240]	20 - 21	31 - 32
Units	mg/L	
Decimals	0.0	
Measure range	0.3 ~ 20.0	
R1 Lim. Abs	3000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

Procedure is linear up to 20 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 (LoQ): 0.26 mg/L
Limit of linearity: 20 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 – 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. Zoecklein BW, Fugelsang KC, Gump BH, Nury FS. Wine analysis and production. Van Nostrand Reinhold, 1st Ed. (1990).